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Research Activities in Dentistry

Petra Bořilová Linhartová, Jan Křivánek, Marcela Buchtová, Zdeněk Daněk, Alena Bryšová, Břetislav Lipový, Serhiy Forostyak, Jan Gajdziok, Zuzana Vranková, Michaela Cvanová, Tomáš Kratochvíl, Martin Vaculík, Filip Hromčík, David Száraz, Lydie Izakovičová Hollá

Objectives of the subject

— to broaden students' horizons in the field of scientific advances in dentistry

— to motivate students and strengthen their scientific ambitions

— and to lay the groundwork for their postgraduate studies and scientific careers

— orientation in the specialized literature in the field of dentistry

— possibility to participate in scientific and research projects of MED MUNI within this field

— familiarization with the principles of scientific work and publishing activities

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Organization of the subject

– Students will become acquainted with scientific advances in various areas of dentistry, including the research of the etiopathogenesis of specific diseases and their therapies.

– The course will take place as a block (1 week) in the form of seminars and excursions to specialized workplaces.

– The course will end with a colloquia – a debate on a student-selected scientific topic, which he/she will elaborate in writing before the colloquia.

Recommended literature

- DAY, Robert A. a Barbara GASTEL. How to write and publish a scientific paper. 6th ed. Cambridge: Cambridge University Press, 2008. xv, 302. ISBN 9780521671675.
- The Oxford textbook of clinical research ethics. Edited by Ezekiel J. Emanuel. New York: Oxford University Press, 2008. xx, 827. ISBN 0195168658.
- LACHIN, John M. Biostatistical methods : the assessment of relative risk. 1st ed. New York: John Wiley & Sons, 2000. xvii, 529. ISBN 0471369969

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Organization of the subject

Day 1

– Introduction (Bořilová Linhartová)

– Basics of methodology in scientific work (Bořilová Linhartová)

– Evidence levels in dentistry (Hromčík)

– Clinical trial methodology (not) only for dentists (Lipový)

– Questionnaires and psychometrics (Kratochvíl, Vaculík)

– Introduction to biostatistics (Cvanová)

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Organization of the subject

Day 2

– Genetic association studies and oral microbiome (Bořilová Linhartová)

• tooth decay (behavioral intervention, lactation)

• aphthous stomatitis

• diseases of the periodontal tissue

• external apical root resorption after the orthodontic treatment

• oral lichen planus, hypodontia and oligodontia, and others

– Molecular etiopathogenesis apical periodontitis and odontogenic cysts (Száraz, Bořilová Linhartová)

– **Excursion to the workplace and practical part I:** DNA isolation (Deissová, Bořilová Linhartová)

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Organization of the subject

Day 3

— Lipoxins and resolvins in the treatment of periodontitis (Hromčík)

— Use of nanofibres for application of bioactive substances in the prevention of oral diseases (Hromčík, Bořilová Linhartová)

— Obstructive sleep apnea (not only) in children from the perspective of a dentist & orthodontist (Vranková, Bořilová Linhartová)

— Congenital developmental anomalies of the orofacial region (cleft defects) (Bryšová)

— **Excursion to the workplace**

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Organization of the subject

Day 3

— Research and development of modern oral pharmaceutical forms (Gajdziok)

— Mucoadhesive films designed to cover defects of the oral mucosa (Daněk)

— Head and neck cancer – clinical studies, therapy, microbiome (Daněk, Bořilová Linhartová)

— 3D virtual planning and reconstruction of the lower jaw (Daněk)

— **Excursion to the workplace**

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Organization of the subject

Day 4

— Modern methods in studying the development and maintenance of the vitality of the tooth (Křivánek)

• methods of genetic mapping of in vivo development lines,

• study and evaluation of transcription at the level of individual cells,

• methods for finding new stem cells

• continuously growing teeth – what do they have to do with human teeth,

• use of modern three-dimensional imaging methods (confocal microscopy, uCT, FIB-SEM) in the study of the development and internal structure of the tooth.

— **Excursion to the workplace**

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Organization of the subject

Day 4

– Odontogenesis – use of alternative model organisms (Buchtová)

• Comparative odontogenesis (conventional and alternative model organisms, their advantages and disadvantages)

• Morphogenesis and regulation of the development of replacement generations of teeth

• Causes of alterations in the development of replacement generations in some model organisms with an emphasis on cellular and molecular processes

– **Excursion to the workplace**

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Organization of the subject

Day 5

— Translational research in life sciences: challenges and possibilities (Forostyak)

— **Excursion to the workplace and practical part II:** qPCR (Deissová, Bořilová Linhartová)

— **Colloquium** (Bořilová Linhartová)

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Basics of methodology in scientific work

Assoc. Prof. RNDr. Petra Bořilová Linhartová, Ph.D., MBA
Clinic of Stomatology, Department of Pathophysiology, Institute of Medical Genetics and Genomics, Faculty of Medicine, Masaryk University Brno
Clinic of Maxillofacial Surgery, University Hospital Brno

peta.linhartova@gmail.com

Content of the lecture

Basics of methodology in scientific work

- Motivation
- Introduction
- Work with scientific literature
- Research methodology
- Quality of research
- Planning
- Intellectual property
- Applied results
- Preparation of a scientific publication

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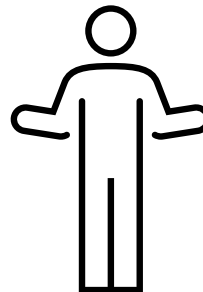
Research methodology

Research

— Basic research, the aim of which is to enrich knowledge, solve principal problems

— Applied research - the aim is to use the results in practice, solves specific practical problems

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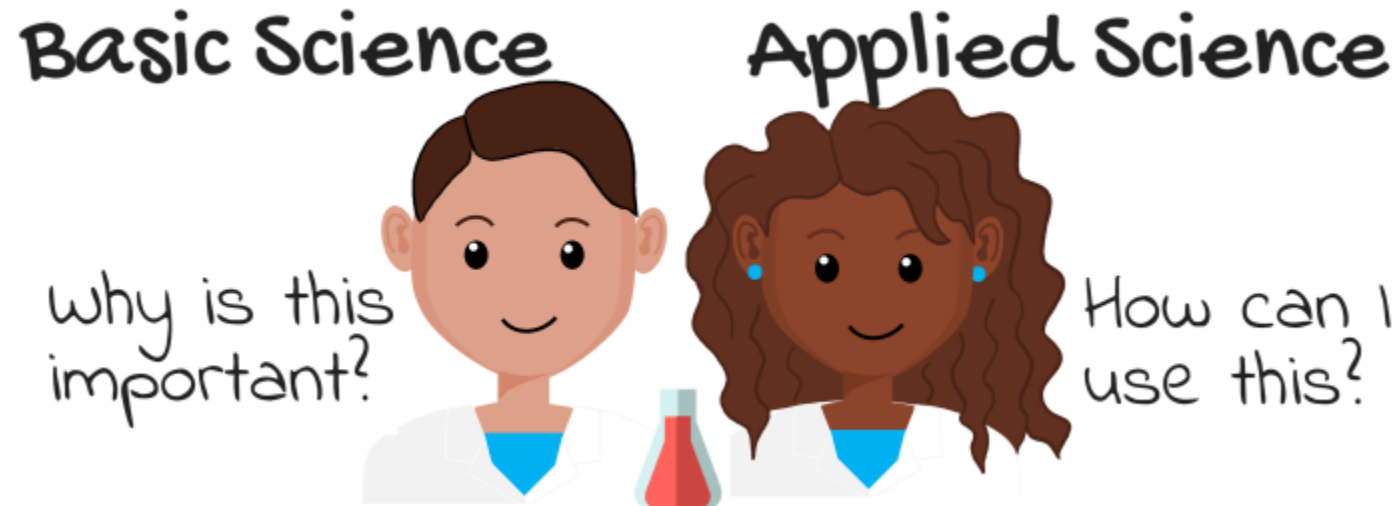
Research methodology

Introduction

— To start: What are we going to explore?

— Literature research: What do we already know about the problem?

— Methodology: How will we conduct the research?

— What data will we collect?

— Who will collect the data and where?

— How will we collect data?

— How will we analyze the data?

https://pubmed.ncbi.nlm.nih.gov/
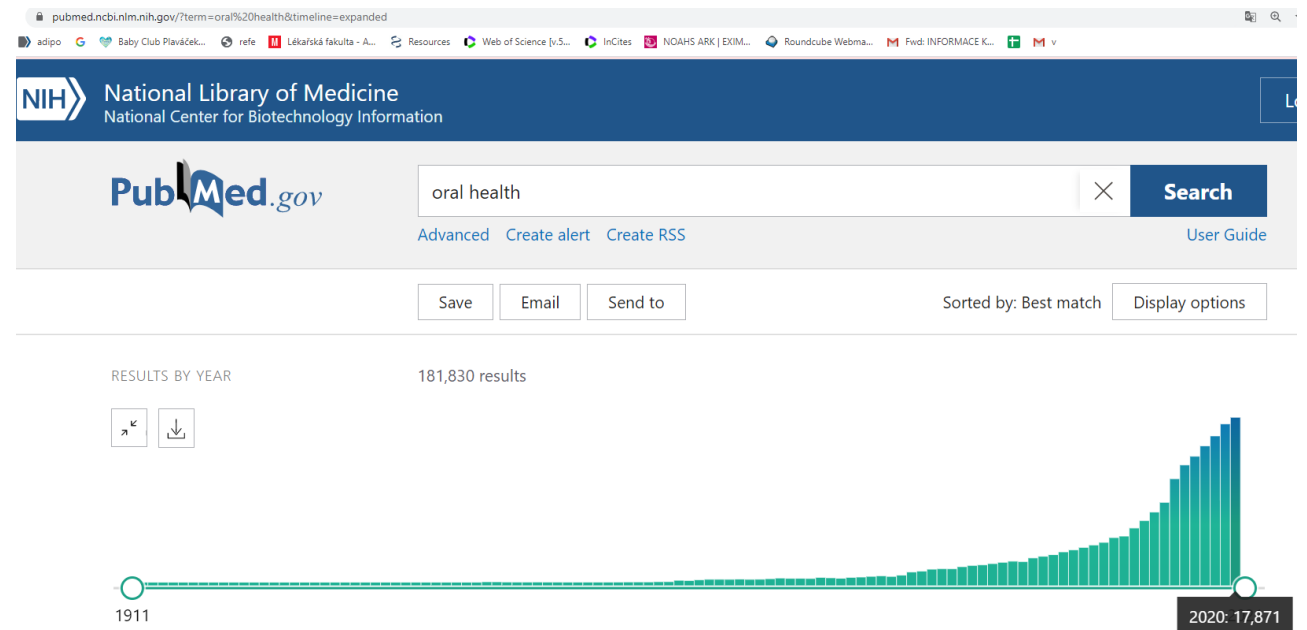
Research methodology

Research Philosophy: Hypothesis - Data - Alternative Hypothesis

— Hypothesis = assumption whose validity must be verified – confirmed or rejected

- Confirmatory research

- Exploratory research

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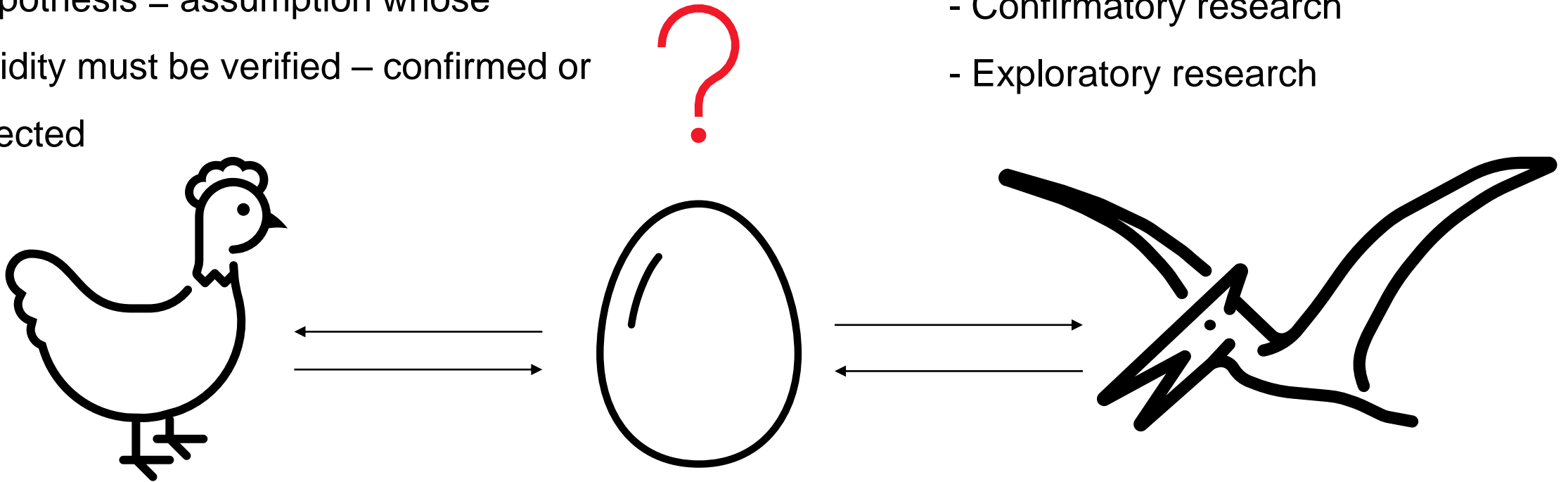
Research methodology

Research approach: Inductive vs. Deductive

— To start: What are we going to

explore?

— Provisional name

— Research questions

— Research objectives

— ... in accordance with the methodology

— **Pilot research**

— **Empirical research**

— Observation

— Measurement

— Experiment

— Questionnaire

— Interview

— Document analysis

— **Theoretical research**

— Analysis

— Synthesis

— Deduction

— Induction

— Analogy Comparison

— Generalization...

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Research methodology

Research strategy: Methods

Words, ideas, concepts

Numbers

Qualitative
Methods

Mixed
Methods

Quantitative
Methods

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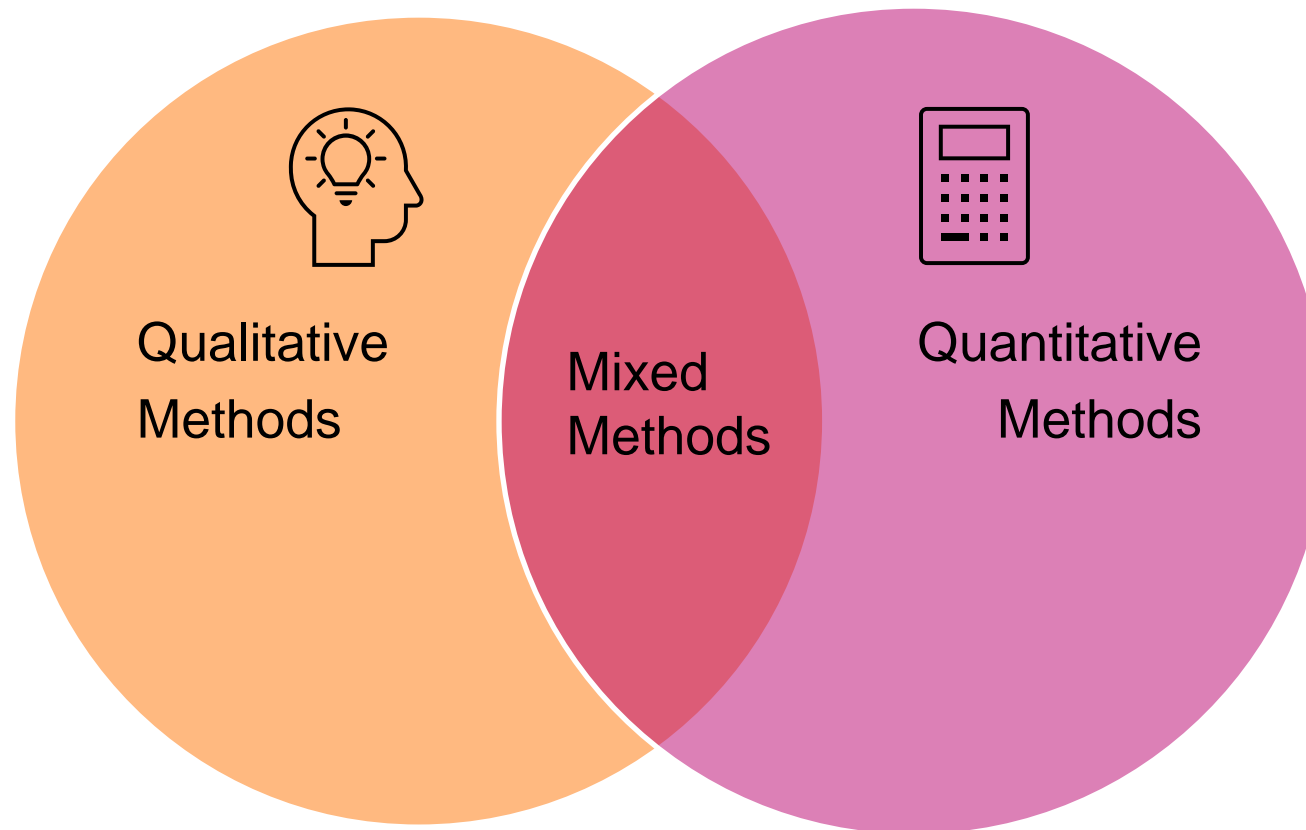
Research methodology

Quality of research

– **Validity** – ability of the research tool to identify or measure precisely what is the intention of the researcher

– **Reliability –** expresses the accuracy and reliability in the application of the research tool

– **Triangulation** – to increase the validity of research in cases where the use of only one method does not guarantee that the results are sufficiently objective and true

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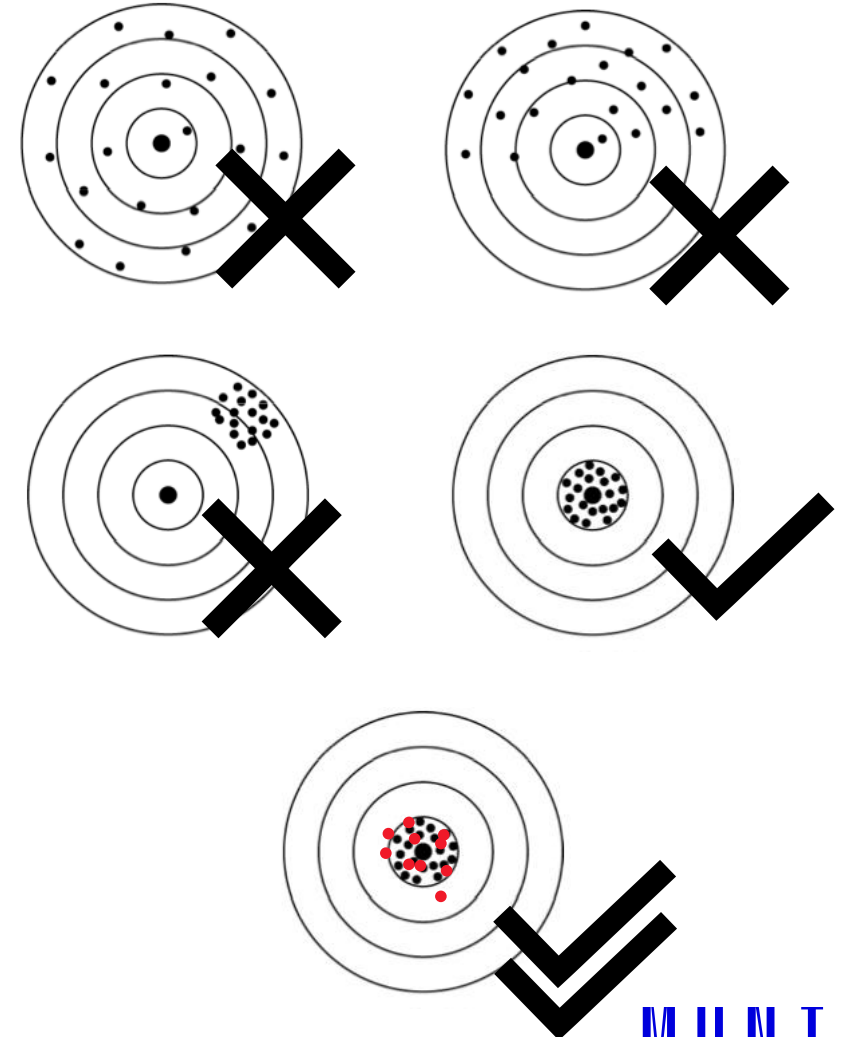
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Research quality: Representativeness (sampling)

Entire population

Population sample

Unrepresentative

Representative

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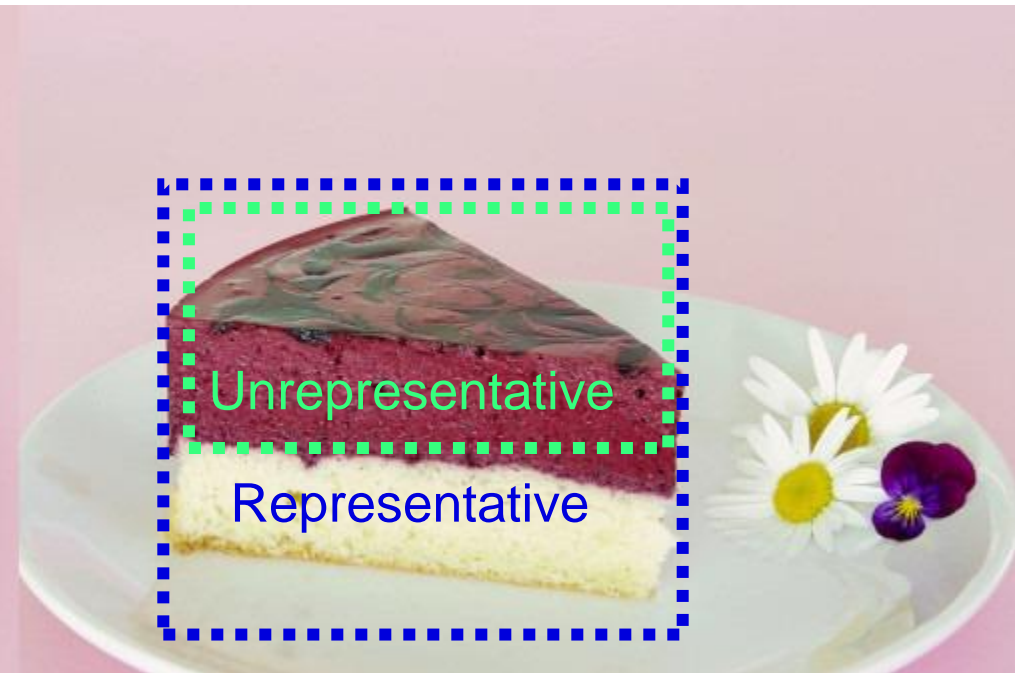
Research methodology

Planning

https://www.projectmanager.com/gantt-chart

GANTT CHART		2020		2021				2022				2023	
		5 - 9	10 - 12	1 - 3	4 - 6	7 - 9	10 - 12	1 - 3	4 - 6	7 - 9	10 - 12	1 - 6	7-12
CLINICAL PART (team ZD)	Clinical examination and collection of samples (blood, teeth, tissue, cystic fluid) from patients with OCs, AP and with ameloblastoma.	██	██	██	██	██	██	██	██	██	██		
	Histological examination of bioptic tissue samples.	██	██	██	██	██	██	██	██	██	██	██	
AIM 1 (I) (team PBL)	Isolation of bacterial DNA from root canal of teeth and from cystic fluid.												
	Analysis of root canals and RC fluids microbiome by metagenome targeting of 16S rRNA gene.		██	██	██	██	██	██	██				
AIM 1 (II) (team PBL)	Isolation of DNA from blood. Genetic association study using TaqMan qPCR assays – determination of genetic predisposition to OC or ameloblastoma (team PBL).			██	██	██	██	██	██	██			
	Multivariate analysis of host genetic background and microbiome.										██	██	
AIM 1 (III) (team MB)	Analysis of gene expression in bioptic tissue samples.		██	██	██	██	██	██	██				
AIM 2 (I) (team MB)	Establishing of mice models.	██	██										
AIM 2 (II) (team MB)	Analysis of odontogenic cyst formation and primary cilia in mice models			██	██								
AIM 2 (III) (team MB)	Gene expression changes of SHH pathway members in cysts.			██	██								
AIM 2 (IV) (team MB)	Application of AHH inhibitors and activators into mice models, their analysis.				██	██	██	██					
AIM 2 (V) (team MB)	Application nanoparticles into mice models, their analysis.				██	██	██	██					

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Research methodology

Intellectual property

– A product or form of expression created by one's own intellect that is new and unique (e.g. literary work, computer software, workflow, chemical formula, circulatory cycle, drug, genetically modified organism, industrial process) resulting from intellectual creative work

– intangible property (intangible assets, intangible goods)

– certain types of intellectual property can be formally protected (in various ways depending on the nature of the intellectual property)

– the results of the activity created by the originator (author) in the performance of tasks arising from his employment relationship with MU (another similar relationship with MU), or in the context of study duties

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Research methodology

Intellectual property

- Industrial technical solutions (e.g. inventions) and their protection (patents, utility models) designs, trademarks...

- Intellectual property protected by special legislation (confidential information, trade secrets, know-how...)

- Author's works and databases of scientific works (publications, scientific works), software...

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Research methodology

Authorship

— **The author** is someone who has made a substantial **intellectual contribution to the creation of the work**.

— Copyright – the rights of the author to his work, rights related to the copyright laws, and the right of the author to the database acquired by him.

— The order of authors should be a joint decision of all authors, reflect the degree of contribution to the text of the publication and the authors should be able to explain it.

— The first author should be the author who contributed the most to the creation and results of the publication.

— The corresponding author is usually the first or last to report, depending on industry practices.

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Research methodology

Applied results

— **Patent**

— **Utility model**

— **Design**

— **Prototype**

— **Functional sample ...**

— **Technology Transfer (TT)**   **https://www.ctt.muni.cz/**

— transfer of research results and development into practice

— the process of transferring various technologies (technical solutions and scientific research knowledge and experience) from the university environment towards practice and vice versa

— forms of TT (a knowledge):

— provision of rights of use (license)

— creation of spin-off companies owned by the university

— sale of rights, contracted research, collaborative research, provision of services and consultations...

Research methodology

Preparation of a scientific publication

— Topic and field?

— Publication output type?

— **Where to publish?**

— **How to start with paper writing?**

- Formal

- Content

- Visual page

- **Revision and language correction**

- **Review procedure**

Web of Knowledge

Web of Science | InCites | Journal Citation Reports | Essential Science Indicators | EndNote | Publons | Kopernio | Master Journal List

Web of Science

Citing Category Data

	Cited Journal	# Citing Journals	All Yrs ▾	2019	2018	2017
1	ALL Journals	91	344,971	3,801	16,820	24,193
2	J ENDODONT	83	8,635	85	377	773
3	CLIN ORAL IMPLAN …	84	8,519	20	378	636
4	J DENT RES	91	7,884	67	279	414
5	J CLIN PERIODONTOL	89	7,641	67	451	471
6	J PERIODONTOL	88	7,430	59	311	261
7	J PROSTHET DENT	83	6,972	58	352	403
8	AM J ORTHOD DENT…	85	6,900	52	154	246
9	DENT MATER	80	6,700	49	317	406
10	J ORAL MAXIL SURG	84	6,156	67	204	334
11	INT J ORAL MAX IMPL	82	5,513	33	160	333
12	OR SURG OR MED …	91	5,197	24	119	135
13	J DENT	90	5,091	67	301	341
14	INT ENDOD J	74	3,988	78	327	339
15	INT J ORAL MAX SU…	80	3,606	59	186	283
16	CLIN ORAL INVEST	90	3,419	190	428	448

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Levels of Evidence in Dentistry

MDDr. Filip Hromčík
Clinic of Stomatology, St. Anne's University Hospital
Faculty of Medicine, Masaryk University Brno

Content of the lecture

Levels of evidence in Dentistry

– Levels of evidence

– Examples of information sources

– Resource evaluation

– Orientation in the article

– Where to find answers, where to publish

– Academic research vs. science in private practice

– Own research questions

– Summary

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Levels of evidence

Systematic Reviews, Meta-analyses, Evidence Synthesis

Randomized Controlled Trials   RCTs

Cohort Studies

Case-Control Studies

Case Series, Case Reports

Expert Opinion

BIAS

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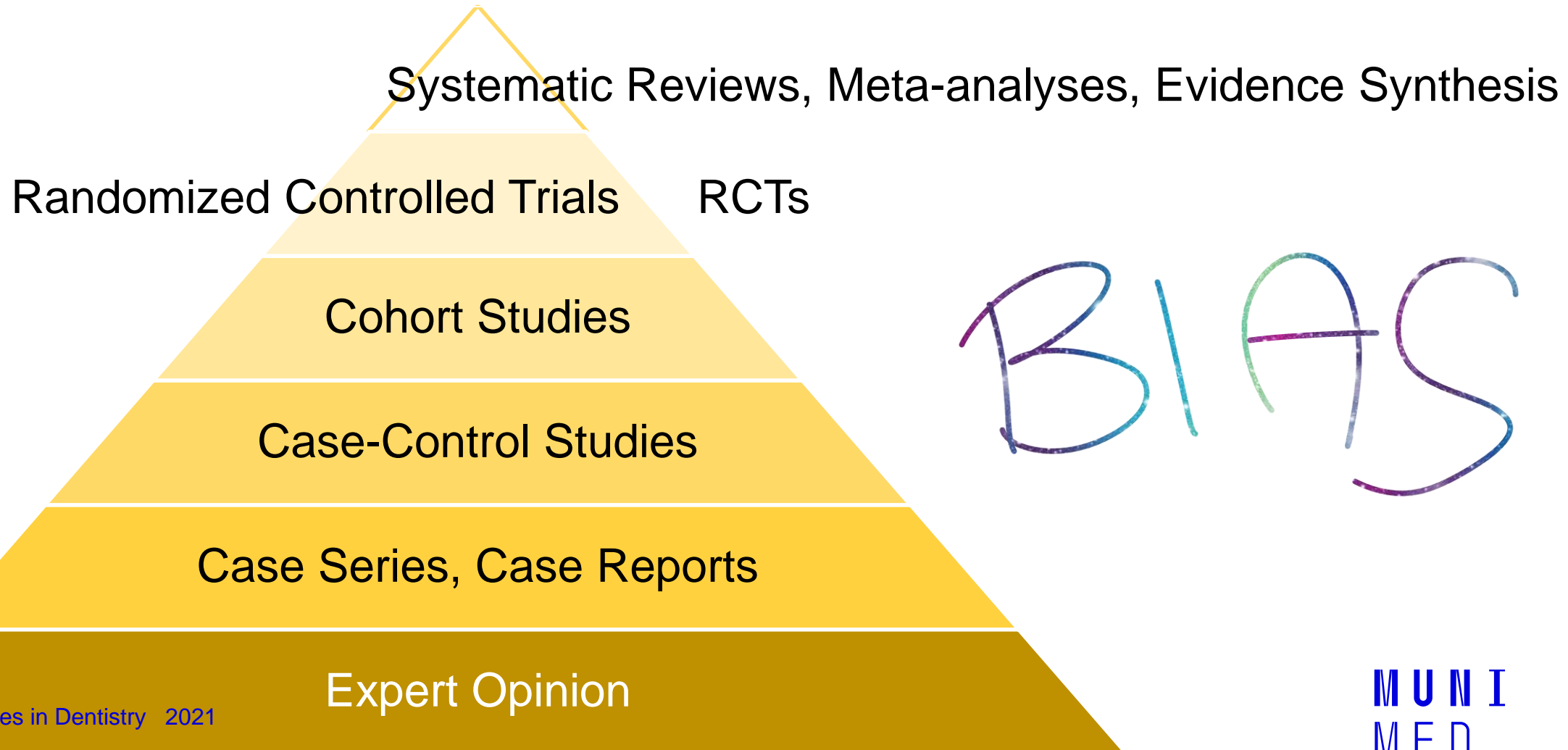
Sources of information for the dentist: Social networks

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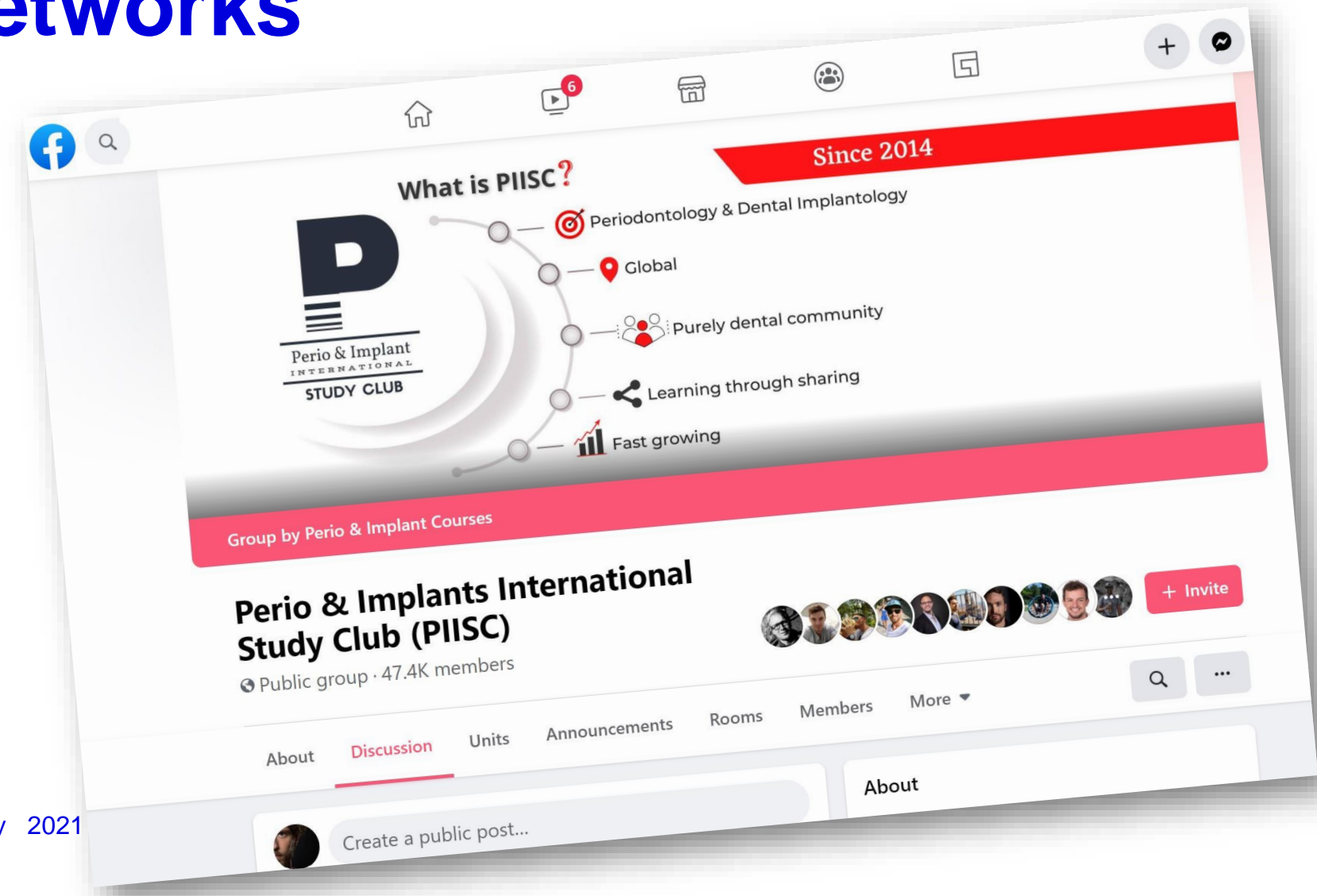
Source:

Lectures and courses

Saturday, March 7, 2020

Time	Session
08:30 – 09:45	**Socket grafting** | Moderator: P. Harrison N. Mardas: Hard tissue grafting R. Jung: Soft tissue grafting Discussion
09:45 – 11:30	**Ridge augmentation** | Moderator: N. Donos R. Allen: Tissue augmentation and aesthetic implant placement M. Schlee: Concepts for lateral augmentation I. Urban: Concepts for vertical augmentation Discussion
11:30 – 12:00	Coffee Break
12:00 – 13:45	**Soft tissue grafting to maintain peri-implant health** | Moderator: M. Sanz M. Roccuzzo: (before implant placement) S. Aroca: (during implant placement) G. Romanos: (after implant placement) Discussion
13:45 – 15:00	Lunch Break
15:00 – 16:55	**Complication management after reconstructive surgery at natural teeth and dental im** Moderators: T. O'Brien, I. Polyzois K. Murphy: Complication management after reconstructive periodontal surgery F. Lambert: Complication management after plastic esthetic peri D. Buser: Management of implant

Case report

International Journal of Oral and Dental [...]

CASE REPORT

Modified Laterally Flap Te[...] gival Phenotype: A Case R[...]

Davi da Silva Barbirato[1,2]*, Natália Ro[...] Magro[3], Mariana Fampa Fogacci[2] and [...]

[1]*Periodontist, Stomatologist, Maxillofacial Sur[...] University of Rio de Janeiro (UFRJ), Brazil*

[2]*Post-Doctoral Researcher, Federal University o[...]*

[3]*Federal University of Rio de Janeiro (UFRJ), Bra[...]*

[4]*Assistant Professor, School of Dentistry, Feder[...]*

***Corresponding author:** *Davi da Silva Barbirat[...] Researcher, External Advisor in Professional Mas[...] Direction, CEP 76800-000, Porto Velho, Rondonia,[...]*

Abstract

The aim of this study was to propose a new modified lateral positioned flap for root coverage of unfavorable gingival phenotype. The patient reported a dentine hypersensitivity and esthetic complaint in tooth 41 related to the gingival recession: "U"-shaped gingival contour, class II and type IV periodontium of "Miller" and "Maynard & Wilson" classifications, respectively. There was neither accident nor surgical complications. We observed a creeping reattachment between 3 and 6 months postoperative with an increasing in the periodontal attachment during the 18 months of follow-up. Total root coverage and the complete remission of dentin hypersensitivity were achieved.

Keywords

Gingival recession, Modified lateral positioned flap, Mucogingival flap, Periodontal aesthetics, Root coverage

Abstract

The aim of this study was to propose a new modified lateral positioned flap for root coverage of unfavorable gingival phenotype. The patient reported a dentine hypersensitivity and esthetic complaint in tooth 41 related to the gingival recession: "U"-shaped gingival contour, class II and type IV periodontium of "Miller" and "Maynard & Wilson" classifications, respectively. There was neither accident nor surgical complications. We observed a creeping reattachment between 3 and 6 months postoperative with an increasing in the periodontal attachment during the 18 months of follow-up. Total root coverage and the complete remission of dentin hypersensitivity were achieved.

thodontic treatment and occlusal trauma are etiological factors for gingival recessions [2-6]. The dental plaque and the consequent periodontal diseases, accompanied by clinical attachment loss, besides the local mechanical trauma, are primary etiological factors of gingival recessions. Among the secondary etiological risk factors, there are periodontitis risk factors, alveolar bone dehiscences and fenestrations. The amount of attached gingiva and the thickness of alveolar bone and gingival tissue can be considered as predisposing factors for gingival recessions too [3-6]. Main causes of recessions includes [...]

Barbirato et al., 2017

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Case series

¹... Dental Medicine, Medical Center –
University of Freiburg, Faculty of Medicine -
University of Freiburg, Freiburg, Germany

²Center for Medical Biometry and Medical
Informatics, Institute for Medical Biometry
and Statistics, Medical Center – University of
Freiburg, Faculty of Medicine - University of
Freiburg, Freiburg, Germany

Correspondence
Benedikt C. Spies, Department of Prosthetic
Dentistry, Medical Center – University of
Freiburg, Freiburg, Germany.
Email: benedikt.spies@uniklinik-freiburg.de

Abstract

Objective: To evaluate the clinical and patient-reported outcome of all-ceramic zirconia implant supported fixed dental prostheses (FDPs) 5 years after implant installation.

Materials and methods: Thirteen patients were treated with two terminally placed one-piece zirconia implants for a three-unit FDP each. The FDPs consisted of a CAD/CAM-fabricated zirconia framework over-pressed with a fluor-apatite veneering ceramic and were adhesively cemented. Survival and success were assessed by applying modified US Public Health Service (USPHS) criteria and preparation of Kaplan–Meier (KM) plots. Alpha and Bravo ratings were accepted for success (among others including small area veneer chippings and occlusal roughness), whereas Charlie ratings allowing for intra-oral correction (e.g., polishing) were accepted for survival. Furthermore, patient-reported outcome measures (PROMs) were analyzed with the help of visual analogue scales (VAS). Wilcoxon matched-pairs signed-rank test (USPHS criteria) and linear mixed models (PROMs) were used to evaluate time effects on response variables.

Results: All patients were available 61.8 ± 1.1 months after implant installation (53.6 ± 3.1 months after final prosthesis insertion). FDP survival was 100%. Significant incidence of veneer chipping ($p = .0096$) and occlusal roughness ($p = .0019$) was observed. Charlie rated extent of both phenomena resulted in a KM success estimate of 38.5% (95% CI: 14.1%–62.8%; seven FDPs with obvious roughness, three of them with extended veneer chipping). Compared with the pre-treatment assessments (30%–81% of satisfaction), all surveys at prosthetic delivery showed significantly improved VAS scores (66%–93%; $p \le .038$), except for speech ($p = .341$). Concerning function, esthetics and self-esteem, no decrease in satisfaction could be observed until the end of follow-up (90%–96%; $p \ge .057$), whereas perception of sense (92%) and speech (95%) increased over time ($p \le .030$). Occurrence of technical complications did not correlate with patient satisfaction.

Conclusions: Bi-layered FDPs made from zirconia/fluor-apatite highly satisfied patients but showed significant incidence of technical complications.

KEYWORDS

CAD-CAM, case series, ceramic...

Spies et al. 2017

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Cohort study

A prospective cohort study of endodontic treatments of 1,369 root canals: results after 5 years

Domenico Ricucci, MD, DDS,[a] John Russo, DMD,[b] Michael Rutberg, DMD,[c] Josef A. Burleson, PhD,[d] and Larz S. W. Spångberg, DDS, PhD,[e] Rome, Italy; and Farmington, Connecticut
UNIVERSITY OF CONNECTICUT

Objective. The purpose of this prospective study was: 1) to follow-up a large number of endodontic treatments performed by a single operator, periodically checked over a 5-year period; and 2) to correlate outcome to a number of clinical variables.

Study design. This prospective study included all consecutive cases during the selected time period. All cases were followed regularly for a 5-year period. At the 5-year end point of the study, 470 patients with 816 treated teeth and with 1,369 treated root canals were available for evaluation.

Results. The overall rate of success among the 816 teeth/1,369 root canals available for evaluation was 88.6%/90.3%. The success rate for 435 teeth/793 root canals undergoing vital pulp therapy was 91.5%/93.1%. Teeth/root canals with necrotic pulp but without detectable periapical bone lesion were successfully treated in 89.5%/92.3%. If the pulp necrosis was complicated by apical periodontitis, the success rate fell to 82.7% for the teeth and 84.1% for the root canals ($P = .037$). Teeth with periapical lesion <5 mm had a success rate of 86.6%, and in cases where the lesion was ≥5 mm the rate of success was 78.2%.

Conclusions. More severe disease conditions negatively affects outcome. An optimal working length was identified. Excess of root canal filling material decreases success. Infected pulp space should be treated with an effective antimicrobial treatment. The quality of the coronal restoration or the placement of intracanal post retentions does not affect treatment outcome. **(Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:825-842)**

RCT

Castro dos Santos, et al. 2020

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HUMAN RANDOMIZED CONTROLLED TRIAL

Omega-3 PUFA and aspirin as adjuncts to periodontal debridement in patients with periodontitis and type 2 diabetes mellitus: Randomized clinical trial

Nidia C. Castro dos Santos[1,2,3] | Naira M. R. B. Andere[1] | Cássia F. Araujo[1] | Marco[1] | Alpdogan Kantarci[2] | Thomas E. Van Dyke[2] | Mauro P. Santamaria

[1]Division of Periodontics, Unesp – São Paulo State University, Institute of Science and Technology, São José dos Campos, São Paulo, Brazil

[2]Center for Clinical and Translational Research, The Forsyth Institute, Cambridge, Massachusetts, United States

[3]Dental Research Division, Guarulhos University, Guarulhos, São Paulo, Brazil

Correspondence
Mauro P. Santamaria, Principal investigator, Division of Periodontics, Institute of Science and Technology, São Paulo State University (Unesp), Av. Eng. Francisco José Longo, 777, São José dos Campos, SP 12245-000, Brazil.
Email: mauro.santamaria@unesp.br

Abstract

Background: Supplementation with omega-3 polyunsaturated fatty acids (ω-3 PUFA) and low-dose aspirin (ASA) have been proposed as a host modulation regimen to control chronic inflammatory diseases. The aim of this study was to investigate the clinical and immunological impact of orally administered ω-3 PUFA and ASA as adjuncts to periodontal debridement for the treatment of periodontitis in patients type 2 diabetes.

Methods: Seventy-five patients (n = 25/group) were randomly assigned to receive placebo and periodontal debridement (CG), ω-3 PUFA + ASA (3 g of fish oil/d + 100 mg ASA/d for 2 months) after periodontal debridement (test group [TG]1), or ω-3 PUFA + ASA (3 g of fish oil/d + 100 mg ASA/d for 2 months) before periodontal debridement (TG2). Periodontal parameters and GCF were collected at baseline (t0), 3 months after periodontal debridement and ω-3 PUFA + ASA or placebo for TG1 and CG (t1), after ω-3 PUFA + ASA (before periodontal debridement) for TG2 (t1), and 6 months after periodontal debridement (all groups) (t2). GCF was analyzed for cytokine levels by multiplex ELISA.

Results: Ten patients (40%) in TG1 and nine patients (36%) in TG2 achieved the clinical endpoint for treatment (less than or equal to four sites with probing depth ≥ 5 mm), as opposed to four (16%) in CG. There was clinical attachment gain in moderate and deep pockets for TG1. IFN-γ and interleukin (IL)-8 levels decreased over time for both test groups. IL-6 levels were lower for TG1. HbA1c levels reduced for TG1.

Conclusion: Adjunctive ω-3 and ASA after periodontal debridement provides clinical and immunological benefits to the treatment of periodontitis in patients with type 2 diabetes.

KEYWORDS
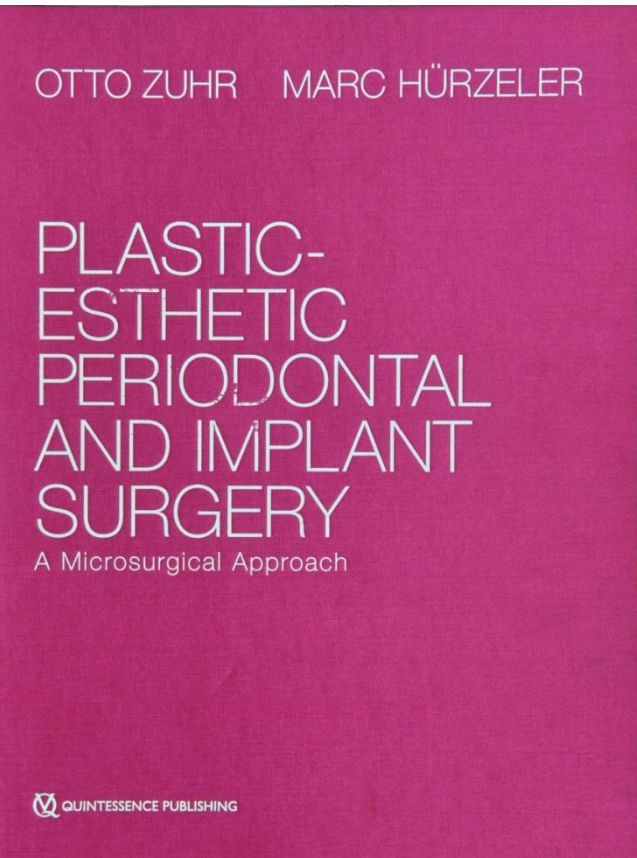aspirin, diabetes, immunomodulation, inflammation, omega-3 fatty acids, periodontitis

Meta-analysis, systematic review

Jepsen et al. 2020

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SYSTEMATIC REVIEW

Journal of Clinical Periodontology | WILEY

Regenerative surgical treatment of furcation defects: A systematic review and Bayesian network meta-analysis of randomized clinical trials

Søren Jepsen[1] | Stefano Gennai[2] | Josefine Hirschfeld[3] | Zamira Kalemaj[4] |
Jacopo Buti[5] | Filippo Graziani[2] |

[1]Department of Periodontology, Operative and Preventive Dentistry, University Hospital Bonn, Bonn, Germany

[2]Sub-Unit of Periodontology, Halitosis and Periodontal Medicine, University Hospital of Pisa, Pisa, Italy

[3]Birmingham Dental School & Hospital, Birmingham, UK

[4]Private Practice, Milano, Italy

[5]Unit of Periodontology, UCL Eastman Dental Institute, London, UK

Correspondence
Søren Jepsen, Department of Periodontology, Operative and Preventive Dentistry, University Hospital Bonn, Welschnonnenstr. 17, 53111 Bonn, Germany.
Email: sjepsen@uni-bonn.de

Abstract

Aims: To investigate the clinical performance of regenerative periodontal surgery in the treatment of furcation defects versus open flap debridement (OFD) and to compare different regenerative modalities.

Material and Methods: A systematic search was conducted to identify RCTs evaluating regenerative surgical treatment of furcations with a minimum of 12-month follow-up. Three authors independently reviewed, selected and extracted data from the search conducted and assessed risk of bias. Primary outcomes were tooth loss, furcation improvement (closure/conversion) (FImp), gain of horizontal bone level (HBL) and attachment level (HCAL). Secondary outcomes were gain in vertical attachment level (VCAL), probing pocket depth (PPD) reduction, PROMs and adverse events. Data were summarized into Bayesian standard and network meta-analysis in order to estimate direct and indirect treatment effects and to establish a ranking of treatments.

Results: The search identified 19 articles, reporting on 20 RCTs (19 on class II, 1 on class III furcations) with a total of 575 patients/787 defects. Tooth loss was not reported. Furcation closure ranged between 0% and 60% (10 trials), and class I conversion from 29% to 100% (six trials). Regenerative techniques were superior to OFD for FImp (OR = 20.9; 90% CrI = 5.81, 69.41), HCAL gain (1.6 mm), VCAL gain (1.3 mm) and PPD reduction (1.3 mm). Bone replacement grafts (BRG) resulted in the highest probability (Pr = 61%) of being the best treatment for HBL gain. Non-resorbable membranes + BRG ranked as the best treatment for VCAL gain (Pr = 75%) and PPD reduction (Pr = 56%).

Conclusions: Regenerative surgery of class II furcations is superior to OFD. FImp (furcation closure or class I conversion) can be expected for the majority of defects. Treatment modalities involving BRG are associated with higher performance.

Textbooks

A mixture of evidence of different levels

MUNI
MED

OTTO ZUHR   MARC HÜRZELER

PLASTIC-
ESTHETIC
PERIODONTAL
AND IMPLANT
SURGERY

A Microsurgical Approach

Zuhr et Hürzeler 2012, s. 30

Individuals with the thin gingival biotype have a high risk of developing gingival recession on buccal tooth surfaces and in the papillary region after prosthetic, orthodontic, and surgical interventions. Conversely, individuals with the thick gingival biotype are less susceptible to gingival recession but have an increased risk of inflammation and pocket formation following treatment.[76,78–80]

The classification of thick and thin gingival biotypes is subjective and operator dependent because the borderline between what constitutes the different biotypes can be unclear (Fig 1-31). Based on clinical experience, there seems to be a direct correlation between tooth morphology and gingival biotype. Therefore, it would seem that tooth morphology could be useful as an objective parameter of gingival biotype, as was proposed by *Olsson* et al.[76,77] However, there is no scientific evidence confirm-
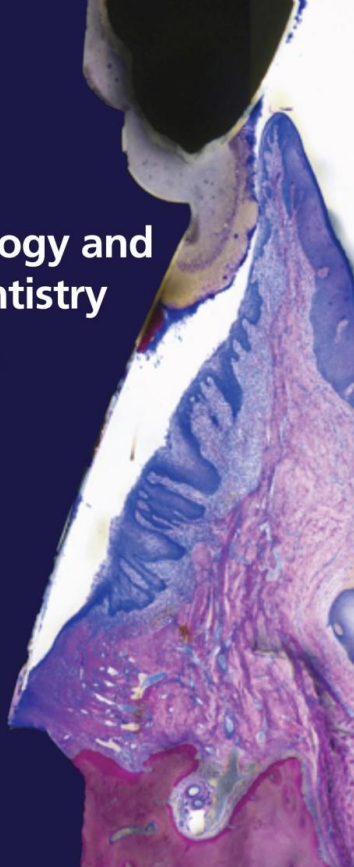
Lang et Lindhe, 2015

smoking is much greater than that due to other systemic predispositions, such as diabetes mellitus. Data derived from the NHANES III study (Tomar & Asma 2000) suggested that as many as 42% of periodontitis cases in the US can be attributed to current smoking and another 11% to former smoking. Similarly, a study from Brazil (Susin *et al.* 2004b) reported that the attributable fraction of clinical attachment loss due to cigarette smoking was 37.7% and 15.6% among heavy and moderate smokers, respectively. In longitudinal studies, smoking has been found to confer a statistically significant increased risk for periodontitis progression after adjustment for other co-variates (Beck *et al.* 1995, 1997; Machtei *et al.* 1999; Norderyd *et al.* 1999; Chen *et al.* 2001; Ogawa *et al.* 2002; Paulander *et al.* 2004b).

Figure 7-4 shows an early *meta-analysis* of data from studies reporting on the association between smoking and periodontal conditions. In essence, meta-analysis is a statistical method which combines results from different studies of similar design, in order to achieve increased *power* to detect epidemiologic associations that may be difficult to identify in smaller, individual studies (Oakes 1993; Chalmers 1993; Proskin & Volpe 1994). This analysis, initially published as part of the 1996 World Workshop in Periodontics (Papapanou 1996), incorporated data from six studies, including a total of 2361 subjects with known smoking habits and periodontal status (Bergström & Eliasson 1989; Haber & Kent 1992; Locker 1992; Haber *et al.* 1993; Stoltenberg *et al.* 1993; Grossi *et al.* 1994). It can be observed that smoking was an overall increased, statistically and clinically, significant risk for severe disease (estimated overall
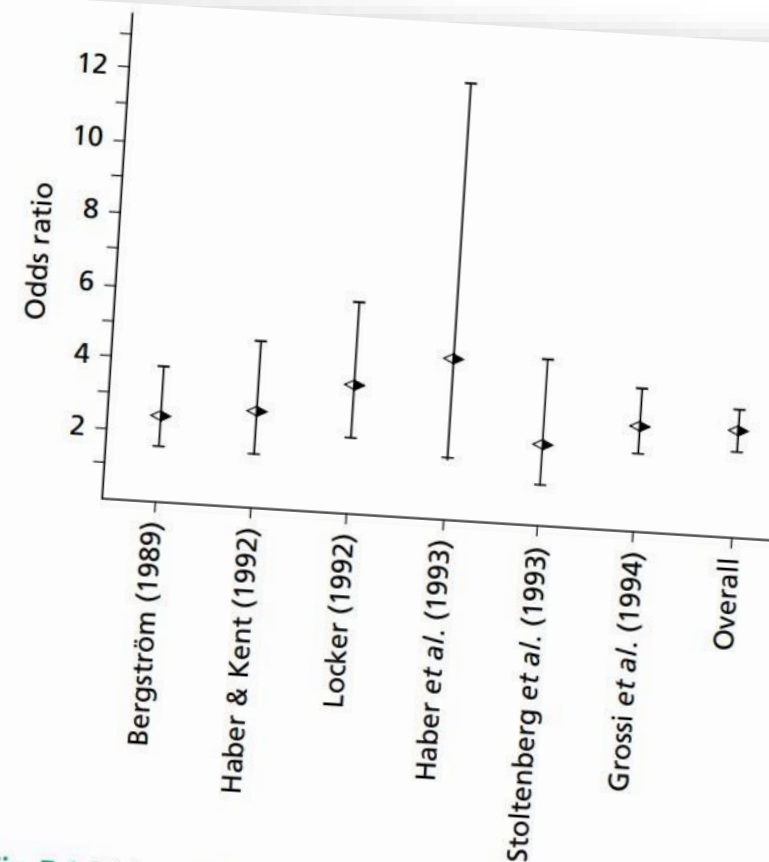
Fig. 7-4 Meta-analysis of smoking as a risk factor for periodontal disease. The studies included are: Bergström (1989), Haber & Kent (1992), Locker (1992), Haber *et al.* (1993), Stoltenberg *et al.* (1993), and Grossi *et al.* (1994). Bars indicate the 95% confidence limits for the depicted odds ratios. (Source: Papapanou 1996. Reproduced from American Academy of Periodontology.)

1994; Kaldahl *et al.* 1996; Grossi *et al.* 1997; Kinane & Radvar 1997; Tonetti *et al.* 1998; Trombelli *et al.* 2003, Stavropoulos *et al.* 2004; Paulander *et al.* 2004a; Rieder *et al.* 2004; Sculean *et al.* 2005; Wan *et al.*, 2009). Notably, these studies have confirmed the negative effect of smoking on the outcome of

Exercise: orientation in an article - RCT

Borges, I., Faveri, M., Figueiredo, L. C., Duarte, P. M., Retamal, V. B., Montenegro, S. C. L., & Feres, M. (2017). Different antibiotic protocols in the treatment of severe chronic periodontitis: A 1-year randomized trial. *Journal of Clinical Periodontology, 44*(8), 822–832. https://doi-org.ezproxy.muni.cz/10.1111/jcpe.12721

1. What was the aim of the study ?

2. How was the control group defined?

3. How many subjects were enrolled?

4. For how long were they followed?

5. What was clinically evaluated, the primary observed parameter?

6. What does the study show?

7. The value of evidence and bias?

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The basic pillar of evidence-based medicine and the biggest difference between literature and opinions in lectures or on social networks?

peer review

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Systematic Reviews,
Meta-analyses,
Evidence Synthesis

Randomized
Controlled Trials (RCTs)

Cohort Studies

Case-Control Studies

Case Series, Case Reports

Expert Opinion

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Where to look for answers?

Am I looking for inspiration and motivation?	Lectures, social networks (be critical!)
I wonder how someone else solved this problem?	Case report, case series
Do I need to understand something?	Textbook, narrative review, expert opinion
Is there consensus in this area?	Systematic review, meta-analysis
Has anyone compared these methods of treatment?	RCT

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At what level to contribute?

Am I a respected expert and would I like to share my views and experiences?

Expert opinion (lecture, workshop), social networks

Would I like to verify a new procedure?   RCT

Do I want to evaluate the influence of various factors over a longer period of time? Cohort study

I like reading and writing, I am systematic, I have a lot of time and I would like to summarize the knowledge in some area?

Systematic review, meta-analysis

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At what level to contribute?

Did I solve an exceptional case and documented it exemplarly?

Case report

Do I want to show that the success of my procedure was no accident?

Case series

Am I a student and would like to discuss a topic, evaluate the current view of this issue and draw practical conclusions?

Narrative review, evidence synthesis

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Own research question

Exercise

— formulate a research question

— prepare design and a possible output

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Academic research
vs.
science in private practice

— scientific question (academic, practical?)

— grants, funding

— systematic evaluation, data collection

— cooperation and equipment

— publication

— a scientist or a clinician?

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Research in private practice

— the right motivation and goal

— determination and perseverance

— source of support, grants???

— connection with a university, library, laboratory

— get to know legislation and ethics

— tools, databases, support

— meaning of the obtained data

více v Hare et al., 2018

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Research at the University

— cooperation, courses, support, patronage

— resources (library), equipment, funding (grants)

— sharing, continuity

— mentors

— doctoral studies (PhD.), academic career

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Key points

— Increase the share of information obtained from peer-reviewed sources with little bias, evaluate others very critically

— Verify information, perceive its different value

— Benefit from EBM and contribute to EBM

— Develop your thoughts at any level

— Have fun with science and be a part of it

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Abbreviations used

- EBM – evidence-based medicine

- RCT – randomized controlled trial, randomized clinical trial

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Clinical trial methodology (not only) for dentists

Assoc Prof. MUDr. Břetislav Lipový, Ph.D., MBA
Department of Burns and Plastic Surgery, University Hospital Brno,
Faculty of Medicine, Masaryk University Brno

Introduction of the researcher and his team

Assoc. Prof. MUDr. Břetislav Lipový, Ph.D., MBA

– **Clinical trials experience**

2007 – Indication of Veloderm® in partial thickness burns - consultant

2008-2009 – Xe-derma® in partial thickness burns (co-investigator)

2010 – Mepilex Ag® in adult and pediatric burns (co-investigator)

2013 – Principal investigator Birken clinical trial (Oleogel-S10)

2014 – Principal investigator Nexobrid™ in adult patients

2014 – Principal investigator Nexobrid™ in pediatric patients

2019 – Principal investigator The RE-ENERGIZE Study: A RandomizEd trial of ENtERal
Glutamine to minimIZE thermal injury (study terminated)

2019 – Principal investigator Reducing microbial burden in acute wounds using non-thermal
plasma.

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Content of the lecture

Clinical trial methodology (not) for dentists only

— Brief history of clinical trials

— Basic principles of a clinical trial

— Human subjects in a clinical trial

— Types of clinical trial

— Ethics Committee and informed consent

— Clinical trial in specific groups (children)

— GCP certificate (training)

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"Biomedical progress is based on research, which necessarily includes studies on human subjects"

„The patient must be treated as the student's teacher, not as a training tool."

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Brief history of the clinical trial

– (Book of DANIEL) one of the books of the Old Testament

– Then Daniel said to the guard whom the master of the eunuchs had put in charge of

  Hananiah, Mishael, Azariah and himself, „*Submit to us this test for ten days. Give us only*

  vegetables to eat and water to drink; then compare our looks with those of the young men

  who have lived on the food assigned by the king, and be guided in your treatment of us by

  what you see". (150BC)

<div align="center">

**Nonrandomized Concurrent Control Study
vs.
Randomized Control Trial**

</div>

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Brief history of the clinical trial

— **Randomization**

— This concept was used for the first time in a clinical trial by R.A. Fisher (farmers) – the distribution of their parcels with crops

—   Fisher RA., 1926

   • Probably the first randomization in biomedical research was used by Amberson and McMahon, who observed the therapeutic effect of sanocrysin in the therapy of the pulmonary form of TB

   •   Amberson B. et al., 1931

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Brief history of the clinical trial

— **Blinding**

— Again, studies related to TB therapy

- Evaluation by two radiologists and an X-ray clinic without knowing if patients are in the control or treated group

Medical Research Council. Streptomycin treatment of pulmonary tuberculosis. *Br. Med J.* 1948;2:769-782

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Three important documents relevant to research ethics

‒ Nuremberg Code (1947)

‒ Declaration of Helsinki (1964) – Revision WMA 1975, 1983, 1989, 1996, 2000, 2002, 2004,

2008

‒ The Belmont Report 1979 (regulates research in the US)

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The Nuremberg Code

Nuremberg process (20. 11. 1945 – 1. 10. 1946)

- A total of ten points contain the following
  - Voluntary consent of the patient
  - Results cannot be obtained by other methods
  - The experiment was successfully conducted on animals
  - Avoid all unnecessary physical and mental suffering
  - If there is a reasonable suspicion that it may lead to death or disability, the experimentmust be terminated.
  - The degree of risk must never outweigh the potential benefit to the patient.
  - Execution only after ensuring the access of sufficient equipment protecting from an even remote possibility of damage, disability or death
  - Scientifically qualified people
  - During the experiment, the human subject can quit anytime
  - The scientist must be prepared to end the experiment if he finds that the continuation may lead to damage of the subject

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Declaration of Helsinki 1964

– Total of 32 sections

– Definition of incompetent human subjects

  • Persons under 18 years of age

  • Entities whose decision-making capacity is compromised as a result of physical and/or mental conditions

– **Does the Helsinki Declaration correspond to the current concept of science?**

– Distinguishing between therapeutic and non-therapeutic research

– Contentiousness in control groups (placebo)

  • Responsibility for the health of the subject lies solely with him (not the researcher)

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The Tuskegee syphilis study

„we learned more about racism
than syphilis"

Participants in the Tuskegee Syphilis Study.
(Credit: National Archives)

Herman Shaw speaks as President Bill Clinton looks on during ceremonies at the
White House on May 16, 1997, during which Clinton apologized to the survivors
and families of the victims of the Tuskegee Syphilis Study.
(Credit: PAUL J. RICHARDS/AFP/Getty Images)

https://www.history.com/news/the-infamous-40-year-tuskegee-study

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Pre-clinical trial procedure

— Preclinical testing (the procedure is regulated in Regulation No. 86/2008 Coll. – procedures for non-clinical testing of drug safety when conducting laboratory tests according to the principles of good laboratory practice)

— The aim is to verify safety at this stage – toxicity, teratogenicity, mutagenicity, carcinogenicity, etc.

— In vitro and in vivo tested

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In vitro testing
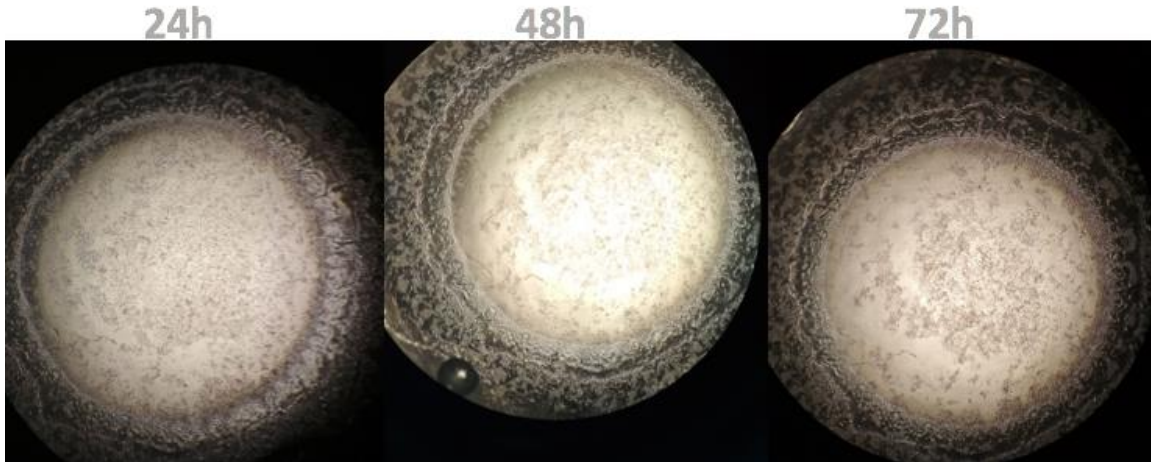
Photograph of a B-lymfocyte during time period
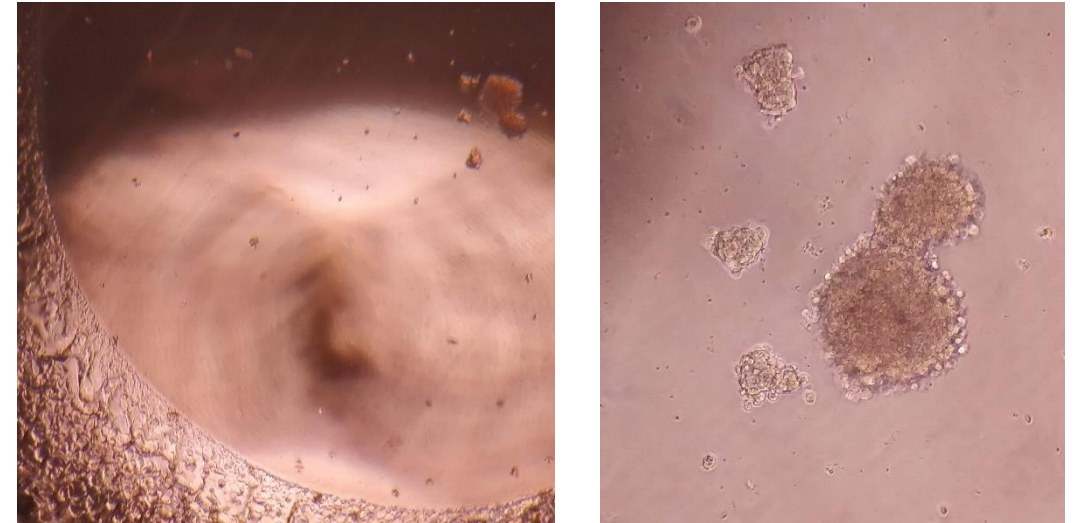
First *in vitro* testing on mouse fibroblasts. The cells clump together to form a stronger structure.

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In vivo testing

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In vivo testing

MUNI
MED

Ex ovo testing

CAM model
(chick chorioallantoic membrane model)

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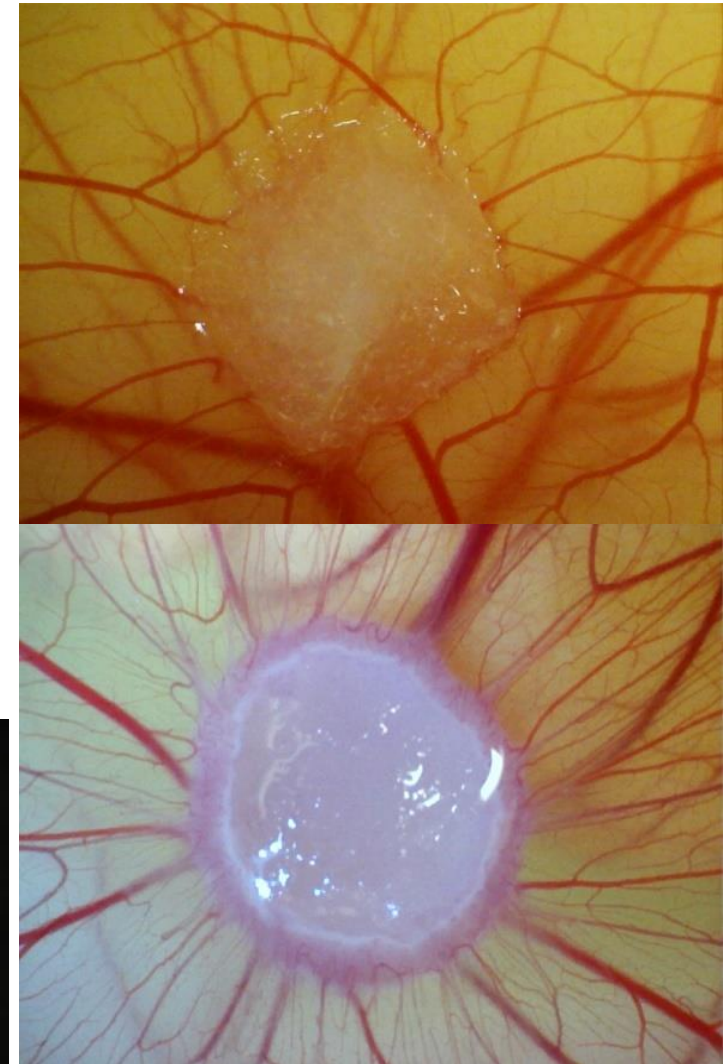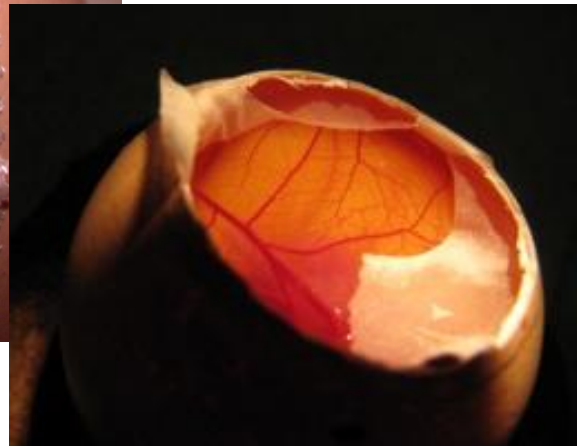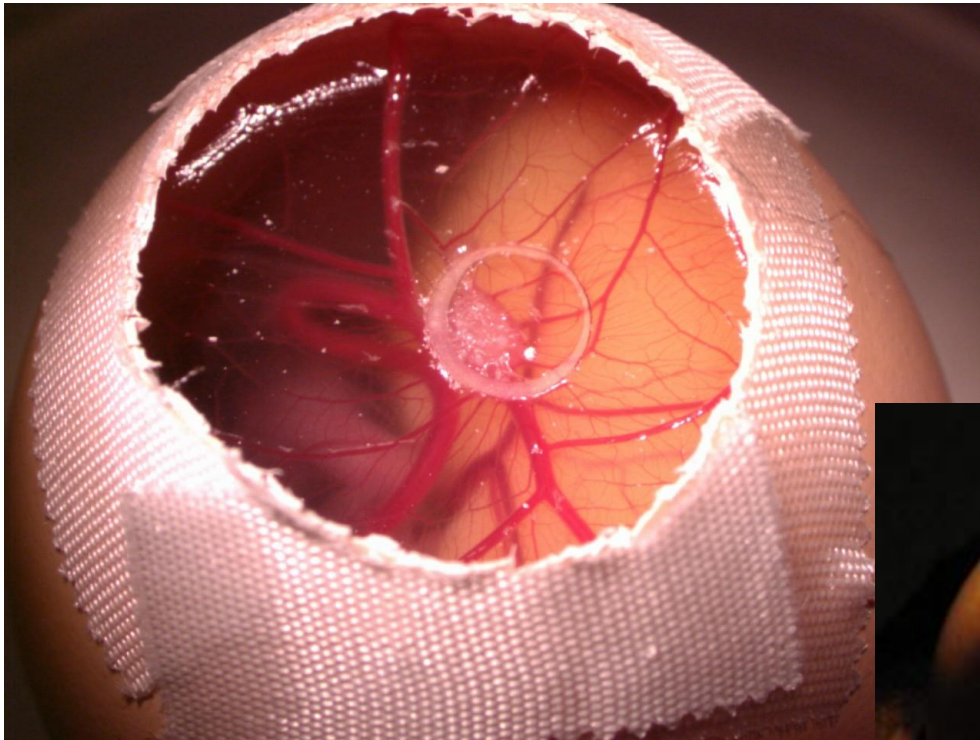
Of every 10,000 substances in the preclinical testing phase, around 5 will reach the clinical trial phase

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Definition of a clinical trial

— A clinical trial of medicinal products for human use is any systematic testing carried out on human subjects in order to detect or verify clinical, pharmacological or other effects, or to identify adverse reactions or to study the pharmacokinetics of a single drug or to compare it with several medicines in order to verify their safety or efficacy (Medicines Act No. 378/2007 Coll.)

— **systematic testing of the drug on patients or healthy volunteers, the main task of which is to:**
  - demonstrate and verify the medical effects of the drug,
  - demonstrate the safety and tolerability of the drug
  - find out what its side effects are,
  - determine the pharmacokinetic parameters and behavior of the drug in the human body.

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Individual parties to the clinical trial

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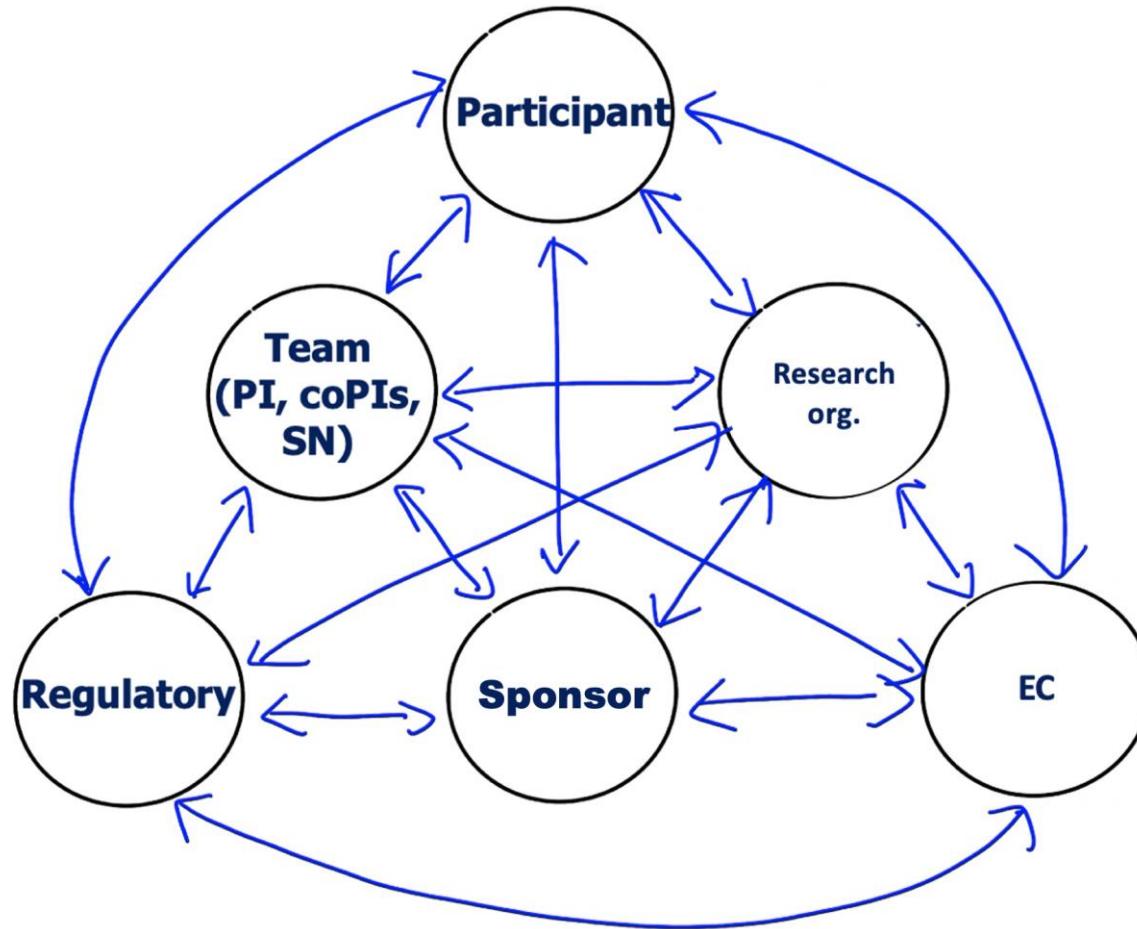
Basic principles of a clinical trial

- **The course of the clinical trial is globally standardized,** subject to strict rules of Good Clinical Practice, Helsinki Declaration, EU and Czech legislation for conducting clinical trials

- Ethical and correct conduct of studies according to the approved study protocol is supervised by an innovative pharmaceutical company (contract owner, sponsor) and control authorities, i.e. SIDC (State Institute for Drug Control) and EMA (FDA) or other regulatory authorities, and independent ethics committees.

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Basic principles of a clinical trial

27.5.2014   CS   Úřední věstník Evropské unie   L 158/1

I

(Legislativní akty)

NAŘÍZENÍ

NAŘÍZENÍ EVROPSKÉHO PARLAMENTU A RADY (EU) č. 536/2014

ze dne 16. dubna 2014

o klinických hodnoceních humánních léčivých přípravků a o zrušení směrnice 2001/20/ES

(Text s významem pro EHP)

| Clinical trial (CT) | Low-intervention clinical trial | Non-interventional studies |

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Good clinical practice (GCP)

— Decree No. 226/2008 Coll. (Decree on good clinical practice and closer conditions of clinical trial of medicinal products)

— GCP – a set of internationally recognised ethical and scientific quality requirements that must be complied with in the design, implementation, documentation and evaluation of CT.

— GCP training

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Good Clinical Practice (GCP)

Base points

— CT to be implemented in accordance with legal and ethical principles (DH)

— Before starting a CT, the risks and disadvantages with the expected benefit to the patient and society should be considered. The study can only be carried out if it justifies the expected benefit of the potential risk

— The most important aspect is always the law, safety and health of the subject(s)

— All information on the investigational medicinal product should be sufficient to carry out the CT

— CT must be scientifically reliable with the protocol described in detail

— CT must always be carried out in accordance with the approved protocol

— A qualified physician is always responsible for the care provided during the CT

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Good Clinical Practice (GCP)

Base points

– Each person conducting a clinical trial should be qualified by its education and relevant experience

– Participation in a clinical trial must be obtained voluntarily for each subject

– All information related to a CT must be recorded in a way ensuring that it is easily and accurately reproduced, reported and verified

– Confidentiality of records in accordance with the law

– The manufacture, handling, transfer and storage of the studied medicinal product must be in accordance with the GMP

– Quality backstop procedures are to be put in place in relation to all aspects of CT

Strnadová V., 2010

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Overview of approved clinical trials

‒ An overview of clinical trials is publicly available in Czech republic in the database on the www.olecich.cz.

‒ The review includes all clinical trials approved since 31 December 2007. Information on clinical trials from 2004 to 2007 is gradually supplemented retroactively.

‒ www.olecich.cz.

‒ **https://www.clinicaltrials.gov**

M U N I
M E D

State Institute for Drug Control (SIDC)

The Act on Pharmaceuticals:

initiation of a clinical trial

without the positive opinion of the ethics committee

without permission / notification of SIDC

is an offense for which

a fine of up to CZK 5,000,000

can be imposed

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Intervention from the point of view of a clinical trial:

Any method of intervention:

— **till the use of the drug/medicinal product**
(predetermined method of administration, dose, dose adjustment... All according to the protocol**)**

— **till investigative procedures**
(new investigative procedures outside normal practice, examination only for CT... All according to the protocol)

— **treatment procedures**
(rounds beyond normal practice... everything defined in advance by the protocol).

Completion of a questionnaire by a patient/healthy volunteer is not considered an intervention.

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State Institute for Drug Control Authority

Medicine	Intervention	Human subject	
YES	YES	YES	it is a CT Medicine regulated by the SIDC MUST BE SENT for assessment at the SIDC - even if registered LPs are administered in accordance with normal practice (Phase IV)
YES	NO	YES	it may be a study regulated by the SIDC MUST ALWAYS BE SENT/ SUBMITTED FOR ASSESSMENT TO THE SIDC even if registered LPs are administered in accordance with normal practice - pharmacoeconomic studies, post-marketing safety studies

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Ethics Committee

— It is an institution consisting of at least 5 members

— Sufficient education, qualifications and experience

— It assesses the submitted clinical trial from the perspective of the Section 3(1) of the Good

   Clinical Practice Decree

   - Scientific

   - Medical

   - Ethical

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Ethics Committee

– *„at least 1 EC member must be a person without medical education and without professional scientific qualifications and at least 1 member must be a person who is not in an employment relationship, a similar employment relationship or in a dependent position with a health service provider'*

–

§ 53 para. 2 of the Medicines Act

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Ethics Committee

– task: to protect the <u>rights, safety and health</u> of all subjects with particular attention to clinical

trials that may be carried out on vulnerable subjects

– multicentric studies:

requests for opinions in the case of multicentre clinical trials are submitted to the Ethics

Committees for Multicentre Trials (11 in total)

– local studies

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Informed consent

According to the SIDC...

– **Informed consent** to participate in a clinical trial means a voluntary and demonstrable expression of the will of the person (or, where appropriate, his legal representative or guardian) to become the subject of the trial to submit to the clinical test, confirmed by the signature of the subject (or, where appropriate, his legal representative or guardian).

– Informed consent must be given in a written form before the start of the clinical trial.

– The subject shall be duly instructed by the investigator on the conditions under which the clinical trial will be conducted, the risks arising from the submission of the clinical test and any withdrawal from the clinical trial.

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Informed consent

- **The instruction needs to be given in writing, it has to be comprehensible and in a language understood by the subject. It shall be a part of the informed consent andit has to include:**

- information on the clinical trial, including the definition of its objective,

- the expected duration of the clinical trial and the estimated duration of the subject's participation in the clinical trial,

- identification and description of the medical device to be tested,

- a list of health procedures to be performed on the subject,

- information on the potential benefit of the clinical trial to the subject,

- information on the foreseeable risks and potential difficulties associated with subjecting to a clinical trial,

- Information on other treatment or diagnosis options,

- information on the processing of the subject's personal data, including information on the security of their confidentiality

- rights and obligations of the subject.

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Informed consent

– given by the person eligible to give it, who can be the legal representative of the minor or guardian of the person with limited incompetence (see also the definition of incompetent human entities from the Helsinki Declaration, already mentioned in the previous slides)

– if that person is unable to write, oral consent given in the presence of at least one witness shall be permitted; a written record must be made of such oral consent

Good Clinical Practice (GCP) and International Conference on Harmonization (ICH) are the basis for writing Informed Consent

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Informed consent

— the text of the informed consent shall be:

— understandable (not to use technical terms when not necessary for explanation)

— in a language that the subject understands well

— it is necessary to adapt to the group of patients to which the document is addressed – i.e. more briefly / simply in specific groups of patients (psychiatric patients, dementia, seniors, painful conditions, post-traumatic and postoperative conditions, heart attack, stroke,...)

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Informed consent - formal requirements

— document range and font size (maximum 8 pages, using a standard font size (e.g. Times New Roman 12, Arial 11, etc.)

— phrasing, language (proper use of the Czech language)

Introduction to the document:

- salutation

- offer to participate in the study

("We would like to invite you to participate ... / We offer you participation ...")

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Informed consent - content requirements

— **warning that the clinical trial is a research activity**

— **objectives of the clinical trial**

— **treatments**

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Informed consent - content requirements

— warning of the likelihood of accidental inclusion in different treatment groups where a randomised clinical trial is involved, i.e.:

- enumeration of individual treatment branches and their size (number of patients / ratio)
- definition of placebo, randomisation, blinding (including justification)

— procedures during the clinical trial, including all invasive procedures:

- it is not necessary to write in detail the description of individual visits - just put in a clear table and describe only the essential examination

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Informed consent - content requirements

— responsibility of the subject

— visits to the centre, use of the drug, adherence to the established regimen, contraception, reporting of adverse reactions / personal injury, etc.

— highlighting those elements of the clinical trial that are of the nature of research

— distinguishing between routine care and what is 'extra' in the study

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Informed consent - content requirements

– foreseeable risks or inconveniences to the subject, including any risk to the foetus or

breastfed child:

- adverse reactions (clearly, comprehensibly, briefly, or in a table, not to mention the results

of animal research, not to write down the details of previous studies)

- information on contraception (always indicate that the only reliable method is a combination

of one highly reliable and one complementary method)

– expected benefits - the subject should be aware even if no clinical benefit is expected for him

or her

– alternative treatments that may be used for the treatment of the subject, their benefits and

risks

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Informed consent - content requirements

- the treatment and conditions of compensation to be granted to the subject in the event of personal injury resulting from his participation in the clinical trial:

      -information about study insurance according to applicable legislation

      -in the event of personal injury resulting from the participation in the CT, adequate medical care will be provided and paid for

      -in case of injury, the patient reports to the examiner - not directly to the insurance company

- the expected amount of the subject's remuneration for participation in the clinical trial;remuneration must not be provided to vulnerable entities (e.g. minors, persons with limited autonomy)

      - where appropriate, explicitly state that no reward will be provided

 the entity's estimated expenses in connection with its participation in the clinical trial

      -compensation of expenses: compensation must be adequate (travel, substantive, loss of time) - can be in the form of vouchers

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Informed consent - content requirements

— information that the subject's participation in the clinical trial is voluntary and that the subject may refuse to participate or withdraw from participation in the clinical trial at any time, without penalty or loss of benefits to which it is otherwise entitled

— if possible in time, it is recommended to indicate that the patient can take the information home and discuss his/her participation in the study with his/her family

— consent that monitors, auditors, the relevant ethics committee and the SIDC will have a granted direct access to the original clinical documentation in order to verify the course of the clinical trial and/or data without breaching the confidentiality of information on subjects

— confidential records and their storage

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Informed consent - content requirements

— foreseeable circumstances and reasons for which the subject's participation in the clinical trial may be terminated

   - (termination by the SH, the investigator and the sponsor, reflects the exclusion criteria defined in the study protocol)

— expected duration of subject participation in the clinical trial

— approximate number of subjects participating in the clinical trial

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Children and
clinical research:
ethical issues
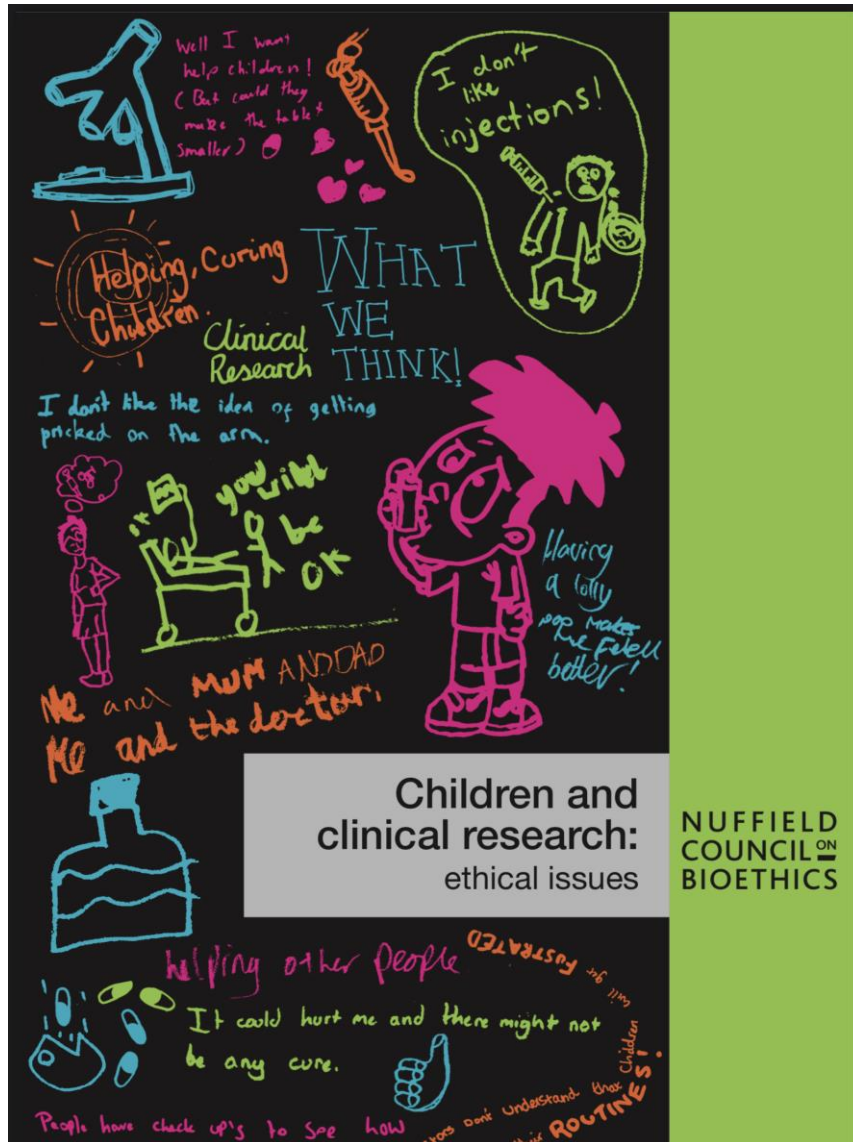
NUFFIELD
COUNCIL ON
BIOETHICS

No consent – no research
No consent – only therapy

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Informed consent - minors

necessary consent of <u>both</u> parents

(was repealed only in the Health Services Act)

<u>Medicines Act</u>:

„…informed consent of the parents or any other legal guardian"

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Informed consent - minors

If the patient reaches 18 years of age during study participation, he/she must re-sign an informed consent for his/her further participation in the study – in the version for adults

By reaching the legal age, consent to the participation of a minor in the CT given by his or her parent or representative ceases to apply.

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Informed consent - minors (information)

— Age groups as instructed by the SIDC:

— 12-14 years - max. 4 pages, simple formulations

— 15-17 years – the scope of information can be identical as in the informed consent for adults

range of information for adults; information about contraception shall be included

It does not replace the consent by legal representatives, which is decisive for the child's participation in the CT, but the child's opinion must be taken into account.

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Informed consent - additional research

– For supplementary research in which not all subjects are involved, a separate informed consent must be established, which:

- does not repeat the information from the primary informed consent (only reference to it)

- voluntary participation - does not affect participation in the main study

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Development of a new drug - conditions for the initiation of a CT

- EUDRA CT "number" generated by the European Medicines Agency (EMA)

- SIDC authorisation (based on supplied documents including proof of insurance)

- EC consent (local or multicentre)

- Informed consent and instruction of the patient

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Phase 1
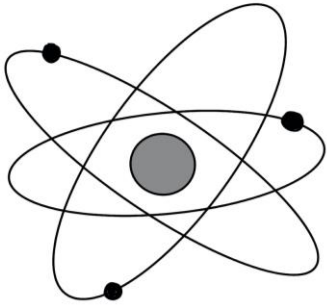
- First administered medicinal product to a human proband

- Very careful / dosing (dose given vs. target dose)

- Healthy volunteers (students)

- Elimination of the risk group (comorbidities, children, pregnant women, etc.)

- Mostly one-time testing - monitored pharmacokinetics of the drug. Usually only at one workplace (monocentric)

- A small number of probands

- **Exceptionally, the drug may also be given to sick patients at this stage (administering it to healthy patients would be inappropriate)**

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- The medicine has proven itself in phase 1 CT (safety)

- Depending on the nature of the drug, it is tested on a population of several tens to hundreds of patients at this stage

- Mostly multicentre (usually one country)

- Typically, it is an administration to patients (meeting the indications of the drug)

- In some types of studies, this phase may be divided into IIA and IIB

- **IIA - Safety**

- **IIB - Efficiency**

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- The pre-marketing phase of the drug

- Hundreds of subjects

- Always multicentre, optimally multinational (Europe vs. USA)

- Demonstration of sufficient population safety and efficacy (superiority) compared to comparator (placebo, SOC)

- **RCT (Randomized Control Trial)**

Randomization/check/blinding

Successful completion – eligibility for registration

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Registration and sales process

Phase 1

Phase 2

Phase 3

Phase 4

SIDC
Registration of the drug

↓

Ministry of Finance
Determination of max price

↓

Ministry of Health
Categorization and payment

↓

Categorization
Amount of remuneration

↓

VZP
Payment, Bulletin

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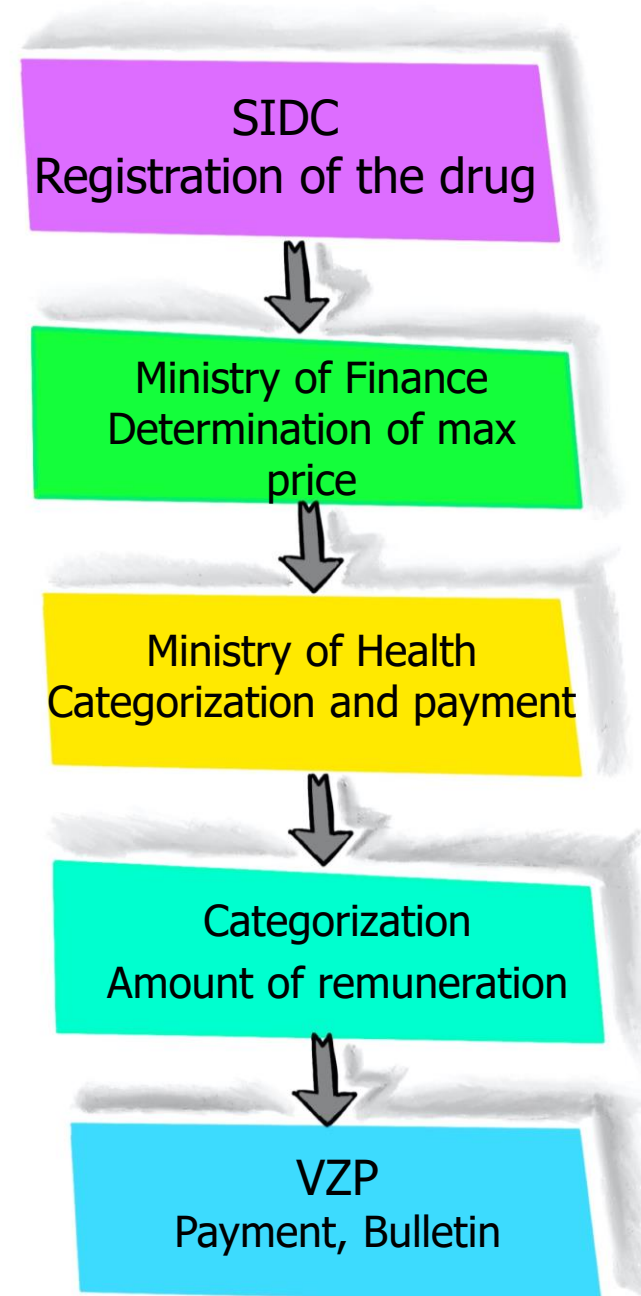
Phase 4

— Post-marketing phase (post-registration phase)

- Obligation to collect information about a newly registered drug for a minimum of 5 years

- The drug is indicated for the therapy of several thousand patients – the potential for rare side effects

- Mandatory reporting of these side effects to SIDC

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Placebo vs. Standard-of-care (SOC) "best available therapy"

– In general, placebo is considered an inert substance or procedure, and the placebo effect (or response) is something that follows placebo administration.

– The paradox of this statement is that if something 'inert' by definition cannot produce the effect

– This can be further confused with terminology such as "active", "true" and "perceived" placebo.

– There is no single placebo effect

– Psychological aspect

– Neurobiological aspect

The role of placebo in RCTs is currently highly discussed!!!

Gupta et al., 2013

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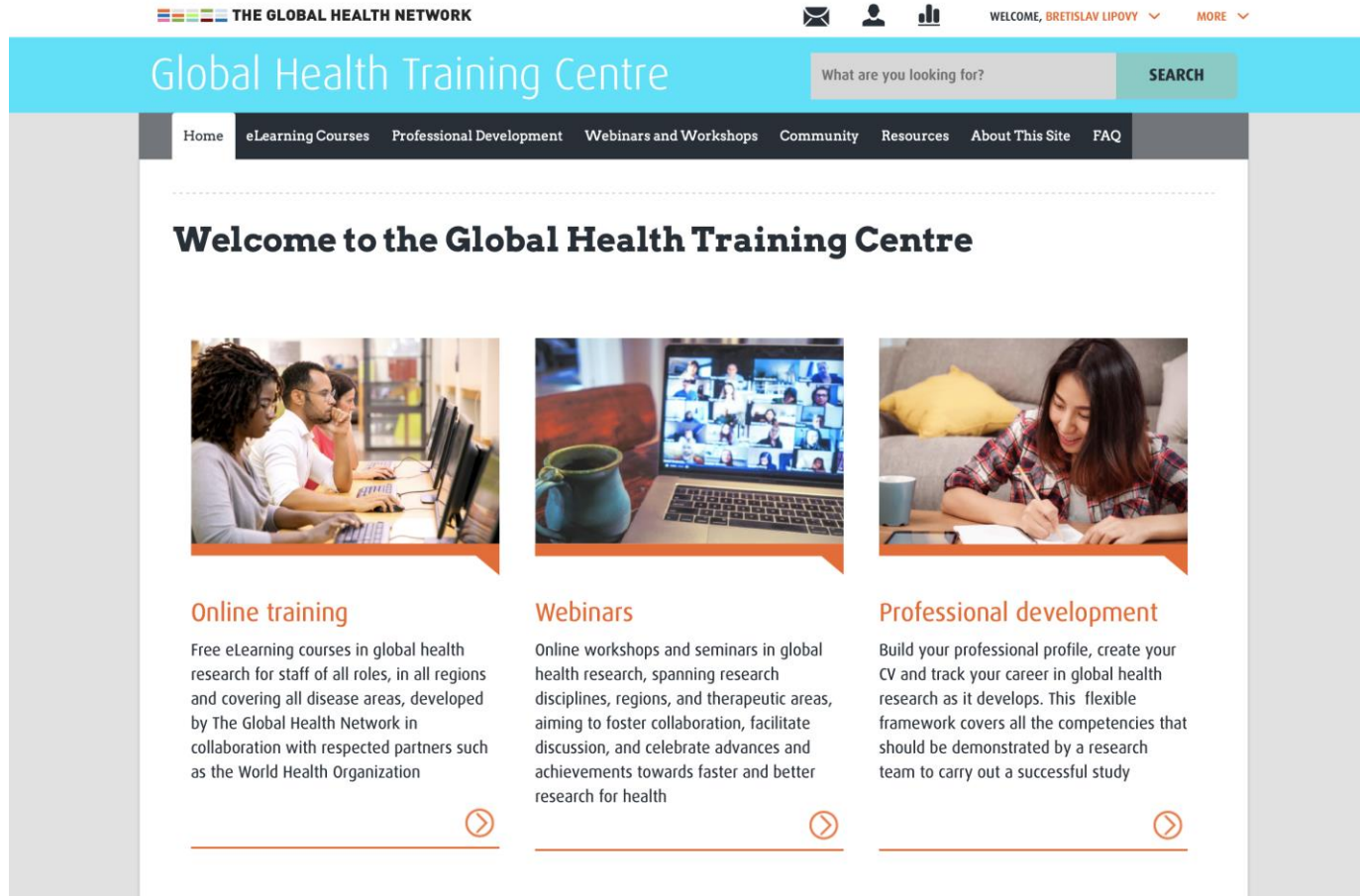
Placebo effect – psychological aspect

– There are many mechanisms that psychologically provoke a placebo effect

- Expectations

- Motivation

- Somatic focus

- Reward

- Reducing anxiety

- Learning and memory

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Placebo effect – neurobiological aspect

— Research on the neurobiology of placebo sensitivity has focused on placebo analgesia

— Potential roles of opioid and non-opioid mechanisms

— The effects of placebo may be partially or completely reversed by opioid antagonists (placebo analgesic effect).

— According to some studies, cholecystokinin (CCK) may play a role - the analgesic placebo effect is potentiated by CCK antagonists.

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GCP training and certificate

https://globalhealthtrainingcentre.tghn.org/elearning/

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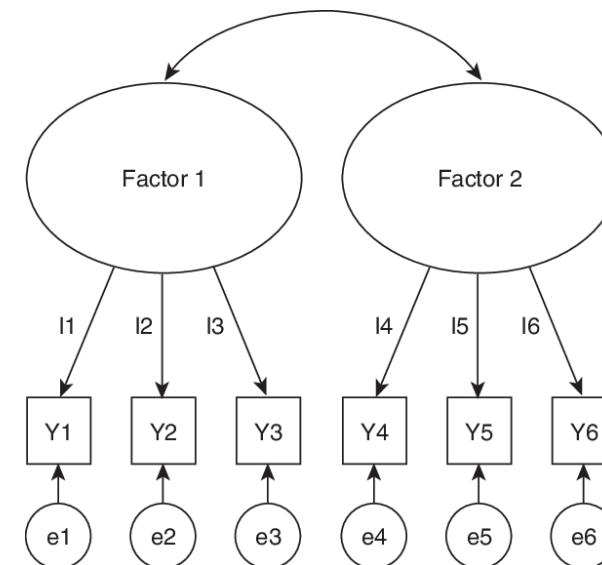
Questionnaires and psychometrics

Mgr. Tomáš Kratochvíl
doc. PhDr. Martin Vaculík, Ph.D.
Department of Psychology
Faculty of Social Studies, Masaryk University Brno

Content of the lecture

Questionnaire surveys and psychometrics

— Motivation. What is a questionnaire and what is it useful for?

— Where and how to get a questionnaire?

— How do we create questionnaires?

— How to adapt a foreign questionnaire?

— How to verify the original and translated questionnaire?

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Introduction of the researcher

Mgr. Tomáš Kratochvíl – work psychologist and data analyst at the Department of Psychology

– **A team of work psychologists led by doc. PhDr. Martin Vaculík, Ph.D.**

– KPSY FSS MU is a workplace providing
  - **scientific research activity**
  - **solutions for scientific projects**
  - related services, especially **psychological experimental, correlational and qualitative research, development and adaptation of psychodiagnostic methods**, organization of studies and data analysis, psychological advice in the period of pandemic and refutation of work-psychology myths

– KPSY FSS MU provides full-time teaching and management of final thesis within the fields of study of single-major and major/minor psychology.

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How is it useful for a dentist?

PAIN MEASUREMENT SCALE

Screening for a problem that is costly to diagnose

— neurological disease (ACE-R), oral cancer…

Finding out the medically important characteristics of a person or their attitude

— „Health Literacy in Dentistry"

Behavior, feelings or attitudes of research participants

— e.g. we want to verify compliance with various hygiene habits

People's feedback on treatment/staff attitude/...

— satisfaction of the "customer" with the service

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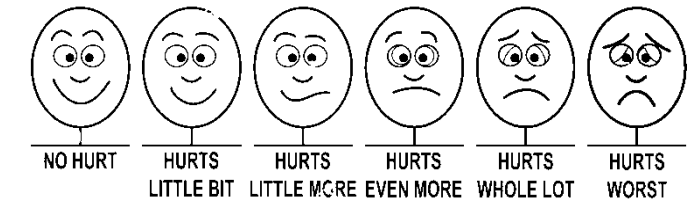
What is a <u>professional</u> questionnaire?

Standardized stimulus material

– has a predetermined purpose

– questions are based on a theoretically based definition

– its functionality and comprehensibility is verified

Standardized method of administration

– instructions are understandable and always the same

– the administrator is trained

Standardised method of evaluation

– there are evaluation guidelines in the manual

– conversion from gross scores to Σ, M, z-score...

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What types of answers exist in a questionnaire

Likert

— usually 5- or 7-point (agree-disagree, always-never…)

   — should be symmetrical (as many negative/low points as positive/high points)

   — the middle value (such as „neither agree nor disagree") does not necessarily have to be included

— one of the most widely used solutions in social sciences
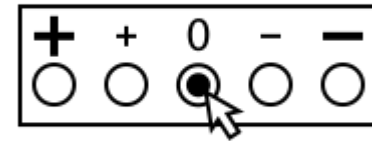
Q-sorting

— sorting statements by their valence

— it is also used for the rater agreement/reliability
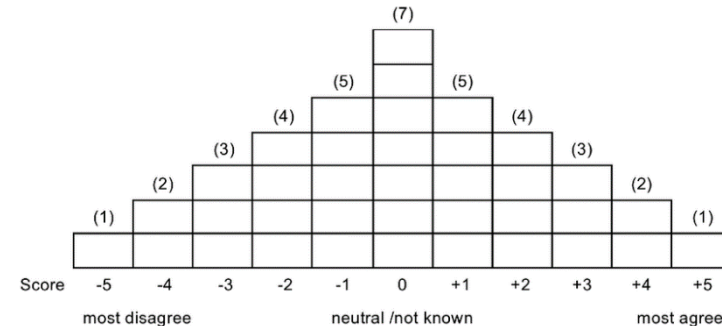
Dichotomic, demographic, verbal and more

— used for economicality, control of intervening phenomena, getting details

— we must consider their use based on our aims

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Dis/advantages of a questionnaire

…or why it may be more useful than to talk with the client/subjects.

Interview		Questionnaire
Lower	standardizability	Higher
Higher	potential validity	Lower
Higher	influence of the interviewer	Lower
Higher	response rate	Lower
Higher	price ($ and time)	Lower
Lower	anonymity	Higher
Higher	the ability to control understanding and answering the questions	Lower

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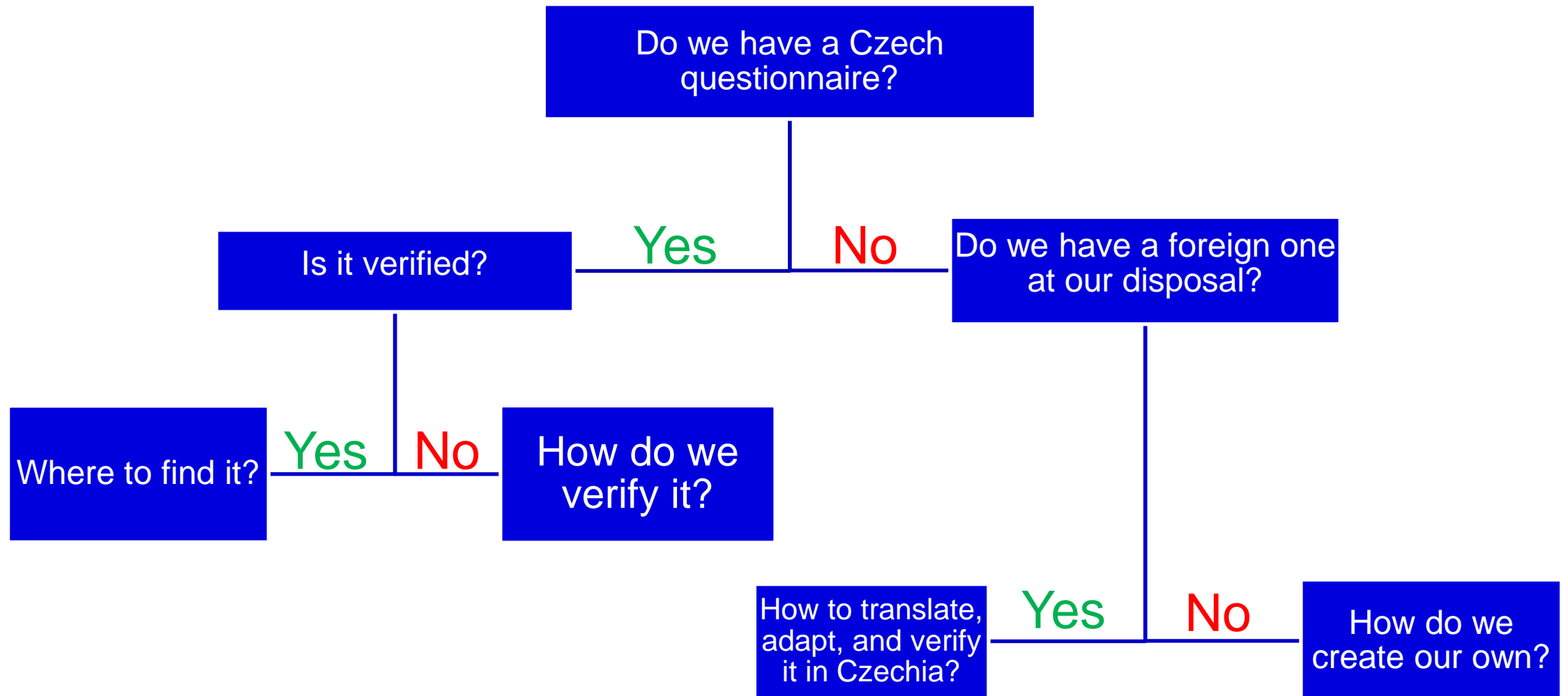
How to obtain a questionnaire method?

If it is a psychodiagnostic test (intelligence test, attention test, depression inventory…)

— in the catalogues of publishers of psychological tests (Testcentrum, Psychodiagnostika)

— in the Kabinet diagnostických metod KPSY FSS MU

— for research purposes, sometimes in earlier studies

If we need just information about a test

— yearbooks (in psychology Buros), books

— magazines focused on method reviews (e.g., Testforum.cz)

If it is a specific questionnaire (attitudes, interests, experience, evaluation...)

— previous research

— Bc./Mgr./Ph.D. theses of students of the given field

How to work with an obtained method

Instructions for administration

— in psychodiagnostics it is usually in the **method manual**

— if it exists, it specifies the use procedure and areas of use

— e.g., what to say before the administration, whether to administer orally/written

Evaluation and standards

— sometimes we just add/average scores, other times we code intricately

— methods tend to have standards for different populations (e.g., men vs. women)

Interpretation

— **recommendations** may be based on manuals and/or articles

— it needs to be written in regard to other information and its sources

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How to create questionnaire items

QUESTIONNAIRE ITEMS

Items with forced answers

— we do not usually use
  - it invalidates the data (one may answer randomly and we cannot know about it)
  - when a person leaves many blank answers, we easily recognize them as those to be removed from the data
  - If there is no "I don't know" option and the participant does not know the answer = they cannot answer properly

— can be meaningful in case of controlling variables

Simple wording

— ideally simple sentences, we avoid coordinate conjunctions
  - "If my gums were bleeding, I'd know what it means and how to react. When brushing my teeth, my gums or my teeth hurt."
  - "If there was blood coming out of my gums, I'd know what it means."; "When brushing my teeth, I have the feeling of a burning mouth."

"Saturation" of the construct

— questionnaire cannot contain all possible items

→ we select key items

→ we cover as many parts of the construct as possible

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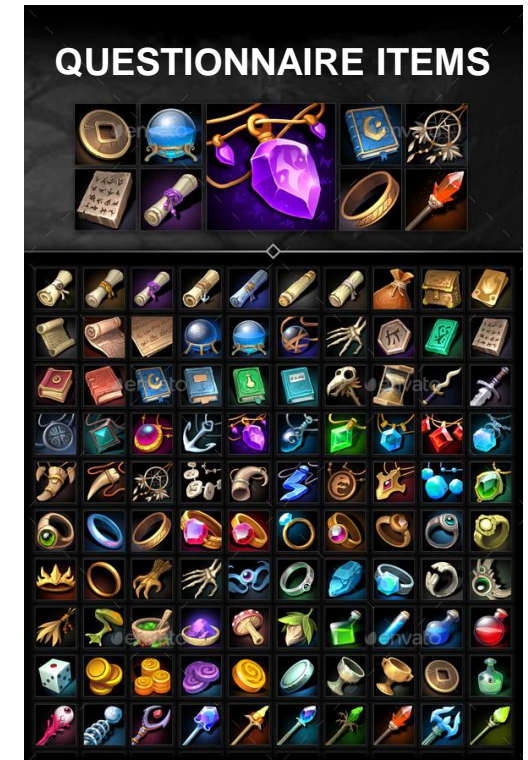
How to "compose" items in a questionnaire

- What are we measuring?
- Each aspect of the given "what" is measured once.

We specify our aim

We determine the questionnaire length

- based on its purpose
- based on the population

- N > 10
- cognitive interview
- sometimes repeatedly

Piloting

Order of questions

- from easier / less sensitive
- Demographics at the end

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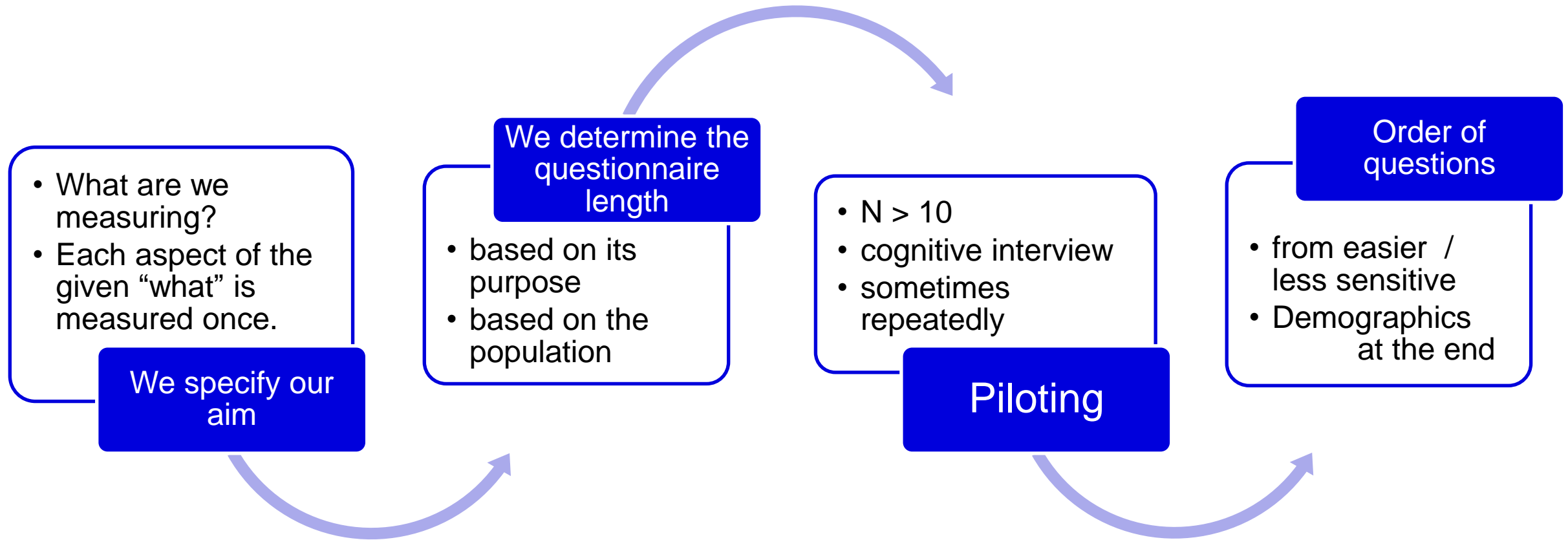
How to adapt a foreign questionnaire to Czech language?

Step I: Translation
- 2x translation
- T1 = knows the field
- T2 = does not know the field

Step II: Synthesis
- T1+T2
- resolve differences

Step III: Back translation
- 2x native speaker
- do not know the original or the field

Step IV: Expert Comparison
- Creators T1, T2, BT1, BT2
- joint analysis of irregularities

Step V: Piloting
- N > 10
- Cognitive interview
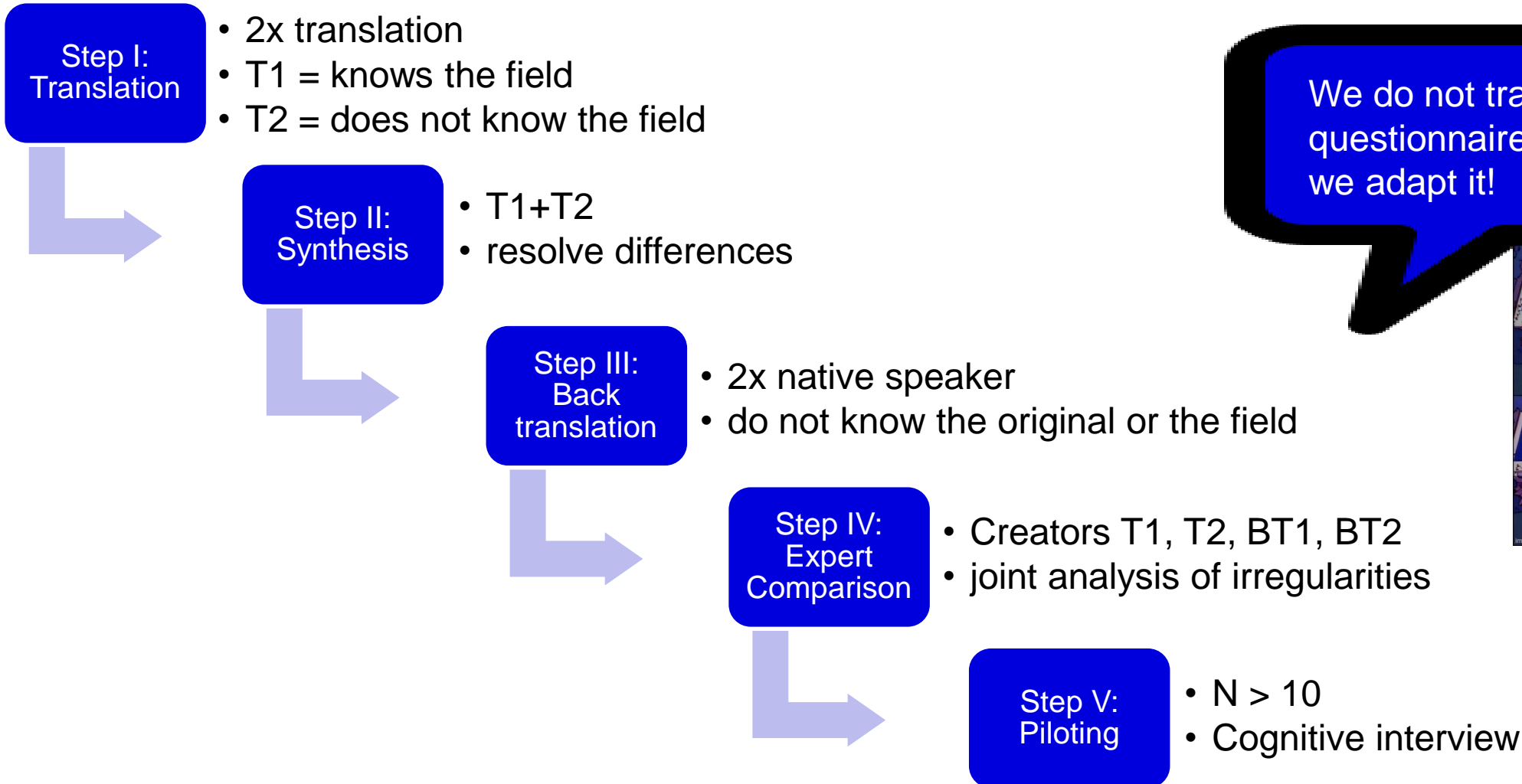
> We do not translate the questionnaire, we adapt it!

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What affects a questionnaire and what to do about it?

social desirability	Order effect	straight-line (1, 1, 1...)	Tendences in responding	memory errors
• reassure of anonymity • multiple sources of information • multiple measurement	• perform piloting • general → specific items	• Rotate the range (yes … no) → (no … yes) • inverted items ("I'm taller than most." "I often tiptoe.") •	• neutral items • selecting a scale	• define the time period • give enough time to respond

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How to verify a questionnaire - validity

Construct validity

— do we measure what we think we measure?

— what are its dimensions?

— it is verified by factor analysis, a relation with a close construct

— **Height questionnaire**: strongly related to weight, weakly to self-esteem

Content validity

— the item "fits" into the questionnaire based on theory and expert opinion

— **Height questionnaire**: "I'm taller than most people." X "I have long legs."

Criterion-related validity

— concurrent or predictive relation to the criterion

— **Height questionnaire:** success in basketball, height measured by the meter

WELCOME MY LORDS TO ISENGARD!

HOW HIGH ARE YOU?!
NO GANDALF,

IT'S HI, HOW ARE YOU

HOBBITS!

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How to verify the questionnaire - reliability

— how exactly do we measure? with what error?

Internal consistency

— the accuracy with which all items find out the same thing

— it is verified by Cronbach's α, McDonald's ω

Reliability in time (test-retest)

— with what error do we measure in time T1 and time T2

— it is verified by correlation of repeated measurements

Split-half reliability

— what is the error in measurement between two halves of one test

— a corrected correlation of two well-chosen halves

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How and in what to analyze it

What analysis do we usually use to verify a questionnaire?

— exploratory and confirmatory factor analysis (CTT)

— Rasch Model (IRT)

— network models

What program to use to calculate the FA

— the basics can be done in SPSS (poorly)

— exploratory: CEFA, Matlab

— confirmation: R Studio (lavaan), AMOS

What program to use to calculate reliability?

— Cronbach's α = SPSS

— McDonald's ω = R Studio (psych)

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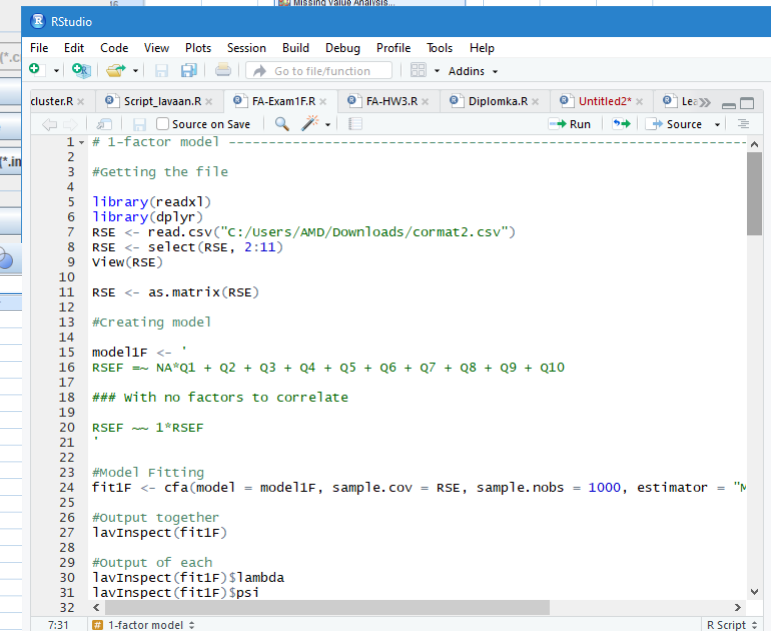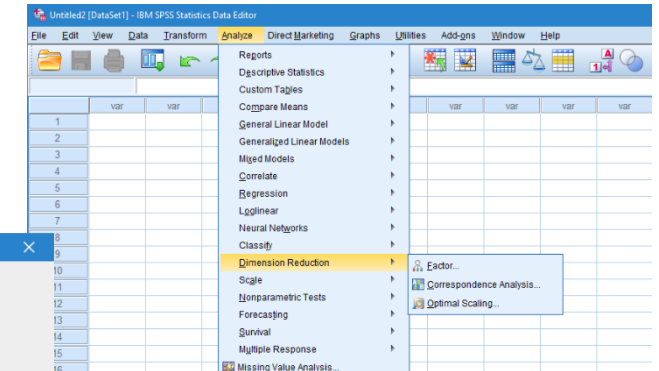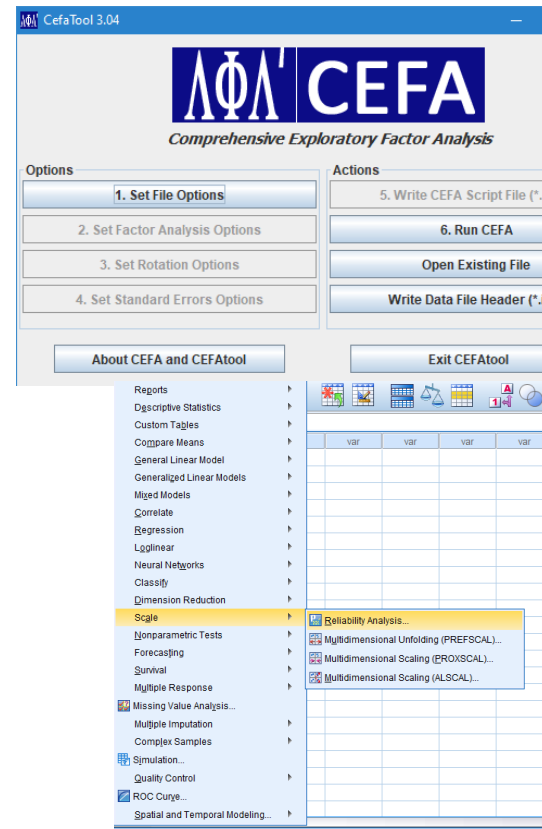
What is the logic behind factor analysis?

Manifest and latent variable

Y1. How fast will they run 50 m?

Y2. How fast will they run 100 m?

Y3. How fast will they run 400 m?

ability to sprint quickly
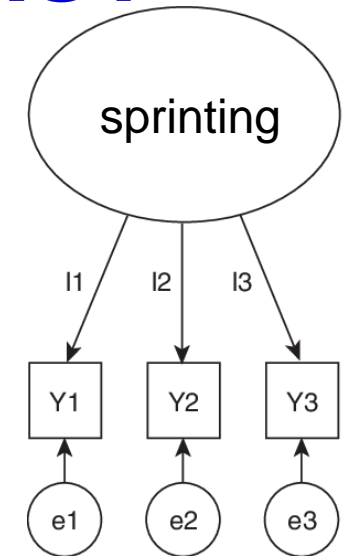
Dental example: Health Literacy in Dentistry

Q1. Are you able to pay to see a dentist?

Q2. Are you able to afford transport to dental clinics?

Q3. Are you able to pay to manage your dental health?

financial hindrances

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Summary of the whole process

EN
Searching for questionnaires → Other studies → Translation → Piloting → Confirmation FA

CZ
Purpose, item generation → Selecting items → Piloting → Exploratory FA → Confirmation FA

if we measure the construct

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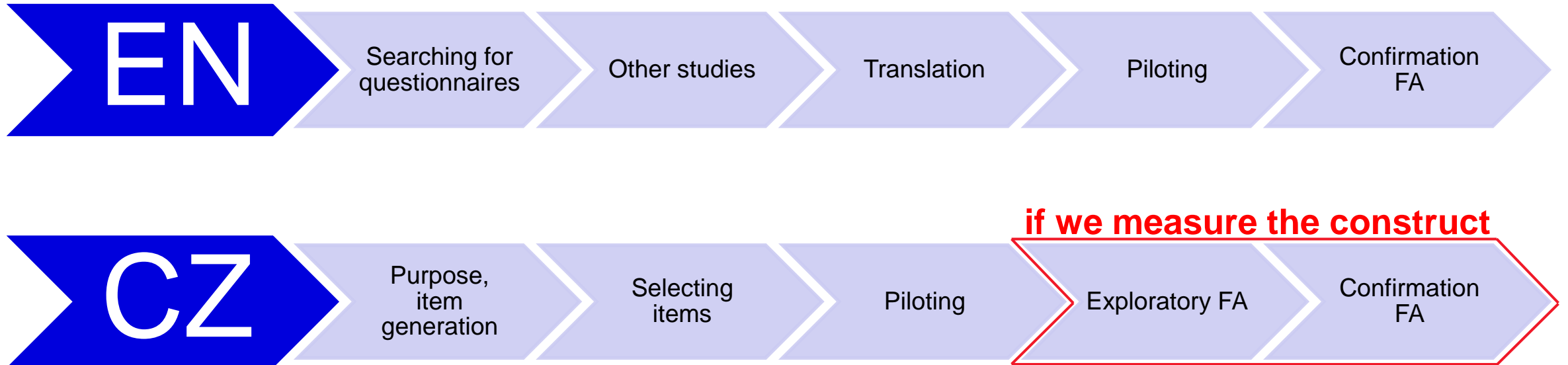
Practical example at the end

Brno Height Questionnaire (shortened version)
— developed as a demonstration of psychometrics

— is evaluated on the basis of the mentioned CTT

— items are summed up to a score

Link

— http://fssvm6.fss.muni.cz/vyska/

Authors

— **validation study:**   Karel Rečka (2018)

— **shortened version:**   Martin Tancoš (2019)

— **web application:**   Hynek Cígler (2019)

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Sources

Research studies

– Jones, K., Parker, E., Mills, H., Brennan, D., & Jamieson, L.M. (2014). Development and psychometric validation of a Health Literacy in Dentistry scale (HeLD). *Community Dental Health, 31*, 37–43. doi: 10.1922/CDH_3269Jones07

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Monographs

– Goodwin, C. J. (2008). *Research in Psychology: Methods and Design* (5. ed.). NJ: Willey & Sons Inc.

– Urbánek, T., Denglerová, D., & Širůček, J. *Psychometrika: Měření v psychologii*. Praha: Portál, 2011.

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Introduction to biostatistics

RNDr. Michaela Cvanová

Danka Haruštiaková

Eva Koriťáková

Institute of Biostatistics and Analyses,

Faculty of Mediciny, Masaryk University Brno

Statistical joke

"Statistician is a person, who lays with his head in an oven and his feet in a deep freeze, stating, 'On the average, I feel comfortable'."

C. Bruce Grossman, approx 1958

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Introduction of the researcher and his team

Michaela Cvanová, RNDr. – analyst at the Institute of Biostatistics and Analyzes, FM MUNI

— **Data analysis team led by Jiří Jarkovský, PhD.**

— IBA FM MU is
  — research institute
  — **oriented to the solution of scientific projects** and
  — providing related services, especially in the field of **biological and clinical data analysis**, organization and management of clinical trials and syntheses, software development and ICT applications.

— Educational activities of the IBA FM MU include teaching at Faculty of Medicine and Faculty of Science and supervision of theses; the institute is also a guarantor of two study programs: Public Health, and **Computational Biology and Biomedicine**.

Content of the lecture

Introduction to the Biostatistics

— Motivation. Biostatistics and its importance

— What is data and what does it look like

— What variables do we have

— How we can describe and visualize the variables

— We can test hypotheses statistically

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Motivation.
Biostatistics and its importance

EFFORT + WRONG TOOLS
= NO RESULTS
@successpictures

What will you take away from the lecture

Ability to better understand the published result

Knowledge: What is the mean, median, confidence interval, standard deviation and more, and the differences between them
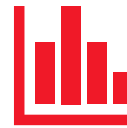
Ability to read tables and graphs

You will improve critical thinking in the field of statistics

The courage to analyze your own data

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What is Biostatistics?

— Biostatistics is **the application of statistical methods** in solving biological and clinical problems.

— The aim is to **obtain useful information from the observed data.**

— It is focused on a **specific problem**, not on theoretical aspects. However, this does not mean that statistical methods can be used headlessly.

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Motivation – an example from practice

The Journal of **EVIDENCE-BASED** DENTAL PRACTICE

FEATURE ARTICLE

EVIDENCE OF DIETARY CALCIUM AND VITAMIN D INADEQUACIES IN A POPULATION OF DENTAL PATIENTS

DANIEL J. PEHOWICH, PhD, MSc[a], AND ENID D. PEHOWICH, MSc[b]

[a]Department of Dentistry, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Canada
[b]Department of Cellular Biology, Faculty of Medicine and Dentistry, University of Alberta, Edmonton, Canada

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Table 1. Dietary calcium and vitamin D intake by sex as percent of daily recommended intake.

Variable	Male (n = 305)		Female (n = 366)	
	Mean ± SD	Median (IQR)	Mean ± SD	Median (IQR)
Age[a]	49.2 ± 19.25	52.0 (25-66)	48.1 ± 19.68	49.0 (27-65)
Body mass index (kg/m^2)[a]	25.1 ± 4.67	24.0 (22-27)	26.0 ± 4.38	26.0 (22-28)
Calcium: all patients[b]	76.3 ± 33.12	72.0 (53-104)	85.8 ± 40.94	70.0 (57-95)
Calcium: < 50 years of age[c]	83.1 ± 35.29[d]	78.0 (58-103)	99.5 ± 46.07[d]	91.0 (70-122)
Calcium: > 50 years of age[a]	70.9 ± 30.91	64.0 (50-87)	71.8 ± 28.87	67.0 (51-92)
Vitamin D: all patients[a]	52.2 ± 43.38	38.0 (22-70)	65.4 ± 61.81	43.0 (23-86)
Vitamin: D < 50 years of age[b]	68.0 ± 50.77[d]	57.0 (29-95)	89.7 ± 71.91[d]	67.5 (34-123)
Vitamin: D > 50 years of age[a]	37.9 ± 29.31	33.0 (18-49)	40.1 ± 34.43	31.0 (16-52)

IQR, interquartile range; n, number of patients; SD, standard deviation. Calcium adequate intakes (AIs) for women and men: age 18-50 years, 1000 mg/day; age > 50 years, 1200 mg/day. Vitamin D adequate intake (AI) for women and men: age 18-50 years, 200 IU/day; age > 50 years, 400 IU/day.

[a] Difference between female and male not significant.
[b] Difference between female and male, $P < .05$.
[c] Difference between female and male, $P < .01$.
[d] Difference between same gender age groups, $P < .01$.

Figure 2. Frequency distribution of dietary vitamin D intake for all patients for the period 2003-2013 as percentage of assumed Adequate Intake (AI; $n = 670$).

Median = 40.5%

Number of Patients

% of Adequate Intake for Vitamin D
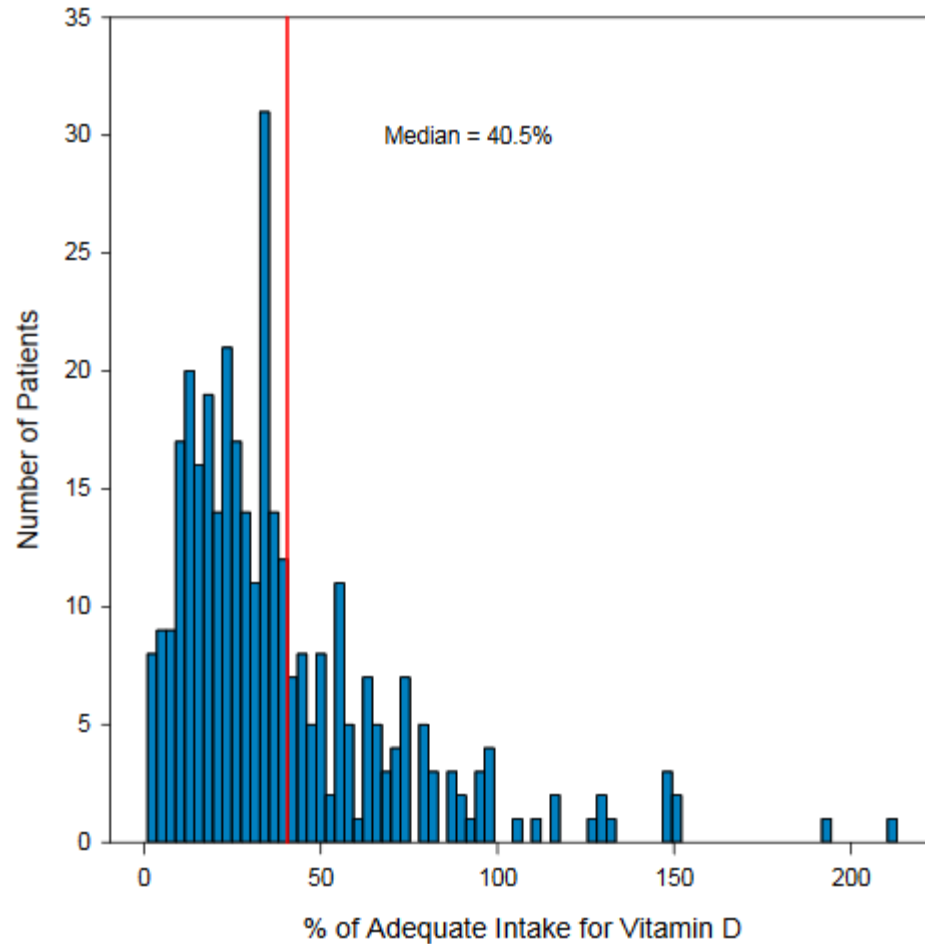
Figure 4. Box plot of means and median dietary vitamin D intake for all patients by year. Estimates of intake are percentage of existent Adequate Intake (AI) for the period 2003-2013 ($n = 670$).

Mean
Median

% Adequate Intake Vitamin D

Year

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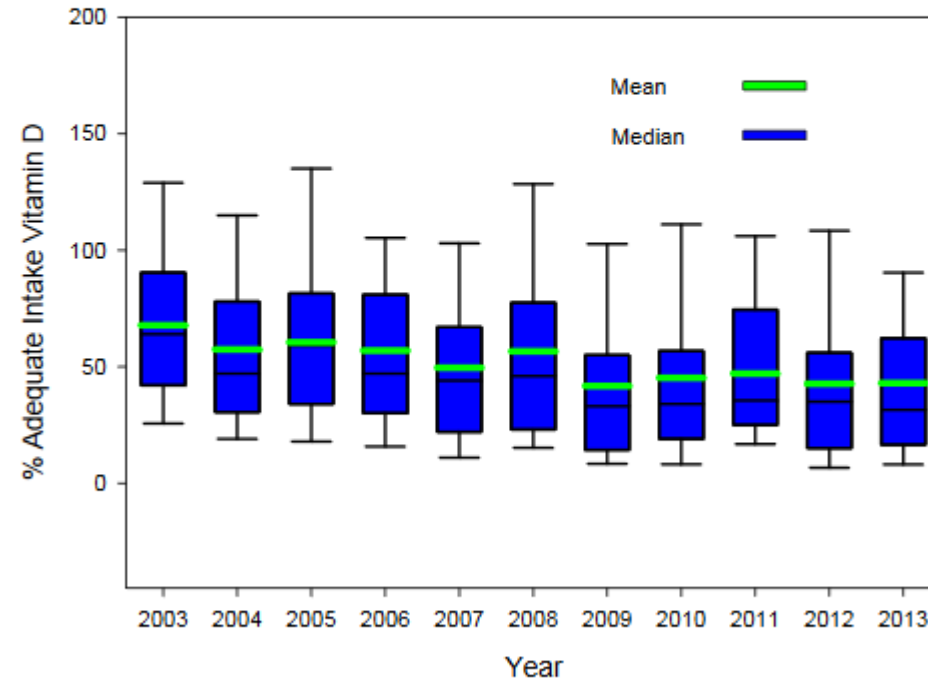
What is data and what does it look like

Data

- **Target population** – a group of subjects about whom we want to find some information (e.g. all patients with a given diagnosis in the Czech Republic).

- Target population = basic set

- **Experimental sample** – a subgroup (selection) from the target population that "is available to us" (observed set).
  - It must match the characteristics of the target population.
  - We want to generalize the results to the entire target population.

- **Data** – numerical or verbal record of information about the observed group of people, medical facilities, etc.

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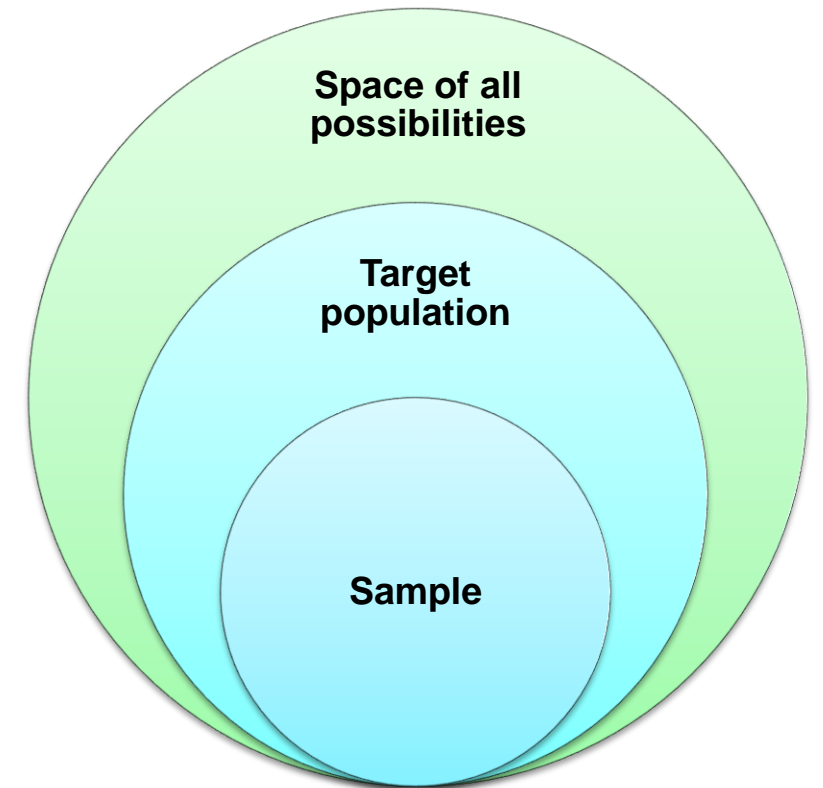
Sample data file

VARIABLE (character) →

Basic data unit (objects, subjects) ↓

	A	B	C	D	E	F	G	H
1	ID	datum odběru a vyšetření	věk v době odběrů a vyšetření	pohlaví_muž =0, žena=1	národnost česká=0, slovenská=1	váha v kg	výška v cm	BMI
2	ZL_01	06.04.2017	21	0	0	63	184	18,6
3	ZL_02	06.04.2017	21	1	1	57	167	20,43
4	ZL_03	06.04.2017	23	1	0	62	163	23,34
5	ZL_04	06.04.2017	22	1	1	64	174	21,14
6	ZL_05	06.04.2017	21	0	0	80.4	177	25,66
7	ZL_06	06.04.2017	21	0	0	90	178	28,41
8	ZL_07	06.04.2017	21	1	0	57	173	19,04
9	ZL_08	06.04.2017	22	1	0	65	169	22,75
10	ZL_09	06.04.2017	22	1	1	75	179	23,4
11	ZL_11	18.12.2017	22	1	0	59	170	20,42
12	ZL_12	18.12.2017	22	1	0	57	164	21,19
13	ZL_14	19.04.2017	21	1	0	62	174	20,48
14	ZL_15	19.04.2017	21	1	0	51	162	19,81
15	ZL_16	19.04.2017	20	1	0	54	165	19,83
16	ZL_17	08.11.2017	21	0	0	76	173	25,39
17	ZL_18	19.04.2017	21	1	1	62	173	20,71
18	ZL_21	19.04.2018	21	1	0	48	156	19,72

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Data storage policies

– Correct and clear data storage is the basis of their later analysis.

– It is advisable to think about how the data will be stored before starting the data collection.

– For computer data processing, it is necessary to store data in tabular form:
  – Each column contains only one type of data, identified by a column header (column headers must be unique).
  – Each row contains a minimum unit of data (e.g. patient, one patient visit, etc.).
  – It is not permissible to combine numeric and text values in one column.
  – Comments are stored in separate columns.
  – For text data, it is necessary to check for typing errors.
  – Dates are a specific type of data and they must be stored in a correct formate

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What variables do we have

Variables described by **two values**

- Only two categories

- They are usually coded using digits 1 (presence of the character / yes) and 0 (absence of the character / no)

- *Example:*

- Equals?

Sex

37.5%

62.5%
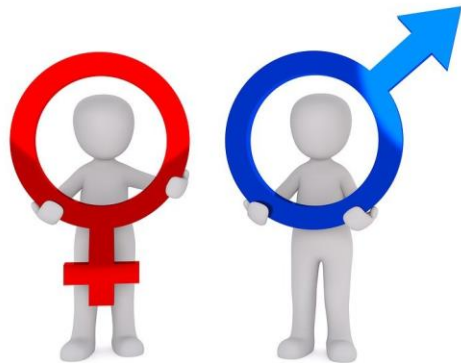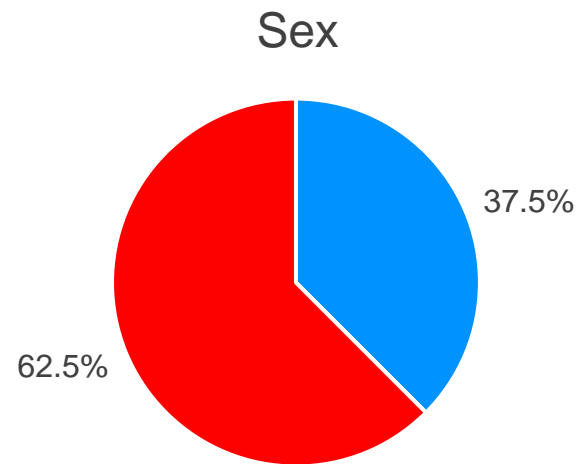
- male (n=39)
- female (n=65)

Pie chart

	n (%)
Sex	
male	39 (37.5)
female	65 (62.5)
total	104 (100.0)

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Variables described by **two values**

- Only two categories

- They are usually coded using digits 1 (presence of the character / yes) and 0 (absence...

- *Example...*

Binary variables (**categorical**)

	n (%)
x	
male	39 (37.5)
female	65 (62.5)
total	104 (100.0)

- Equals?

62.5%

■ male (n=39)   ■ female (n=65)

Pie chart

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Variables described by **multiple values that cannot be sorted**

– Multiple categories that cannot be sorted (there

is no natural order of individual values)

– Usually text values

– *Example:*

– Equals?

	n (%)
Nationality	
Czech	67 (64.4)
Slovak	37 (35.6)
other	0 (0.0)
total	104 (100.0)
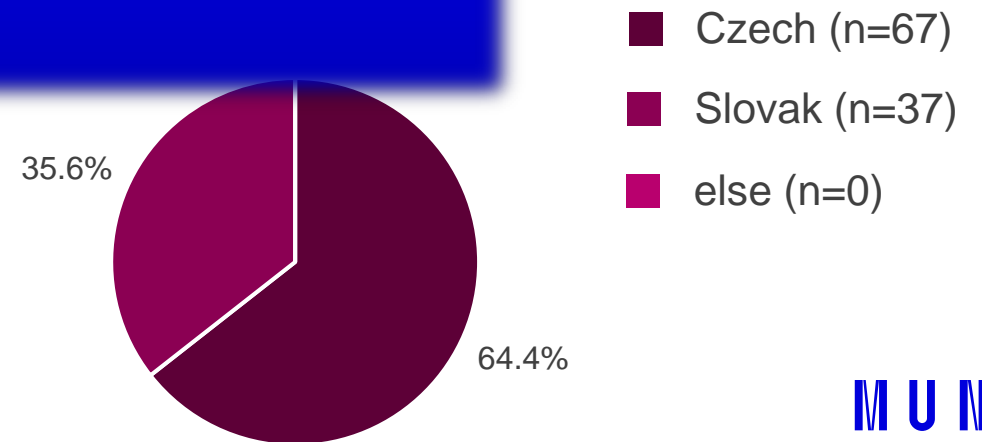
Nationality

- ■ Czech (n=67)
- ■ Slovak (n=37)
- ■ other (n=0)

0.0%

35.6%

64.4%

Pie chart

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Variables described by **more values that cannot be sorted**

- Multiple categories that cannot be sorted (there is no natural order of individual values)

- Usually

- *Example*

	n (%)
Nationality	
	67 (64.4)
	37 (35.6)
	0 (0.0)
	104 (100.0)

Nominal variables (**categorical**)

- Equals?

Pie chart

35.6%

64.4%

■ Czech (n=67)

■ Slovak (n=37)

■ else (n=0)

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Variables described by **more values that can be sorted**

– Several categories that can be sorted each other out

– *Example:* frequency of teeth cleaning

– Equals? Bigger x smaller?
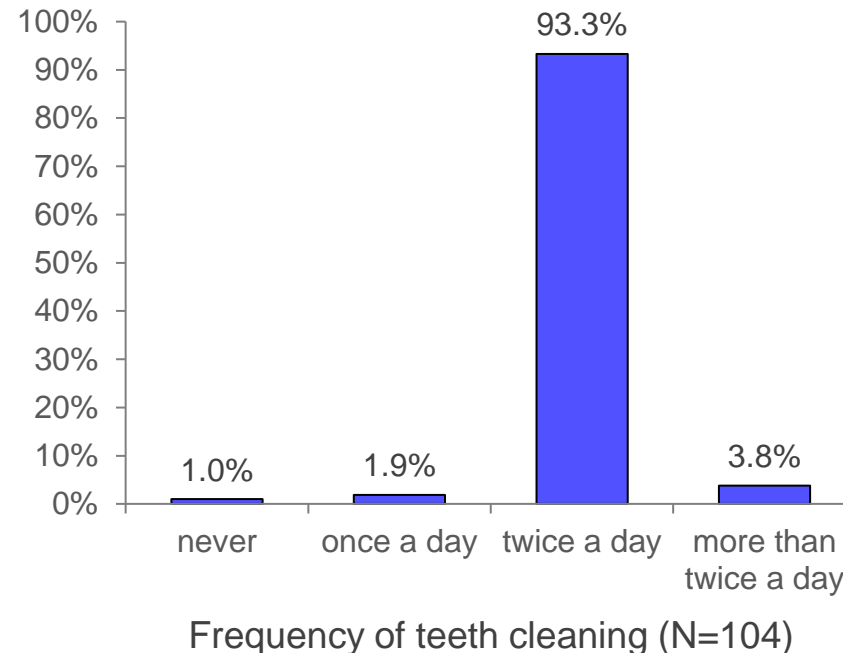
	n (%)
Frequency of teeth cleaning	
never	1 (1.0)
once a day	2 (1.9)
twice a day	97 (93.3)
more than twice a day	4 (3.8)
total	104 (100.0)

Bar chart

Frequency of teeth cleaning (N=104)

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Variables described by **more values that can be sorted**

- Several categories that can be sorted each other out

- *Example: frequency of teeth cleaning*

- Equals?

Ordinal variables (**categorical**)
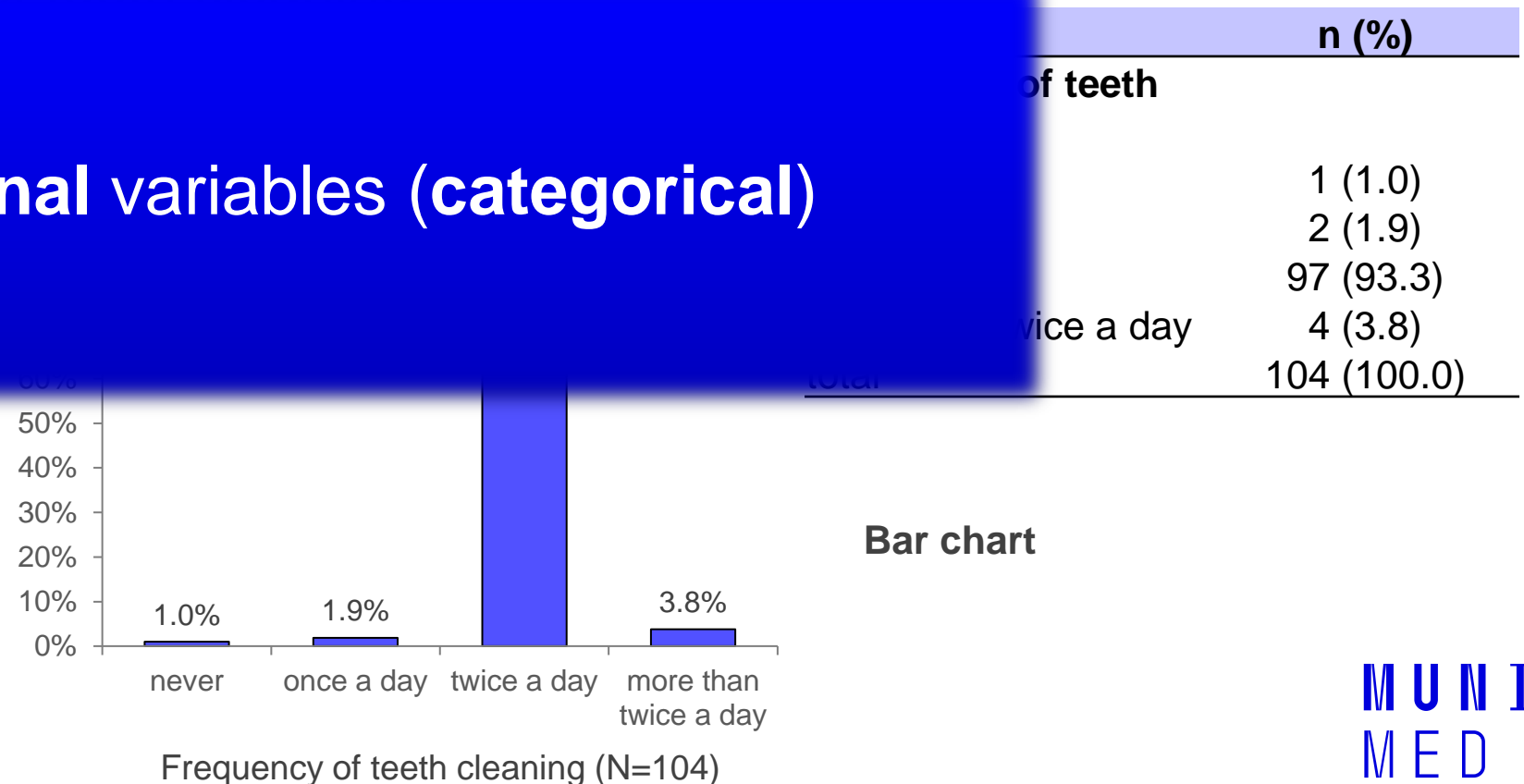
	n (%)
of teeth	
	1 (1.0)
	2 (1.9)
	97 (93.3)
...ice a day	4 (3.8)
total	104 (100.0)

Bar chart

Frequency of teeth cleaning (N=104)

- 50%
- 40%
- 30%
- 20%
- 10%
- 0%

never 1.0% once a day 1.9% twice a day more than twice a day 3.8%

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Variables described by **arbitrary values (within a certain range)**

— Measurements, the result of which takes arbitrary values in a certain range (interval)

— *Example:*

— Equals? Bigger x smaller? By how much? How many times?

Box Plot
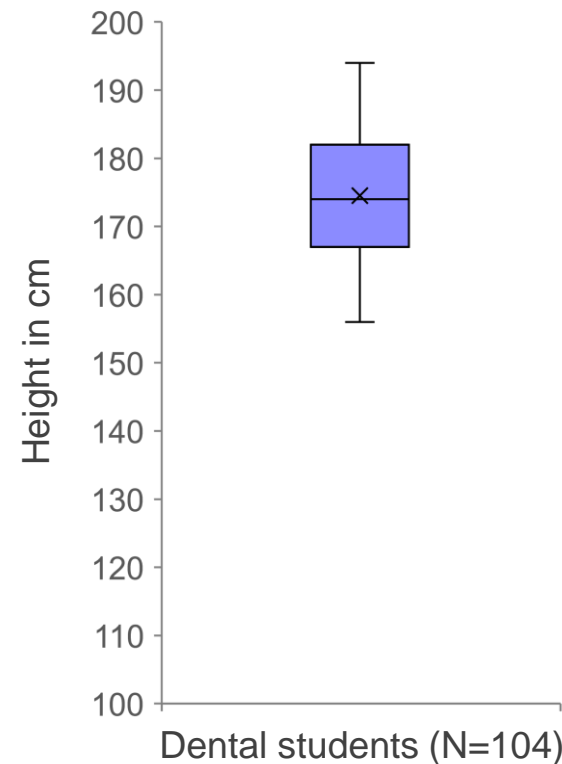
	Height in cm
median	174
5%-95% percentile	162-191
mean	174.5
SD	9.4

maximum
75% percentile
median × *mean*
25% percentile
minimum

Dental students (N=104)

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Variables described by **arbitrary values (within a certain range)**

— Measurements, the result of which

takes on arbitrary values in a certain

range (i...

— *Exampl...*

Continuous variables

Box Plot

	Height in cm
...edian	174
%-95% percentile	162-191
...ean	174.5
...D	9.4

maximum
75% percentile
median ✕ *mean*
25% percentile
minimum

— Equals? Bigger x smaller? About how

much? How many times?

Dental students (N=104)

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Variables described by **only countably many values**

- Limited number of values

- *Example:*

- Equals? Bigger x smaller?
  About how much? How
  many times?

DMFT	frequency
0	8
1	11
2	11
3	10
4	5
5	11
6	11
7	6
8	5
9	2
10	5
11	4
12	2
13	1
14	4
15	1
16	1
17	1
18	1
19	0
20	1
missing	3
total	104

Box Plot

Dental students (n=101)

75% percentile
median   × *mean*
25% percentile

range of non-outliers

• *outliers*

	DMFT index
median	5
5%-95% percentile	0-14
mean	5.7
SD	4.6

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Variables described by **only a limited (countable) number of values**

- Limited number of values

- *Example:*

- Equals? Bigger x smaller?
  By how much? How many
  times?

DMFT	frequency
0	8
1	11
2	11
3	10
4	5
5	11
6	11
7	6
8	5
9	2
10	5
11	4
12	2
13	1
14	4
15	1
16	1
17	1
18	1
19	0
20	1
missing	3
total	104

Bar chart

Bar chart: DMFT index (N=104)
- 0-4: 43.3%
- 5-9: 33.7%
- 10-14: 15.4%
- ≥15: 7.7%

25% percentile
median   × *mean*
75% percentile

Range of non-outliers

• *outliers*

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Variables described by **only countably many values**

- Limited number of values

- *Example:*

Discrete variables

- Equals? Bigger x smaller? About how much? How many times?

DMFT	frequency
0	8
1	11
2	11
3	10
4	5
14	4
15	1
16	1
17	1
18	1
19	0
20	1
missing	3
total	104

Box Plot

Dental students (n=101)

75% percentile
median   × *mean*
25% percentile

range of non-outliers

outliers

	DMFT index
median	5
5%-95% percentile	0-14
mean	5.7
SD	4.6

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Nominal variables

Binary variables

Ordinal variables

Continuous variables

Discrete variables

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Nominal variables

Binary variables

Ordinal variables

Discrete variables

Can be treated as **categorical variables**

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Can be treated as **continuous variables**

Ordinal variables

Continuous variables

Discrete variables

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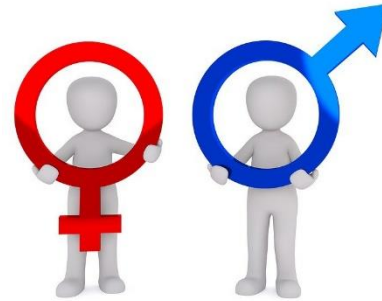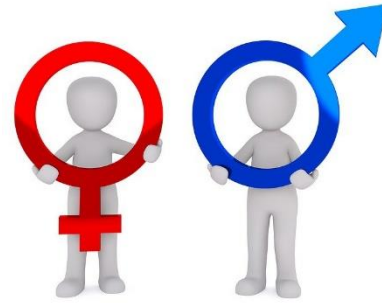
Possibility of data conversion

How we can describe and visualize the variables

Motivation
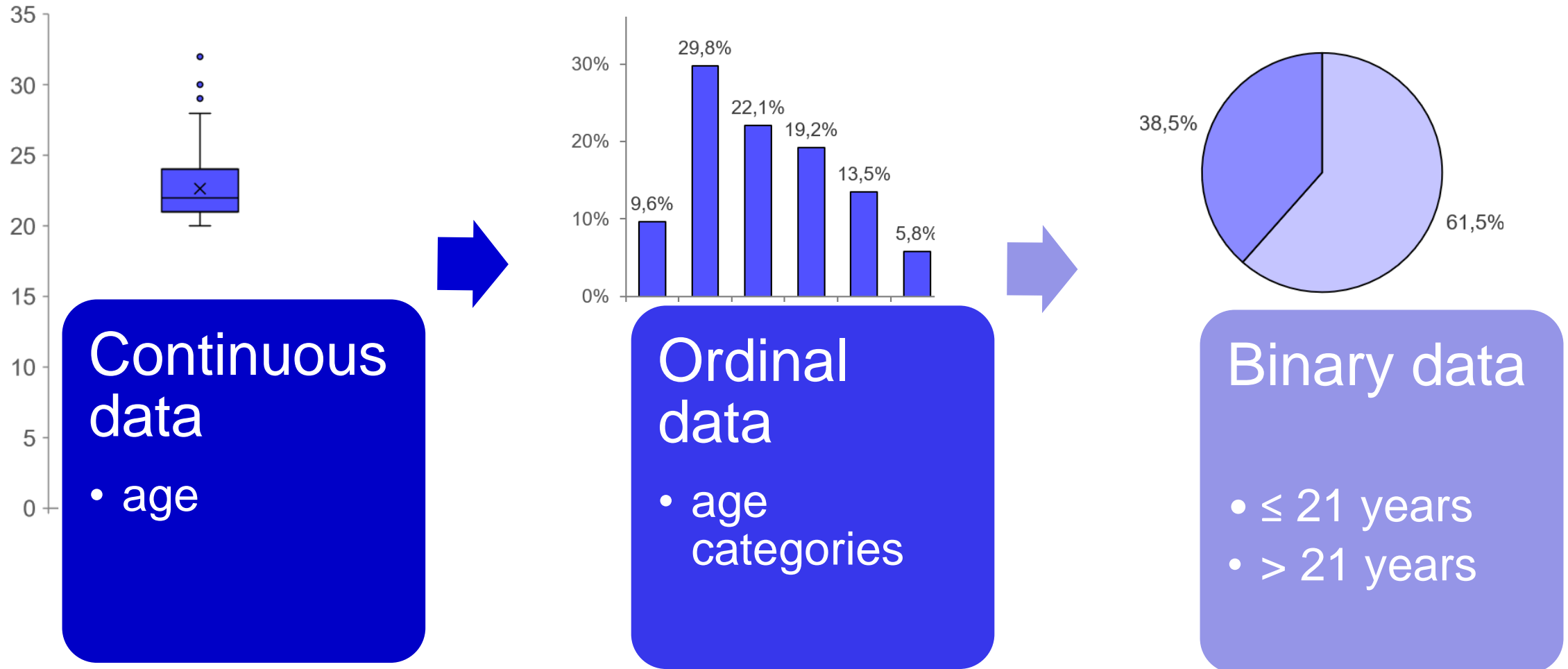
Objectives of descriptive data summarization

| Make it clearer | Summarize | Background for determining hypotheses | Detect outliers and incorrect values | Detect missing values |

— **summary of categorical data** → the aim is to describe the absolute and relative frequencies of individual categories

— **summary of continuous data** → the aim is to describe the center of gravity (measures of location) and the range (measures of variability) of the observed values

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Descriptive summary of categorical data

Nominal variables

Binary variables

Ordinal variables

Discrete variables

Can be treated as categorical variables

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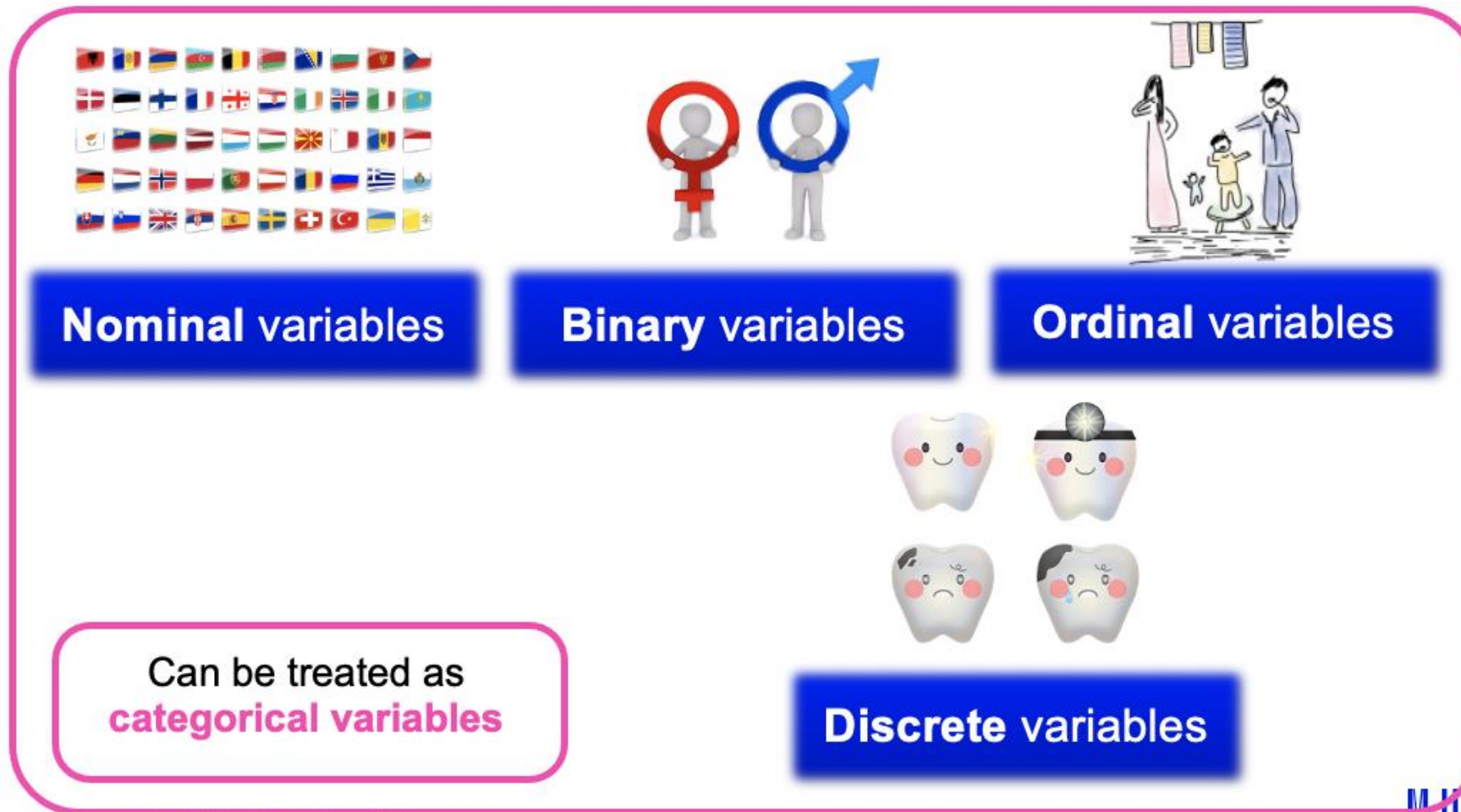
Descriptive summary of categorical data

Primary data

How often you brush your teeth

2
0
2
2
2
2
2
2
1
2
3
.
.
.
.
N=104

Coding:

0 = never
1 = once a day
2 = twice a day
3 = more than twice a day

Frequency table

Frequency of teeth cleaning	n (%)
never	1 (1.0)
once a day	2 (1.9)
twice a day	97 (93.3)
more than twice a day	4 (3.8)
total	104 (100.0)

n – the absolute frequency of the category
% – the relative frequency; calculation as n/N*100

Visualization

Frequency of teeth cleaning (N=104)

- never (n=1)
- once a day (n=2)
- twice a day (n=93)
- more than twice a day (n=4)

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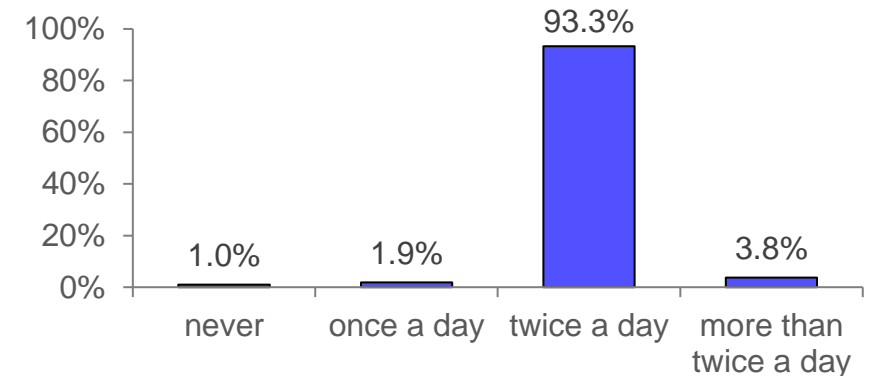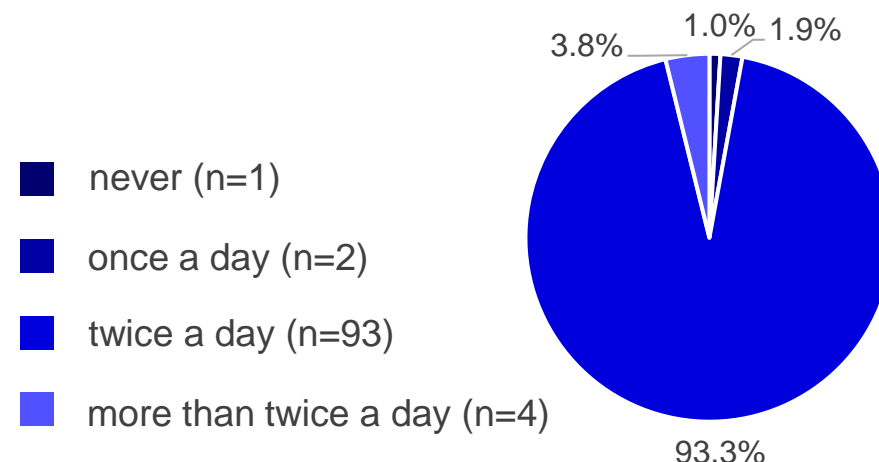
Descriptive summary of continuous data

Can be treated as **continuous variables**

Ordinal variables

Continuous variables

Discrete variables

Descriptive summary of continuous data

Primary data

Height
184
167
163
174
177
178
173
169
179
185
.
.
.
.
N=104

Table of descriptive statistics

	Height in cm
N	104
Mean	174.5
95% confidence interval	172.7-176.3
Median	174
Standard Deviation; SD	9.4
Minimum	156
Maximum	194
Lower Quartile	167
Upper Quartile	182
5%-95% percentile	162-191

Visualization

Box Plot

Dental students (N=104)

- maximum
- 75% percentile
- median   ✕ *mean*
- 25% percentile
- minimum
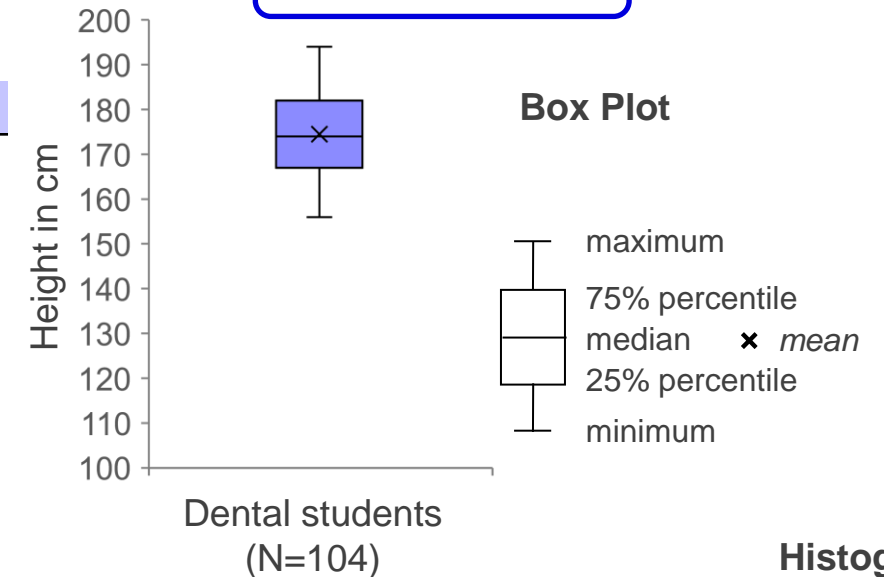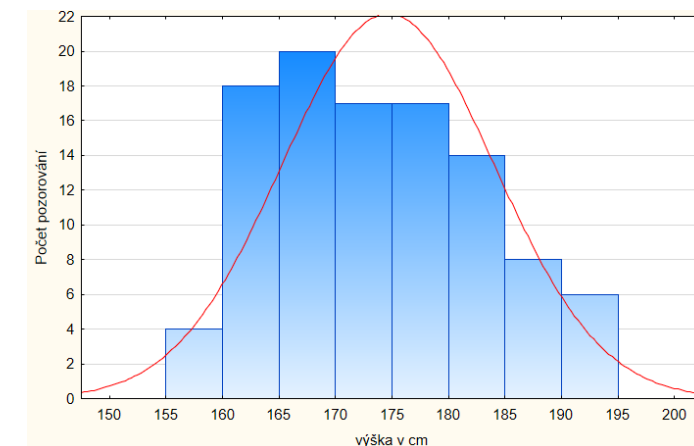
Histogram

Visualization of continuous data

– **Visualization of quantitative data:** most often using a box plot or histogram

Example: Description of patient height (cm)

Box Plot

maximum (100% quantile)

upper quantile (75% quantile)
median (50% quantile)
lower quantile (25% quantile)

Is the data symmetrical?

minimum (0% quantile)

Height in cm

Histogram

Height in cm

Outlier?

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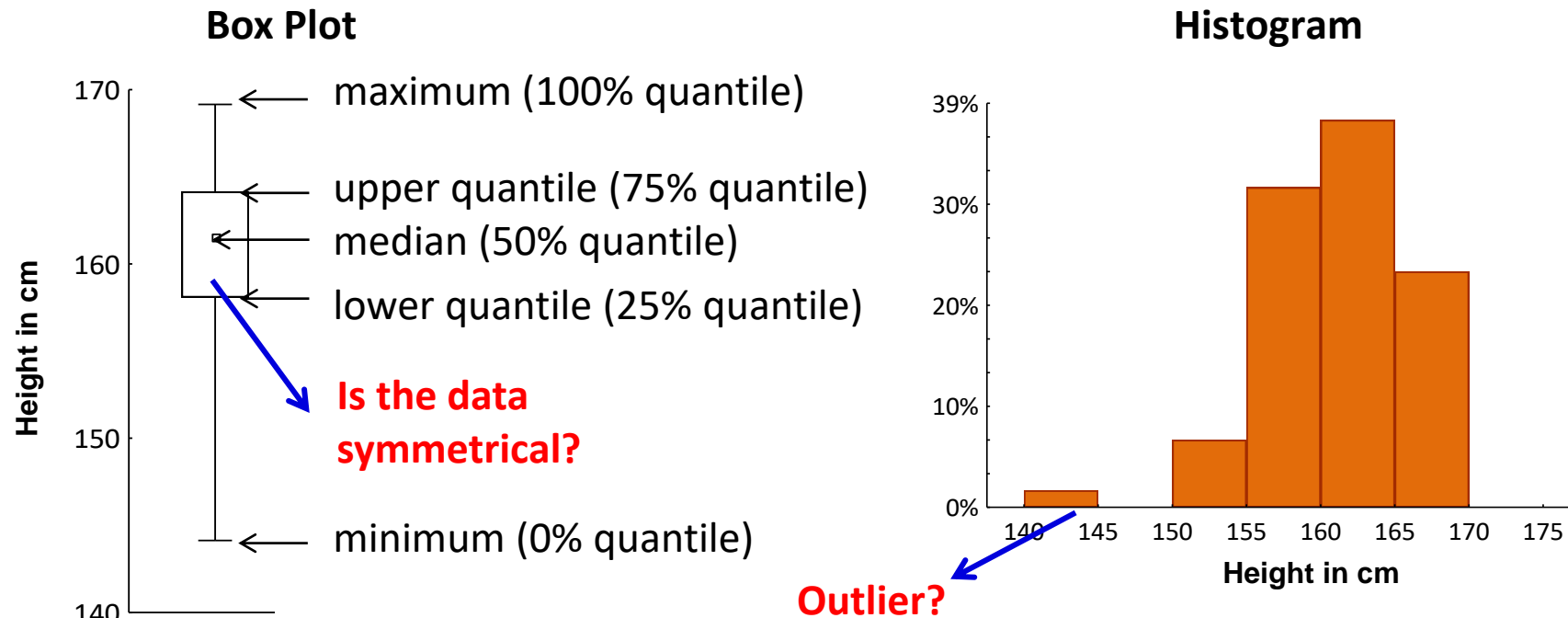
Continuous data – measures of location I

- **Minimum and maximum** – the smallest and largest observed value give us an image of the range of values.

- **Mean ($\bar{x}$)** – characterizes the value around which other observed values fluctuate. It is the "center of gravity" of the data.

Visulization:

$$\bar{x} = (25+64+73) / 3 = 54$$

Example: N = 8

Data = 6 1 7 4 3 2 7 8

Sum of the data = 6+1+7+4+3+2+7+8 = 38

Mean = 38 / N = 38 / 8 = 4.75

$$\bar{x} = \frac{1}{N}\sum_{i=1}^{N} x_i$$

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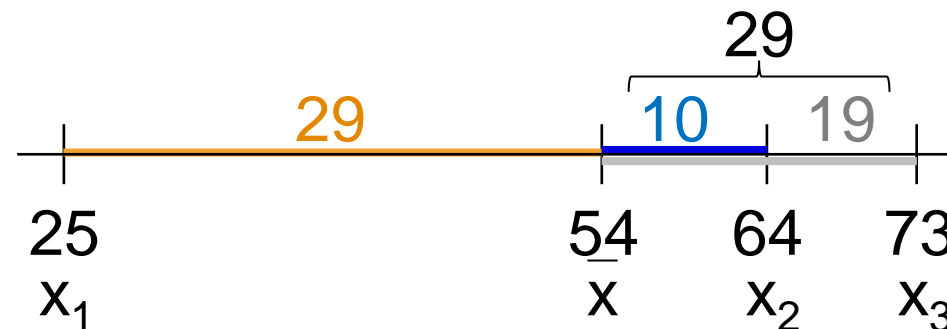
Continuous data – measures of location II

– **Median** – the middle observed value

– divides the observed values into two halves. Half the values are smaller, half the values are bigger than the median.

– **Example 1:**

N = 9

N is odd -> (N + 1) / 2 position. Here, 5th position after sorting

Data = 3.0 4.2 1.1 2.5 2.2 3.8 5.6 2.7 1.7

Sorted data = 1.1 1.7 2.2 2.5 2.7 3.0 3.8 4.2 5.6

Median = 2.7

– **Example 2:**

N = 8

N is even -> we calculate the value "between" 4th (N/2) a 5th (N/2+1) value after sorting

Data = 6 1 7 4 3 2 7 8

Sorted data = 1 2 3 4 6 7 7 8

Median = (4 + 6) / 2 = 5

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Mean vs. median

Symmetric data
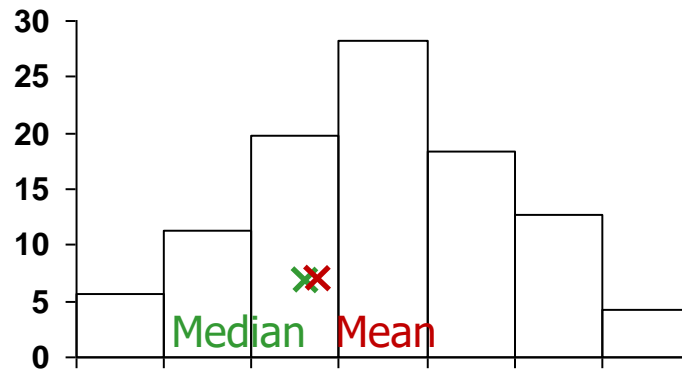
Asymmetric data

— the median and mean are almost identical

— median and mean are good estimates of the frequency center of the data
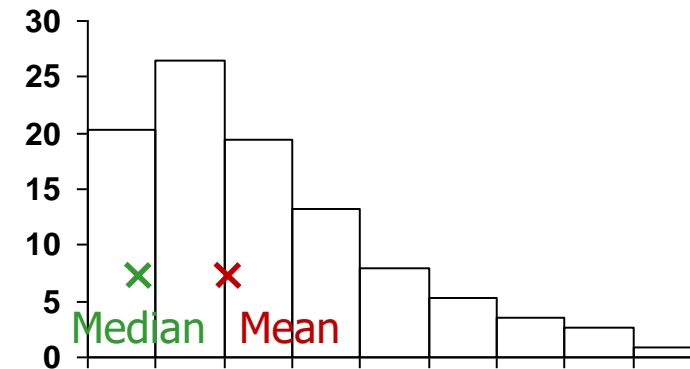
— the median and mean differ

— mean IS NOT a good estimate of the frequency center of the data

— mean is suitable in a situation where we want to characterize consumption (drugs, money, etc.)

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Quantile

— The quantile can be defined as a number on the real axis

that divides the observed data into two parts:

the p% quantile divides the data into p% values

and (100-p)% values.

80% of values

20% of values
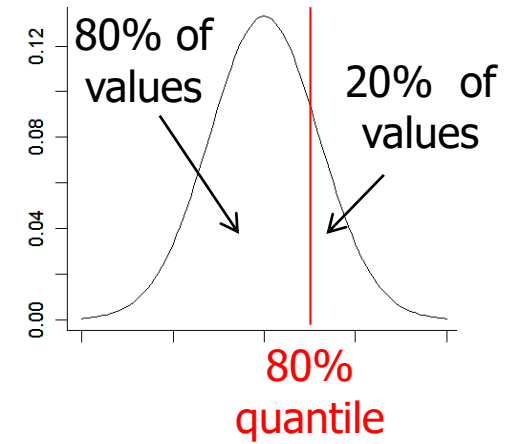
80% quantile

— We have a set of 20 people in whom we measure height. We want to find the 80% quantile of the set of observed data.

Mean of these two values = 80% quantile

n = 20

16 / 20 = 80% values

4 / 20 = 20% values

110 cm   140 cm   170 cm   200 cm   230 cm

Height in cm

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Important quantiles

Age

Maximum = 100% quantile   90

Upper quartile= 75% quantile   80
Median = 50% quantile   **75**
Lower quartile= 25% quantile   71

Minimum = 0% quantile   54

□ Median = 75
▢ 25%-75%
= (71, 80)
⊥ Min-Max
= (54, 90)

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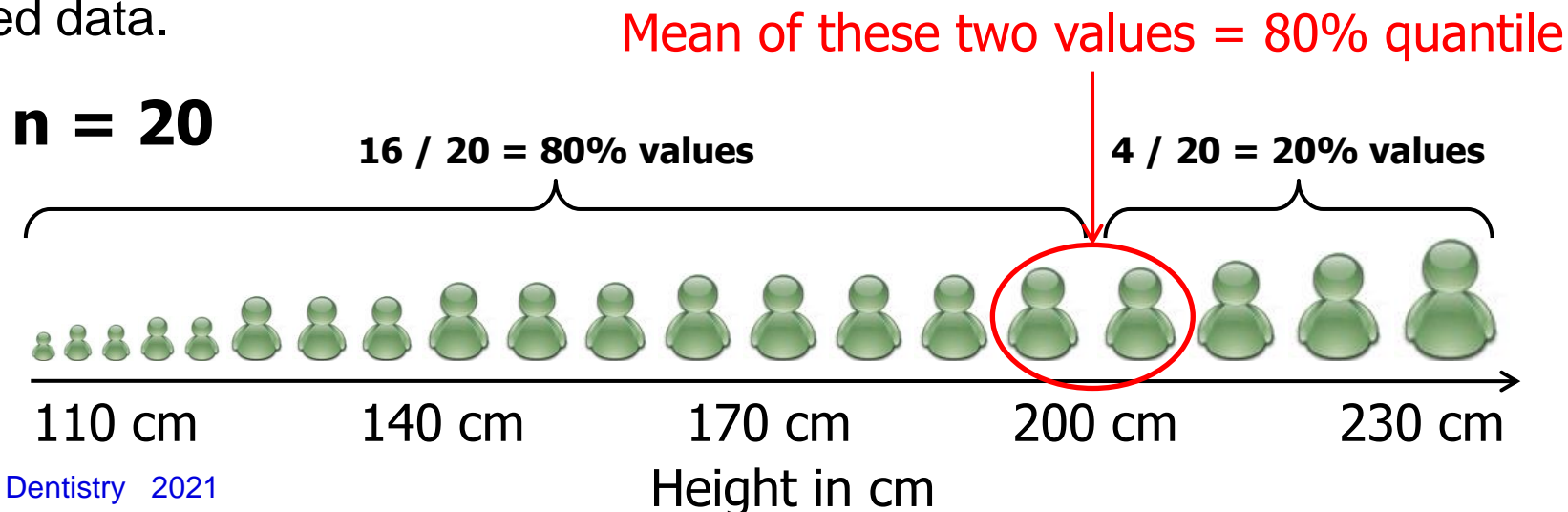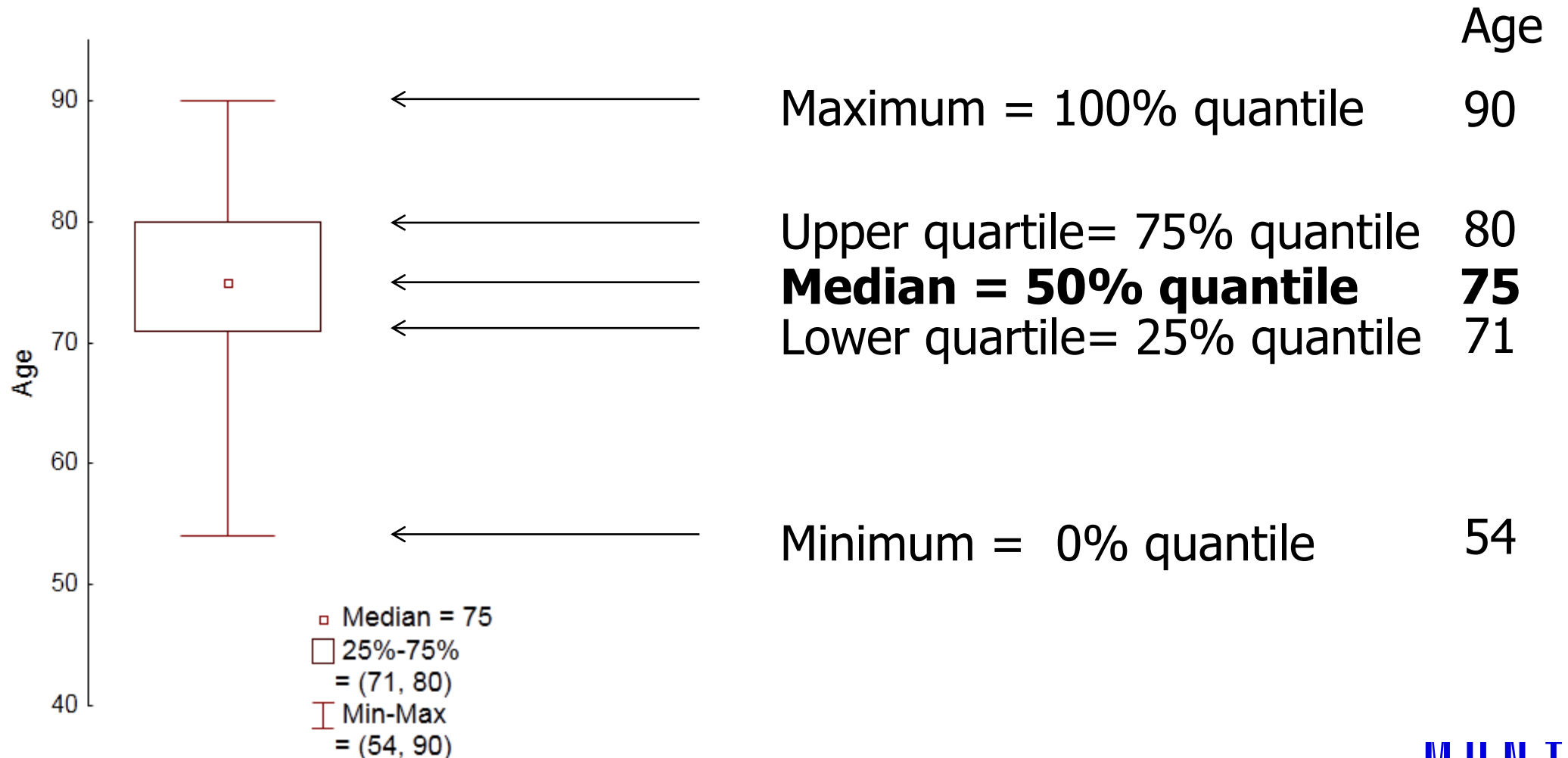
Continuous data – measures of variability I
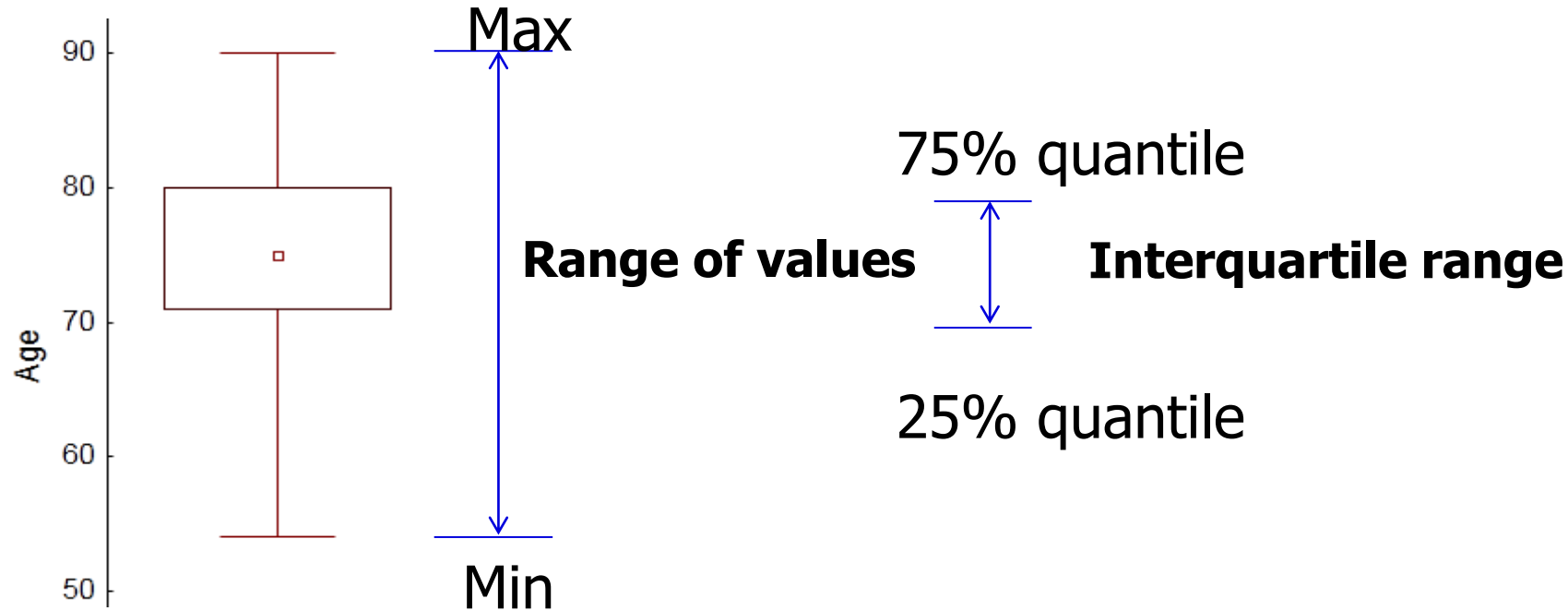
- **Range of values** = maximum – minimum. It is the simplest characteristics of the variability of the observed data. It can be easily influenced by atypical values (outliers).

- **The quantile range** is defined by the p% quantile and the (100-p)% quantile and is less affected by outliers. A special case is **the interquartile range** (25%-75% quantile), which covers 50% of the observed data.

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Continuous data – measures of variability II

— **Variance** - the average square of the deviation from the mean. Very influenced by outliers.

$$s^2 = \frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2$$

— **Standard deviation, SD** - square root of variance. The advantage of the standard deviation is that it has the same units as the observed data.

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Calculation of variance and standard deviation - example

– Example of squares of deviations from the mean for $n = 3$.

– The variance can be greatly affected by outliers.
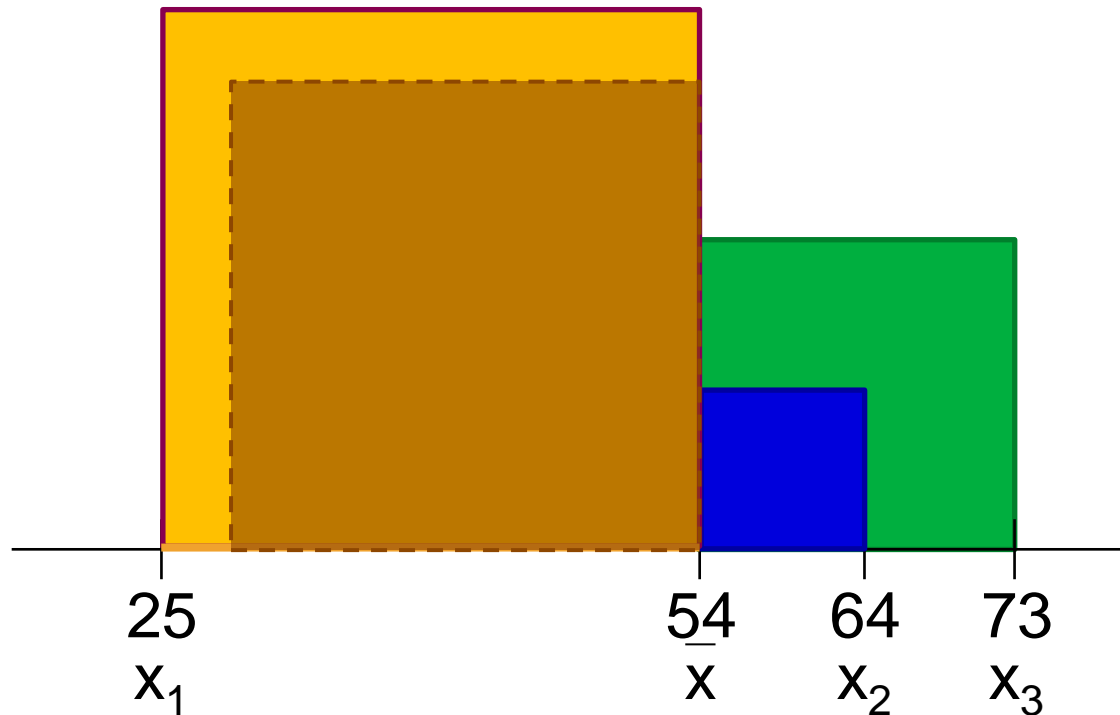
Variance:

$$s^2 = \frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2$$

Standard deviation:

$$s = \sqrt{\frac{1}{n-1} \sum_{i=1}^{n} (x_i - \bar{x})^2}$$

$$s = \sqrt{\frac{(25-54)^2 + (64-54)^2 + (73-54)^2}{2}} = \sqrt{651} = 25.5$$

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Normal distribution

— The most classical model distribution, from which several statistical analyzes are derived, is the so-called **normal distribution**, also known as the **Gaussian curve**.

— Describes the probability distribution of a continuous variable, e.g. height in a population

— Is completely described by two parameters:

μ – mean value

σ^2 – variance

Designation: **N(μ, σ^2)**

NORMALITY is a key assumption for several statistical methods

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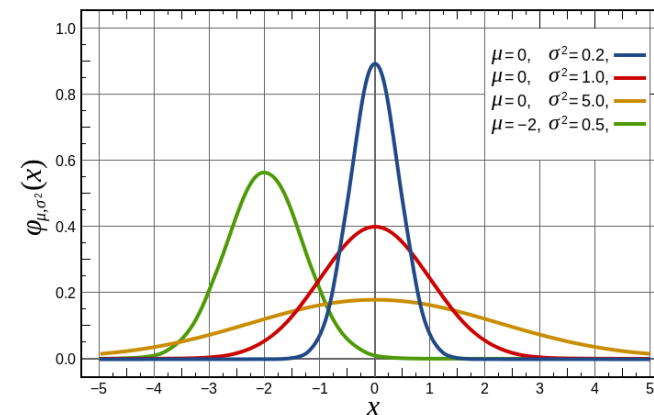
Rule ± 3 SD (sigma)

– **99.7% of all values should occur within the mean ± 3 SD**

– Application:
  – orientation verification of normality
  – identification of outliers

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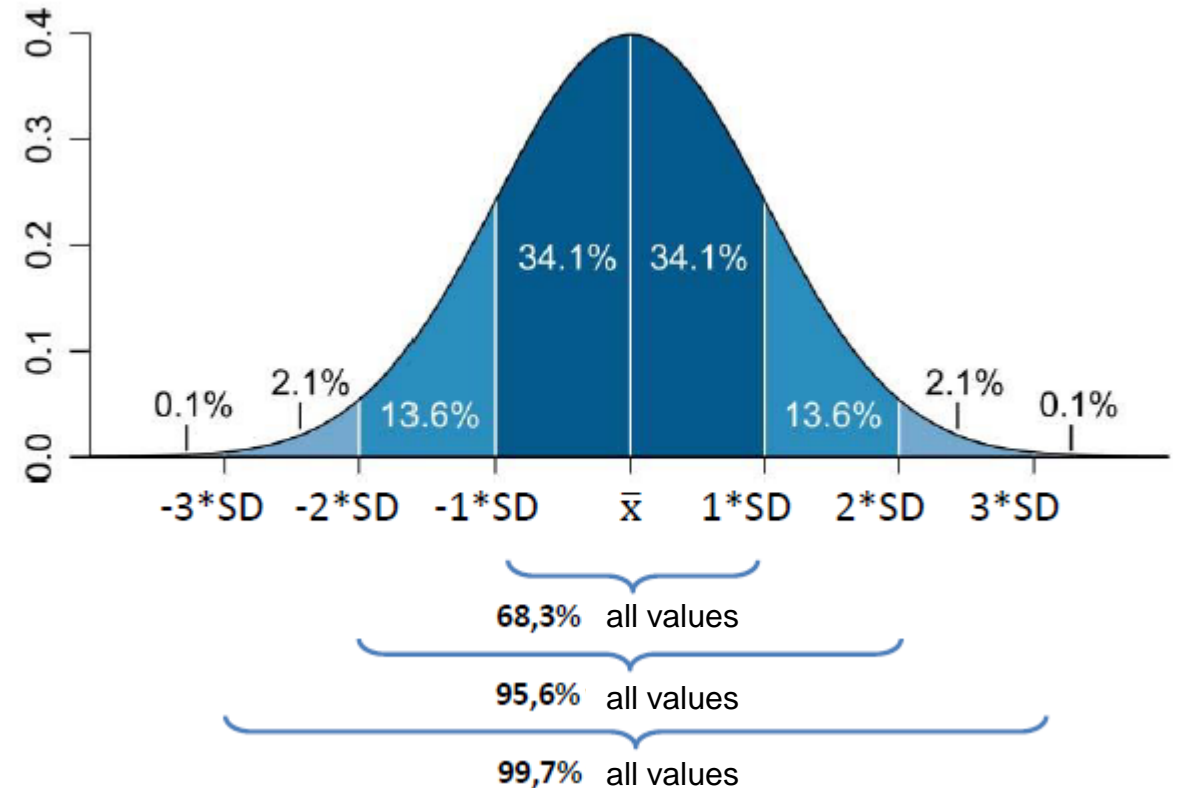
Visual verification of normality

— A **histogram** can be used to evaluate the shape of the distribution (disadvantage: it is necessary to determine the "appropriate" number of columns)

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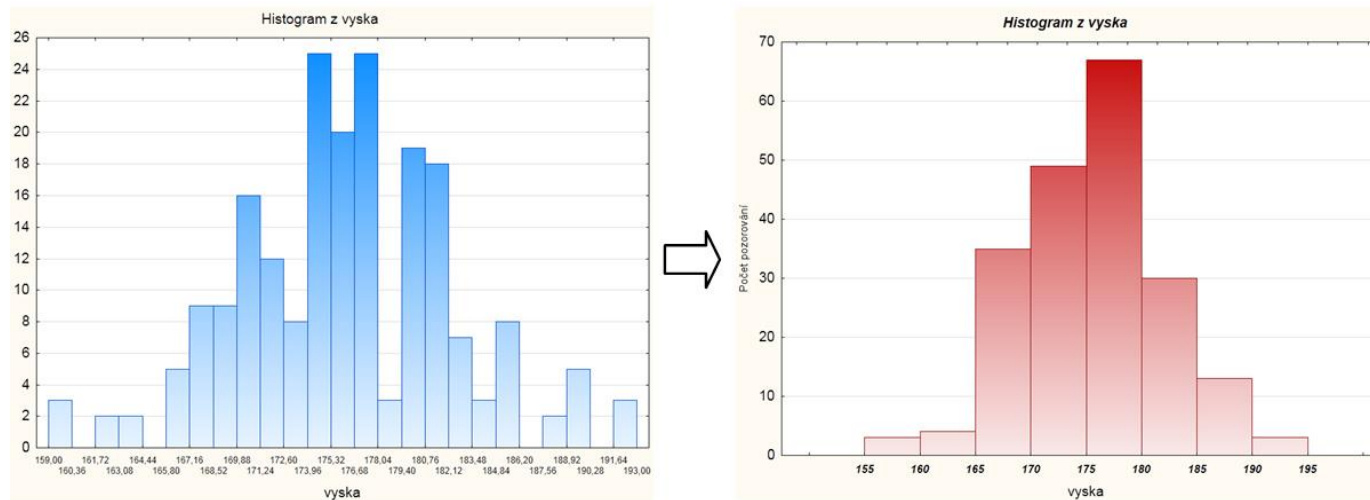
We can test hypotheses statistically

Principle of hypothesis testing

- Hypothesis formulation
- Selection of the target population and a representative sample from it
- Measurement of monitored variables
- Use the appropriate test $\longrightarrow$ conclusion of the test
- Interpretation of the results
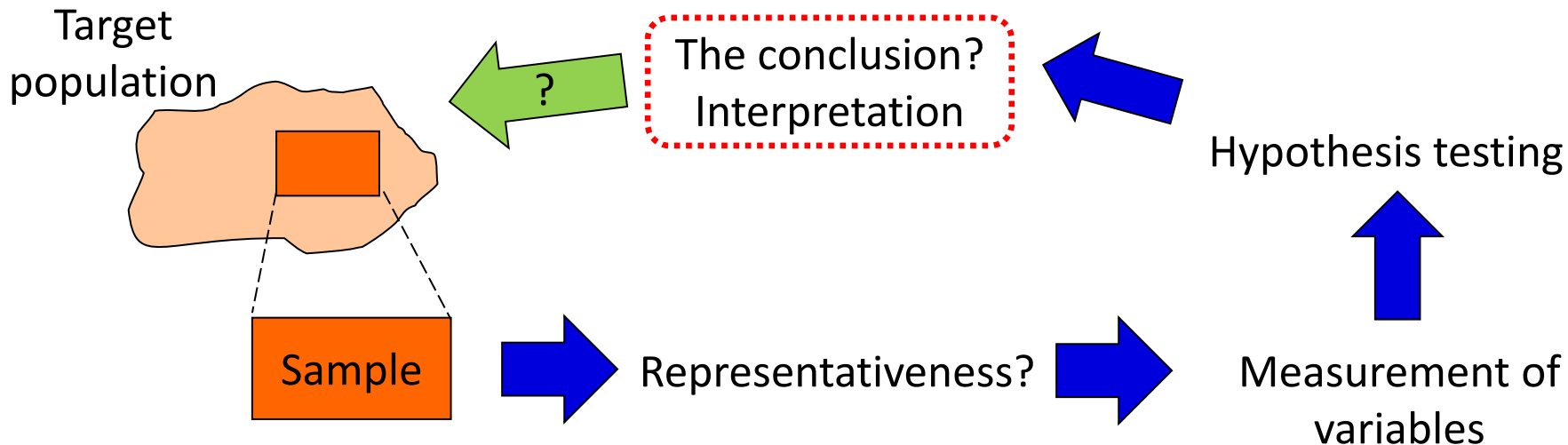
Statistical testing – basic concepts

— Null hypothesis H_0

H_0: the observed effect is zero

— Alternative hypothesis H_A

H_A: the observed effect varies between groups

— Test statistics

$$\text{Test statistics} = \frac{\text{Observed value} - \text{Expected value}}{\text{Data variability}} * \sqrt{\text{Sample size}}$$

— Critical field of test statistics
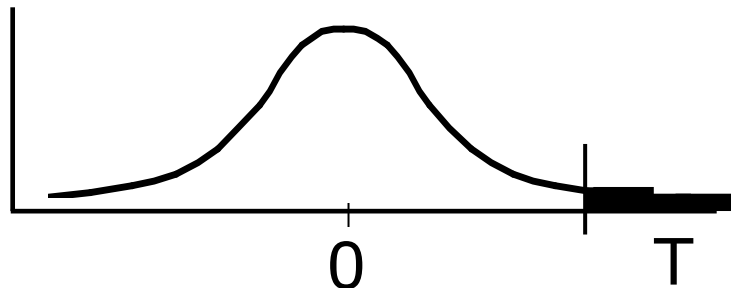
Statistical testing answers the question of **whether the observed difference is random or not**.
The statistical model - test statistics - is used to answer the question.

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Hypothesis creation
Possible errors in hypothesis testing

– Despite the sufficient sample size and quality design of the experiment, we can make

mistakes when deciding to (not to) reject the null hypothesis.
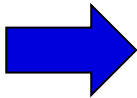
		Conclusion from statistical analysis	
		not to reject H_0	reject H_0
The true state of nature	H_0 is TRUE	Correct $1-\alpha$	α — Type I error: reject a true null hypothesis – **false positive**
	H_0 is FALSE	β — Type II error: not to reject a false null hypothesis – **false negative**	$1-\beta$ Correct

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Meaning of errors in hypothesis testing
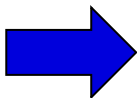
— **Probability of type I error**

α ➡ Probability of a false rejection of the null hypothesis, **significance level**

— **Probability of type II error**

β ➡ Probability of not recognizing a false null hypothesis

— **Power of a test**

$1-\beta$ ➡ Probabilistic ability to recognize the invalidity of the null hypothesis

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Ways of hypothesis testing

— Testing H_0 against H_A at the **α** significance level can be performed in three different ways:

1. **Rejection region** or critical region H_0,

2. **Confidence interval**,

3. ***P*-value** (expresses the probability under the validity of H_0, with which we would obtain the same or more extreme value of the test statistic).

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Testing methods: P-value

‒ We evaluate the significance of the hypothesis according to the obtained **p-value**, which expresses the probability with which the numerical realizations of the sample set support H_0, if it is true.

‒ We compare the *p*-value with the significance level **α** (we set it to 0.05, i.e. we allow a 5% error of the test, that is, we reject H_0, although it is true).

‒ The *p*-value is obtained by testing hypotheses in statistical software.

If $p \leq α$, then we **reject H_0** at the significance level α and **accept H_A**.

If $p > α$, then we **do not reject H_0** at the significance level α.

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Notes on hypothesis testing

– **Failure to reject the null hypothesis does not automatically mean its acceptance!** This may be a situation where we do not have enough information to reject the null hypothesis.

– **The achieved level of significance of the test** (whether 5%, 1% or 10%) **must not be blindly taken as the limit for the (non)existence of the tested effect**.

– **A small p-value does not necessarily mean a large effect**. The value of the test statistic and the p-value can be affected by the large sample size and the small variability of the observed data.

– **Testing results must be viewed critically** - this is a conclusion based "only" on one sample.

– **Statistical significance** indicates that the observed difference is not random but does not necessarily mean that it is significant even in reality. **Practical (clinical) significance is also important**.

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Basic statistical tests

Data type

- **Continuous x continuous data**
  - **Pearson's correlation coefficient**
  - **Spearman's correlation coefficient**

- **Continuous x categorical data**
  - **One-sample**
    - **One-sample t test**
    - **One-sample Wilcoxon test**
  - **Two-sample**
    - **Paired data**
      - **Paired t test**
      - **Wilcoxon / sign test**
    - **Independent data**
      - **Two-sample t test**
      - **Mann-Whitney test**
  - **More samples (independent)**
    - **ANOVA**
    - **Kruskal-Wallis test**

- **Categorical x categorical data**
  - **One-sample**
    - **One-sample binomic test**
  - **More samples**
    - **Paired data**
      - **McNemar test**
    - **Independent data**
      - **Chi-square test**
      - **Fisher's exact test**

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Used shortcuts

- SD, standard deviation

- DMFT, Decayed, Missing and Filled Permanent Teeth

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Genetic association studies and oral microbiome

Assoc. Prof. RNDr. Petra Bořilová Linhartová, Ph.D., MBA
Clinic of Stomatology, Department of Pathophysiology, Institute of Medical Genetics and Genomics, Faculty of Medicine, Masaryk University Brno
Clinic of Maxillofacial Surgery, University Hospital Brno

peta.linhartova@gmail.com

Content of the lecture

Genetic association studies and oral microbiome

— Genetic association studies

— Oral microbiome

— Diseases:

— tooth decay (behavioral intervention) (Bořilová Linhartová)

— aphthosis stomatitis (clinical experimental study) (Bořilová Linhartová)

— periodontopathy (Bořilová Linhartová)

— apical periodontitis and odontogenic cysts (Szaraz, Daněk, Bořilová Linhartová)

— external apical root resorption after orthodontic treatment (Bořilová Linhartová)

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Presentation of the research team

Medical Molecular Genetics and Pharmacogenetics – Petra Bořilová Linhartová RG

– RG deals with basic and applied research of diseases of the orofacial area, respiratory and gastrointestinal tract, as well as the immune system, so we solve several research topics that can be collectively defined as research in the field of medical molecular genetics and pharmacogenetics.

• We mainly focus on the analysis of candidate genes and their products related to the predisposition of an individual to a complex disease using molecular biological methods (qPCR, NGS, MS). We focus on immunogenetics

• At the same time, we also study the human microbiome, the relationship between the host and its microbiota in health and disease.

• We are also working on pharmacogenetic studies.

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Projects

Medical Molecular Genetics and Pharmacogenetics – Petra Bořilová Linhartová RG

— **Advanced biotechnological and behavioral approaches in dental caries research and prevention strategies (17-30439A)**

— **Molecular etiopathogenesis of apical periodontitis and odontogenic cystic lesions (NU20-08-00205)**

— **Host microbiome in relation to Barrett´s esophagus and esophageal adenocarcinoma development (NU20-03-00126)**

— **Use of nanofibres for application of bioactive substances using dental floss (FW02020042)**

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Presentation of the research team

Medical Molecular Genetics and Pharmacogenetics – Petra Bořilová Linhartová RG

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Genetics of complex oral diseases

Oral diseases

– Recurrent aphthous stomatitis

– External apical root resorption

– Dental caries

– Gingivitis and periodontitis

– Etiopathogenesis: immune factors

Interleukins
NOD-like receptor
Purinergic receptor
Vitamin D receptor
ACE

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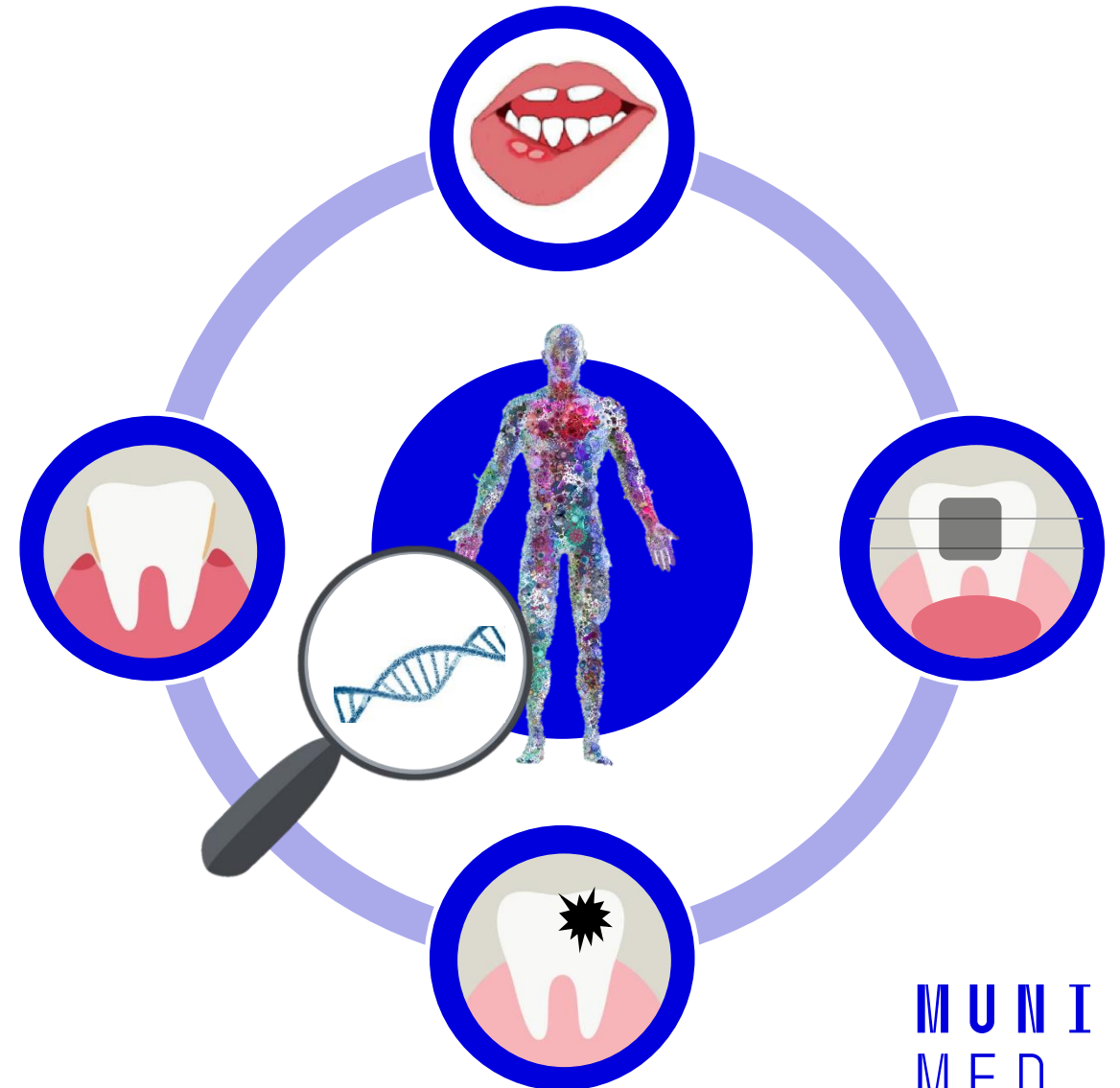
Recurrent aphthous stomatitis

Studies I and II.

– ACC haplotype and CC genotype was protective for RAS (Iran)

Najafi et al., 2014

– ↓ transcriptional activity of IL-10 in carriers of ATA haplotype and ↓ IL-10 production in carriers of ATA/ATA haplogenotype

– ↓ c IL-10 in the tissue of the aphthous lesion as well as in the serum of patients with RAS

Oleksyk et al., 2009

IL-10 178 HC 74 RAS

P<0.05

Association study of *interleukin-1* family, *interleukin-6*, and *its receptor* gene polymorphisms in patients with recurrent aphthous stomatitis

Lydie Izakovicova Holla[1,2] | Simona Valova[1,2] | Petra Borilova Linhartova[1,2] | Jirina Bartova[3] | Jitka Petanova[4] | Pavel Kuklinek[5] | Antonin Fassmann[1]

IF 2,237
Q1, DENTISTRY, ORAL SURGERY & MEDICINE
Q2 PATHOLOGY

Recurrent aphthous stomatitis and gene variability in selected interleukins: a case–control study

IF 1,81
Q2 DENTISTRY, ORAL SURGERY & MEDICINE

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Recurrent aphthous stomatitis

Study III.

– SNP *NLRP3* rs3806265 associated with RAS (Iran)

– SNP *NLRP3* rs3806265 and rs4612666 MAF (26% vs. 14% and 16% vs. 33%)   Bidoki et al., 2016

– not NLRP3 inflamasome activity BUT ↓ *NLPR*3 expression in carriers of TT genotype rs4612666 may affect CD4+ Th1/Th2 balance

Albanidou-Farmaki et al., 2007

NLRP3 143 HC   64 RAS

P<0.05
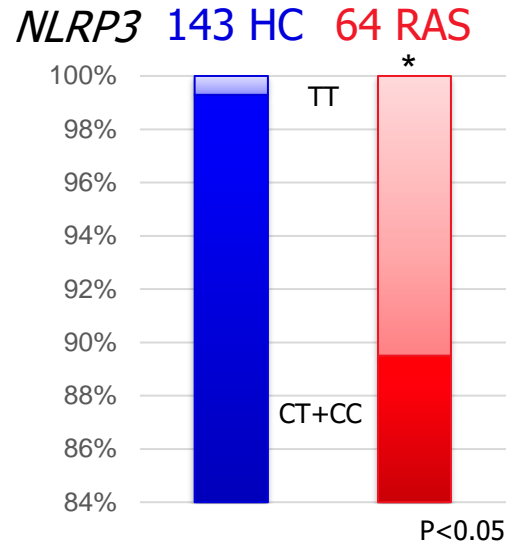
WILEY Journal of Oral Pathology & Medicine

ORIGINAL ARTICLE

Association of the NOD-like receptor 3 (*NLRP3*) gene variability with recurrent aphthous stomatitis in the Czech population

Simona Slezakova[1,2] | Petra Borilova Linhartova[1,2] | Lucie Masopustova[3] | Jirina Bartova[4] | Jitka Petanova[5] | Pavel Kuklinek[6] | Antonin Fassmann[1] | Ladislav Dusek[7] | Lydie Izakovicova Holla[1,2]

IF 2,03
Q2 DENTISTRY, ORAL SURGERY & MEDICINE
Q3 PATHOLOGY
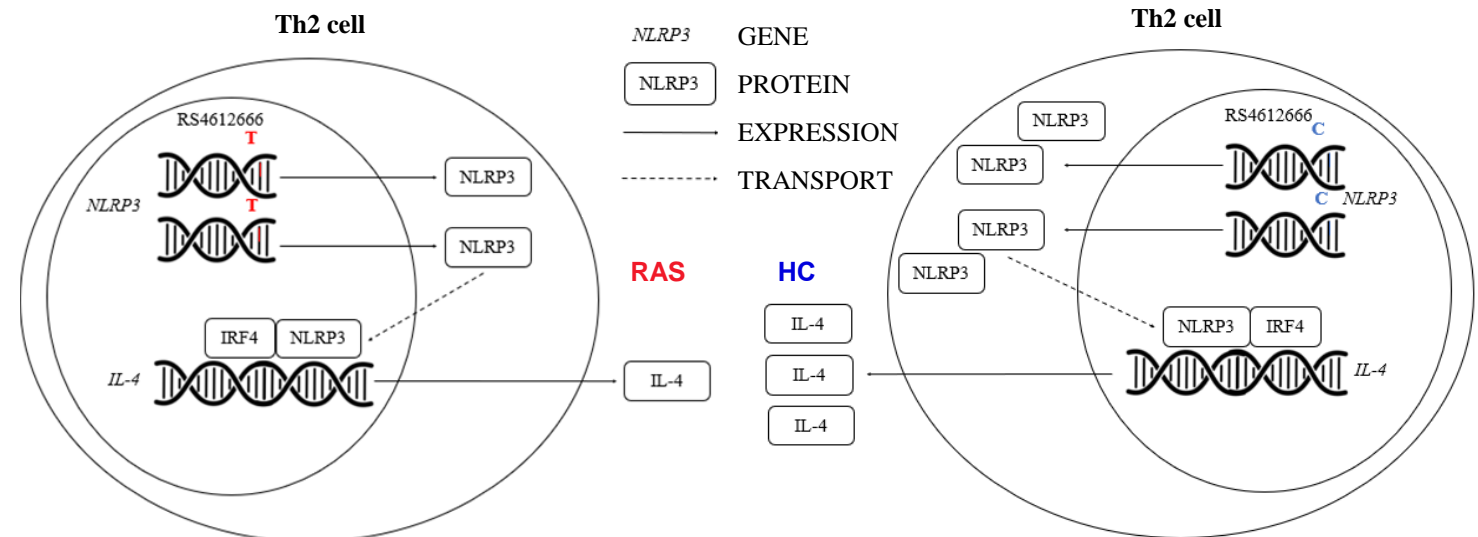
External apical root resorption

Study I.

– the duration of orthodontic therapy is positively correlated with the presence of EARR

– SNP *P2RX7* rs208294 (USA) and *P2RX7* rs1718119 (Portugal) associated with EARR

<u>Sharab et al., 2015</u>
<u>Pereira et al., 2014</u>

– ↑ secretion of proinflammatory IL-1β in carriers of the G allele *P2RX7* rs1718119 (gain of function)

<u>Stokes et al., 2010</u>

P<0.05

P2RX7   69 HC   30 RAS

P<0.05
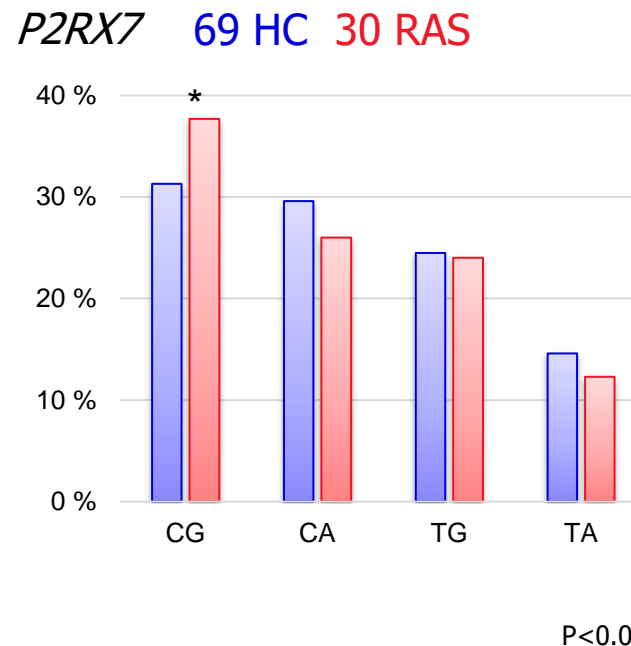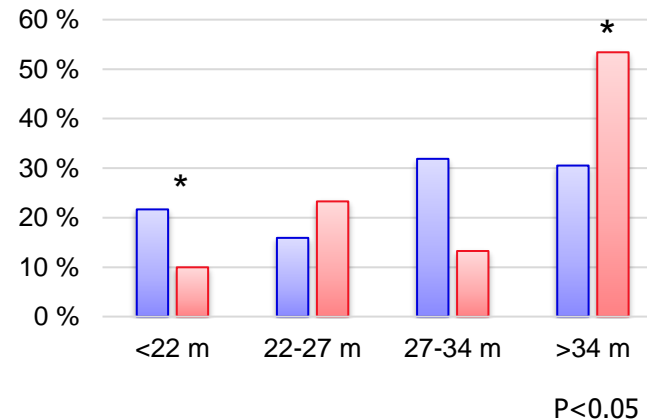
ORIGINAL ARTICLE

Genetic determinants and postorthodontic external apical root resorption in Czech children

P Borilova Linhartova[1,2], P Cernochova[1], J Kastovsky[3,*], Z Vrankova[3,*], M Sirotkova[3,*], L Izakovicova Holla[1,2]

<u>Surprenant and North, 2009</u>

Dental caries and gingivititis

Study I.

- SNP *ACE* I/D associated with dental caries (Poland, 5-13 years), DD genotype was protective

  Olszowski et al., 2015

- ↑ c ACE in serum of carriers of DD genotype Rigat et al., 1990

- *ACE* I/D influence on the release of proinflammatory mediators by immune cells, DD genotype risk for CP

  Holla et al., 2001
  Kang et al., 2015
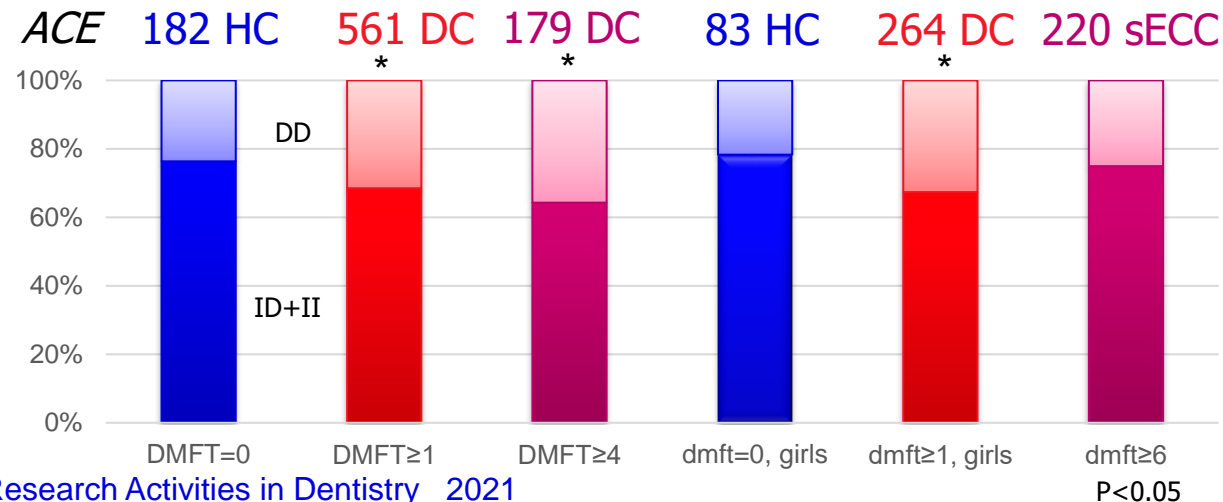
Caries Research

ACE Insertion/Deletion Polymorphism Associated with Caries in Permanent but Not Primary Dentition in Czech Children

Petra Borilova Linhartova[a,b] Jakub Kastovsky[a,b] Michaela Bartosova[a,d]
Kristina Musilova[a] Lenka Zackova[c] Martina Kukletova[a] Lubomir Kukla[d]
Lydie Izakovicova Holla[a,b]

IF 1,811
Q2 DENTISTRY, ORAL SURGERY & MEDICINE

Wang et al., 2010

199 Research Activities in Dentistry   2021

P<0.05

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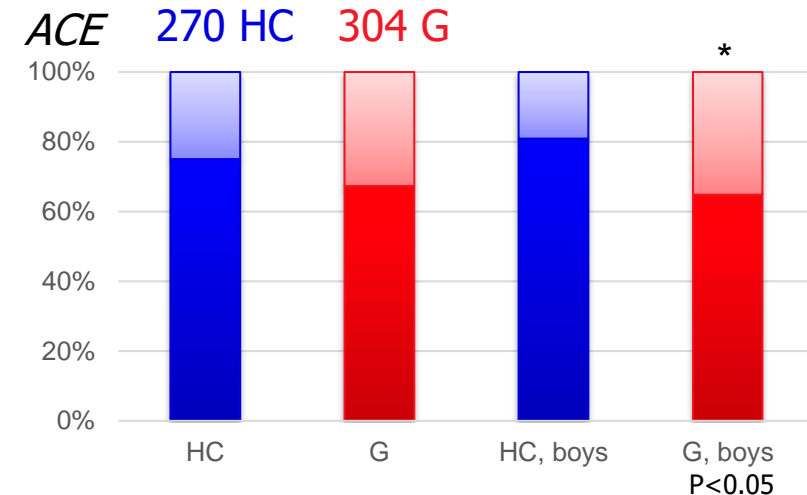
Dental caries and gingivitis

Study II.

– SNP *VDR Taq*I was associated with dental caries (Turkey, China) and with CP (in meta-analysis)

Hu et al., 2015

Cogulu et al., 2016

– ↑ transcriptional activity, stability of mRNA and $1,25(OH)_2D_3$ in serum in carriers of C allele

Deng et al. J Clin Periodontol. 2011.

Martelli et al., 2014

– VDRE

• Upregulation: immunomodulation, apoptosis, differentiation, autophagy

• Downregulation: inflammation, proliferation, angiogenesis
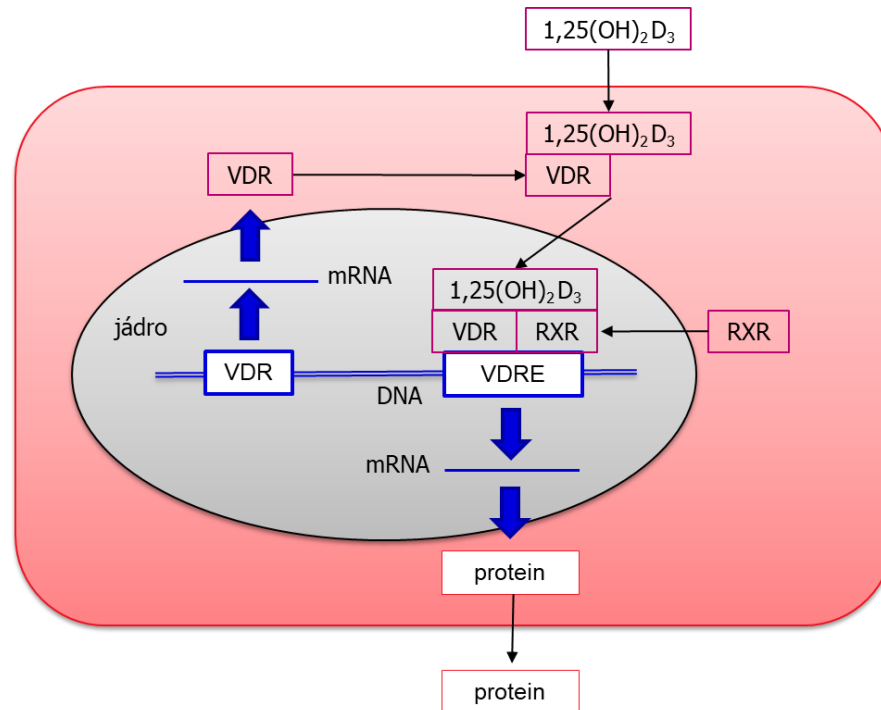
Vitamin D Receptor *Taq*I Gene Polymorphism and Dental Caries in Czech Children

Lydie Izakovicova Holla[a, b]   Petra Borilova Linhartova[a, b]   Jakub Kastovsky[a, b]
Michaela Bartosova[a, c]   Kristina Musilova[a]   Lubomir Kukla[c]   Martina Kukletova[a]

P<0.05

MUNI
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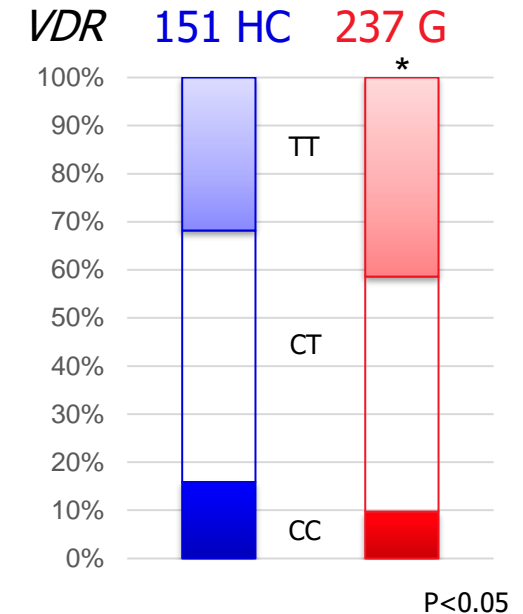
Periodontitis

Study I.

– Meta-analysis for SNP, not haplotypes

– ↑ c IL-1α and IL-1β in the gingival tissue in patients with CP

– ↑ c IL-1β in gingival tissues in rats with DM

Jiang et al., 2013

– 10x ↑ c IL-1RN in the serum of the carriers of S allele

Danis et al., 1995

IL1RI IL1 IL1RAcP IL1RN
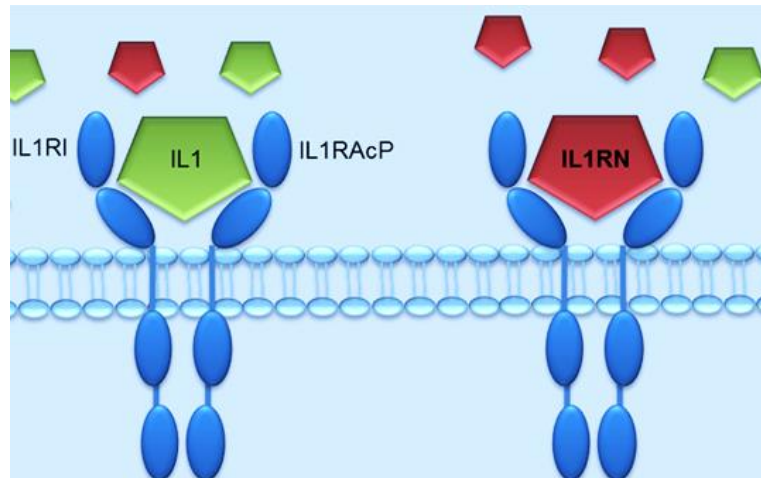
Gómez-Flores-Ramos et al., 2014

– **SNP *IL-1B* and *IL-1RA* do not affect c IL-1β in serum**

P>0.05

Research Article

Interleukin-1 Gene Variability and Plasma Levels in Czech Patients with Chronic Periodontitis and Diabetes Mellitus

Petra Borilova Linhartova,[1,2] Hana Poskerova,[1] Marie Tomandlova,[3] Jirina Bartova,[4] Katerina Kankova,[2] Antonin Fassmann,[1] and Lydie Izakovicova Holla[1,2]

IL-1 225 HC 264 CP 132 T1DM 395 T2DM 60 HC

↑c IL-1β in PBMCs stimulated by

*A. actinomycetemcomitans**

*P. intermedia**

*T. forsythia**

P<0.05

P<0.05

MUNI MED
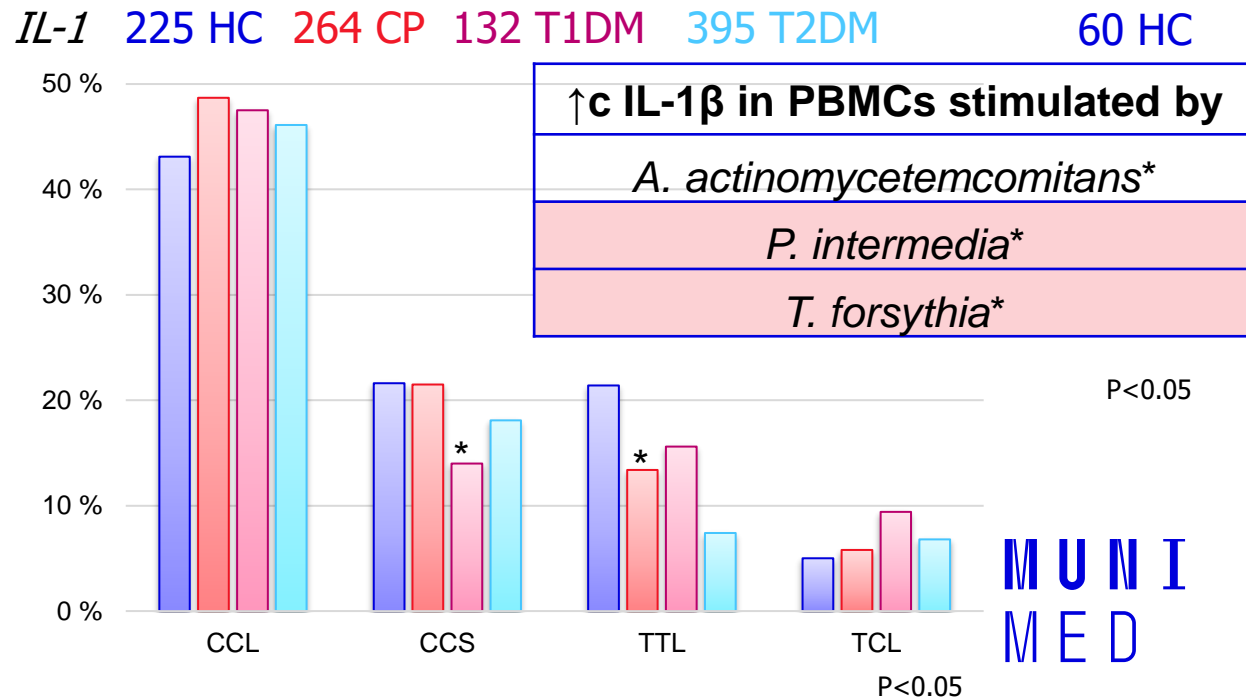
Periodontitis

Studies II and III.

– SNP *IL-8* was not associated with CP (CZ)

Borilova Linhartova et al., 2013
Viana et al., 2010

– haplotypes *CXCR2* were associated with CP (Brasil)
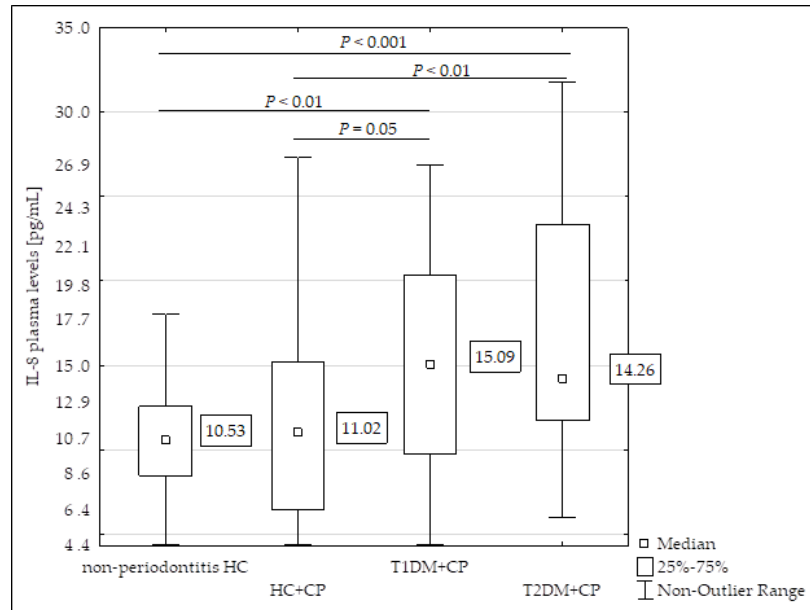
– **SNP *IL-8* and *CXCR2* do not affect c IL-8 in plasma** $P>0.05$

International Journal of **Molecular Sciences**

Article

Differences in Interleukin-8 Plasma Levels between Diabetic Patients and Healthy Individuals Independently on Their Periodontal Status

Petra Borilova Linhartova [1,2,†], Denisa Kavrikova [1,†], Marie Tomandlova [3], Hana Poskerova [1], Vaclav Rehka [4], Ladislav Dušek [5] and Lydie Izakovicova Holla [1,2,*]

IF 3,687
Q2 BIOCHEMISTRY & MOLECULAR BIOLOGY
Q2 CHEMISTRY, MULTIDISCIPLINARY

SNPs *CXCR2* +785/+1208
*A. actinomycetemcomitans**
*P. micra**

P<0.05

Research Article

Chemokine Receptor 2 (*CXCR2*) Gene Variants and Their Association with Periodontal Bacteria in Patients with Chronic Periodontitis

Denisa Kavrikova,[1] Petra Borilova Linhartova [1,2] Svetlana Lucanova,[1] Hana Poskerova [1] Antonin Fassmann,[1] and Lydie Izakovicova Holla [1,2]

IF 3,549
Q2 CELL BIOLOGY
Q2 IMMUNOLOGY

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Periodontitis

Study IV.

- SNP *IL- 17A* -197A/G (rs2275913) associated with CP (Brazil, Iran) Corrêa et al., 2012. Saraiva et al., 2013

  Zacarias et al., 2015
  Kadkhodazadeh et al., 2013

- ↑ c IL-17 after *in vitro* stimulation of T cells from carriers of A allele, upregulation of IL-17A in patients with CP   Espinoza et al., 2011

- ↑ monocyte IL-17 production in patients with T1DM

  Mitani et al., 2015

Research Article

Interleukin-17A Gene Variability in Patients with Type 1 Diabetes Mellitus and Chronic Periodontitis: Its Correlation with IL-17 Levels and the Occurrence of Periodontopathic Bacteria

Petra Borilova Linhartova,[1,2] Jakub Kastovsky,[1,2] Svetlana Lucanova,[1] Jirina Bartova,[3] Hana Poskerova,[1] Jan Vokurka,[1] Antonin Fassmann,[1] Katerina Kankova,[2] and Lydie Izakovicova Holla[1,2]

IF 3,232
Q2 CELL BIOLOGY
Q2 IMMUNOLOGY

IL-17A -197	CP	c IL-17 PBMCs stimulated by *P. gingivalis* [pg/ml]	c IL-17 unstimulated PBMCs [pg/ml]
AA+AG	19	1.51 (0.50-4.56)*	0.98 (0.25-8.53)*
GG	11	0.10 (0.00-1.51)	0.27 (0.00-0.54)

203 Research Activities in Dentistry   2021

P<0,05

IL-17A   182 CP   154 HC 244 CP 125 T1DM

P<0.05   P<0.05
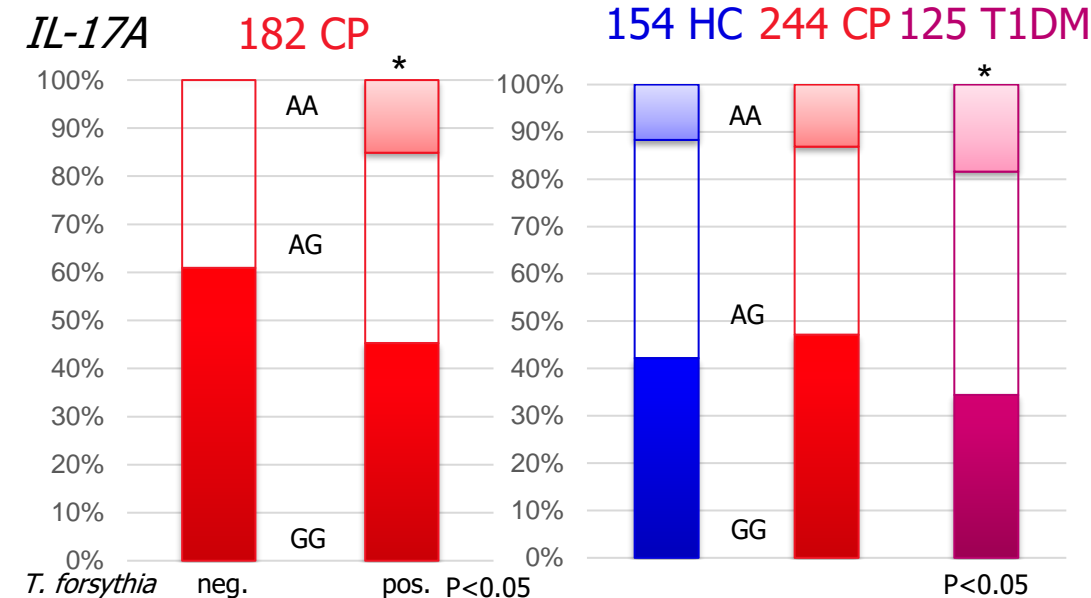
Complex diseases

Genetic Association Study

– Whole genome studies (GWAS)

– Study of candidate genes

– positional x functional

• studies of controls and cases

– Intergene interactions, gene and environmental interactions

– Algorithms

– Interdisciplinary cooperation

Himmelstein et al., 2015

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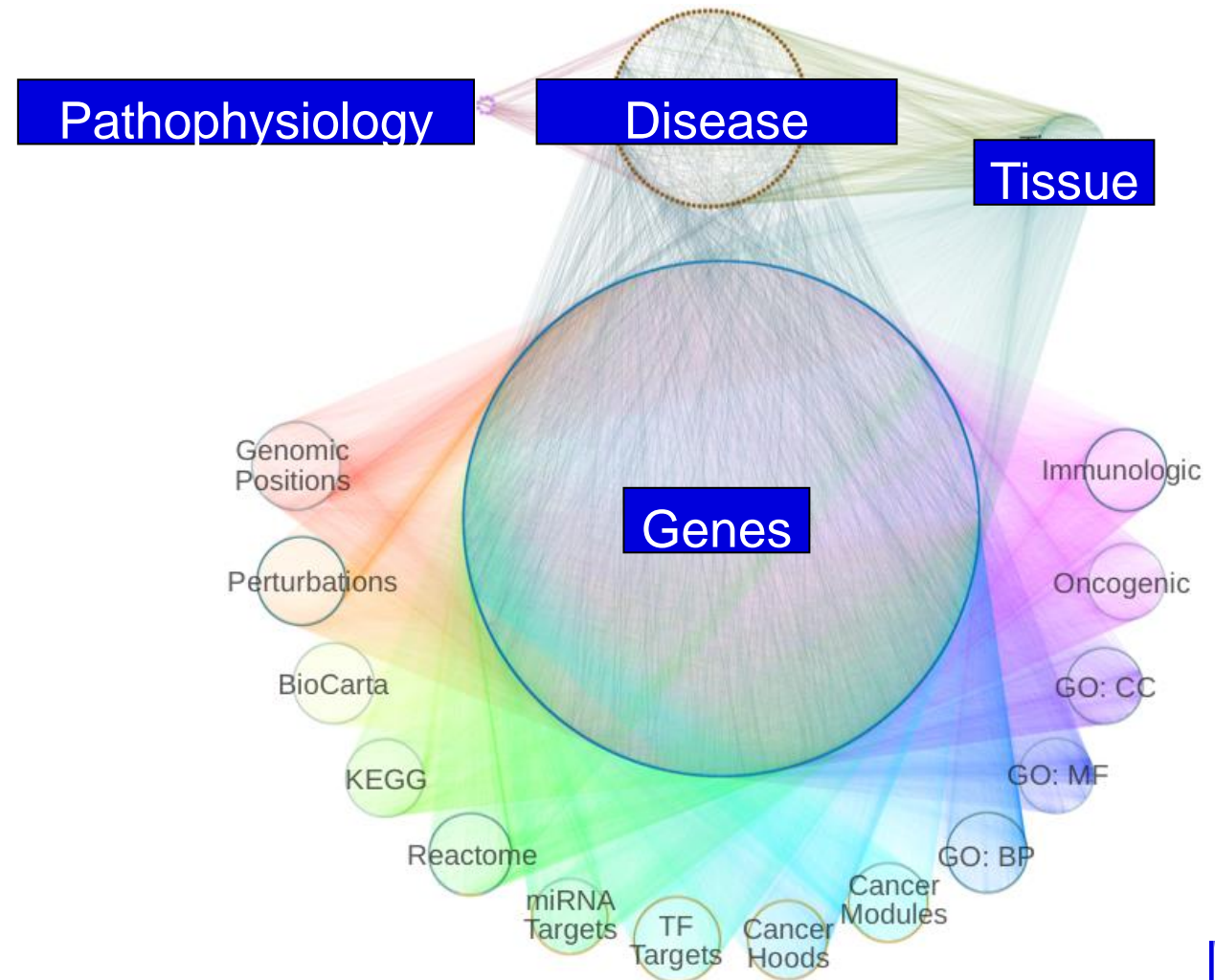
Dental caries in temporary dentition– ECC

Behavioral interventions

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ECC

Behavioral interventions

Median; Box: 25%-75%; Whisker: Non-Outlier Range

Valence
Arousal
Dominance
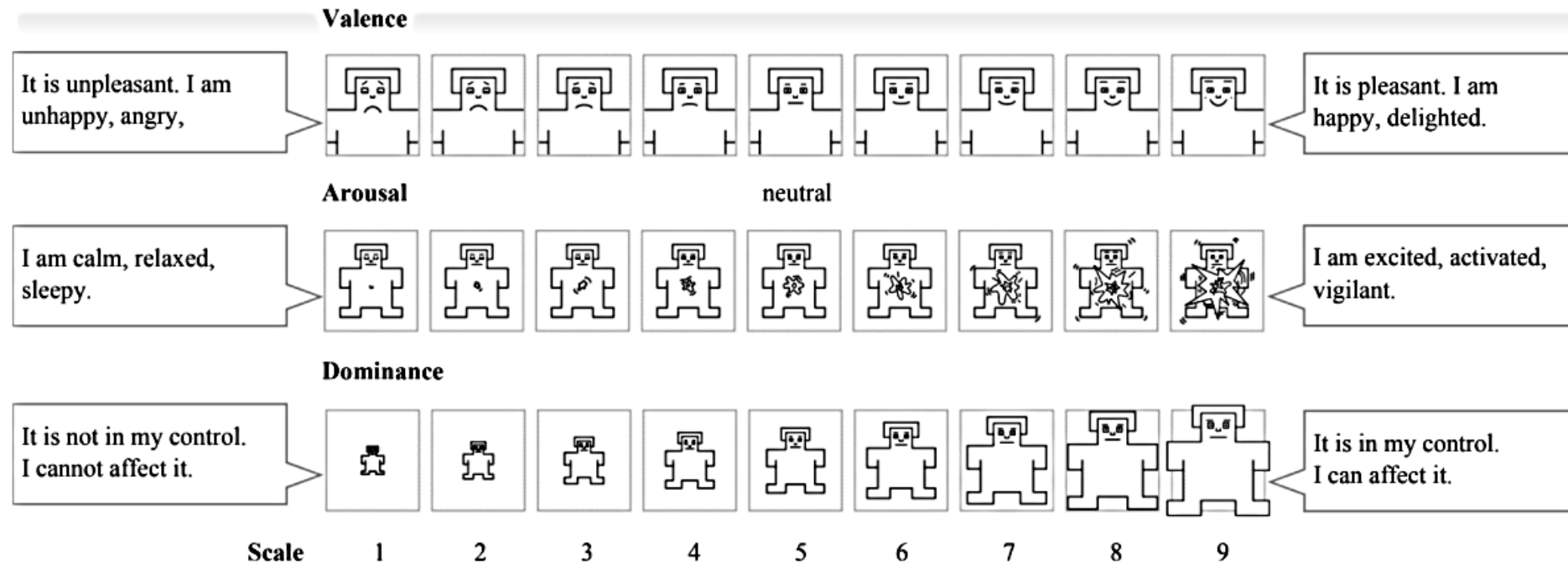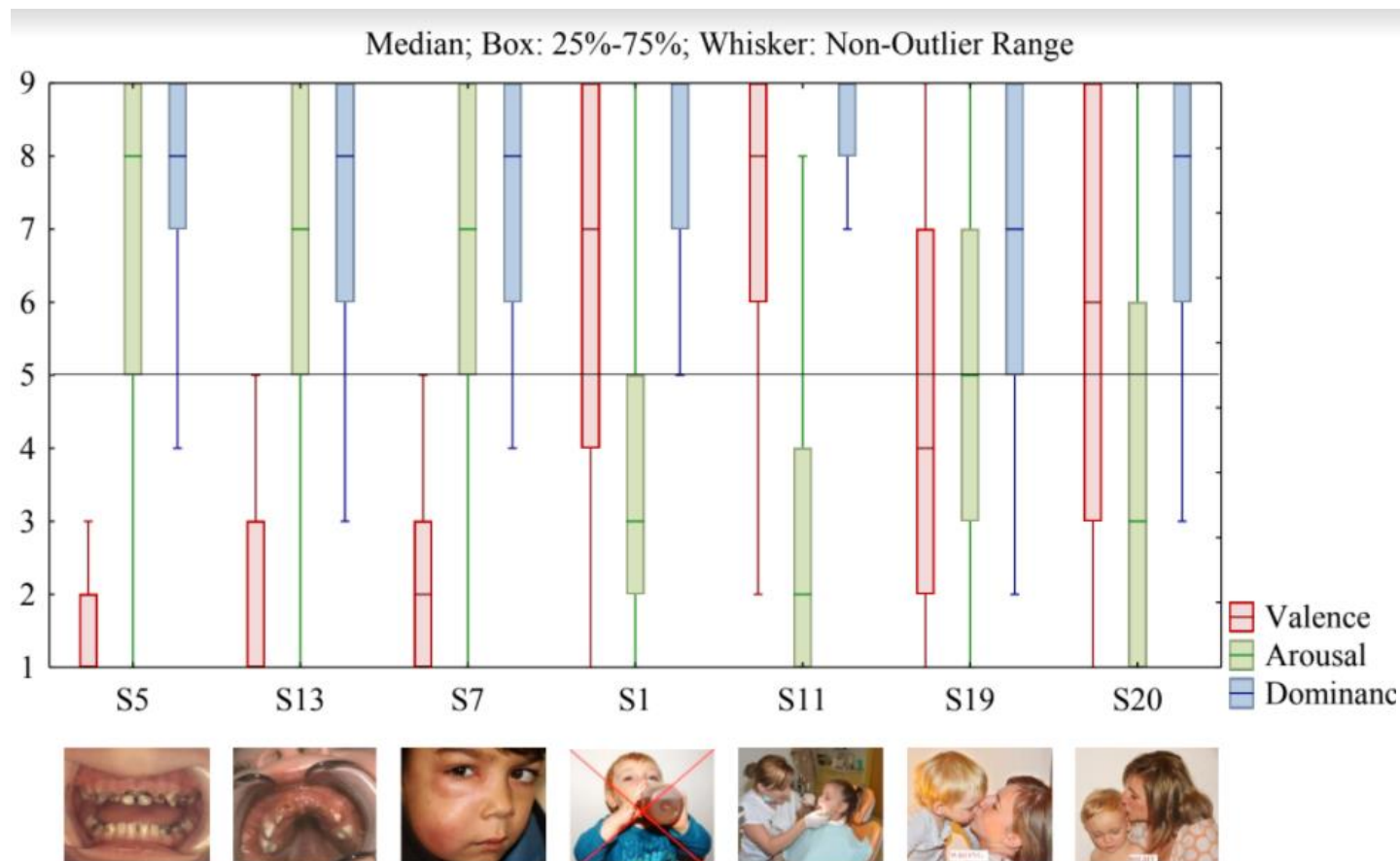
BMC Oral Health

RESEARCH ARTICLE   Open Access

Emotional stimuli candidates for behavioural intervention in the prevention of early childhood caries: a pilot study

Michaela Bartosova[1], Miroslav Svetlak[2], Martina Kukletova[1], Petra Borilova Linhartova[1,3], Ladislav Dusek[4] and Lydie Izakovicova Holla[1,3,5*]

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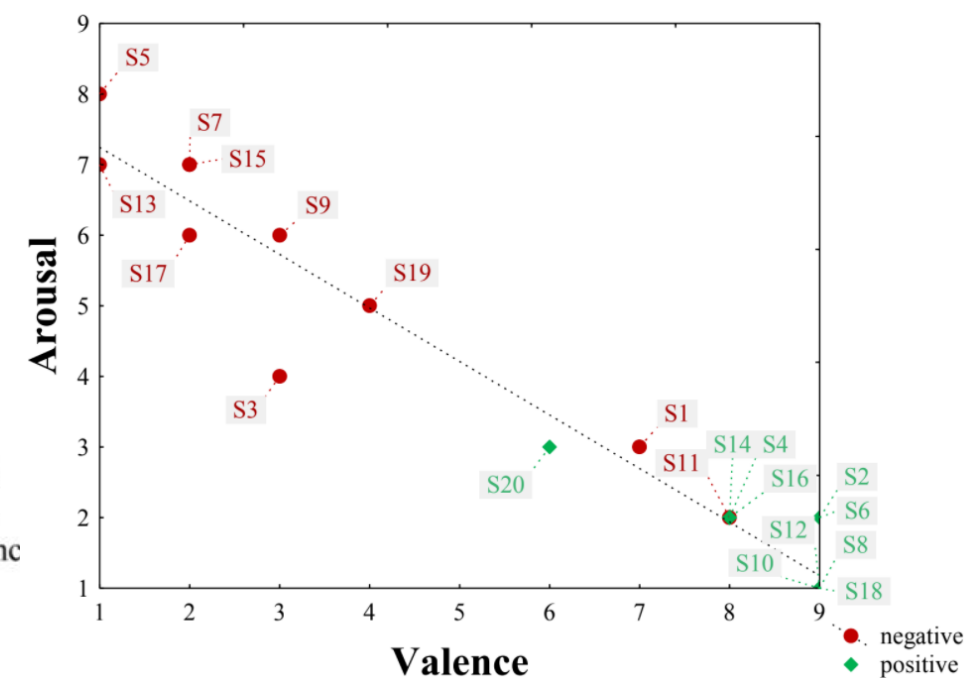
ECC
Breastfeeding
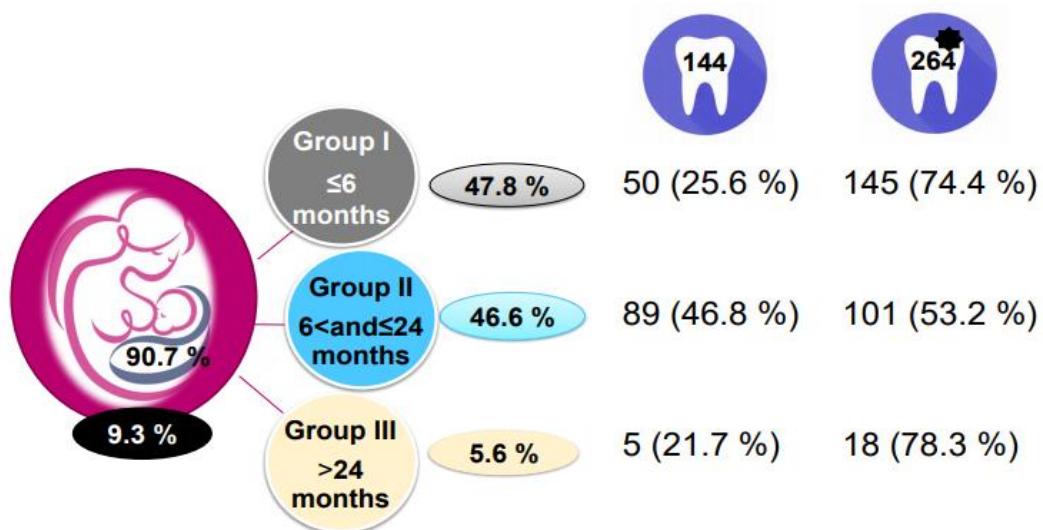
Frequency of sECC occurence in relation to the duration of breastfeeding.
*vs. group I (P<0.001), **vs. group III (P<0.01)

- tea 68.2 %
- syrup 23.4 %
- juice 15.6 %
- milk 12.3 %
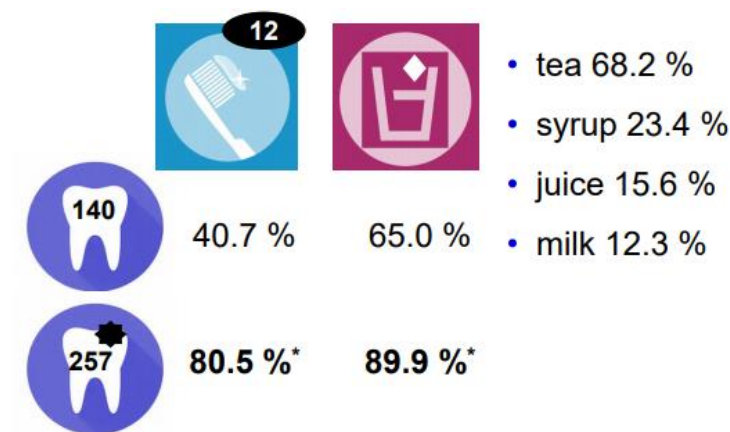
Association of oral hygiene or sweetened soft drinks intake with the frequency of sECC occurence.   *P<0.0001

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Human microbiota and microbiome

Introduction

– Microbiota

- It makes up for 1-3% of a person's body weight

- Complex ecosystem that is location- and conditions specific

- 10 times more bacteria than cells in the human body

A microbiome is a set of genes for all microorganisms that inhabit human tissues and fluids

- Unique imprint

- Project

- 100 times more genesin the microbiome than in the human genome (27,000 genes)

Characterization of the microbiomes of healthy human subjects at five major body sites, using 16S and metagenomic shotgun sequencing.

Enter HMP1
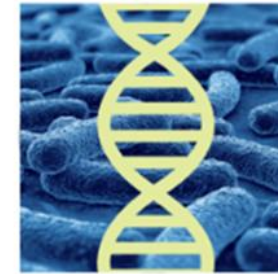
Characterization of microbiome and human host from three cohorts of microbiome-associated conditions, using multiple 'omics technologies.

Enter iHMP

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Human

Philosophical view

- Brain
  - Personality and thinking
- Immune system
  - Adaptive IS
- Genome
  - Phenotypes

Human microbiota

- Eukaryotes
  - Fungi
  - Protozoa
- Bacteria
- Archaea
- Viruses

- Conjuctiva
- Oral cavity
- Airways
- Mammary glands
- GIT
- Skin
- Urogenital tract

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Human microbiota

Brain

- **Brain-gut axis**

- Stress, Depression, Autism, PD, AD

- Regulation with pre- and probiotics

- SCFAs regulate GPR41-mediated activity of sympathetic NS-E release

- Tryptophan has a direct effect on sleep

- Neurotransmitters

- Cytokines

Nature Reviews | Neuroscience

Kimura I. et al., 2011   Jenkins T. A. et al., 2016   Cryan J. F. et Dinan T. G., 2012

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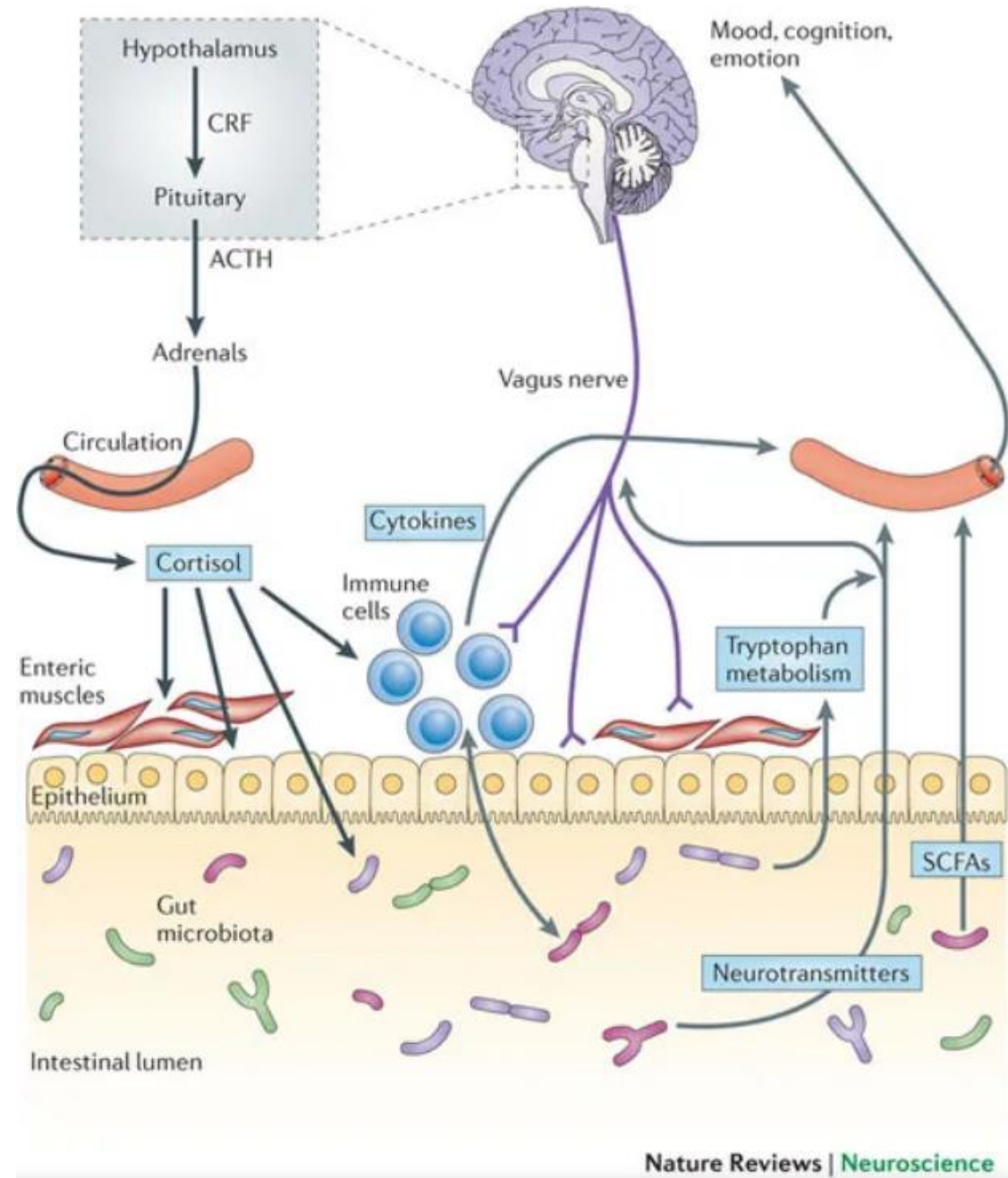
Human microbiota

Immune system

- SCFA

  - Stimulating the production of intestinal mucus

  - Differentiation of B cells to plasma cells producing Ig

  - Associated with anti-inflammatory phenotype (inhibition of histone deacetylases)

  - *Bacteroides fragilis* PSA

  - Induction of CD4^{+} T lym profile – induction of Treg and supression of Th17 and Th1

- Niacin, tryptophan, taurine (inflammasome)

Nature Reviews | Immunology

Rooks M. G. et Garrett W. S., 2016   Belkaid Y. et Hand T. W., 2014

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Human microbiome

"Second genome "

— Epigenetic modifications

— Metabolization of xenobiotics (indirectly/directly)

⬇
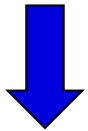
— **Personalized therapy**

— *Eggerthella lenta* – normally, only a specific strain with cgr1 and cgr2 genes inactivates digoxin (their operon inhibited by Arg)

Sayers E. et al., 2018

Spanogiannopoulos P. et al., 2016

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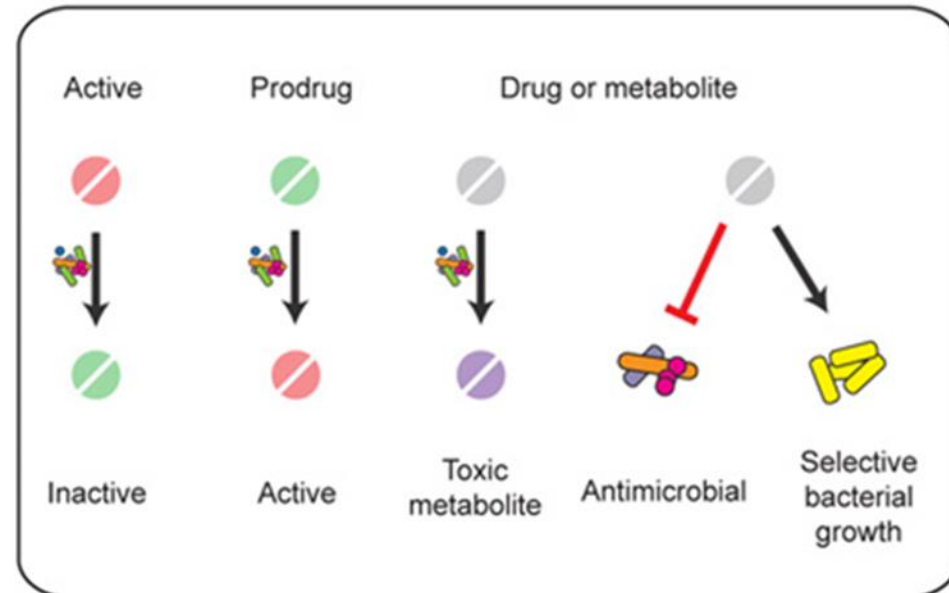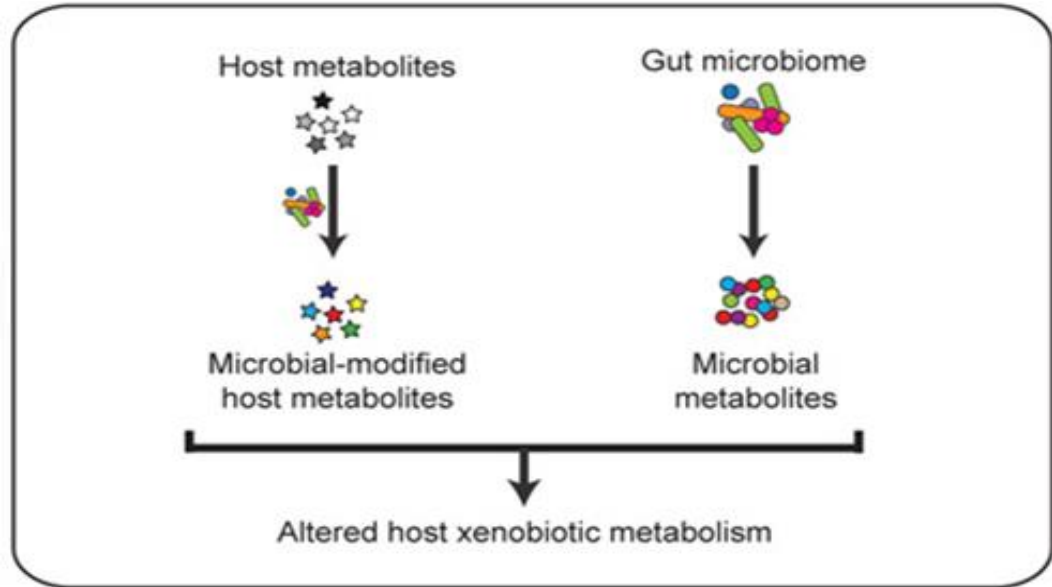
Comparison of microbiomes

Oral vs. intestinal

– Similarity at the genus level but differ in species diversity and relative abundance

Generic Diversity

Candida

Oral mycobiome

Oral microbiome

MICROBIOME = BACTERIOM

Intestinal mycobiome

Intestinal microbiome

Firmicutes

Bacteroides

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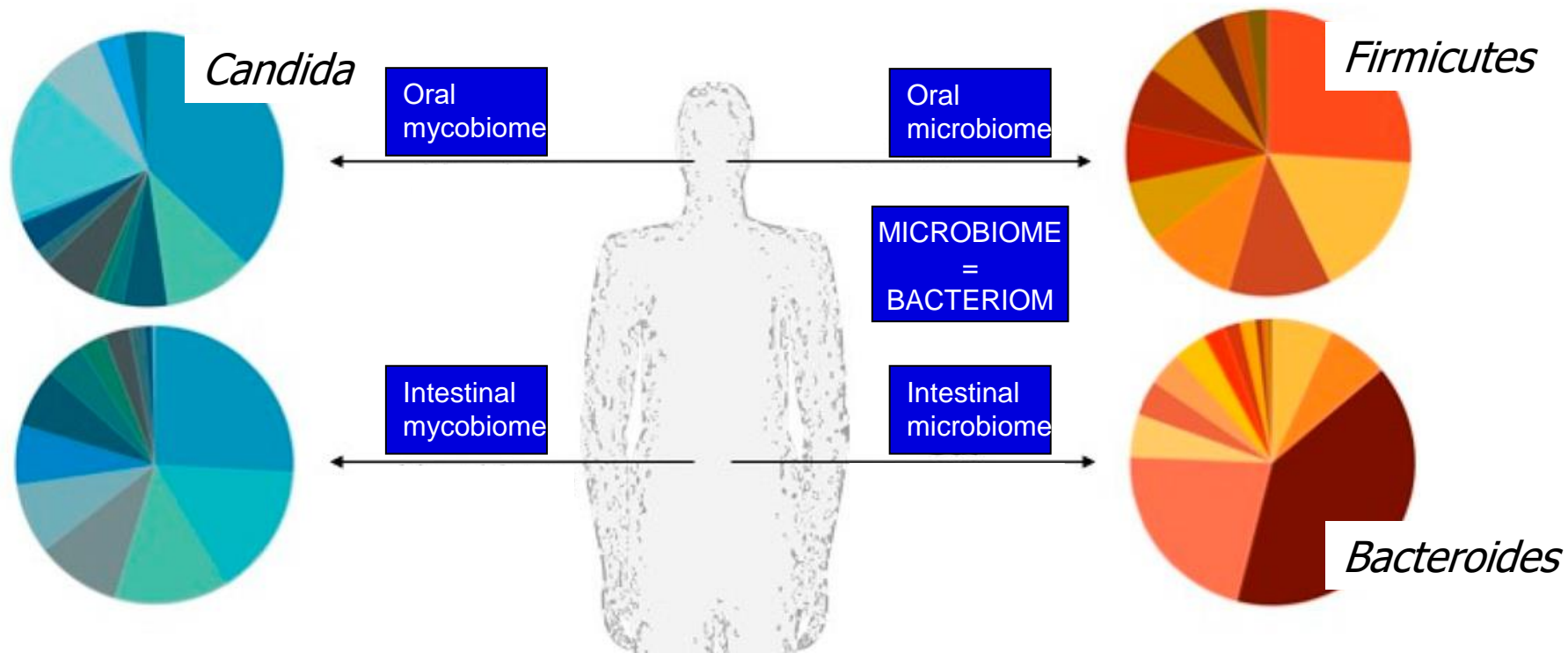
Comparison of microbiomes

Oral microbiome – diagnostics

— Oral microbiome in patients with Barrett's esophagus

— Increased relative incidence of *Firmicutes* and reduced incidence of *Proteobacteria*

— Possibility to identify patients with BE on the basis of the oral microbiome with 96.9% sensitivity and 88.2% specificity

— *Lautropia, Streptococcus, Enterobacteriaceae*

Snider E. J. et al., 2018
Eslick G. D., 2010

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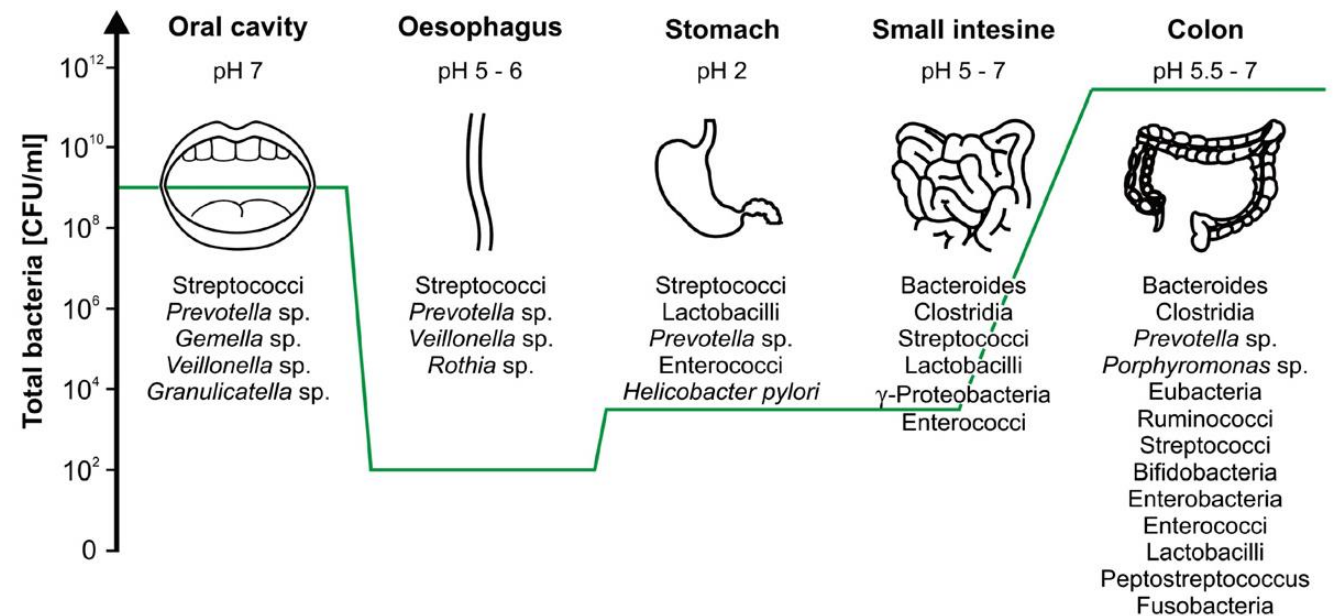
Oral microbiome

Ecosystems

— 700 to 1000 bacterial species

   (HOMD, www.homd.org)

• c O_2, pH, endogenous nutrients

— Soft and hard tissue surfaces

• Creation of biofilms (graduating)

• Mucosal shealation

— Fluid

• Saliva, gingival fluid

Supragingival plaque
• Streptococcus
• Capnocytophaga
• Corynebacterium
• Uncl. Pasteurellaceae
• Uncl. Neisseriaceae
• Fusobacterium

Subgingival plaque
• Streptococcus
• Fusobacterium
• Capnocytophaga
• Prevotella
• Corynebacterium
• Uncl. Pasteurellaceae

Keratinized gingiva
• Streptococcus
• Uncl. Pasteurellaceae

Hard palate
• Streptococcus
• Uncl. Pasteurellaceae
• Veillonella
• Prevotella
• Uncl. Lactobacillales
• Gemella

Buccal mucosa
• Streptococcus
• Uncl. Pasteurellaceae
• Gemella

Throat
• Streptococcus
• Veillonella
• Prevotella
• Uncl. Pasteurellaceae
• Actinomyces
• Fusobacterium
• Uncl. Lachnospiraceae

Tongue dorsum
• Streptococcus
• Veillonella
• Prevotella
• Uncl. Pasteurellaceae
• Actinomyces
• Fusobacterium
• Uncl. Lactobacillales
• Neisseria

Palatine tonsils
• Streptococcus
• Veillonella
• Prevotella
• Fusobacterium
• Uncl. Pasteurellaceae

Saliva
• Prevotella   • Prevotella
• Streptococcus   • Streptococcus
• Veillonella   • Veillonella
• Uncl. Pasteurellaceae

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Sampaio-Maia B. et al., 2016

Oral microbiota

Function

— Effect on the host immune system

- Development and maturation of IS cells (SCFA, molecular patterns associated with bacteria)

- Downregulation of proinflammatory and upregulation of interferon response

- Barriers - creation, integrity, support (mucus)

- Competition with potential pathogens

— Contribution to food metabolism and xenobiotics

- Fermentation, modification of bile acids

- Enterosalivation circuit (NO)

- Vitamin production

Modification of bile acids
metabolic functions
glycaemia
cholesterolemia
local inflammatory response
integrity of gi barrier

Molecular patterns associated with microbes
development of the immune system
immune response to pathogens
immune programming
komenzals

Production of short-chain fatty acids
immune function
anti-inflammatory functions
metabolic functions
integrity of gi barrier

Oral microbiome

Production of vitamins
enzymatic functions

Degradation of mucous polysaccharides
integrity of GI barrier
formation of the microbiome

Production of antimicrobial peptides
formation of the microbiome

Journal of the California Dental Association (10/2017)
Jones M. L. et al., 2014

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Oral microbiota

Function - enterosalivation circuit

— ¼ nitrates are recycled in the salivary glands (sialin – active transport)

— NO Importance

- Vasodilatation of blood vessels (antihypertensius)
- Stimulation of gastric mucus secretion
- Antimicrobial properties

Streptococci
Veillonella
Actinomyces naeslundi,
Actinomyces odpntolyticus

NO_3^-
NO_2^-
NO

Ma L. et al., 2018

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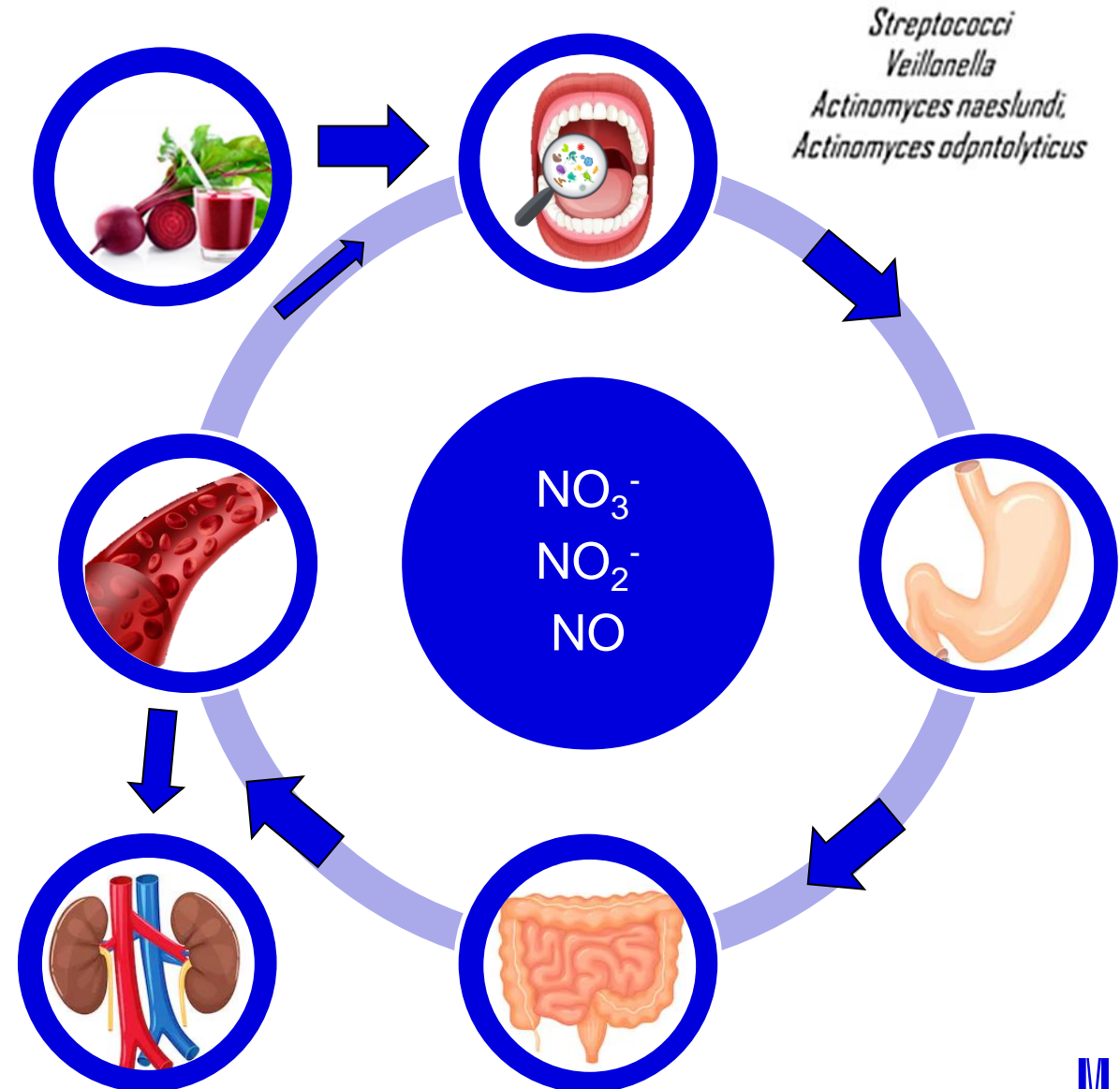
Symbiosis and dysbiosis

— Microbiota in general is not harmful to humans

— Homeostasis disruption – competition

— The change in the composition of the microbiome correlates with many diseases

— Controlled handling of the microbiota has therapeutic potential

Symbiosis

Factors involved is the shift

Dysbiosis

- Immunosuppression
- Post-antibiotic condition
- Reduced saliva function

- Prebiotics
- Probiotics
- Increase in local immunocompetence
- Oral hygiene

Oral candidiasis

Mutans Streptococcus

Mitis Streptococcus

Other Streptococcus spp.

Veillonella parvula

Bifidobacterium spp.

Lactobacillus spp.

Actinomyces spp.

Capnocytophaga spp.

Rothia spp.

Black-pigmented anaerobes

Spirochetes

Fusobacterium spp.

Filifactor alocis

Aggregatibacter actinomycetemcomitans

Neisseria spp.

Candida albicans

Non-albicans Candida spp.

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Factors affecting the oral microbiota

Host

— **Sex**

— **Immune system**

— **Health**

— **Host Age**

— Teeth eruption

  • Transmission

  • Microevolution of microbiota

— **Genome**

  • twins MZ vs. DZ

  • interpopulation variability

— **Use of xenobiotics**

  • Pharmaceuticals

  • Alcohol

  • Smokig

  • **Oral hygiene**

  • **Diet**

  • Fermentable carbohydrates

Unrelated   Dizygotic   Monozygotic

Less similar ← **Oral** microbiome similarity → More similar

External environment

MUNI
MED

Gao L. et al., 2018
Gomez A. et al., 2017
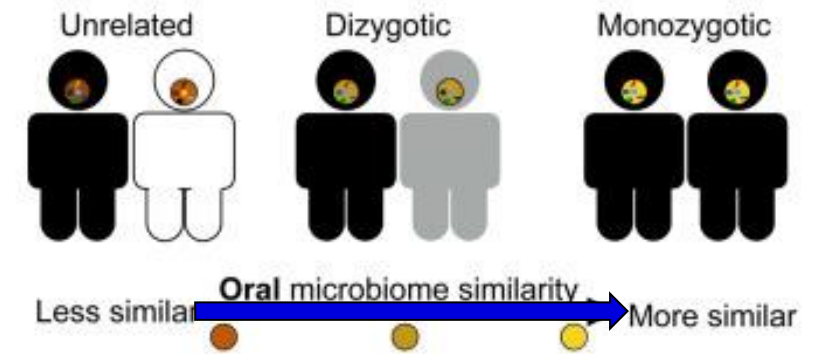
Oral microbiome

Mouthwash

– Saliva

– Mouthwash with 12% alcohol

Number of operational taxonomic units

Shannon Diversity Index

Mouthwash
Saliva

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Fan X. et al., 2018
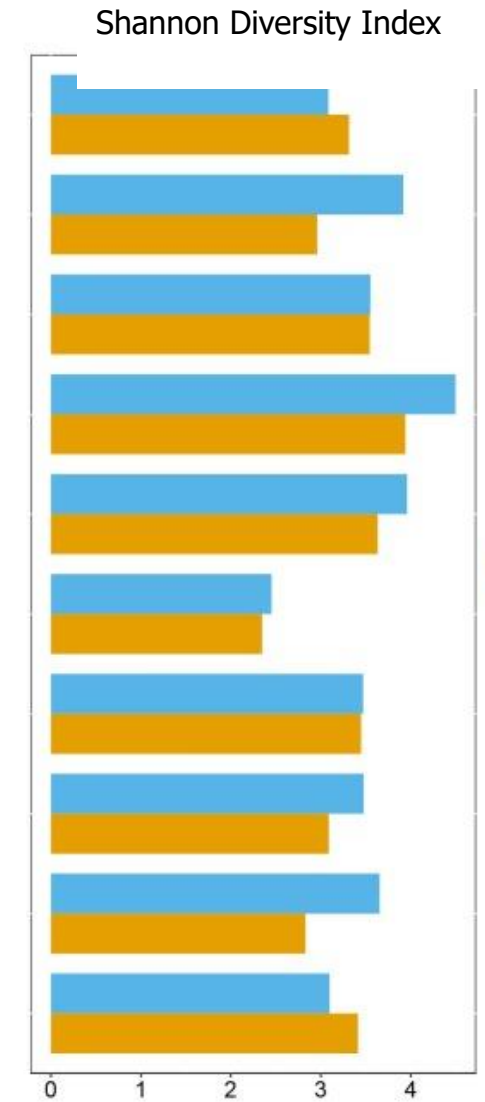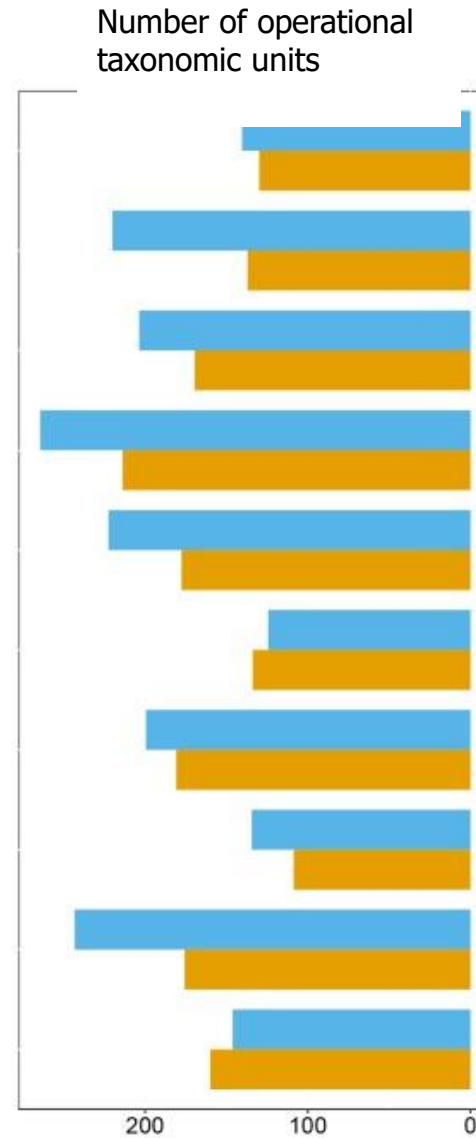
Oral dysbiosis

Oral diseases

- Dental caries

- Apical periodontitis, radical cyst

- Periodontitis

- Perimplantitis

- Diseases of the oral mucosa

  • Oral lichen planus, leukoplakia, SLE

- Cancer of the orofacial region

- Halitosis

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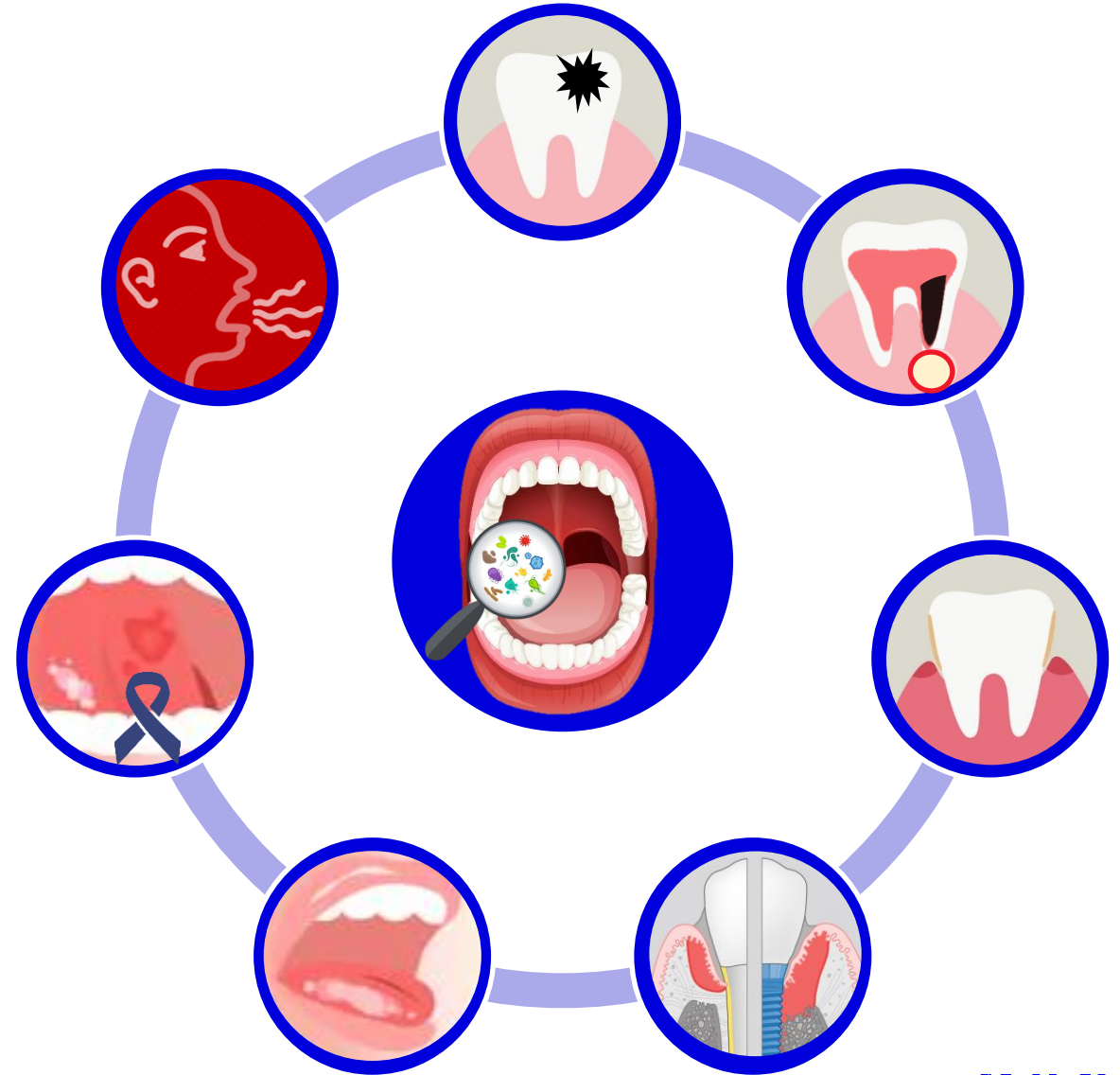
Oral dysbiosis

Dental caries

— Acidogenic/aciduric bacteria

- *Streptococcus mutans*
- *Lactobacillus* sp.
- *Bifidocterium* sp.
- Other *Streptococcus* sp.

— Key features

- Acid production
- Acid pH tolerance
- Production of exopolymers
- Production of intracellular polysaccharides

Symbiosis

Factors involved is the shift

- Frequent income of fermentable carbohydrates
- Reduced pH
- Reduced saliva function

Dysbiosis

Dental caries

- Mutans Streptococcus
- Mitis Streptococcus
- Other Streptococcus spp.
- *Veillonella parvula*
- *Bifidobacterium* spp.
- *Lactobacillus* spp.
- *Actinomyces* spp.
- *Capnocytophaga* spp.
- *Rothia* spp.
- Black-pigmented anaerobes
- Spirochetes
- *Fusobacterium* spp.
- *Filifactor alocis*
- *Aggregatibacter actinomycetemcomitans*
- *Neisseria* spp.
- *Candida albicans*
- Non-albicans *Candida* spp.

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Journal of the California Dental Association (10/2017)
Hoare A. et al. (2017) Microbiol Spectr.

Oral dysbiosis

Dental caries

Kilian M. et al., 2016
Costalonga M. et Herzberg M. C., 2014

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Oral dysbiosis

Dental caries

– sECC

  • *Streptococcus (mutans)*,

    Porphyromonas and *Actinomyces*

– Synergistic action of microbiots with

  acidogenic properties

– Host-microbiota interactions

Enamel

Dentin

Gao L. et al., 2018   Gomez A. et al., 2017

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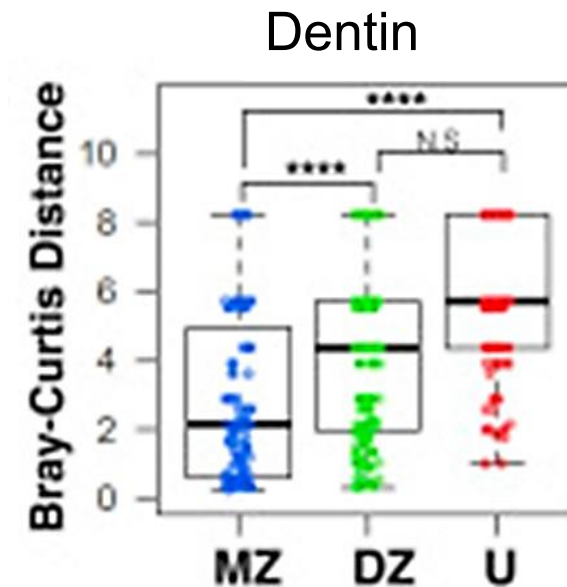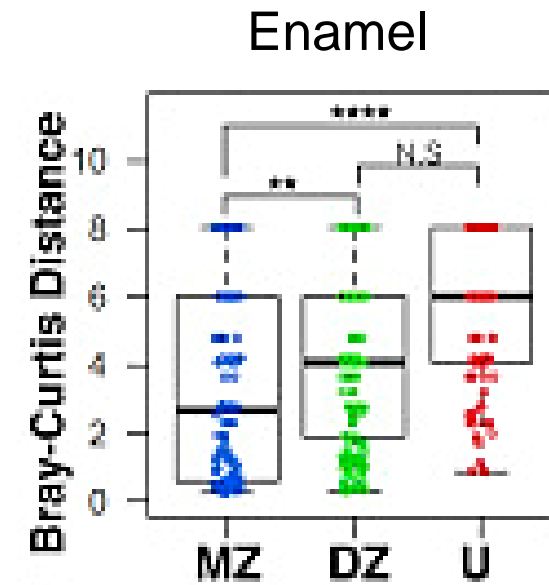
Oral dysbiosis

Periodontitis

— Anaerobic and proteolytic bacteria

- *P. gingivalis*
- *T. forsythia*
- *T. denticola*
- and others

— Key features

- Protease production
- LPS production
- Cytotoxins
- Induction of proinflammatory response (cytokines)

Symbiosis → **Factors involved in the shift** → Dysbiosis

- Dysregulation of immune system
- Infalammatory reaction

Periodontitis

Legend:
- Mutans *Streptococcus*
- Mitis *Streptococcus*
- Other *Streptococcus* spp.
- *Veillonella parvula*
- *Bifidobacterium* spp.
- *Lactobacillus* spp.
- *Actinomyces* spp.
- *Capnocytophaga* spp.
- *Rothia* spp.
- Black-pigmented anaerobes
- Spirochetes
- *Fusobacterium* spp.
- *Filifactor alocis*
- *Aggregatibacter actinomycetemcomitans*
- *Neisseria* spp.
- *Candida albicans*
- Non-albicans *Candida* spp.

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Oral dysbiosis
Periodontitis

Kilian M. et al., 2016
Costalonga M. et Herzberg M. C., 2014

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Oral dysbiosis

Periodontitis

— Metagenome and metatranscriptome (differential expression analysis)

— 9 virulence factors expressed 500 times more in dysbiosis

Virulence factors	Organism
fimA type 1b	Po. gingivalis
fimA type 1	Po. gingivalis
Immunoreactive 47 kD antigen	Po. gingivalis
Hemagglutinin A (hagA)	Po. gingivalis
Arginine deiminase (arcA)	Po. gingivalis
Serine protease dentilisin	Tr. denticola
Flagellar motor switch protein (FliG)	Tr. denticola
fimA type 3	Po. gingivalis
Arginine deiminase (arcA)	My. arginini
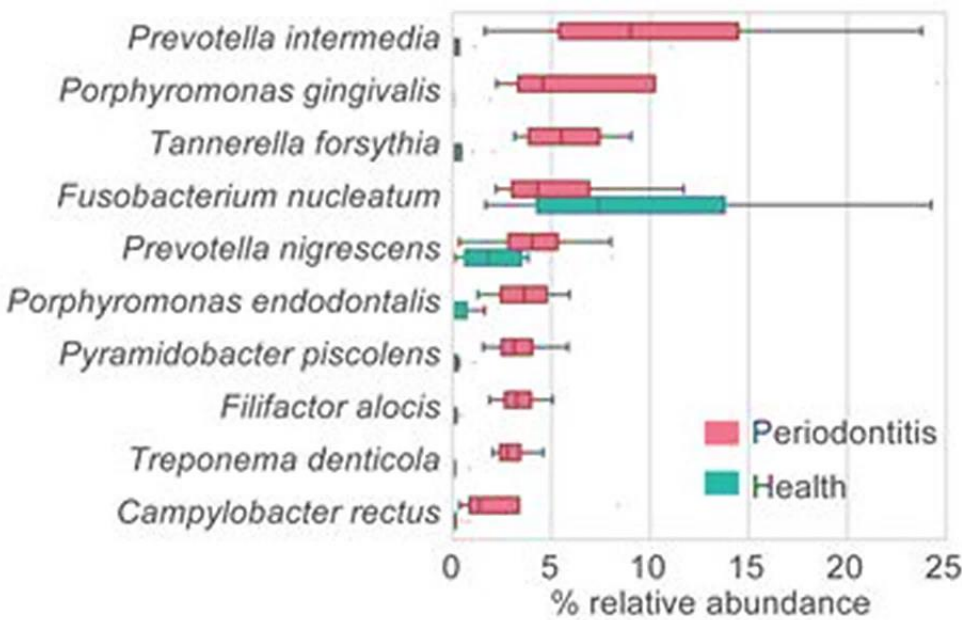
Adhesion and colonization

Surface protease that hydrolyzes pro-inflammatory cytokines

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Oral dysbiosis

Perimplantitis

- *Prevotella nigrescens* significantly increased in periimplantitis than in periodontitis

- *Treponema* sp. associated with 4 clinical parameters

Peri-Implantitis: Gum Disease Can Cause Dental Implant Failure

Maruyama N. et al., 2014

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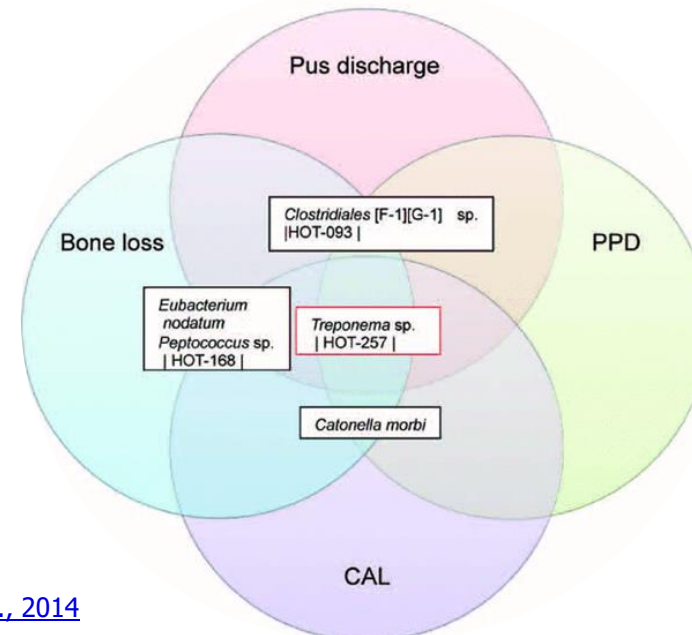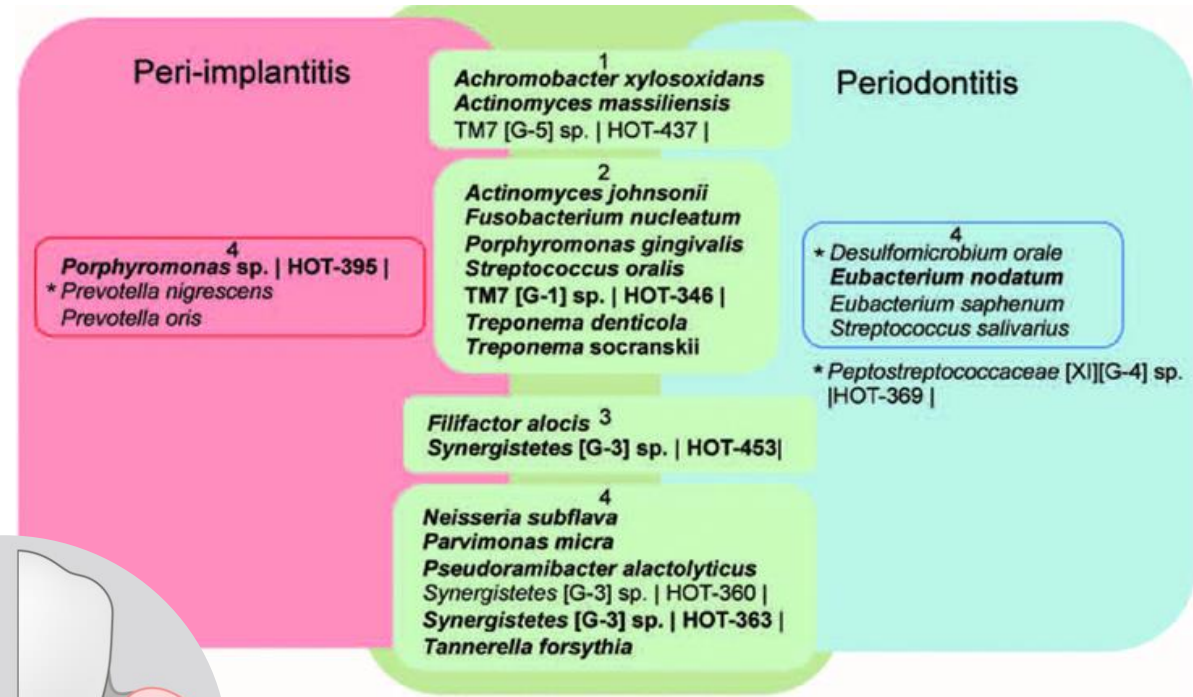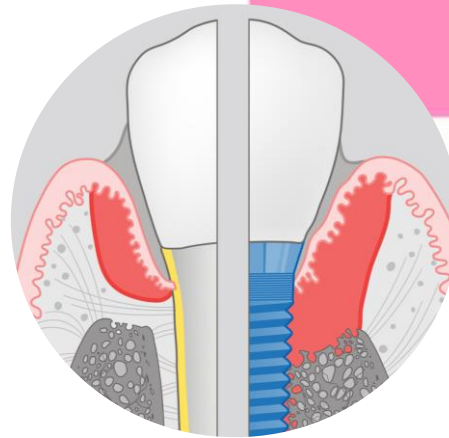
Oral dysbiosis

Diseases of the oral mucosa

— Oral lichen planus – erosive, reticular
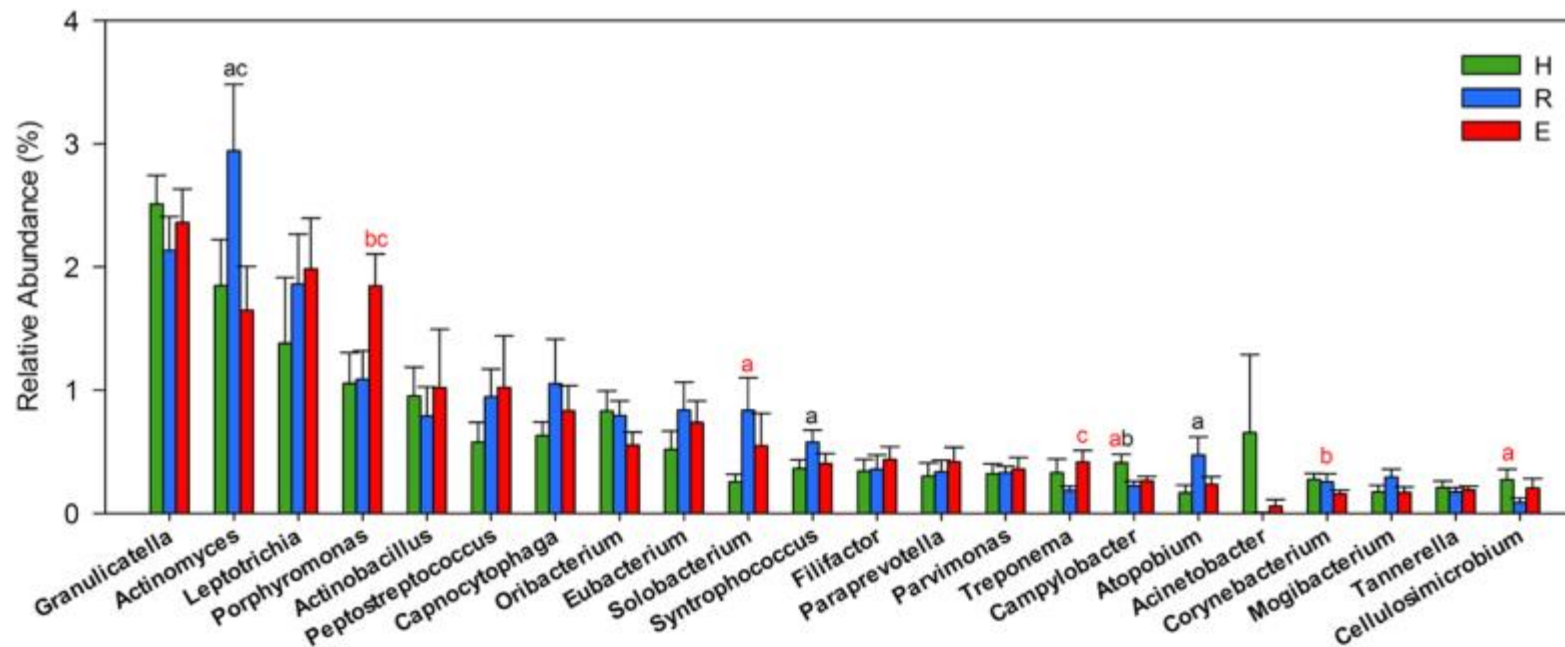
Negative correlation
Positive correlation
with clinical data

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Wang K. et al., 2016
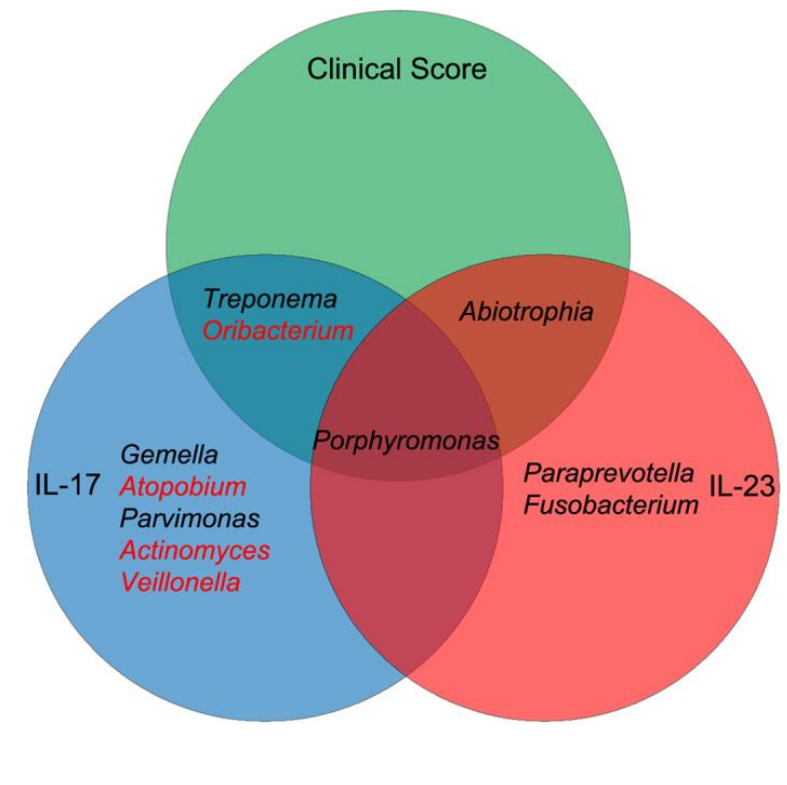
Oral dysbiosis

Diseases of the oral mucosa

— Oral leukoplakia

— Increased amount of *Fusobacteria* and reduced amount of *Firmicutes*

— In dysplasia, *Leptotrichia spp*. and *Campylobacter concisus*... similar profile to colorectal cancer

— Associations with specific changes in host gene expression (e.g. increased expression of pro-inflammatory IL-8)

— Systemic lupus erythematosus (SLE)

• Increased amount of *Fretibacterium*, *Prevotella nigrescens* a *Selenomonas*

Gao L. et al., 2018
Amer A. et al., 2017

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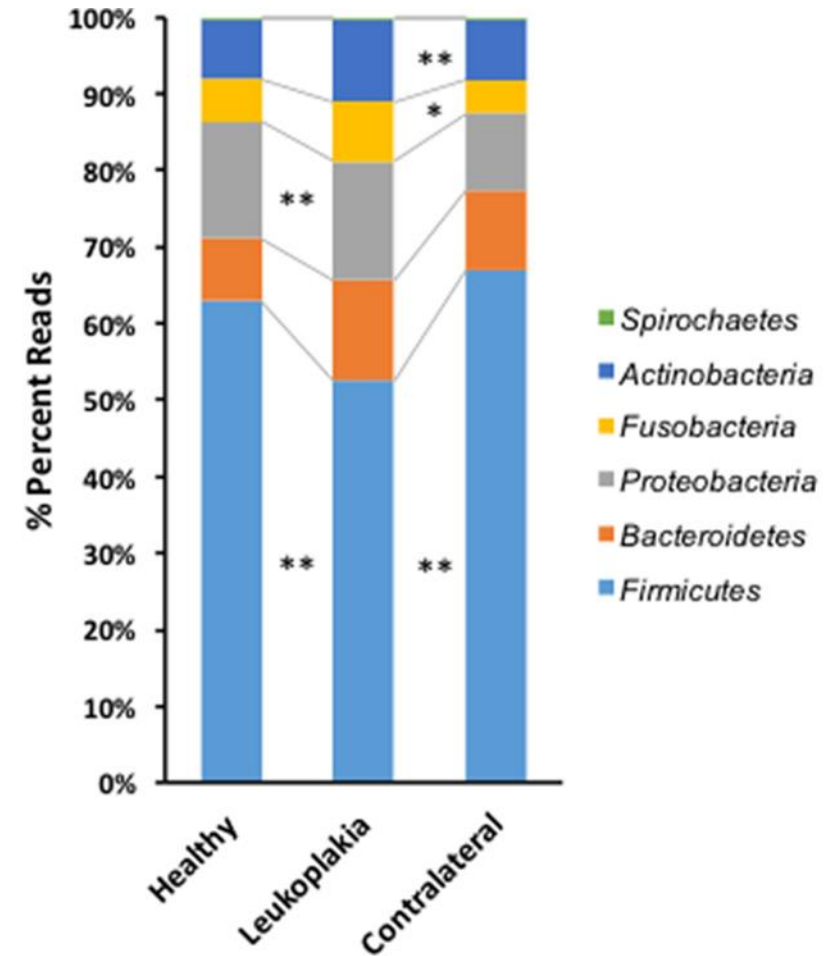
Oral dysbiosis

Halitosis

— Greater frequency of *Leptotrichia wadei* and *Peptostreptococcus stomatis* in children with halitosis

   • On the tongue, the differences in α diversity in saliva do not

— Up/down-regulation of genes in the pathway associated with H2S metabolism

—   Higher microbial production

   • Less consumption of H_2S in children with halitosis

Dorsum of the tongue

A

Saliva

B

Ren W. et al., 2016
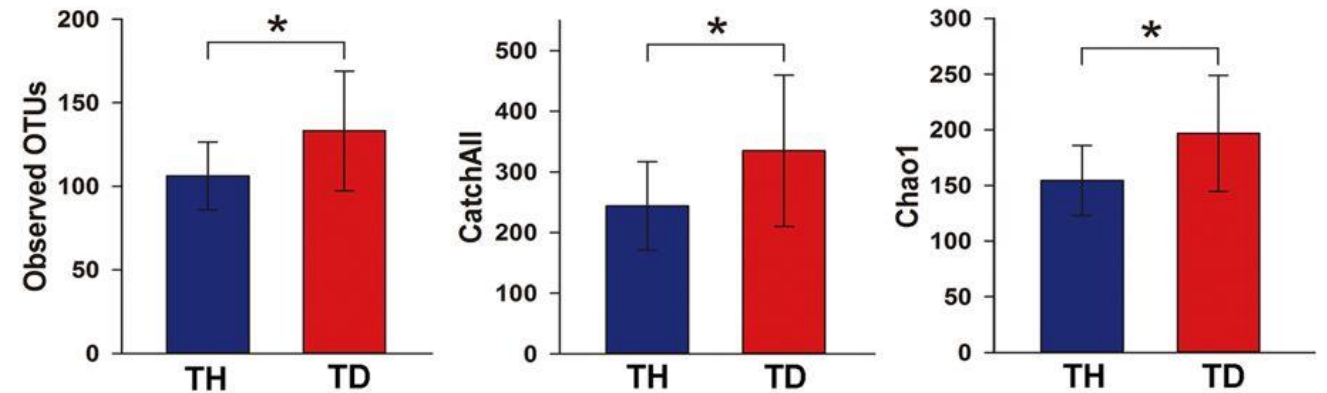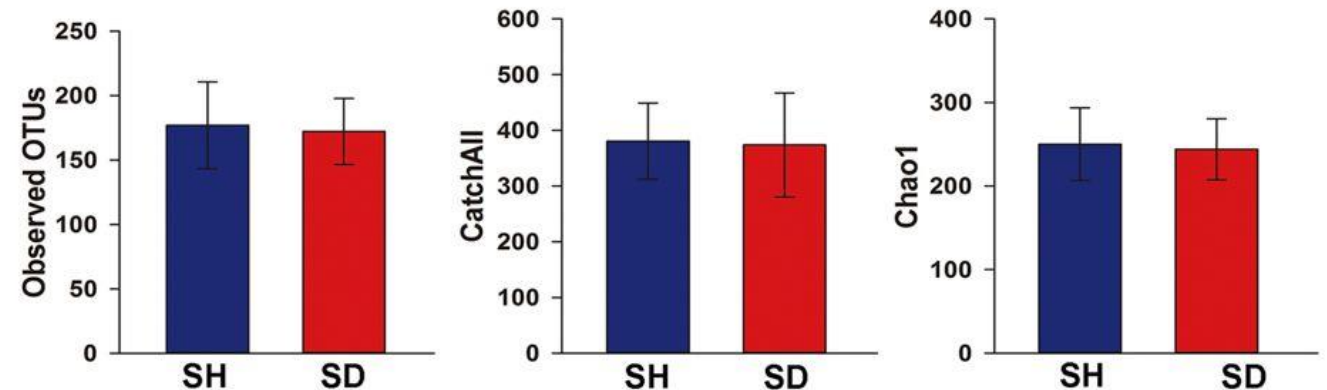
Oral microbiota

Prevention and treatment of oral dysbiosis

— Proper oral hygiene and dental care

— Diet, smoking, alcohol

— Prebiotics and probiotics

— Vaccination

  • 2nd generation fusion protein from *S. mutans* (rPAc) and *E. coli* (flagelin KF) proteins – intranasal vaccine in animal model

— Antibiotics, antimycotics

— Predators BALOs

— *Bdellovibrio, Bacteriovorax* a *Peredibacter = Bdellovibrio*-and similar organisms for killing of anaerobic G- bacteria
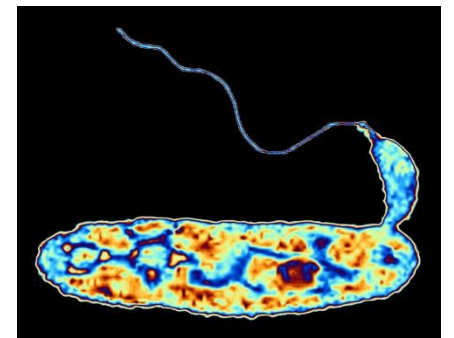
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Oral microbiome

Systemic diseases

- *Streptococcus mutans*

- *Porphyromonas gingivalis*

- *Aggregatibacter actinomycetemcomitans*

- *Fusobacterium nucleatum*

Obesity

Barrett's esophagus

Esophagus cancer

Sjögren syndrome

AD

HIV infection

CAD Atherosclerosis

Cirrhosis of the liver

Pancreatic cancer DM

IBD

CD

Colorectal cancer

RA

PCOS

Systemic lupus erythematodes

Jia G. et al., 2018   Nikitakis N. G. et al., 2017   Gao L. et al., 2018.   Obrenovich M. E. M., 2018.   Olsen I. et Yamazaki K., 2019.
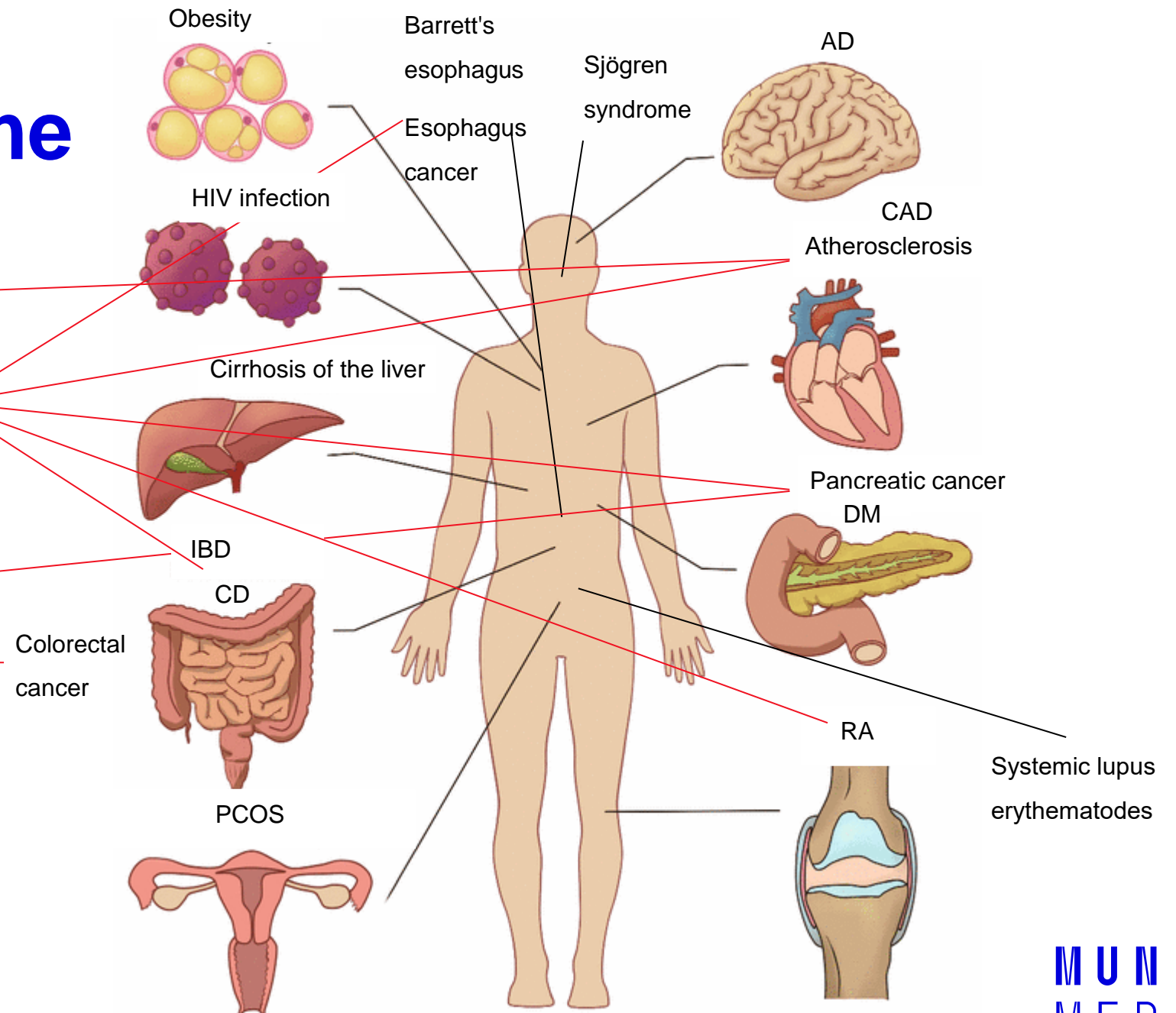
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Oral microbiome

„Leaky mouth"?

Konkel J. E. et al., 2019
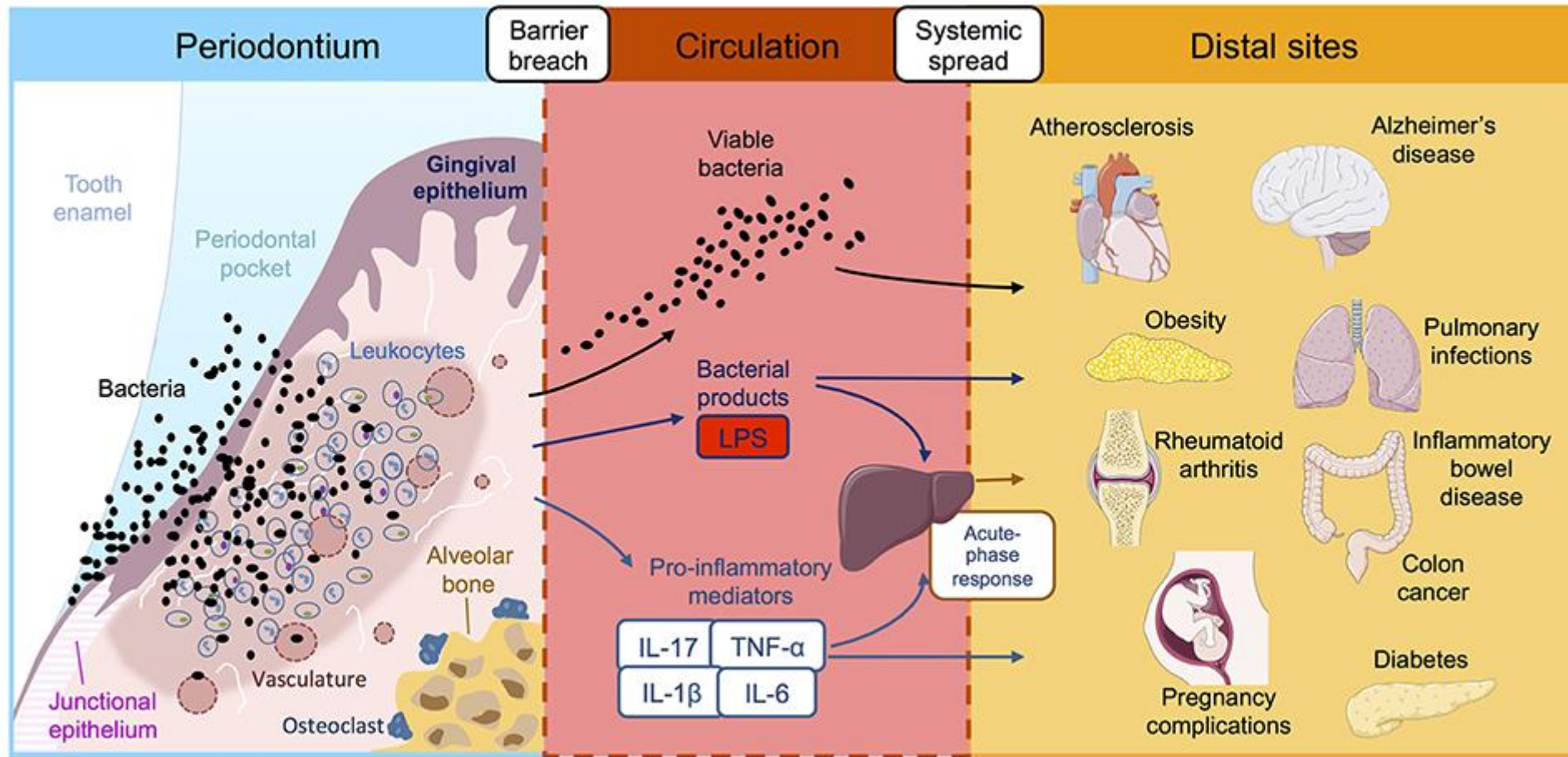
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Human microbiome

Questions

— How do microbial communities form and how do they regenerate?

— What mechanisms regulate microbial composition?

— Which MOs are involved in health and disease?

— To what extent does the microbial community differ between unrelated healthy individuals?

— Is there a basic microbiome in humans in a given locality?

— How does microbial composition change over time, between environments or body locations?

— How can the microbial composition be manipulated with respect to treatment?

The human microbiome
Questions de la maladie

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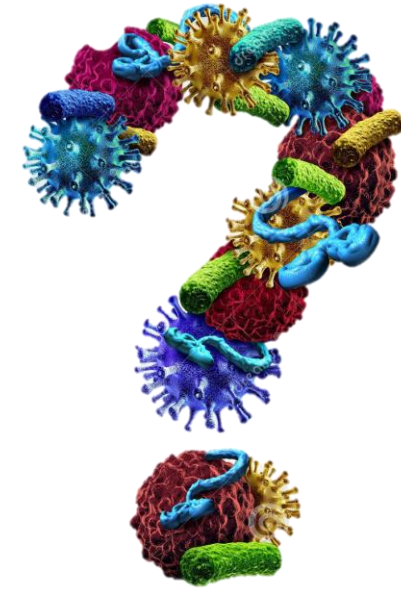
Oral microbiome

Vision

– Interaction between the host and oral microbiome – a combination of approaches

Current Methods for Studying the Human Microbiome (2017)

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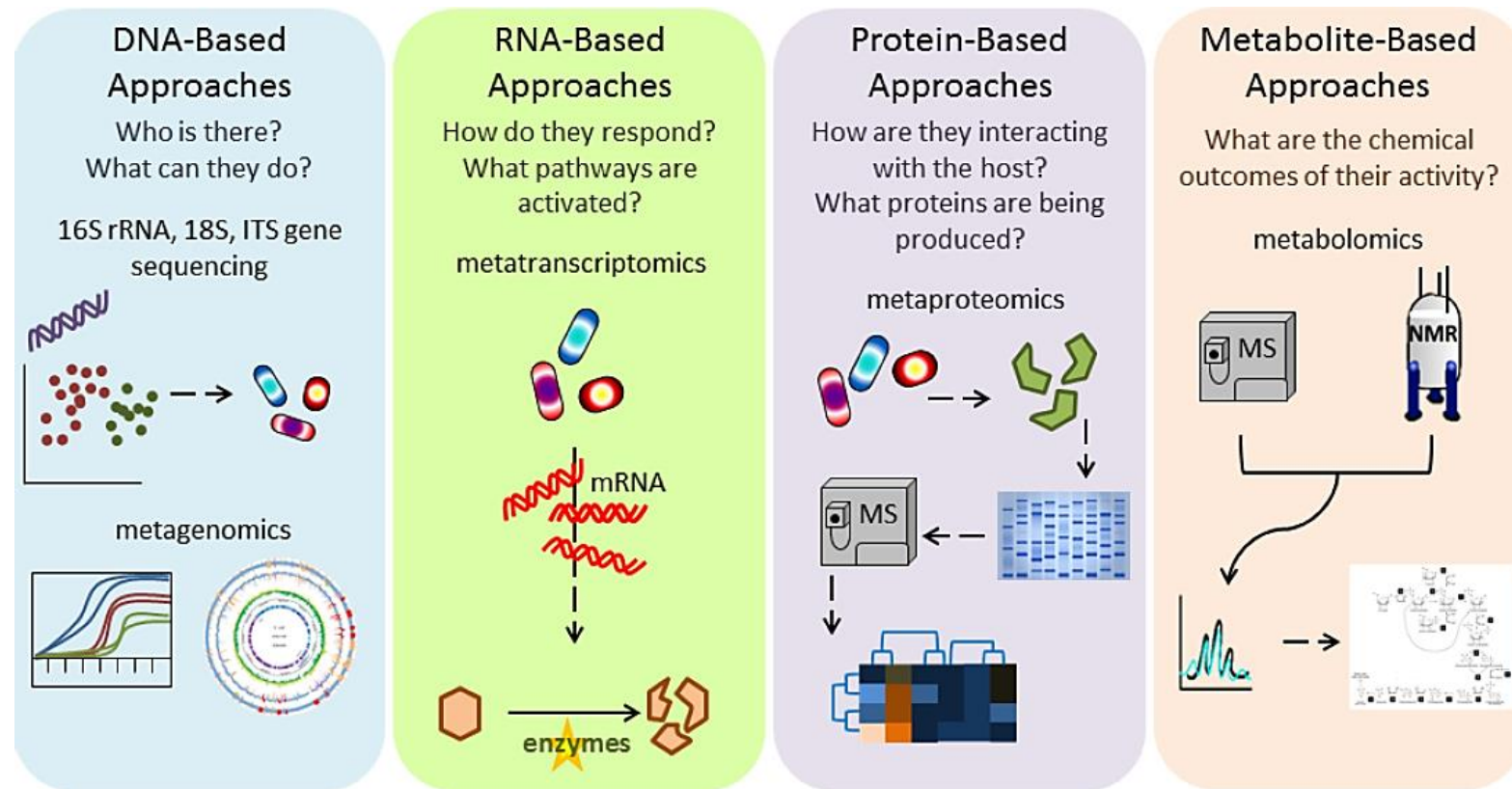
Molecular etiopathogenesis apical periodontitis and odontogenic cysts

MDDr. MUDr. David Száraz
Assoc. Prof. RNDr. Petra Bořilová Linhartová, Ph.D., MBA
Clinic of Stomatology, Department of Pathophysiology, Institute of Medical Genetics and Genomics, Faculty of Medicine, Masaryk University Brno
Clinic of Maxillofacial Surgery, University Hospital Brno

peta.linhartova@gmail.com

Molecular etiopathogenesis AP and OC

Odontogenic cysts

- 1) gingival
- 2) eruption
- 3) periodontal
- 4) residual
- 5) radical
- 6) dentigerous
- 7) odontogenic keratocyst
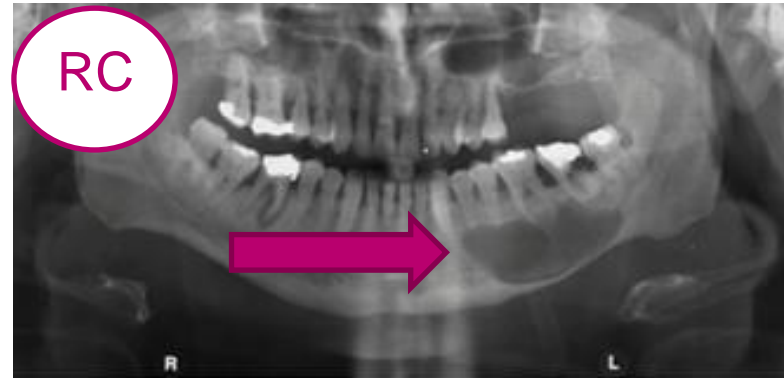
Inflammatory OC
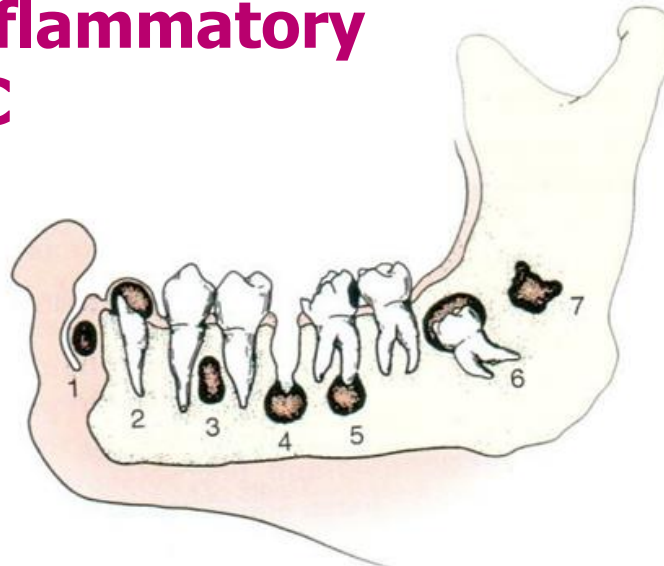
Development OC

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Introduction

Apical periodontitis and radical cyst

– Reduced wealth and diversity of the AP community compared to controls

Relative Abundance of Bacterial Phyla

The number of operational taxonomic units

Shannon Diversity Index

Oral Pathology: Gene Expression in Odontogenic Cysts   Siqueira Jr J. F. et al., 2016   Qian W. et al., 2019

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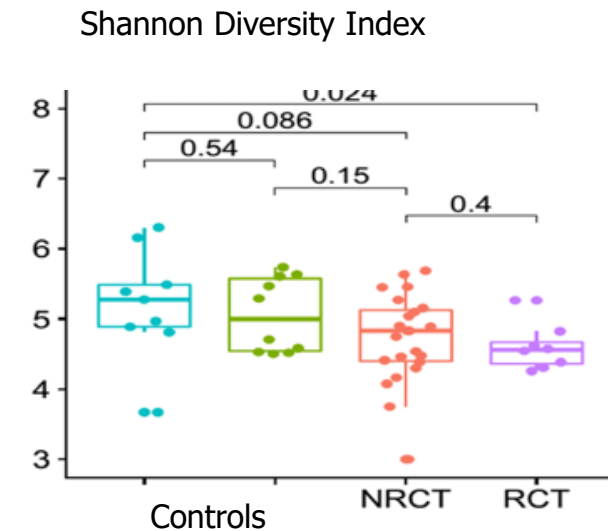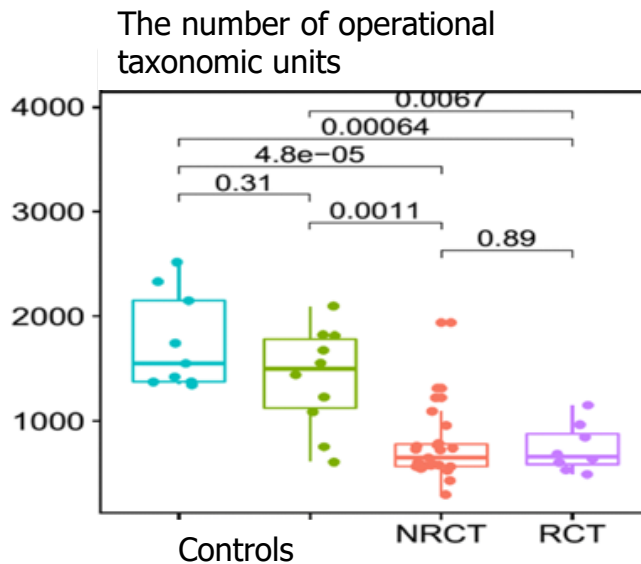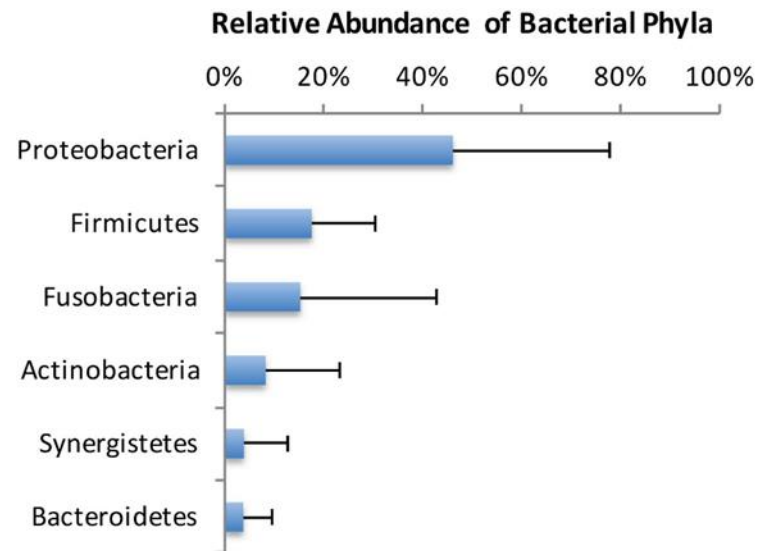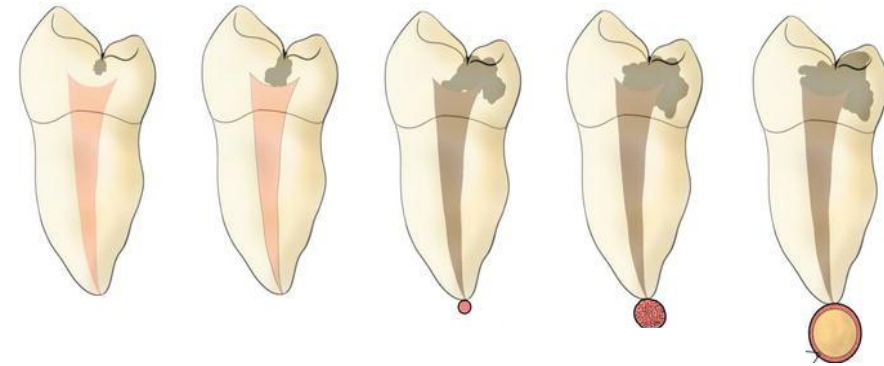
Oral dysbiosis

Apical periodontitis and radical cyst

— *Lactococcus lactis* associated with the formation of periapical granuloma

— Anaerobic taxa (*F. nucleatum*, *P. intermedia*) most pre-used in formulas RC

— In RC samples, a greater number of expected metabolic pathways (G-bacteria) associated with LPS biosynthesis

– subclinical pro-inflammatory marker

Siqueira Jr J. F. et al., 2016   Mussano F. et al., 2018.   Bernardi L. et al., 2015.   Queiroz-Juninor C. M. et al., 2010

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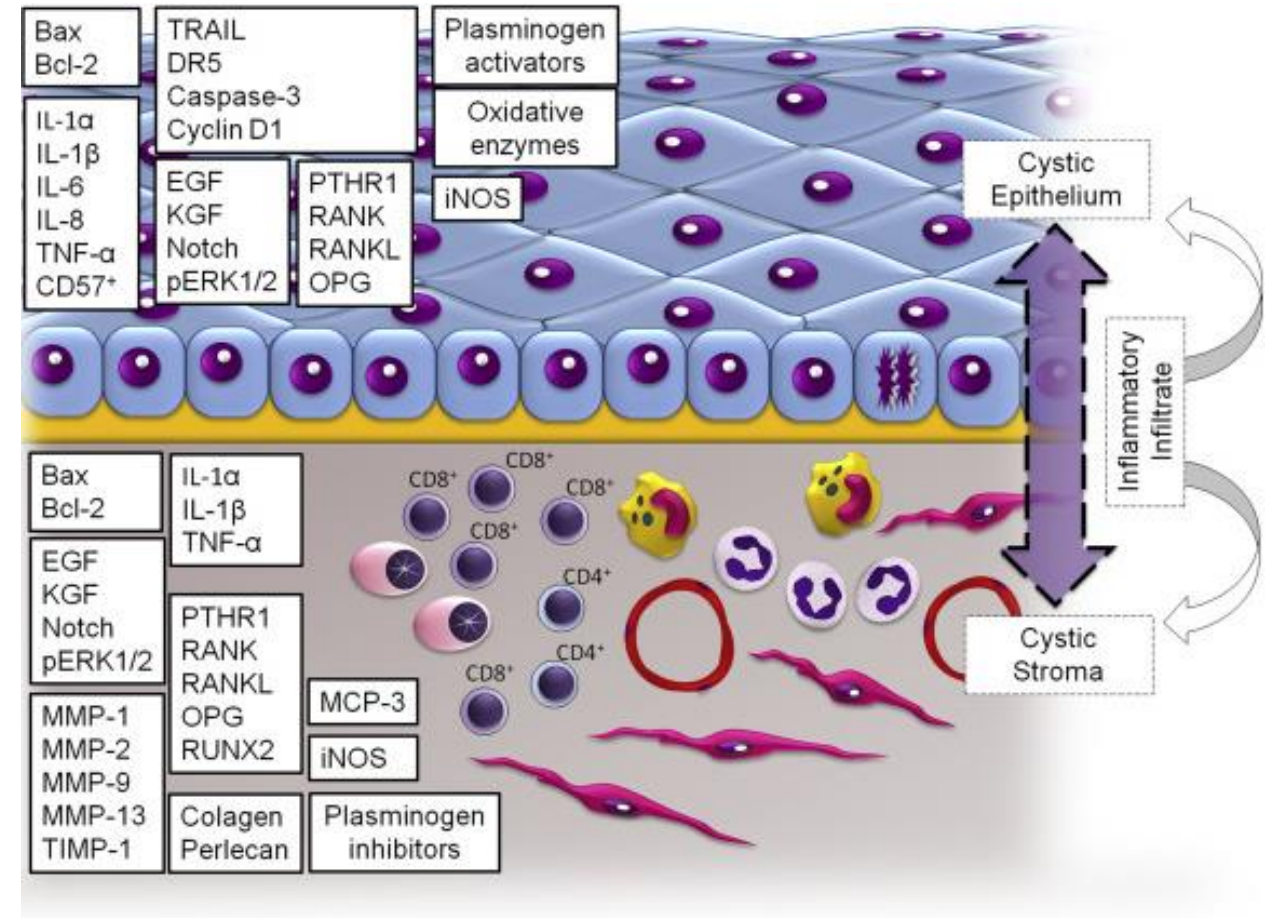
Candidate genes

AP and RC

MMP-1
MMP-2
MMP-3
MMP-8
RANK
RANKL

IL-1
IL-6
IL-8
TLR-4
Fc receptor
TBX21
HSPA1L
HSPA6
VDR

IL-6
IL-17A
IL-22
SSP1
NOS
MMP-9
TLR-2
CB2
MYD88

Developmental OCs

PTCH1
PKD1
GLI1
MMP-2

Ameloblastoma

MMP-9

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Cooperation

Animal research

Animal model
SHH signalling (gene expression, activation and inhibition of the path)
Nanoparticle therapy

Human research

Gene expression in tissue and cystic fluid

Clinical part

Selection of patient
Examination of patients
Sampling
Examination of tissue for pathology

Human research

Microbiome analysis
Genetic association studyies

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Project

Annotation

— **determination of risk factors for development of RC, DC and OKC**

— **finding new markers** for the prediction of their origin and for differential diagnosis using modern biomedical technologies

— **design of a panel of markers for predicting the formation of OCs and their differential diagnostics**

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Project

Objective – study of etiopathogenesis AP and OC: 1) Human research

— (i) monitoring of the root canal microbiome and fluid in the RC to determine risky microbial profiles and possible association with RC formation in patients with AP

— (ii) to search for a genetic predisposition to the formation of RC or OKC in patients with AP, including a multivariate analysis of relationships with the microbial profile (the design of a case-case study)

— (iii) determination and comparison of express profiles of genes involved in immunoregulation, odontogenesis and cancerogenesis in OC, or selection of markers for aggressive forms of OCs

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Project

Design 1) Human Research

— **Criteria for inclusion in the study:**

— Czech / Slovak nationality, age over 18, signing an informed consent

— **Exclusion criteria:**

• AP / RC arm: systemic diseases (diabetes mellitus, cardiovascular diseases, autoimmune diseases, immunodeficiency, cancer), pregnancy and lactation in women

• Microbiome subproject: ATB use less than 2 months before study entry

• Developmental OCs arm and ameloblastoma + retained tooth: cancer, pregnancy and lactation in women

• Except: patients with polycystic diseases and Gorlin-Goltz syndrome

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Project

Design 1) Humane Research

- (i) microbiome (bacteriome and mycobiome) – metagenomic analysis, qPCR dental plaque vs. buccal plaque vs. root canal: 50 patients with AP without RC vs. 50 patients with AP with RC root canal vs. cystic fluid: 50 patients with AP with RC

- (ii) genetic association study (case-case study) – qPCR, SNapShot, sequencing 100 patients with AP without RC vs. 100 patients with AP with RC 50 patients with FC vs. 10 patients with OKC vs. 10 patients with ameloblastoma,

- (iii) determination and comparison of expression profiles – RNAScope 50 patients with RC / 50 patients with DC / 10 patients with OKC / 10 patients with ameloblastoma, vs. in patients with AP without RC in sac tissue / in tissue around the retained tooth in patients without OC

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Project

Pilot data

- (i) development of an animal (mouse) model for the study of OC,

- (ii) analysis of the causes of OC with emphasis on disorders of primary ciliary function,

- (iii) detection of changes in signaling involved in the pathogenesis of OC with emphasis on the Sonic hedgehog (SHH) pathway,

- (iv) testing the effect of targeted changes in SHH signaling on OC formation in our model animals,

- (v) application of nanodiamonds to animal models and determination of their influence on the initiation and progression of OC in order to find new potential treatment options for this disease.

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Project

Summary

- **The novelty of the project lies in the**

- complexity and monitoring of interactions between the host microbiome and the genetic predisposition

- In the determination of the effect of nanodiamonds on the formation and development of OCs in the animal model – new therapeutic possibilities

His Wife Loves Him Just The Way He Is!

YOUR DAD'S BEEN UNDER A LOT OF PRESSURE LATELY.

Imgur

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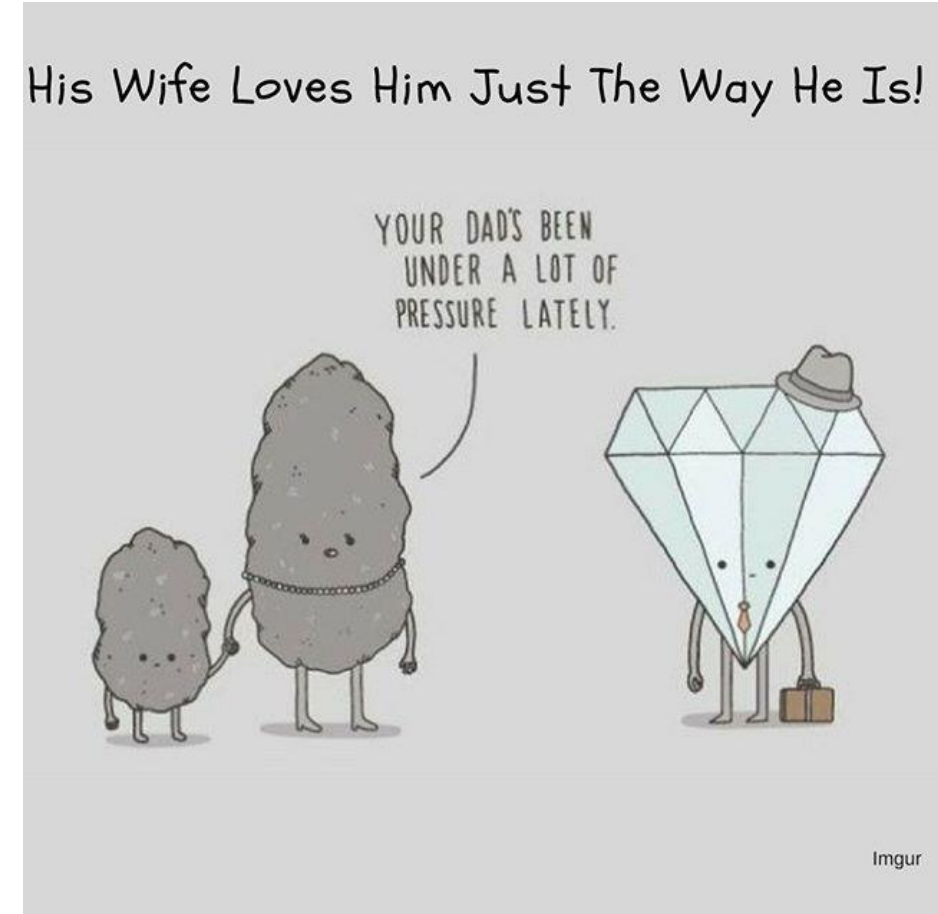
Lipoxins and resolvins in the treatment of periodontitis
Use of nanofibers for application of bioactive substances using dental floss

MDDr. Filip Hromčík
Assoc Prof. RNDr. Petra Bořilová Linhartová, Ph.D., MBA

Introduction of the researcher

Dental Clinic FNUSA, Veterinary Research Institute, externalists

FAKULTNÍ NEMOCNICE U SV. ANNY V BRNĚ ®

Lydie Izakovičová Hollá

Filip Hromčík

Jak Vokurka

VRI VETERINARY RESEARCH INSTITUTE

Martin Faldyna

Eduard Göpfert

Monika Vicenová

I. ústav patologie FN USA   Markéta Hermanová

Klinika dětské onkologie FN Brno   Michal Kýr

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Lipoxins and resolvins in the treatment of periodontitis

Granulation tissue (GT) is not bad

insufficient "resolution phase"

lack of lipoxins and resolvins

Graphics: Hromčík 2020

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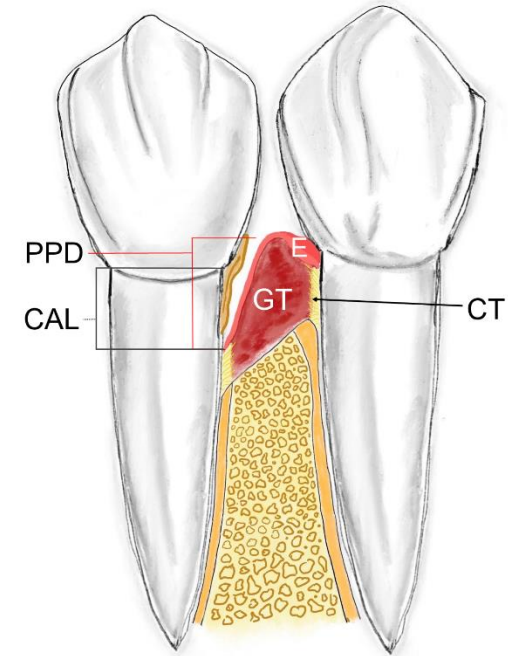
Lipoxins and resolvins in the treatment of periodontitis

ASA + omega-3 fatty acids + GT
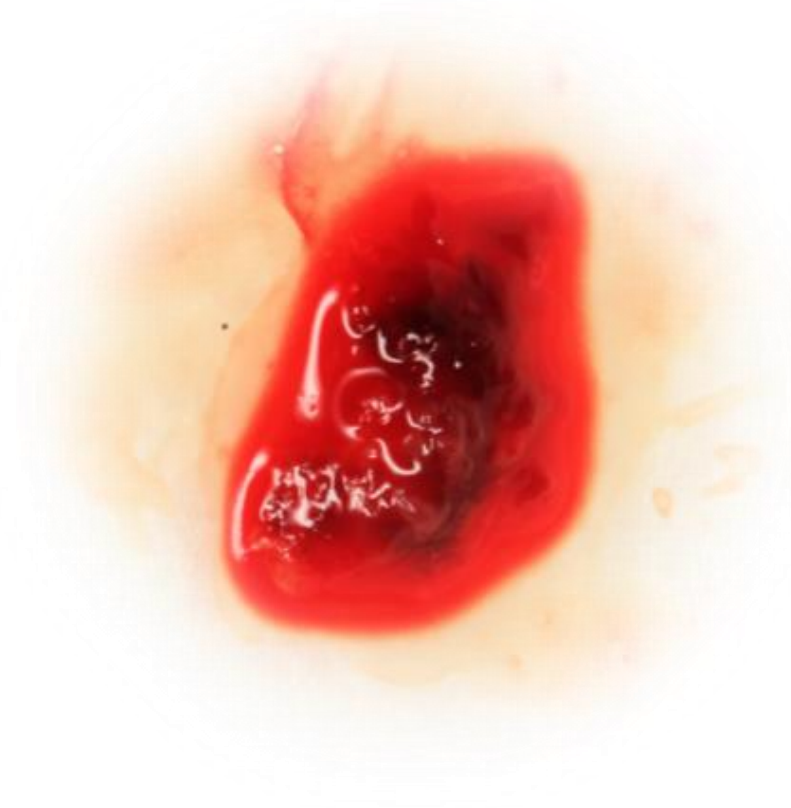
induction of endogenous lipoxin and resolvin synthesis

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Treatment of intrabony periodontal defects

Photo and x-ray: Filip Hromčík 2020

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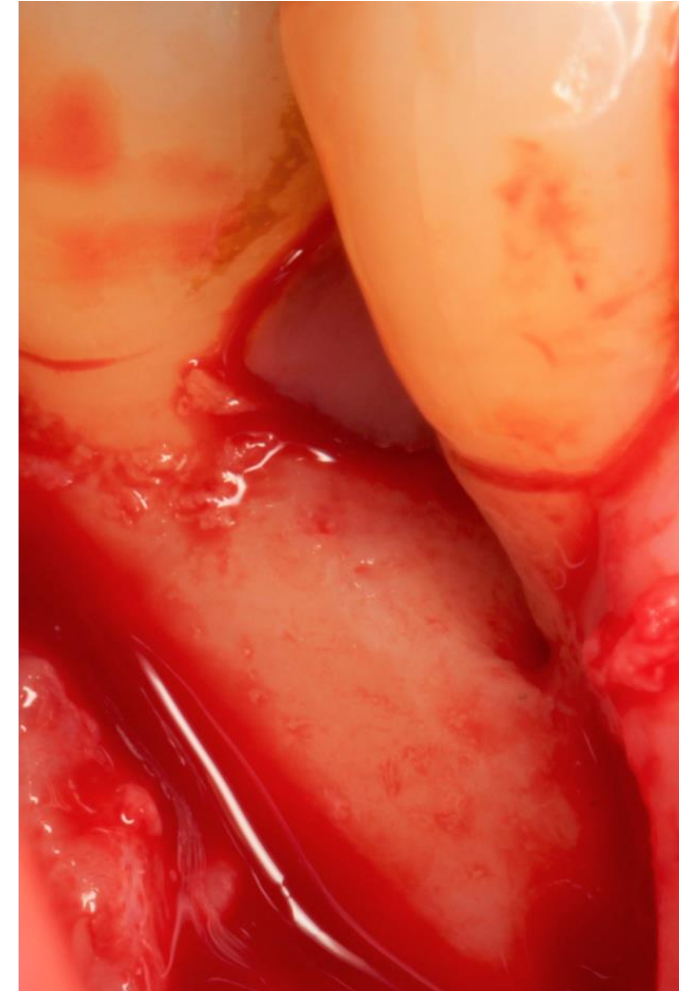
Enhanced granulation tissue = EGT

EGT

GT

OMEGA-3

4 minutes

ASA

4 minutes

Graphics: Hromčík 2020

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EGT

Inflammed Epithelium

Granulation Tissue

Intrabony Defect

Root surface after scaling and planing

Flap

EGT

Graphics: Hromčík 2020

255 Research Activities in Dentistry   2021

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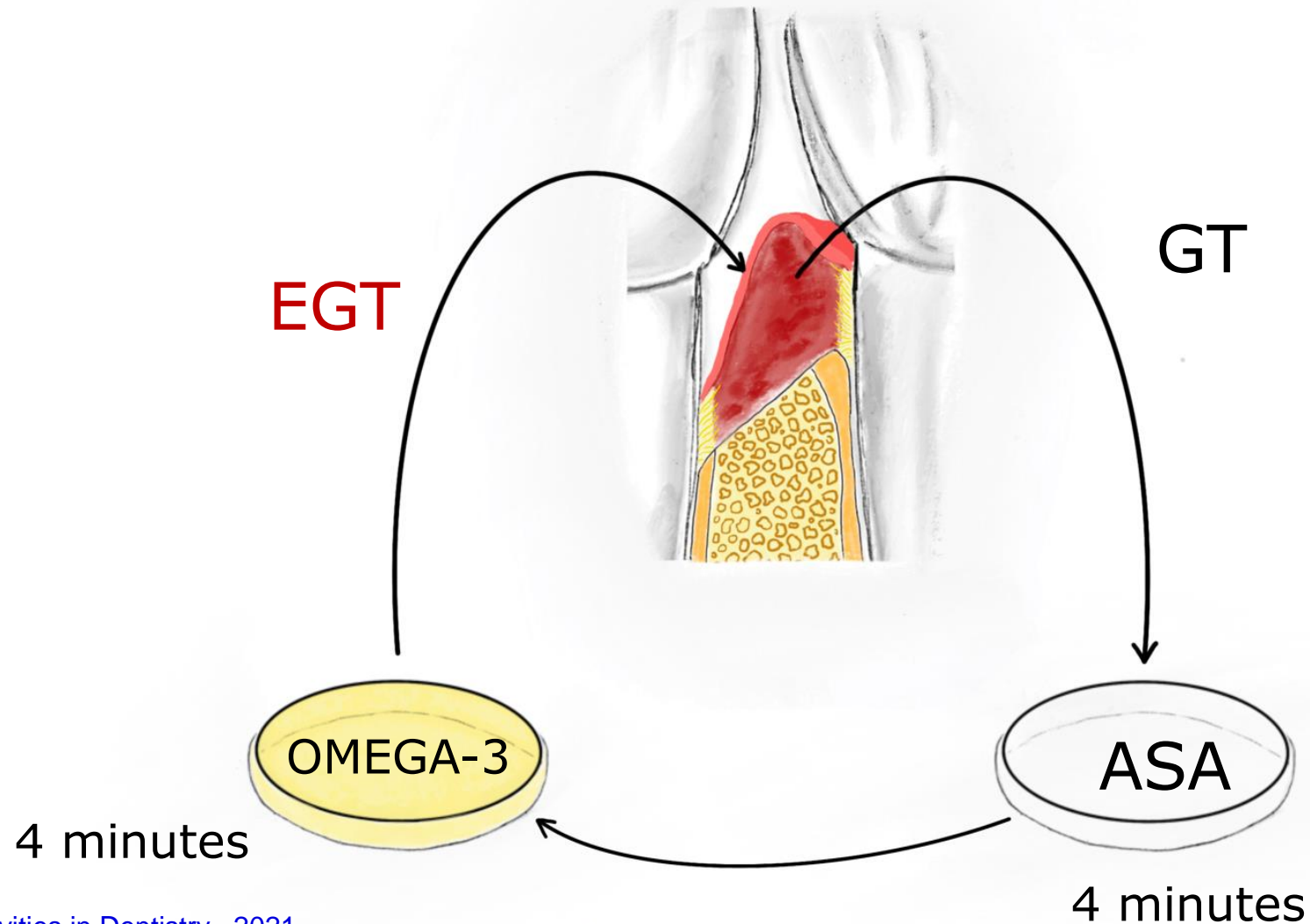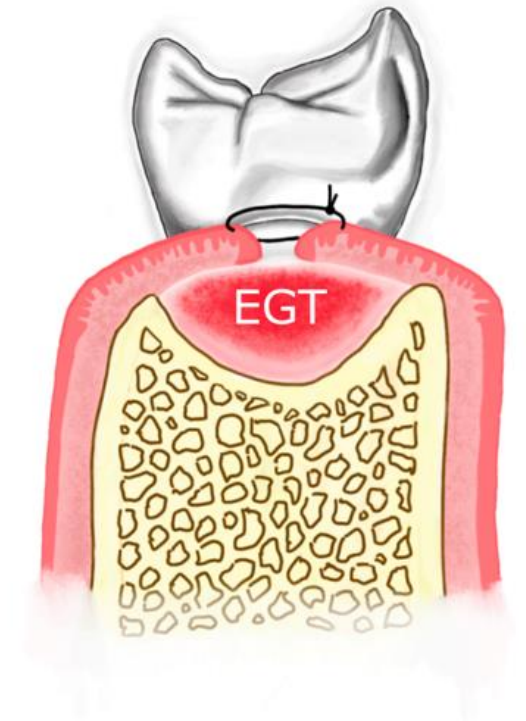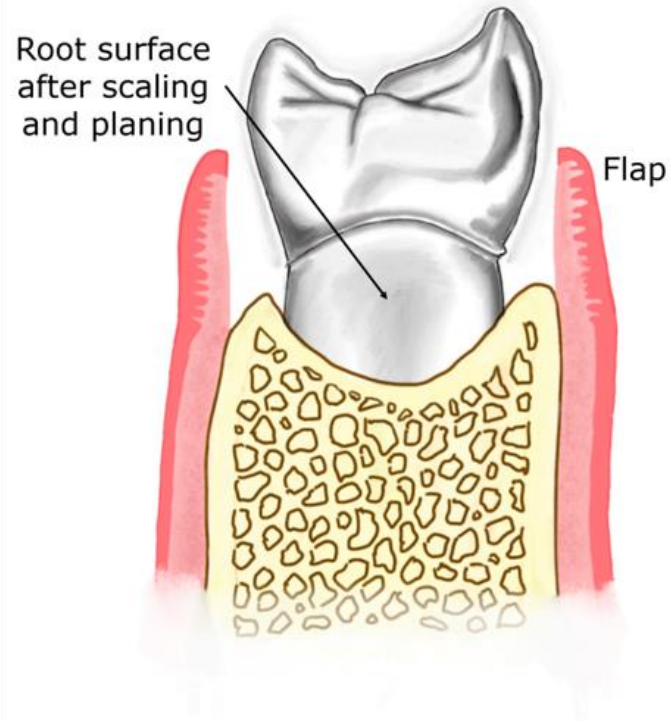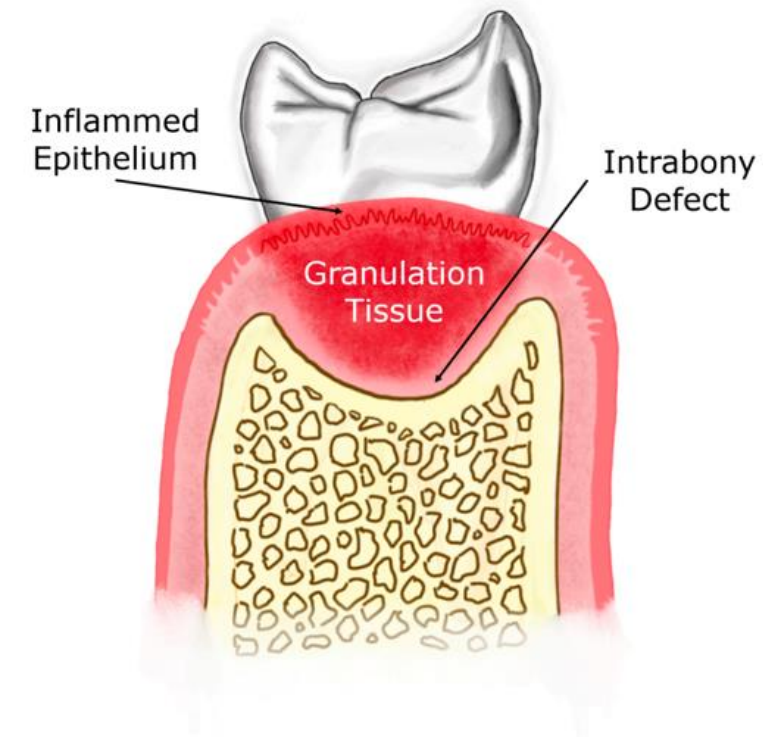
Experimental phase

- 48 rabbits

- ligature-induced periodontitis
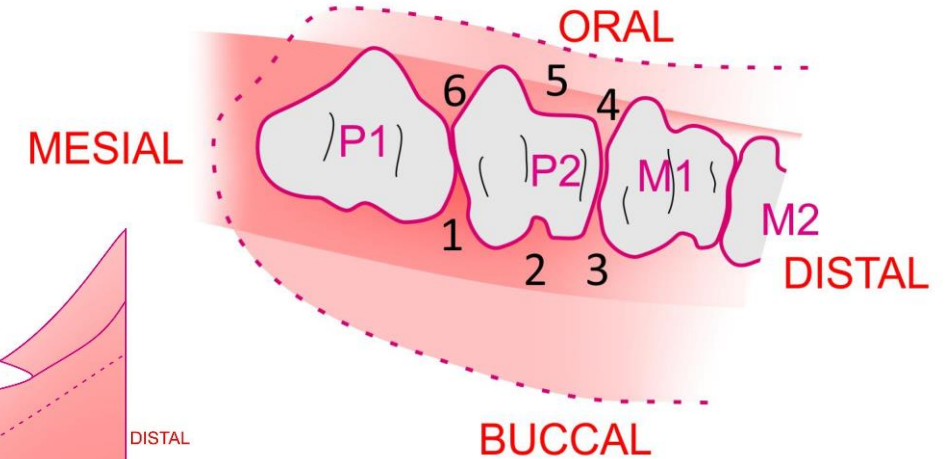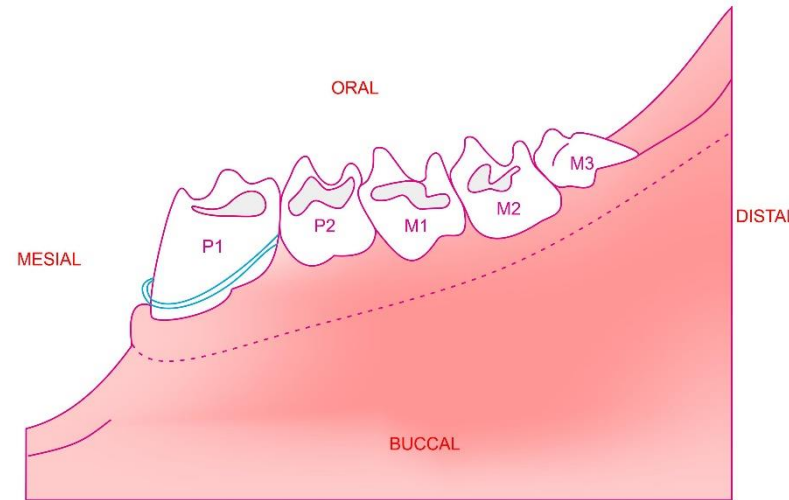
Graphics: Hromčík 2019

Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub. 2019; 163:XX.

Granulation tissue enriched by aspirin and omega-3 fatty acids in healing experimental periodontal lesion

Filip Hromcik[a,b], Jan Vokurka[a,b,†], Eduard Gopfert[c], Martin Faldyna[c], Marketa Hermanova[b,d,#], Michal Kyr[b,e,#], Monika Vicenova[c,#], Lydie Izakovicova Holla[a,b]

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Clinical phase

— 19 patients, 38 treated defects

— primary observed parameters: probing
pocket depth (PPD), clinical attachment
level (CAL)

— result: 1 mm greater CAL gain in the EGT
group (p < 0.05)

— published

RESEARCH ARTICLE

European Journal of
Lipid Science and Technology
www.ejlst.com

Granulation Tissue Enhanced with Aspirin and Omega-3
PUFAs as a Local Adjunct to the Surgical Treatment of
Periodontitis

Filip Hromcik, Jan Vokurka, Michal Kyr, and Lydie Izakovicova Holla*
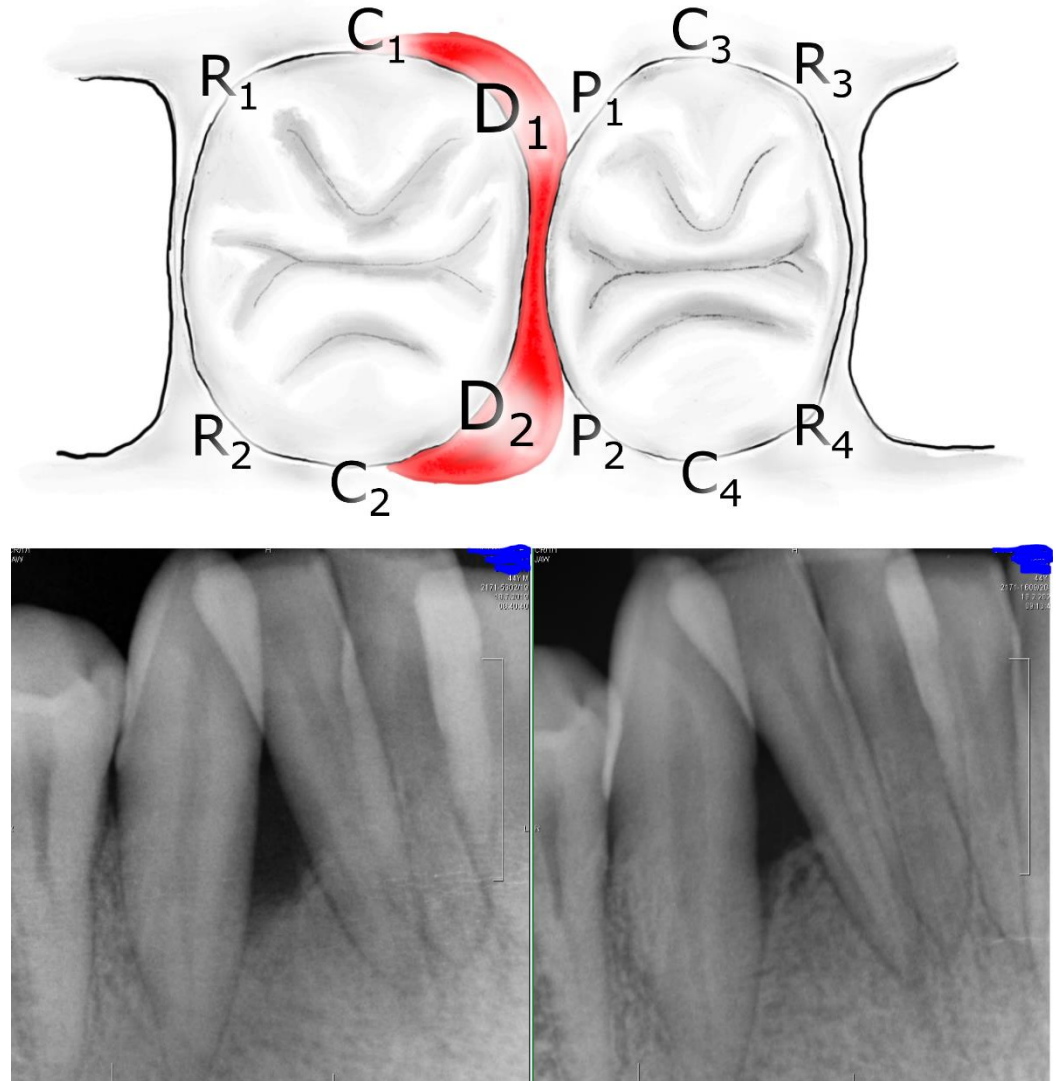
Graphics: Hromčík 2020, X-ray: Hromčík 2019

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Use of nanofibers for application of bioactive substances using dental floss

Wikinomist, s. r. o., Faculty of Medicine at Masaryk Univesity, Technical University of Liberec, University of Defence

Wikinomist — František Janda · Pavel Nešetřil · Magdaléna Chupíková

MUNI MED — Petra Bořilová Linhartová · Zdeněk Daněk · Filip Hromčík · Jan Křivánek · Jiří Kučera · Filip Růžička · Lydie Izakovičová Hollá

TECHNICKÁ UNIVERZITA V LIBERCI www.tul.cz — Eva Kuželová-Košťáková · David Lukáš · Petr Mikeš · Věra Jenčová · Jan Valtera

Zdeněk Pokorný · Zdeněk Joska · David Dobrocký

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TAČR TREND

Objectives

— technology and material for the production of nanofiber yarn

— antimicrobial properties of bioactive substances and probiotics

— enrichment of nanomaterial with bioactive substances, simulation of in vivo conditions

— clinical testing

— prototype and its production

— changes in enamel and dentin after flossing

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Use of nanofibers for application of bioactive substances using dental floss

– project for 4 years

– candidate substances – the body's own bioactive molecules:

• secretion immunoglobulin A, lactoferin, osteopontin

• Probiotics:

• *Lactobacillus reuteri*

• *Streptococcus salivarius*

• *S. dentisani…*

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Outputs

– Prototype?

– Patent?

– market application, sales
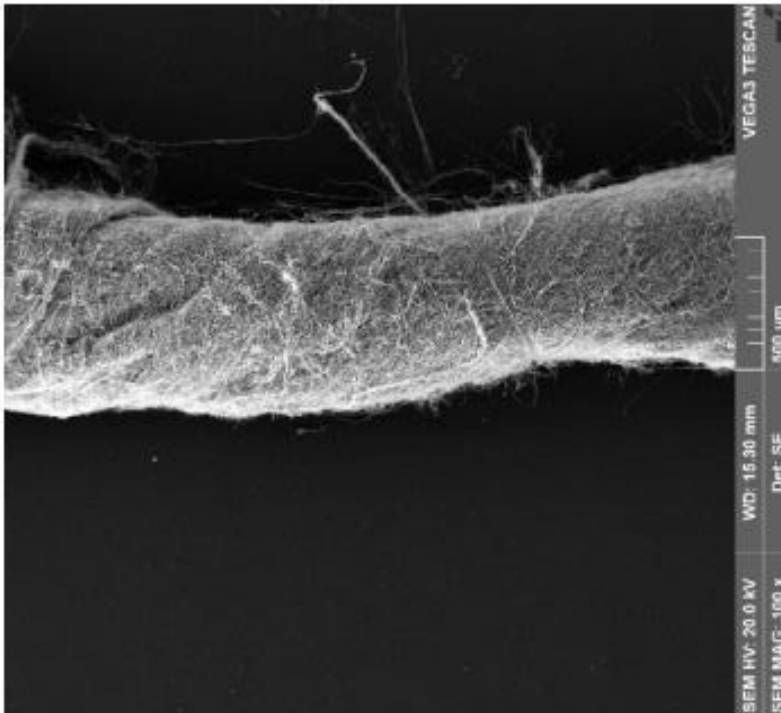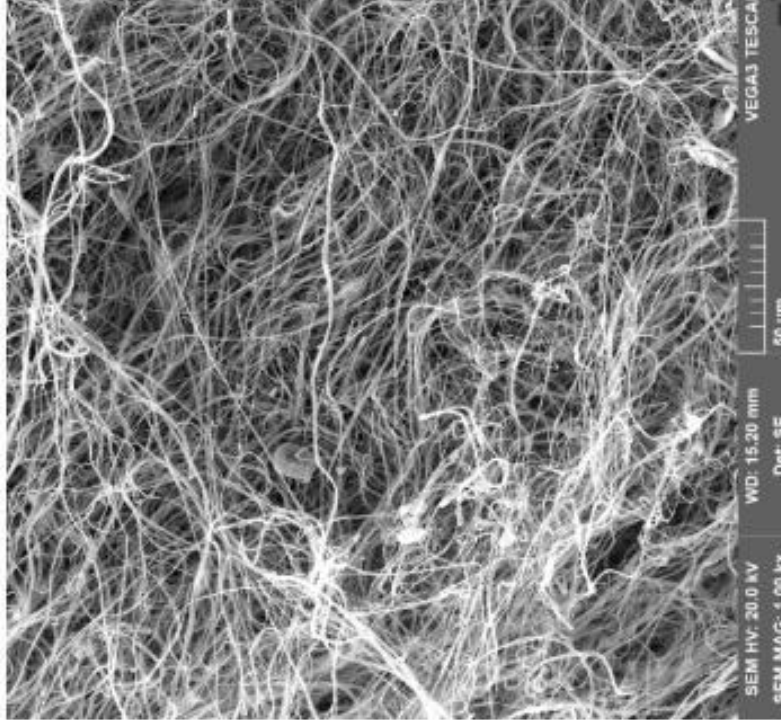
snímek: Technická univerzita Liberec

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Abbreviations used

- FNUSA   St. Anne's University Hospital

- FN Brno   University Hospital Brno

- GT   Granulation tissue

- ASA   Acetylsalycilic Acid

- EGT   Enhanced Granulation Tissue

- PPD   Pocket Probing Depth

- CAL   Clinical Attachment Level

- TAČR   Technology Agency of the Czech Republic

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(Pediatric) Obstructive sleep apnea from dentist's & orthodontist's point of view

MDDr. Zuzana Vranková

doc. RNDr. Petra Bořilová Linhartová, Ph.D., MBA

Clinic of Stomatology, St. Anne's University Hospital

Faculty of Medicine, Masaryk University Brno

Introduction of the Doctoral candidate

MDDr. Zuzana Vranková

— from 2/2019 - Pre-certification preparations in the field of Orthodontics, St. Anne's University

Hospital, Brno

— from 9/2019 - Doctoral studies, MED MUNI Research focus: Congenital predispositions to

orthodontic anomalies, their complications and treatment

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???

What do **OSA** and **SCIENCE** have in common ?
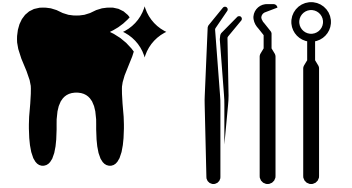
… much more than it may seem at first glance…

… and therefore the first glance should not always be sufficient for you…

Obstructive sleep apnea in dentist's office

Content of the lecture

1. What is obstructive sleep apnea (OSA) and why is it important to know about it

2. Mechanism of OSA and risk factors

3. OSA in adults and children

4. Symptoms of OSA

5. Diagnosis and screening

6. Interdisciplinary cooperation and involvement of dentists

7. OSA from the perspective of an orthodontist

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What is obstructive sleep apnea?

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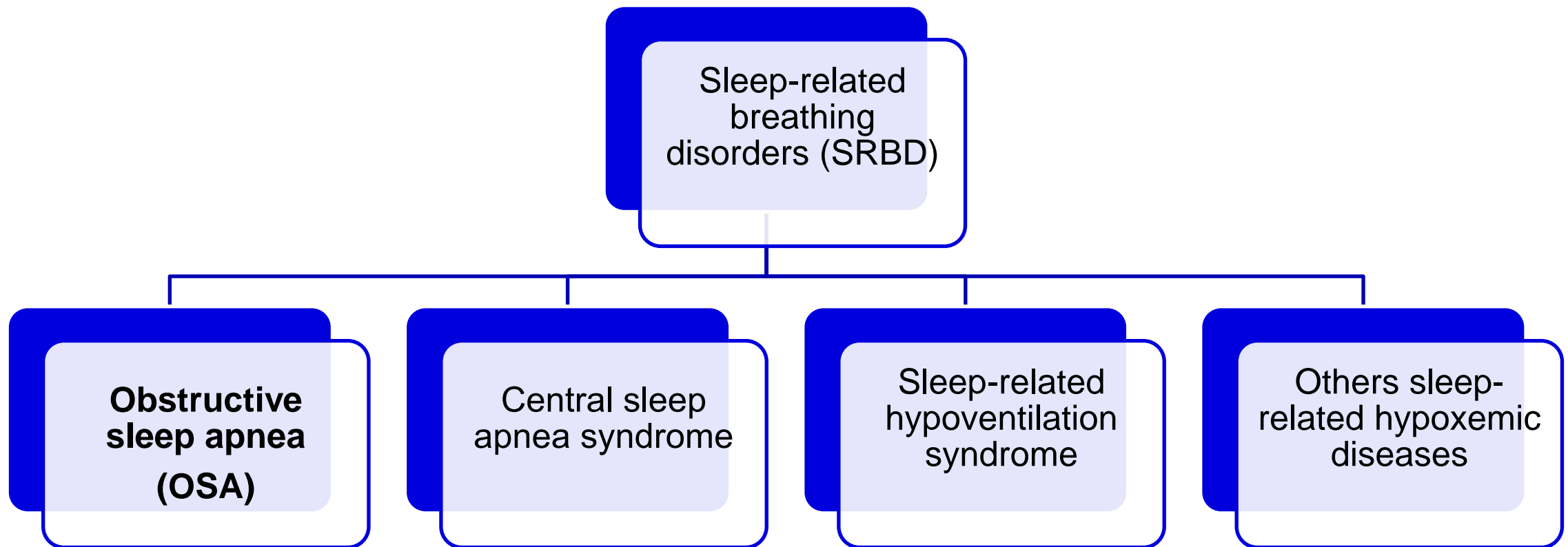
OSA can be life-threatening!

OSA

— What is apnea?

— What is hypopnea?

Absence of air flow for at least 10 seconds or 2 breath cycles

Reduction of air flow - at the level of the nose, mouth and especially pharynx. It is often defined as a decrease in airflow of at least 30% or associated with a reduction in hemoglobin saturation of at least 3%.

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OSA

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OSA

change in the muscle tone control in sleep + horizontal position + risk factors

Upper airways collapse - partial / complete occlusion

hypopnea /apnea

hypoxia + hypercapnia + increasing inspirational muscle effort

awakening reactions (partial / complete awakening)

end of apnea + breath + decrease in airway resistance

https://my.clevelandclinic.org/health/diseases/8718-sleep-apnea

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Risk factors involved in OSA etiopathogenesis

— Overweight / obesity / fat distribution

— Neuromuscular factors – abnormally reduced muscle tone during sleep

— Adenoid hypertrophy

— Craniofacial malformations contributing to the narrowing of upper airways

— Genetic predisposition

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Genetic predisposition

– The genetic background of an individual plays a role, to some extent, in all the mentioned factors

– The importance of genetics is also confirmed by the familial occurrence of OSA (observed since 1978)

– Heredity is also manifested in craniofacial traits - most notably in the size, position and growth of the jaws

– The significant prevalence of OSA in syndromic patients suggests the possibility of linking the genetic basis of both diseases

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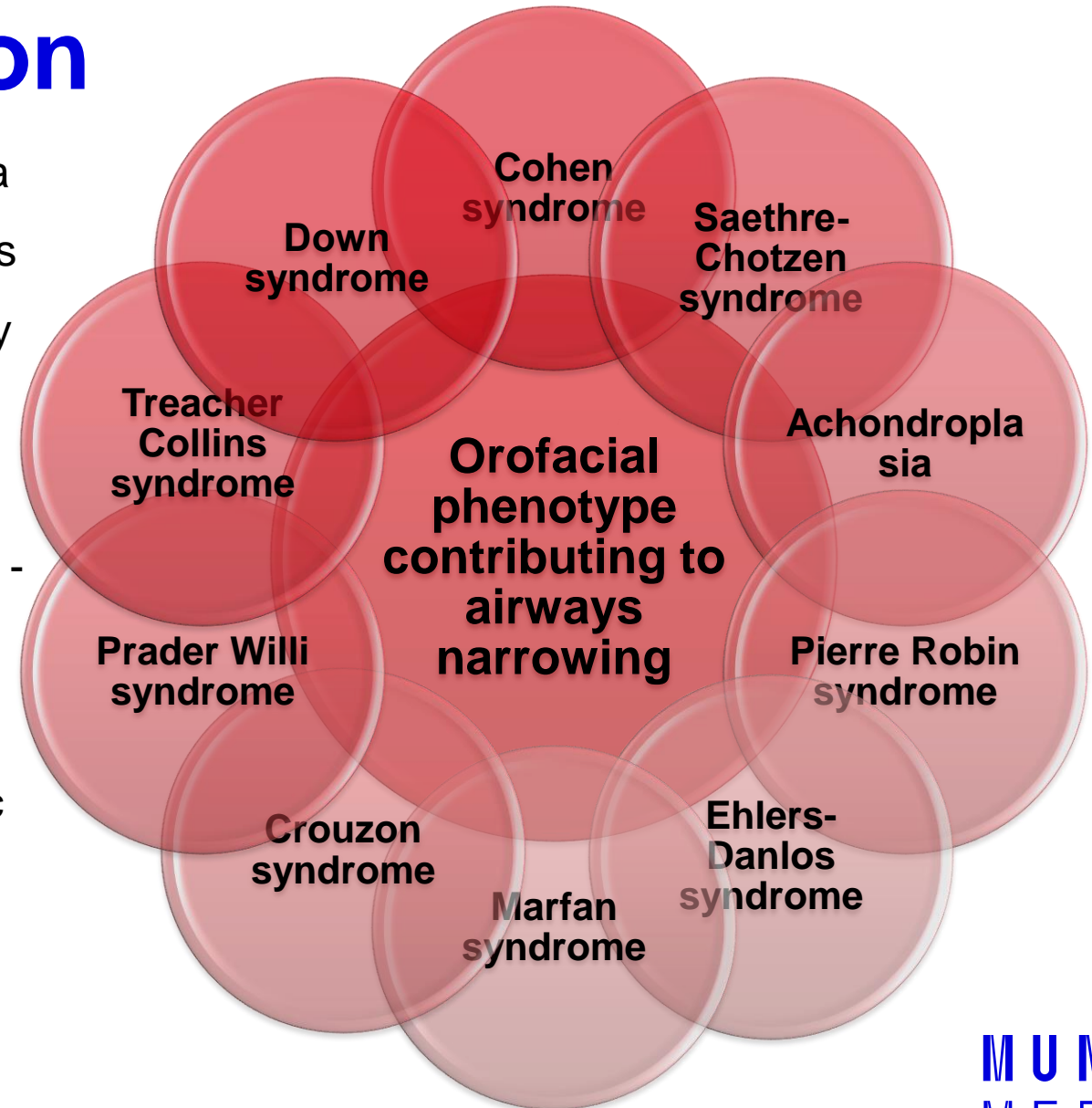
OSA diagnostics

- Sleep questionnaires (Epworth sleepiness scale, Berlin questionnaire, STOP-BANG questionnaire…)
- Polysomnography and polygraphy (sleep laboratory)
- Home monitoring sleep devices
- Night pulse oximetry
- Audio and video recordings
- ENT examination
- Orthodontic examination

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Children's Sleep OSA Questionnaire

There are several ways to primarily detect a sleep disorder – a popular way is represented by **questionnaires**, such as:

- The Sleep-Related Breathing Scale of the Pediatric Sleep Questionnaire, an 18-item Obstructive Sleep apnea QoL ...
- In the Czech Republic, the most commonly used one is the Epworth Sleepiness Scale, which exists abroad also in a modified version for children.
- In the Czech Republic, there is no questionnaire applicable for OSA screening in the pediatric population.
- In cooperation with the pediatric ENT and neurologists and on the basis of the fusion of existing foreign language questionnaires, we have created and are currently working on the validation of the Pediatric OSA questionnaire.

Obstructive sleep apnea

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OSA in adult patients

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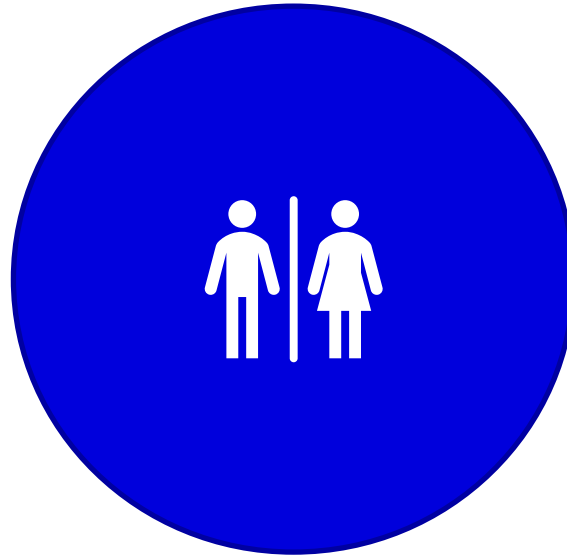
OSA definition

— **AHI** (apnea hypopnea index) – number of apnea and hypopnea per hour of time

— In adults:

— **AHI = 0–5** standard

AHI = 5–15 lighter form of OSA

AHI = 15–30 moderate OSA ⎫ **pathology**

— **AHI > 30** severe OSA ⎭

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Symptoms of OSA in adult patients

➤ Drowsiness – especially in

monotonous activities

➤ Microsleep

➤ Headaches

➤ Sexual dysfunction

➤ Snoring

➤ Gasping for breath

➤ The sounds of choking

➤ Unrefreshing sleep

➤ Aggravated after alcohol,

muscle relaxants, sedatives

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OSA

Arterial hypertension

Disease of a coronary artery

Comorbidities of OSA

Diabetes mellitus type 2

Stroke

Atrial fibrilation

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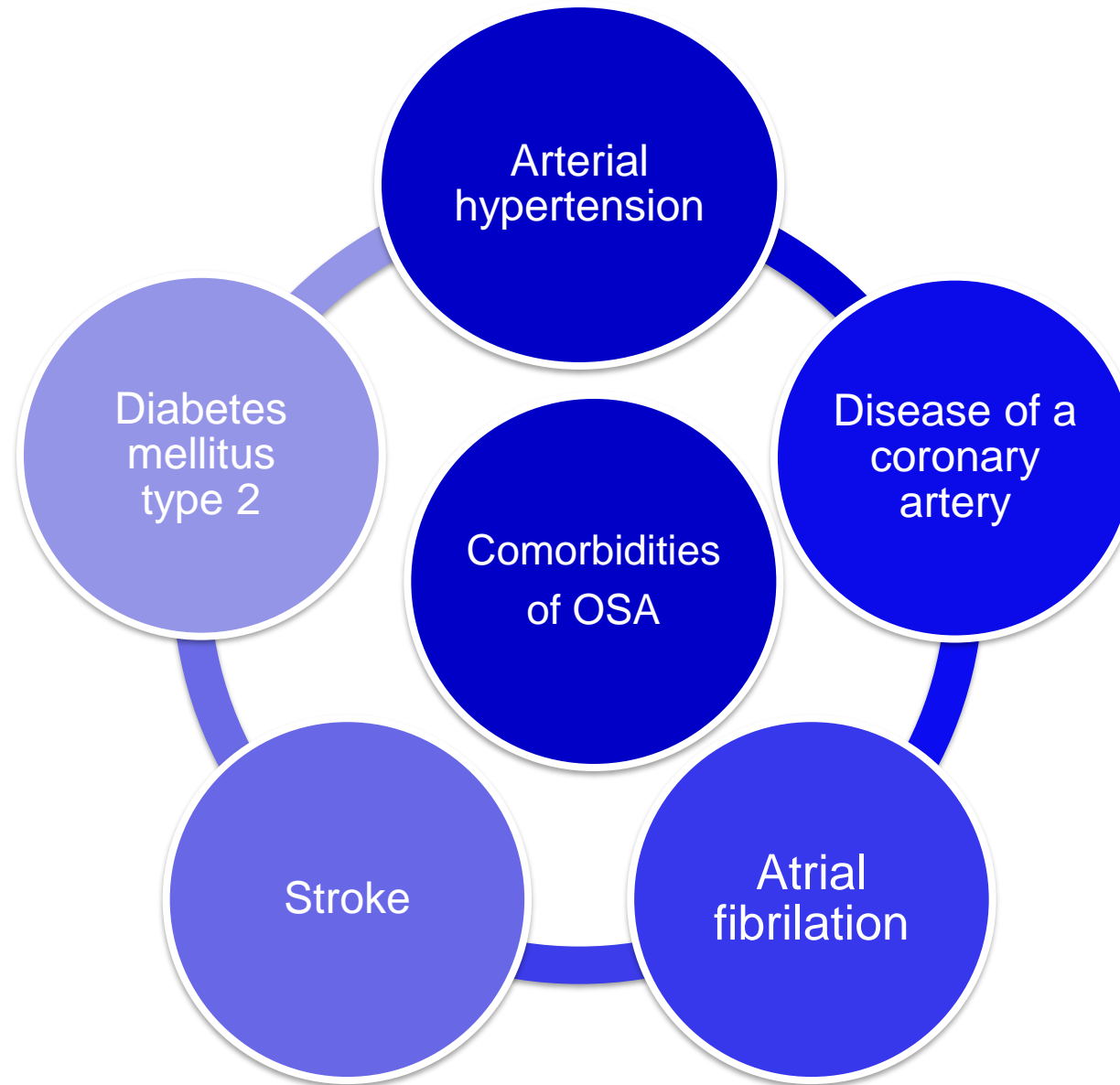
OSA in pediatric patients
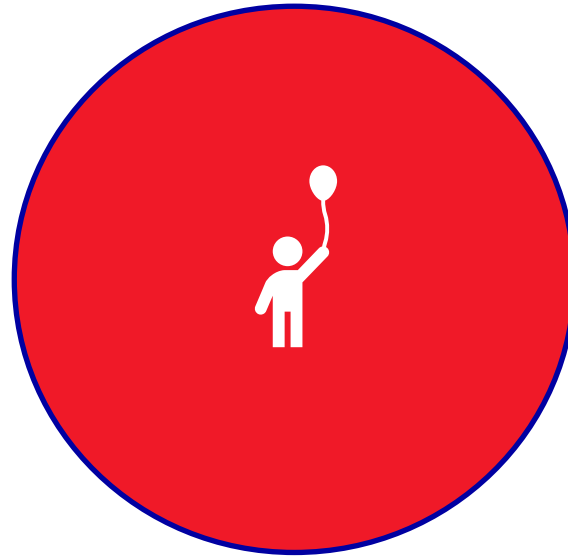
OSA definition

— In 1976, prof. Guilleminault diagnosed obstructive sleep apnea in 8 children –

specific manifestations and risks factors

— Interruption of breathing in sleep for 2 respiratory cycles, accompanied by a

decrease in saturation and/or a wake-up reaction

— **AHI = 1- 4** – lighter form of OSA

— **AHI = 5 -10** – moderate OSA

— **AHI > 10** – severe OSA

— **already AHI = 1 is considered pathological**

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Symptoms of OSA in children

- dry mouth, dry lips

- difficult to wake up

- the child wakes up unrefreshed, tired

- early headaches

- daily inattention and hyperactivity

- snoring

- apneic pauses

- abnormal sleep positions

- excessive sweating

- restless sleep

- night wetting

- NREM parasomnia

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Symptoms of OSA in children

Fragmentation of sleep & hypoxia

affect:

- **Prefrontal cortex**

- **Cognitive executive functions**

- **Information processing**

Beebe et al., 2002

Information processing failure

Impaired cognitive function

Disrupted organization

Hyperactivity

Decision-making disorder

Emotional lability

Attention deficit maintenance disorder

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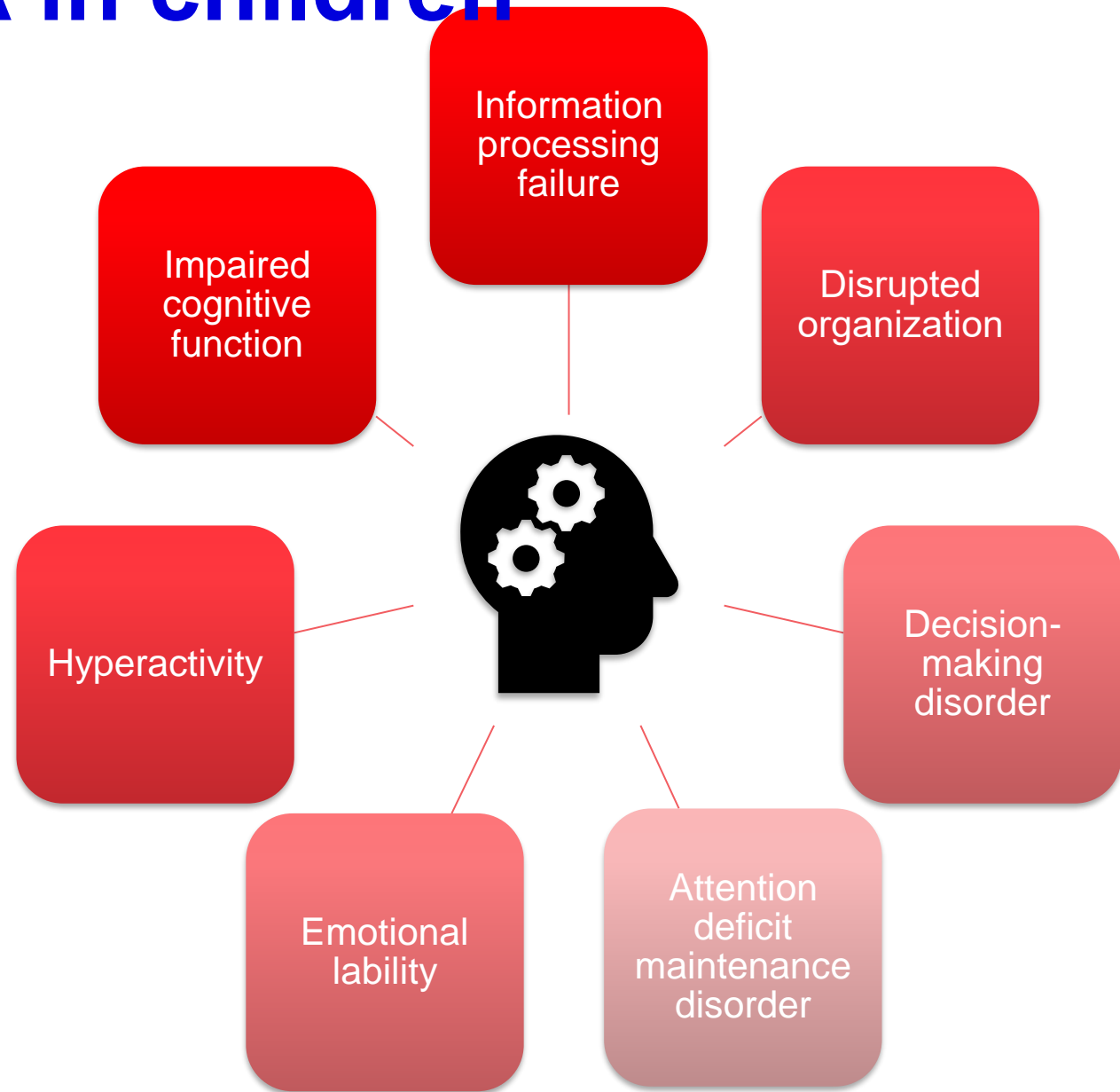
... other possible consequences of OSA in children

- **Growth slowdown** and thriving problems

- Systemic inflammation - an increase in pro-inflammatory cytokines (**OSA is a form of inflammation processes**)

- Dysfunction of the autonomic system - activation of the **sympathetic nervous system**

- **Increase in blood pressure** compared to normal

- Remodeling of heart chambers

- **Endothelial dysfunction**, increased vascular resistance

- **Metabolic syndrome** - increase in insulin levels

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Prevalence of pediatric OSA

— Significant discrepancy in previously published studies: **0.1 - 13% (most commonly around 2-4%)**

— Agreement on **significant underdiagnosability**

— Children suffering from **obesity or craniofacial malformations** – **a significantly higher risk of OSA development - up to 68%**

Caron et al., 2015

Verhulst et al., 2008

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What not to forget in the initial examination

— Risk factors for OSA in children that can&should be diagnosed by dentists include:

— **short sublingual frenulum, mouth breathing, agenesis or premature tooth loss, risk-bearing craniofacial malformations.**

— As **orthodontists are specialists in the orofacial area,** treating the anomalies of the facial skeleton in growing pediatric patients, they can significantly contribute to both **early diagnosis** and **non-invasive treatment** of patients with pediatric OSA.

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Craniofacial features representing risk for OSA development in children

Pediatric patient at higher risk of OSA development

- Overweight / obesity
- Tonsillar hypertrophy
- Preference for oral breathing
- Agenesis or premature loss of teeth
- Insufficient lip seal
- Retrognathic type of facial skelet
- Short sublingual frenulum
- Micrognathism /microgenesis
- Neuro-muscular disorders
- Narrowed hard palate
- Posteriorotation of mandibule
- Lateral crossbite
- Frontal open biite
- Increased overjet
- II. Skeletal class

Vranková et al., 2020

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Imaging methods used in dental office

- **X-ray** – as a part of the initial orthodontic examination, **a cephalometric image**, showing the patient's skull in side projection, is a standard; the image is limited by **2D dimension**, but with a **complex description** of the image, it is possible to observe the **influence of skeletal orthodontic anomaly and jaw position on the upper airways**

- **CBCT** – (CBCT, cone beam computer tomography) – possibility of **3D display**, segmentation and analysis of individual upper airway sections – more precise & detailed information

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Cephalometric image

Lateral cephalogram – arrows indicate risk factors:

- insufficient lip seal (1)
- increased overjet (2)
- posteriorotation of the mandible (3)
- tonsila lingualis (4)
- hypertrophy of the adenoid (5)

xelu: 0.127 mm

128

F

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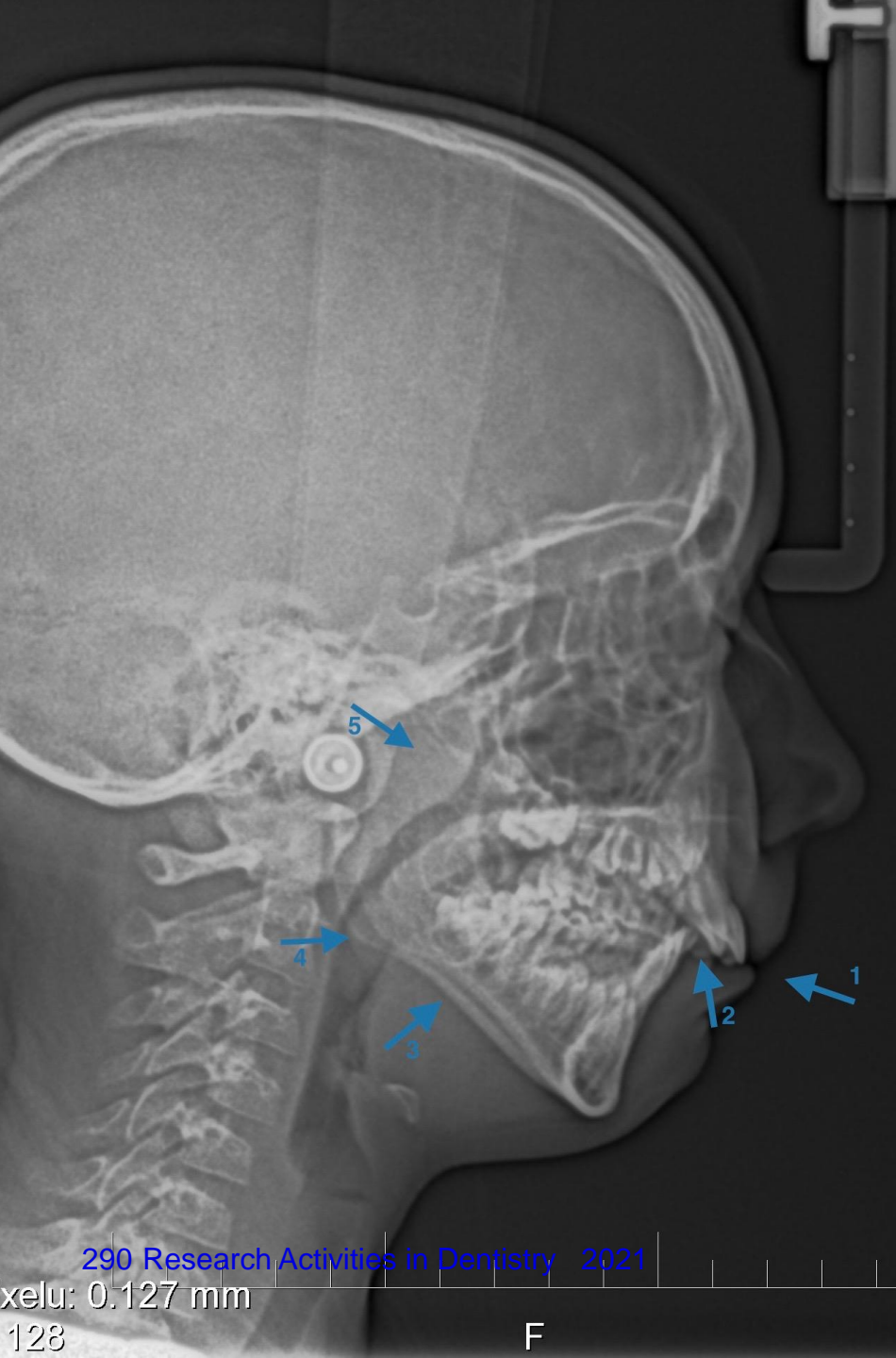
CBCT – image and analysis of upper airways
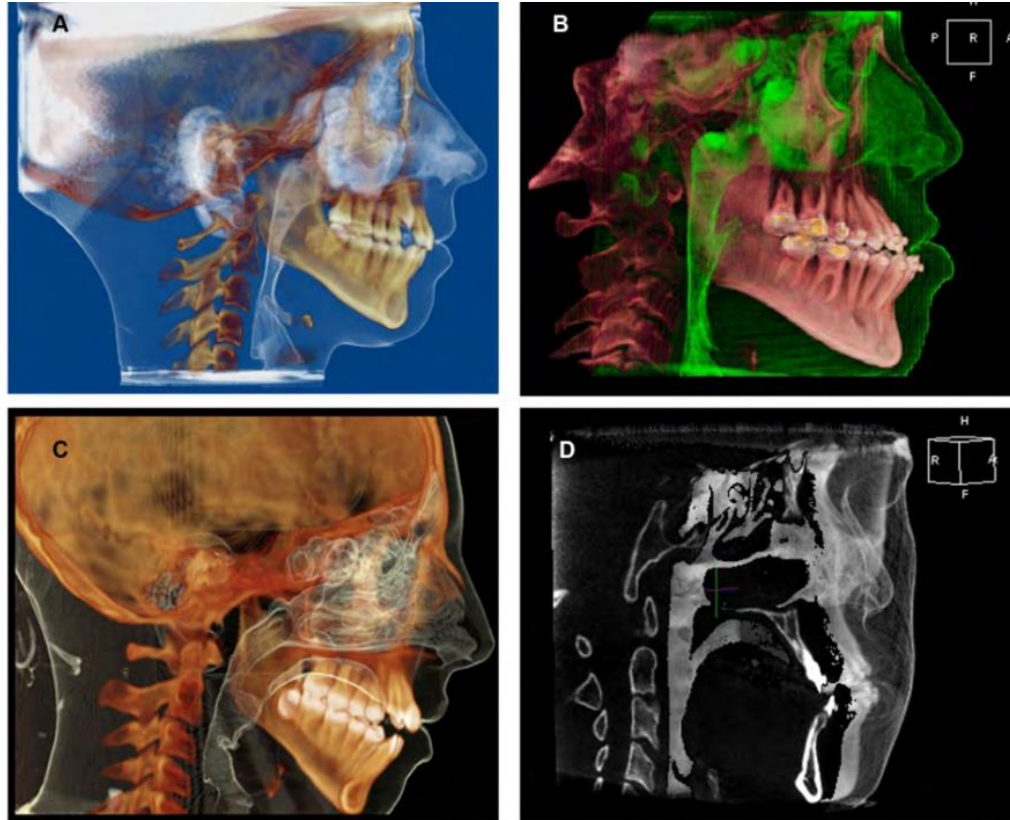
In 3D analysis of the airways, we obtain information about airways length, their total and segmental volumes as well as MCSA

Ghoneima et al., 2012
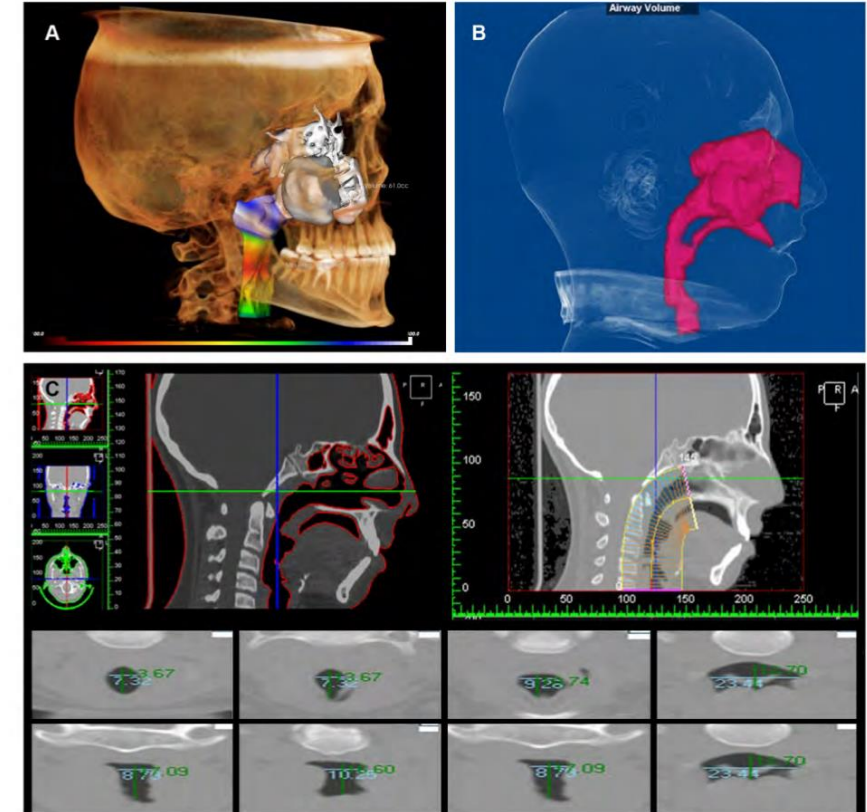
Volume — showing CBCT images used for airway analysis — several different softwares (Dolphin, 3dMD, InVivoDental..) are used to analyze and measure DC volumes.

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CBCT – image and analysis of upper airways
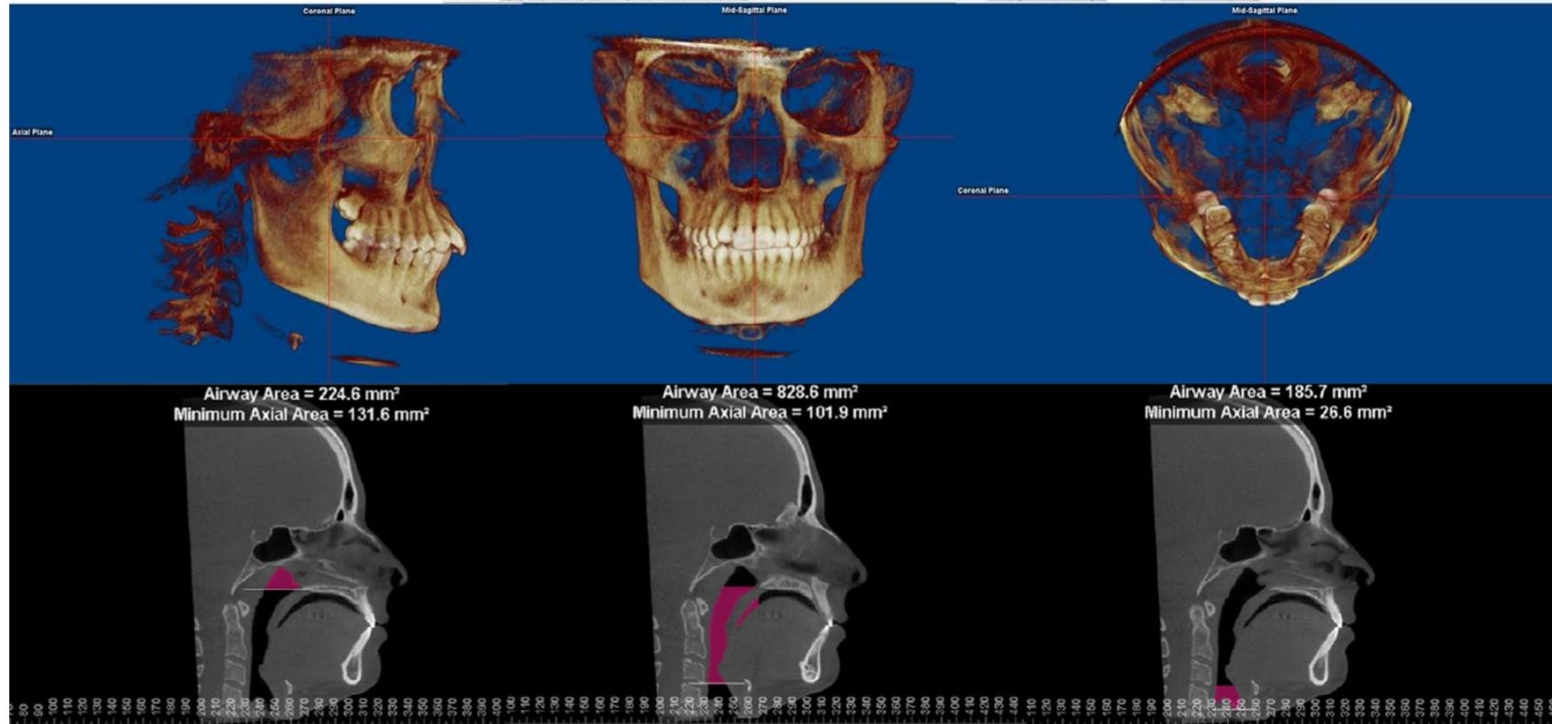
FIGURE 1 Skull orientation in the sagittal, frontal and transverse views, and airway subregions: nasopharynx, oropharynx and hypopharynx. Images rendered using Dolphin (dolphinimaging.com)

Masoud et al., 2020

The reconstruction of analyzed sections of upper airways
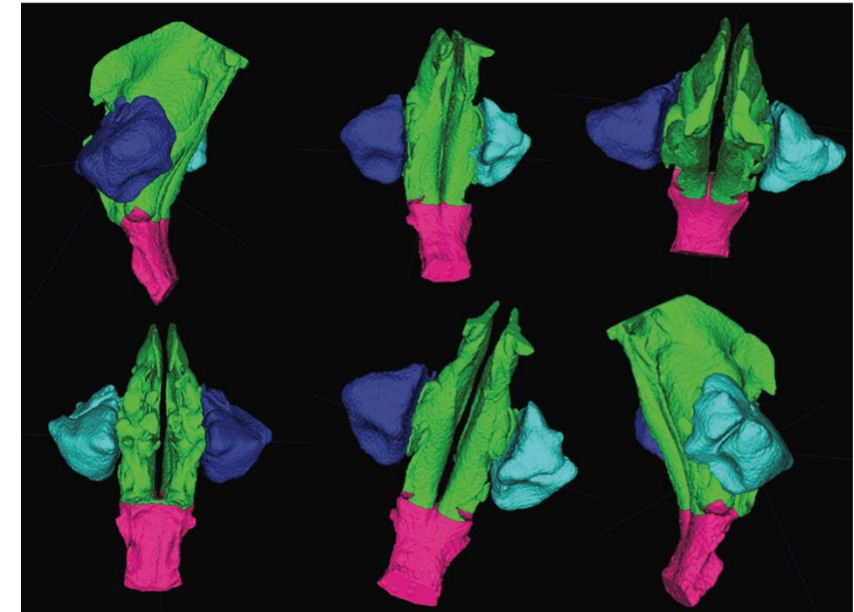
Figure 2. Multiple view of studied reconstructed volumes segmented into the different portions analyzed in this study: Nasal cavity, maxillary sinuses, and rhynopharynx.

Lanteri et al., 2020

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CBCT – image and analysis of upper airways

HDC Volume Analysis - Determining the "MCSA" (minimum cross-sectional area) – the area within the upper airways with the narrowest diameter and therefore a place of high risk for collapse and occlusion of airways

Airway Volume = 13967.5 mm³
Minimum Axial Area = 108.4 mm²

Masoud et al., 2020

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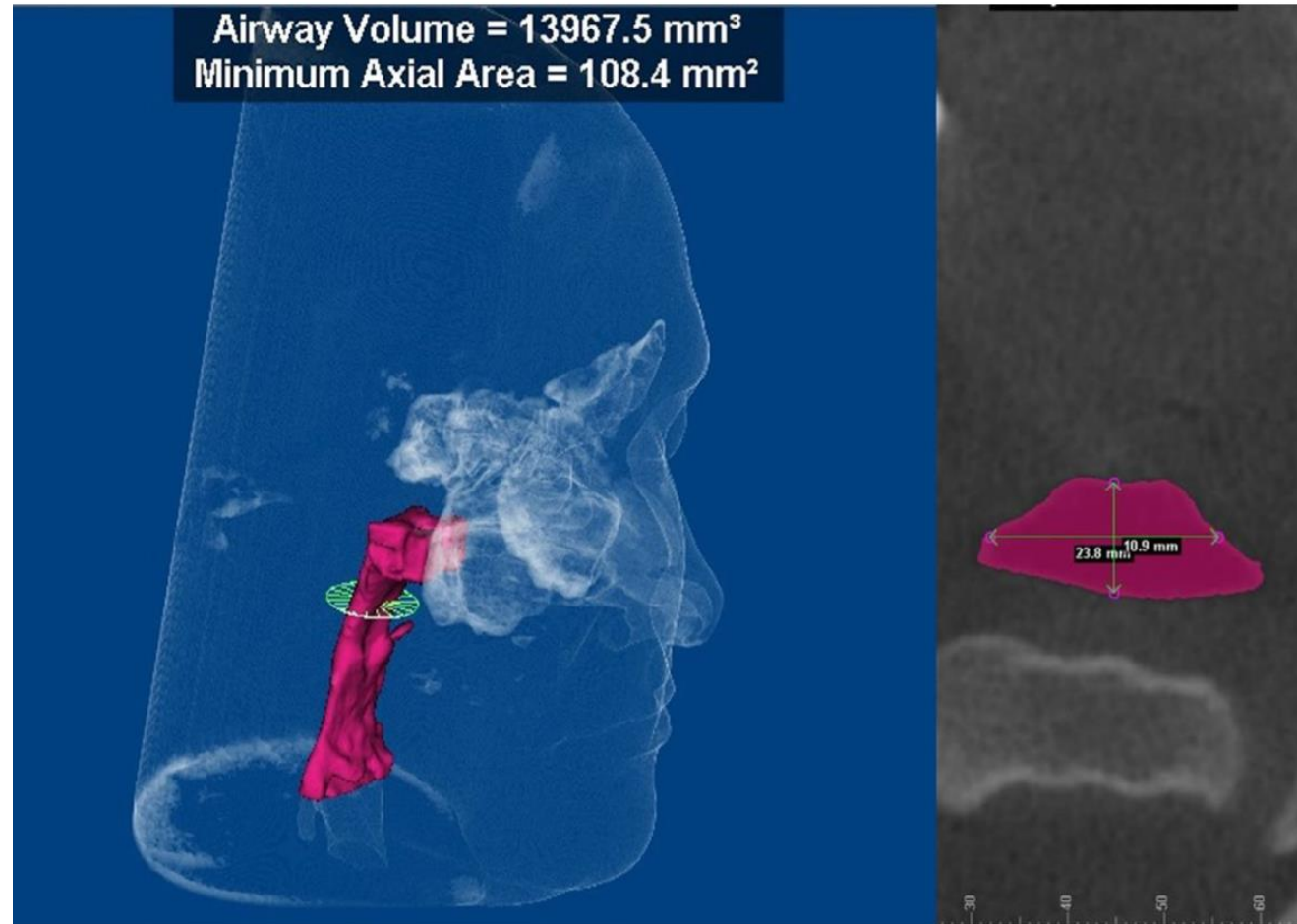
CBCT
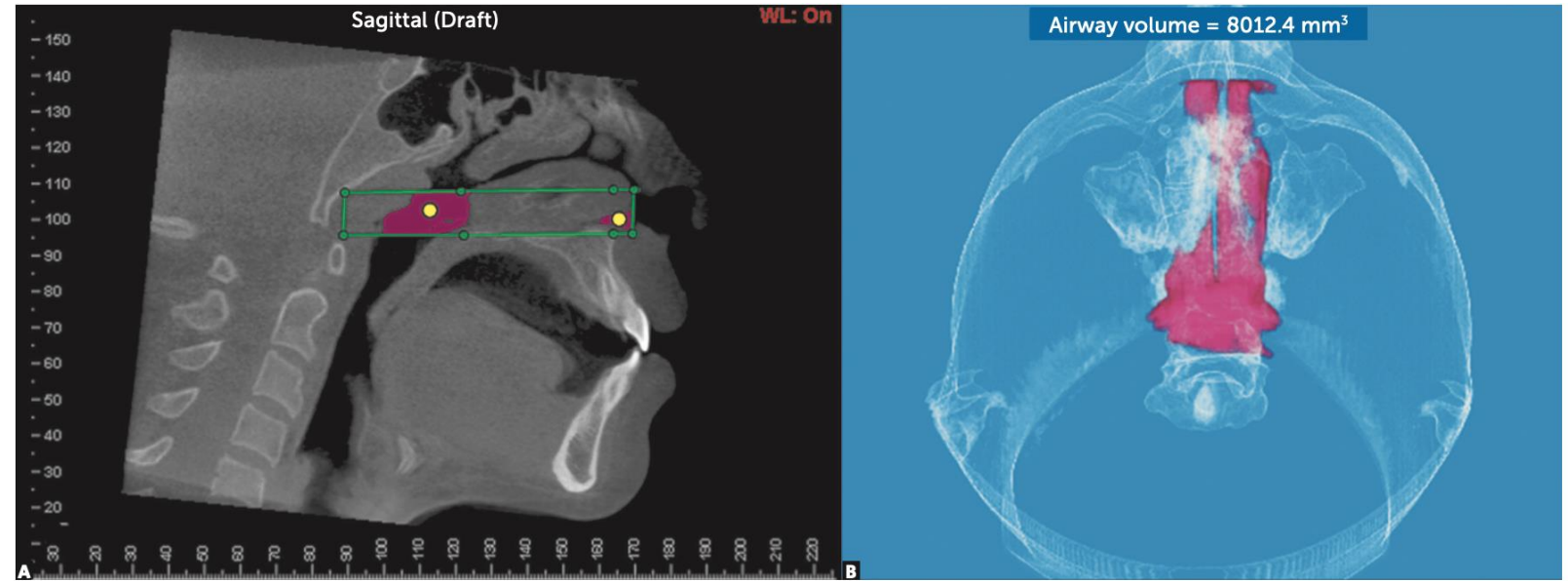
Figure 2 - Measurement of VNN: **A**) limits of the nasopharynx and nasal cavities (green), and **B**) airway volume calculation (pink).
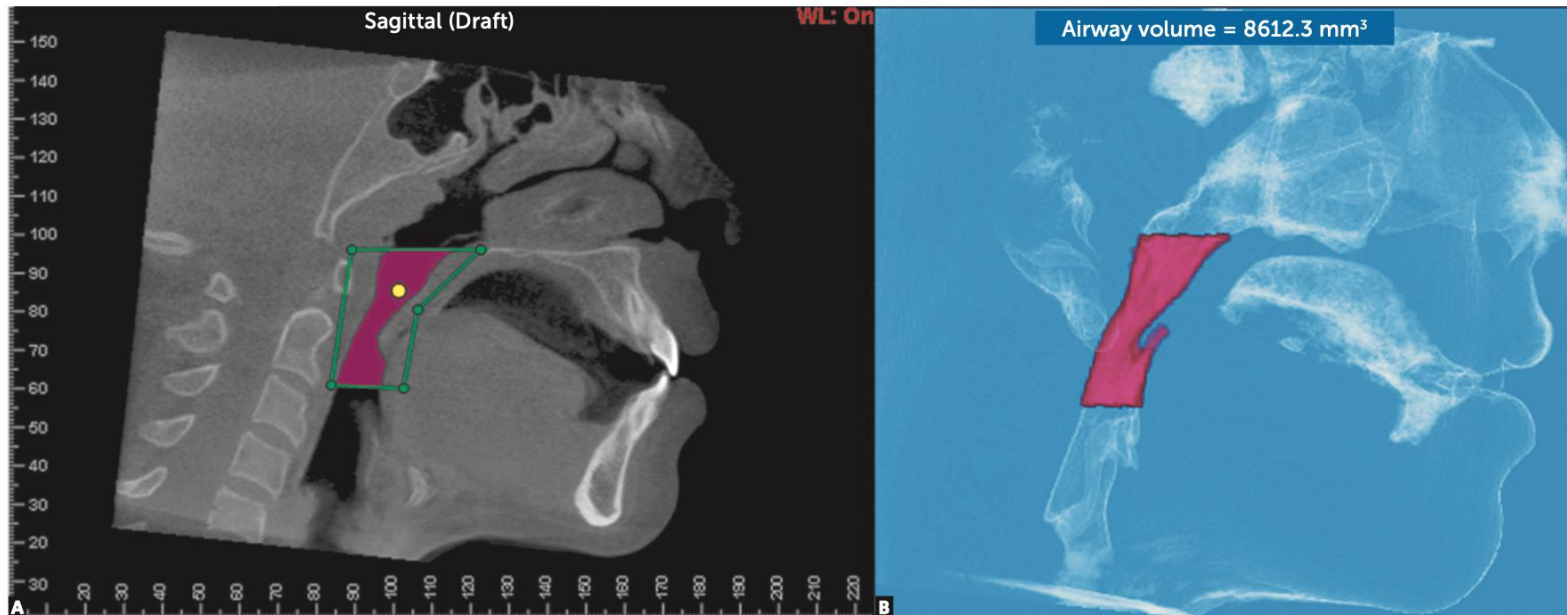
Figure 3 - Measurement of VO: **A**): Limits of the oropharynx (green), and **B**): Airway volume calculation (pink).

Izuka et al., 2015

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Development of the orofacial system

— The key activities for the proper development of the orofacial system in children are:

nasal breathing, mastication, swallowing, sucking (synchronization)

— The muscles involved in these activities stimulate the postnatally active **intermaxillary suture** as well as the **formation of the alveolar processu**s

— Failure or malfunction in these activities disrupt the physiological development of the craniofacial system and lead to the risk of skeletal abnormalities, often contributing to easier collapse of the upper airways

— The development of the facial area is most significant between the **birth and the 6th year,** followed by a **pubertal growth spurt**

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Development of the orofacial system

Short sublingual frenulum

— if, in the indicated case, the short frenulum is not released in time, it almost always disturbs the development of **key functions** (sucking, swallowing, chewing or speech),

— there is **also insufficient stimulation of the palate suture**

— **Agenesis and premature tooth loss**

— many authors in their studies focus on the influence of agenesis or premature loss of teeth on the development of craniofacial structures and the possible emergence of OSA

— there is a **consensus that the loss of teeth or their failure affects the anatomy of the alveolar processus**, regardless of the cause of the loss

— abnormal orofacial anatomy is described in these children - in a form posing a risk for collapse of upper airways in sleep

Huang et al., 2017
Ben-Bassat et al., 2009

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Oral breathing

— In 1981 and 1984 - Study on monkeys - **a demonstration of the consequences of oral respiration on the facial skeleton**

— Artificially **induced nasal obstruction** - **compensatory changes** with the aim of adaptation to oral respiration

— In growing monkeys, craniofacial anomalies improved again after removal of nasal obstruction

Harvold et al., 1981
Vargervik et al., 1984

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Adaptive changes

— delayed maxillary growth

— narrowing of the maxilla and mandible

— open mouth

— mandible advancement – depending on the type of compensation, either II. or III. Angle class occurred in animals

— often a tendency to double-bite

— tongue – adaptive changes in animals differed, but they led to ensuring the patency of the oropharynx

— tongue extrusion
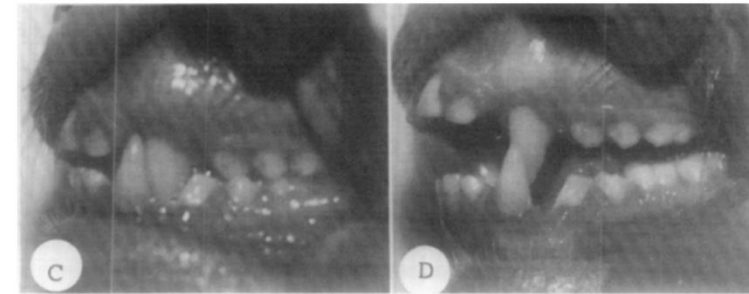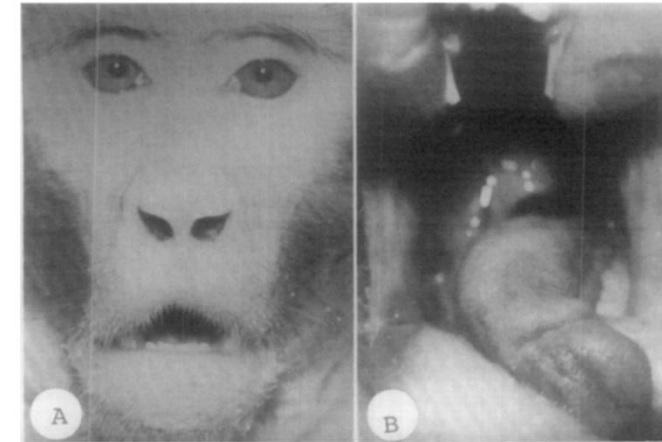
Fig. 2. Three years of oral respiration caused a notch in the upper lip and an open mouth posture (A), as well as a long and slender tongue with midline groove, an open pharyngeal port, and a dental malocclusion (B). The forward positioning and the rhythmic movements of the mandible produced a dual bite (C and D).

Harvold et al., 1981

Fig. 4. The animal lowered the mandible and protruded the tongue. Eighteen months of mouth breathing produced a notch in the upper lip (A) and a severe open-bite (B).

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Orthodontic therapy in pediatric OSA patients

— **Maxillary expansion -** the most commonly used orthodontic approach in the treatment of

   OSA in children

— **Mandibular advancement -** affecting the mandibular position

— **Myofunctional therapy -** active and passive treatment

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Orthodontic expansion of upper jaw

— According to **anchoring**, we divide orthodontic expansion appliances into: "**bone – borne**" – bone anchored (MARPE – miniimplant assisted rapid palatal expansion), "**tooth-borne**" - dentally anchored, and "**hybrid**" (bone and dentally anchored)

— **Types of expansion** – depending on the expansion protocol and force:

— **rapid maxillary expansion (RME)** – faster expansion/greater power in less time

— **slow maxillary expansion (SME)** – weaker forces and longer time

— **surgically assisted rapid maxillary expansion (SARME)**

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Lee et al., 2012

https://www.leone.it/english/orthodontics/

https://www.accutechortho.com/orthodontic-laboratory-products/expansion/rpe

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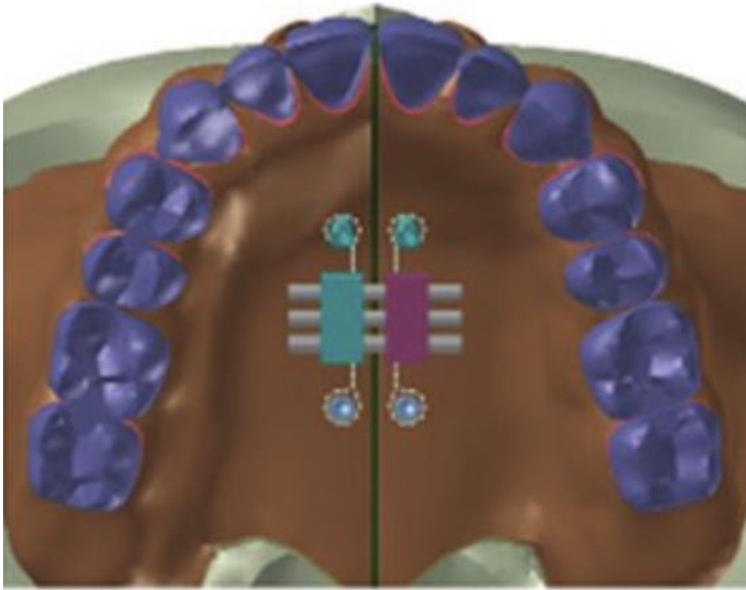
RME

- **RME** – first introduced in 1860 **by Dr. Angell** and was especially popularized by **Dr. Haas**

  During expansion, the maxillary suture ruptures - **triangular extension** - the maximum is in

  the anterior part and the expansion decreases in the dorsal direction - ratio 2: 1 (higher

  resistance of the skull bones)

- Other sutures around the maxilla also partially expand - (zygomatic-maxillary, frontomaxillary,

  zygomaticotemporal, pterygopalatal ..)

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RME

— many studies have shown the effect of maxillary expansion on:

— **significant increase in the volume of the nasal cavity**, **nasopharynx, rhinopharyx and paranasal cavities**

— **reduction of nasal resistence**

— improvement of **ventilation and patency of airways** (examination using imaging techniques, rhinomanometry or PSG)

— **Decrease in AHI and improvement of OSA symptoms in children**

Haas, 1961
Abdalla et al., 2019
Caprioglio et al., 2014
Maspero et al., 2020

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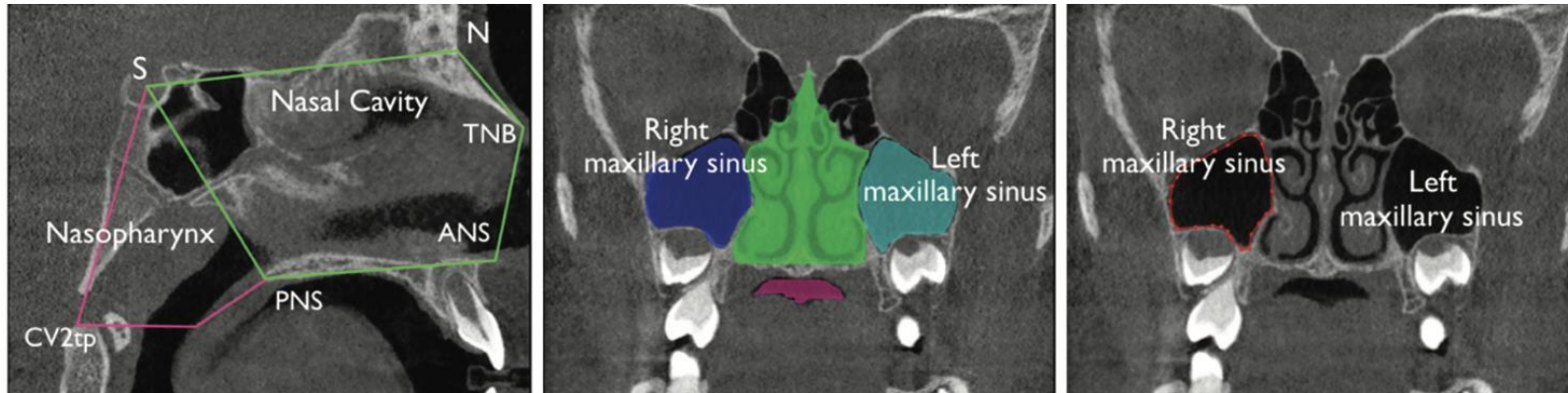
Lanteri et al., 2020

Sagittal and coronal sections CBCT – showing segments of nasal cavity and maxillary sinuses with the possibility of measuring individual volumes

Measurement of the width of the nasal base in the front (ANF) (canine area) and back (PNF) (area of the first permanent molars)

Izuka et al., 2015

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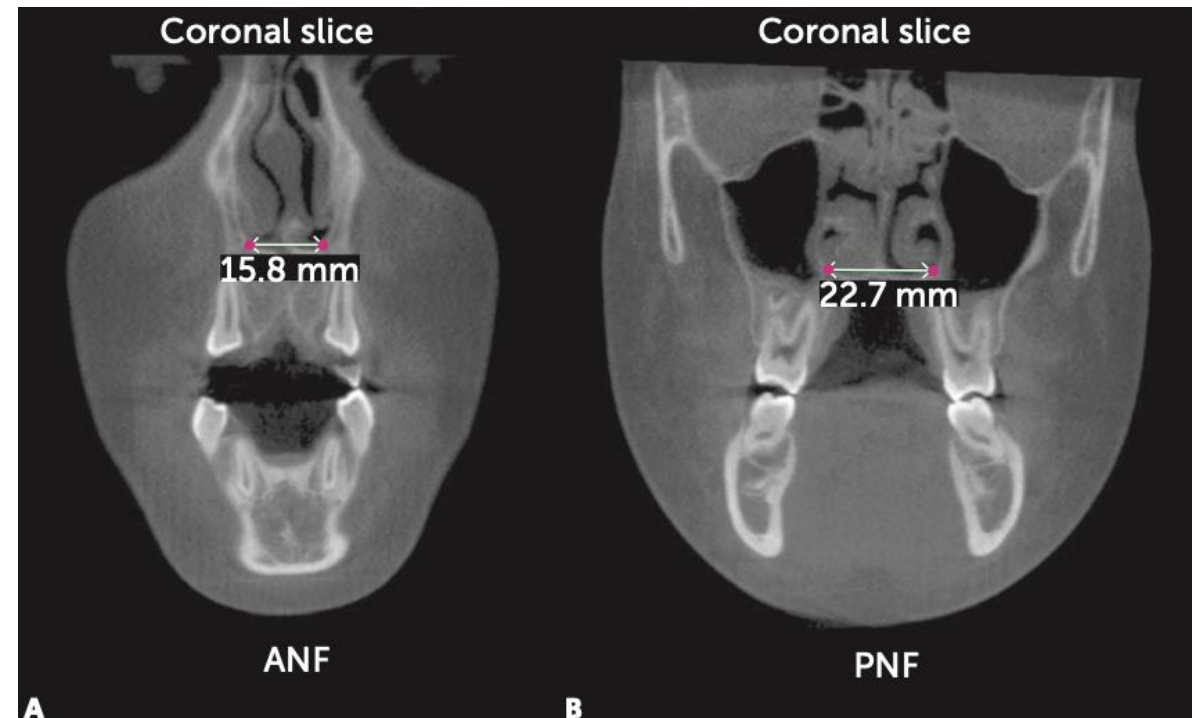
Myofunctional therapy (MFT)

— **Since 1918** - the correct position of the tongue, achieved by MFT, is **described as the main factor leading to the stimulation of maxillary and mandibular growth and nasal breathing**

— MFT is a collection of **isotonic and isometric** exercises affecting the lips, tongue, oropharyngeal structures such as the soft palate or lateral wall of the pharynx

— The task is to ensure the correct posture of the head, the position of the tongue on the palate in relation to the upper teeth, swallowing, mastication, speech and articulation

— **Active and daily provided MFT** in combination with further treatment can lead to **complete remission of OSA in up to 60% of children**

— The **absence of MFT may be associated with recurrence of SRBD**

Huang et al., 2017   Guilleminault et al., 2015   Guilleminault et al., 2013

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Mandibular advancement

— Several studies focused on the analysis of changes in the upper airways after the use of orthodontic appliances used for the advancement of mandible have shown that:

— after the **Twin Block therapy**, the sagittal dimension of the **oro- and hypopharynx increased** in children by correcting the position of **the mandible; the length, thickness and inclination of the soft palate were also improved.**

— there was an **improvement in the symptoms of OSA**, as well as the profile of patients and the results of their sleep questionnaires and PSG

— cephalometric analyzes showed a **significant increase in airway space, a change in the position of the mandible relative to the cranial base** and convexity of the face, **which indicate an increase in mandibular growth and soft palate length, a reduction in maxillo-mandibular discrepancy in the sagittal and vertical planes**

Zhang et al., 2013   Ghodke et al., 2014   Galeotti et al., 2016

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TWIN BLOCK

https://www.youtube.com/watch?v=UI_-wfutrQw

TWIN BLOCK APPLIANCE

BLOCK A

BLOCK B

BOW

CRIB

https://www.orthodonticsuites.co.uk/blogs/news/twin-blocks-or-functional-appliances

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Herbst appliance

– https://www.youtube.com/watch?v=yB8ps1Z1EgU
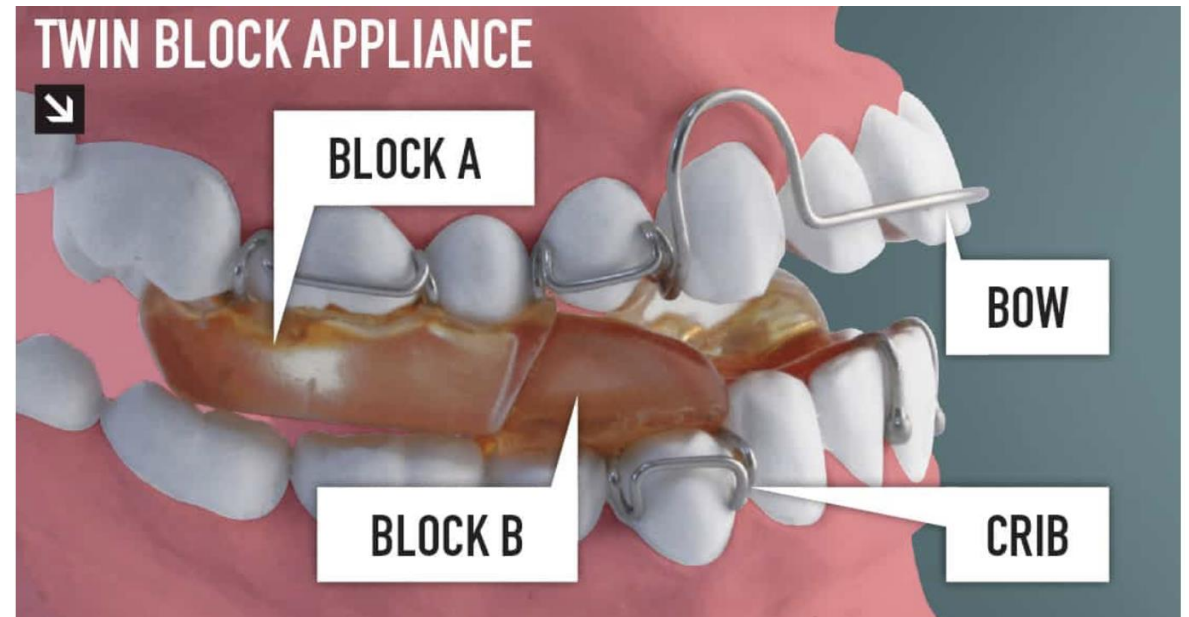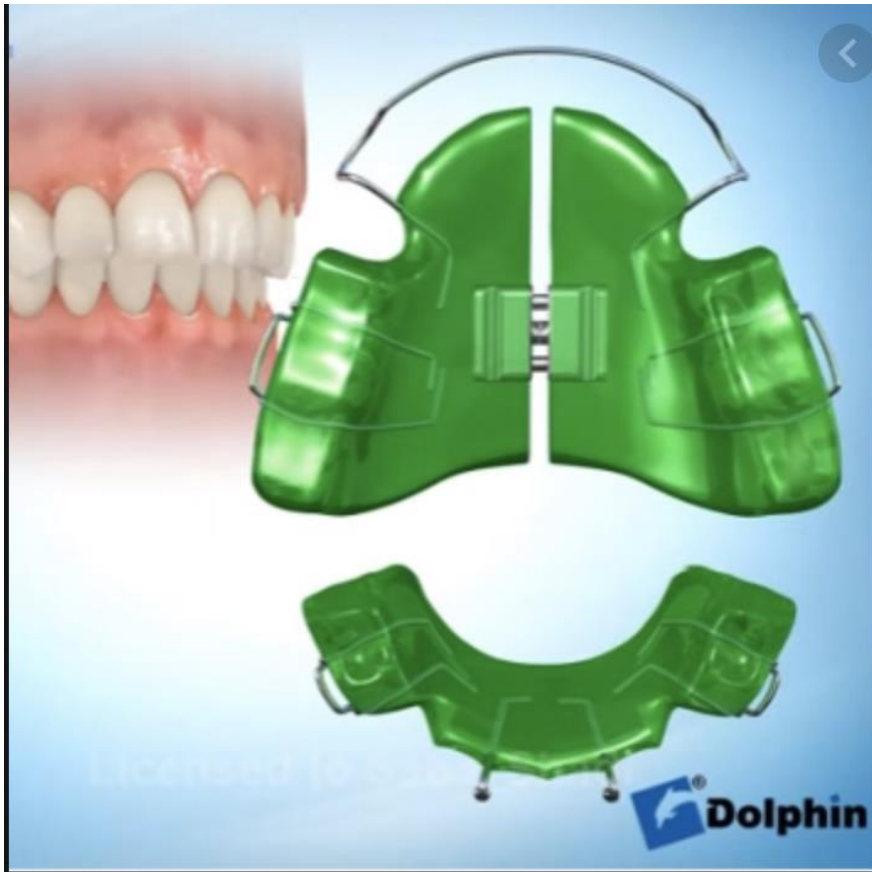
– after therapy, the following has been shown:

– increase in the volume of airways in parts of the oropharynx and larynx, reduced frequency of awakening

– in children with II. skeletal class and OSA - improved respiration

– reduction of OSA symptoms in patients after treatment

Schütz et al., 2014
Iwasaki et al., 2014
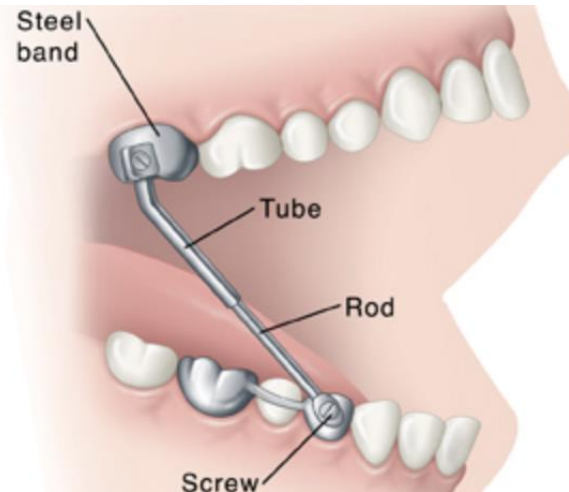
Fig 6. Example of a subject treated with the Herbst appliance: **A,** before treatment, the retropositioned mandible was associated with a reduced pharyngeal airway; **B,** after treatment, a more anterior placement of the mandible significantly (*yellow arrow*) enlarged the pharyngeal airway (*blue arrow*).

Steel band
Tube
Rod
Screw

https://www.fairview.org/patient-education/40051
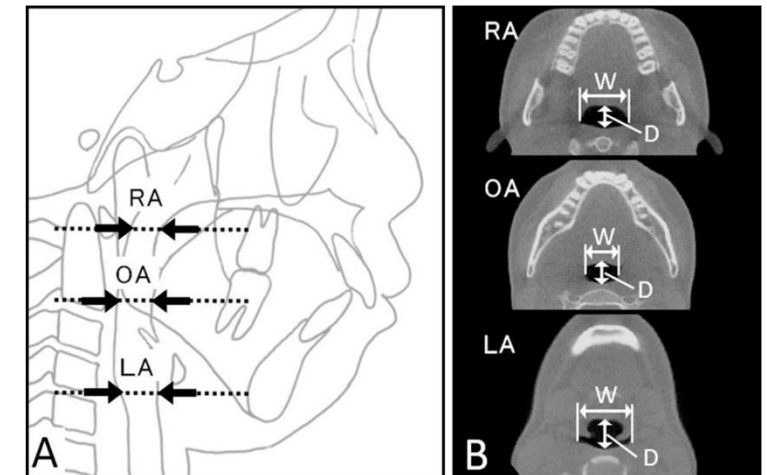
Fig 5. Measurement of the pharyngeal airway cross-sections. **A,** *RA*, Retropalatal airway; *OA*, oropharyngeal airway; *LA*, laryngopharyngeal airway. **B,** Cross-sectional areas of the pharyngeal airway. *D,* Depth; *W,* width.

Iwasaki et al., 2014

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Case reports

Sofia, 7 years old
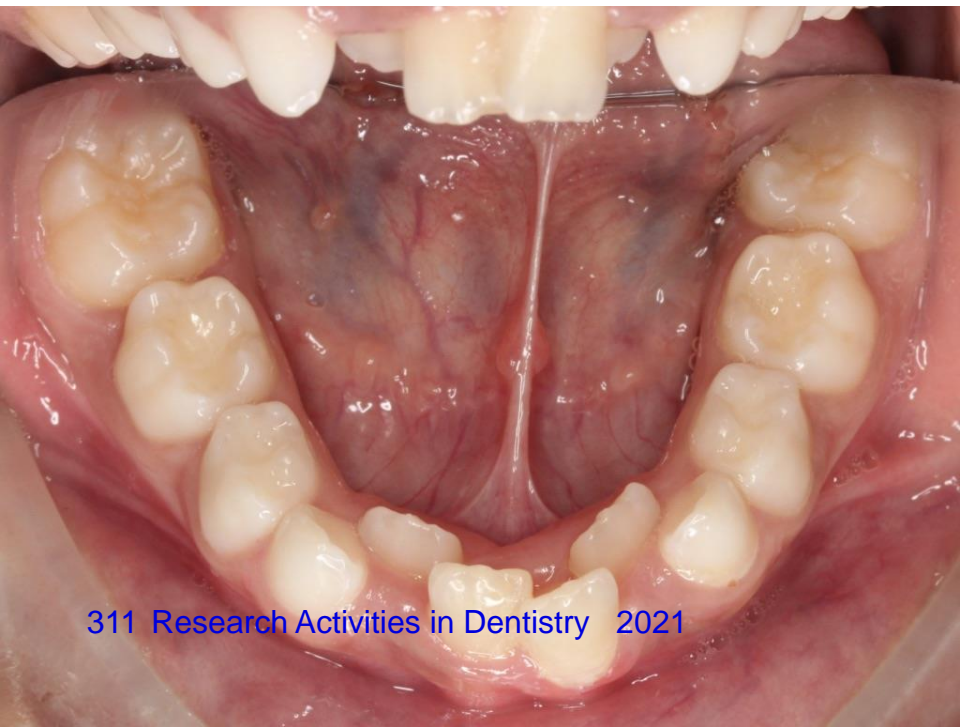
5/8/19
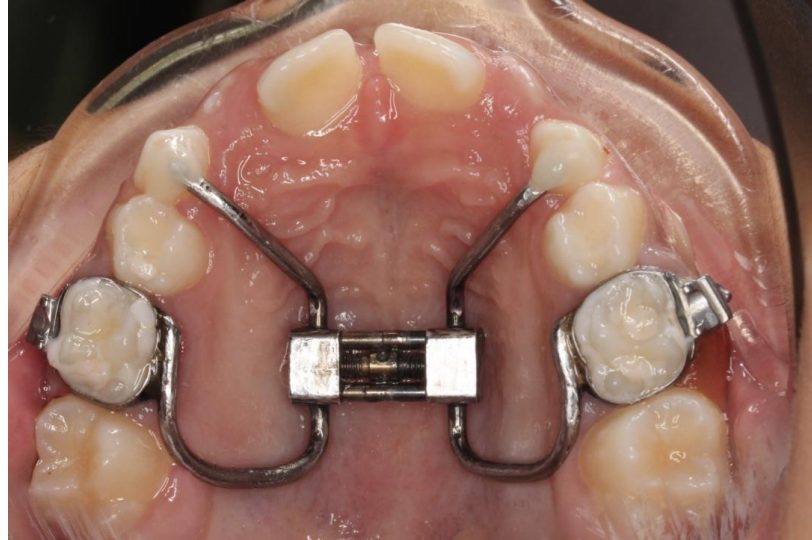
26/8/19
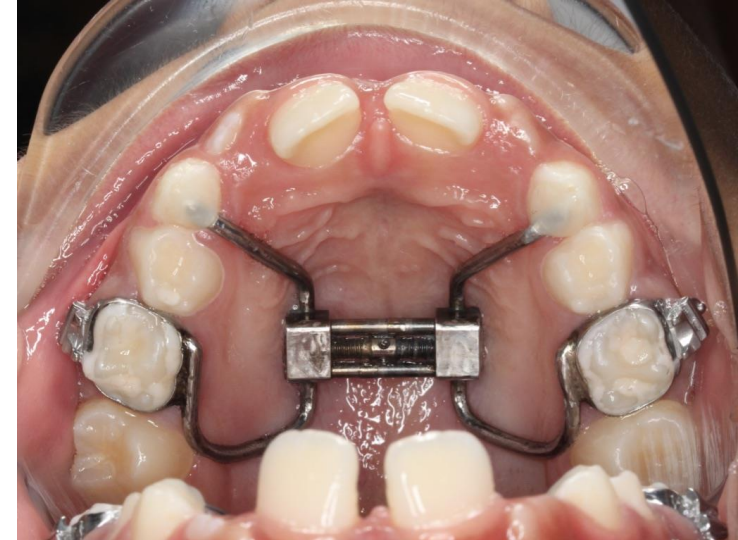
2/9/19

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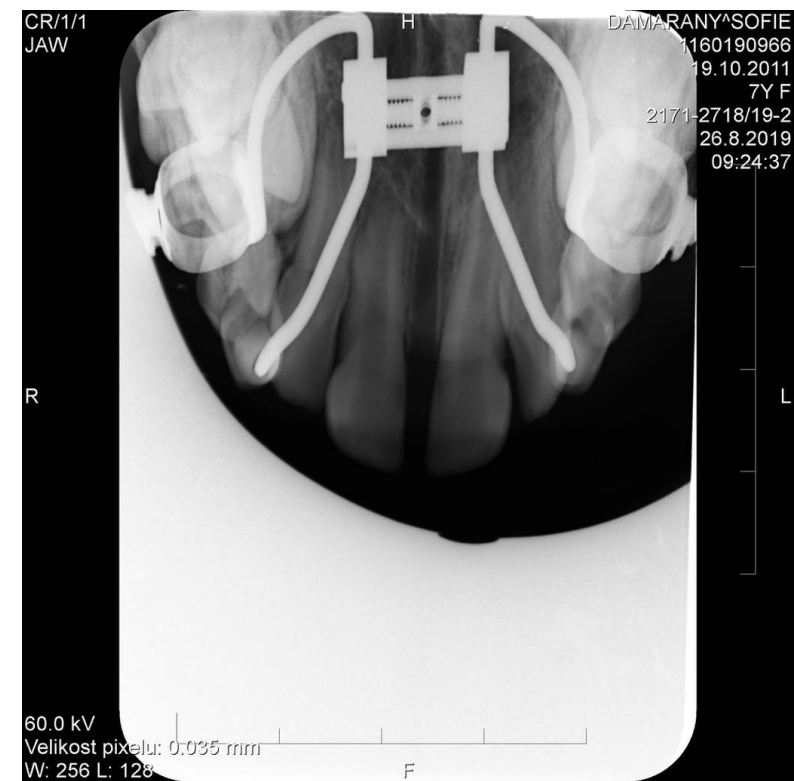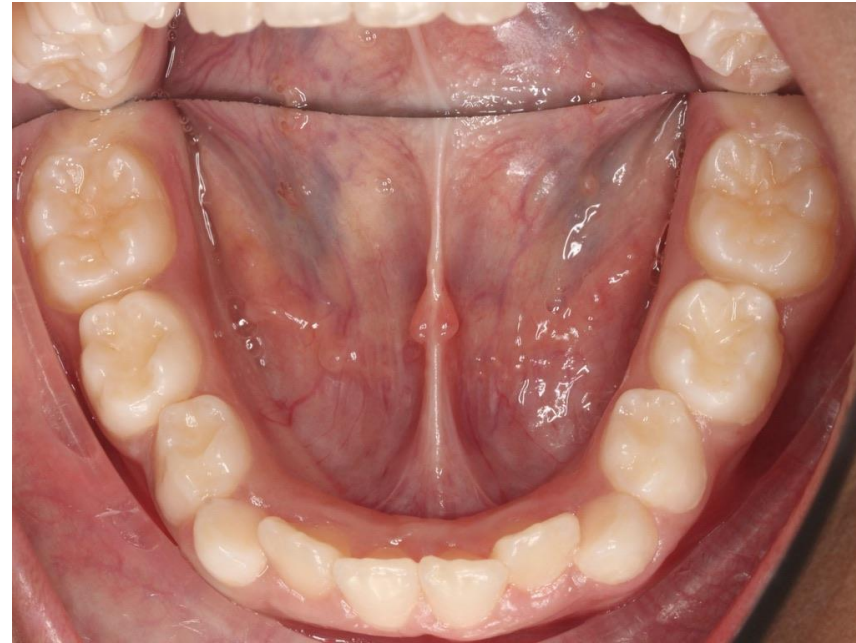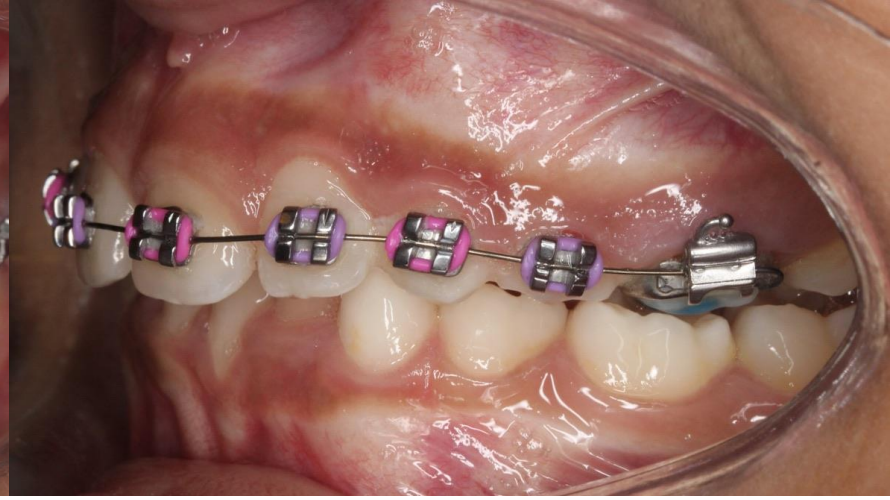
Adam, 8 years old
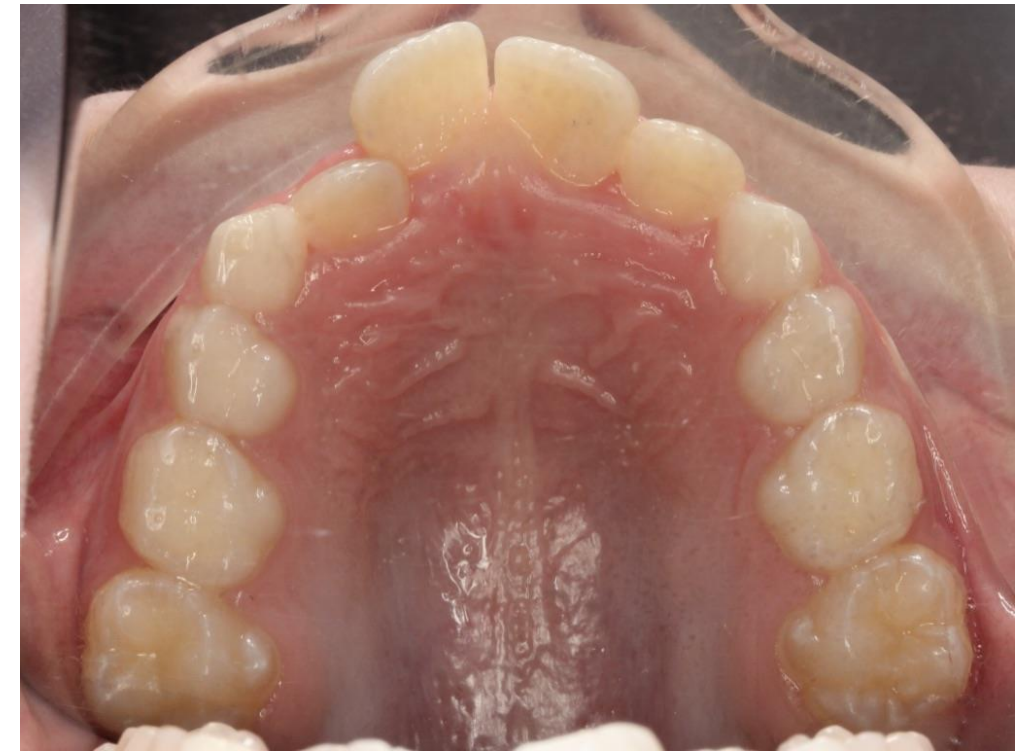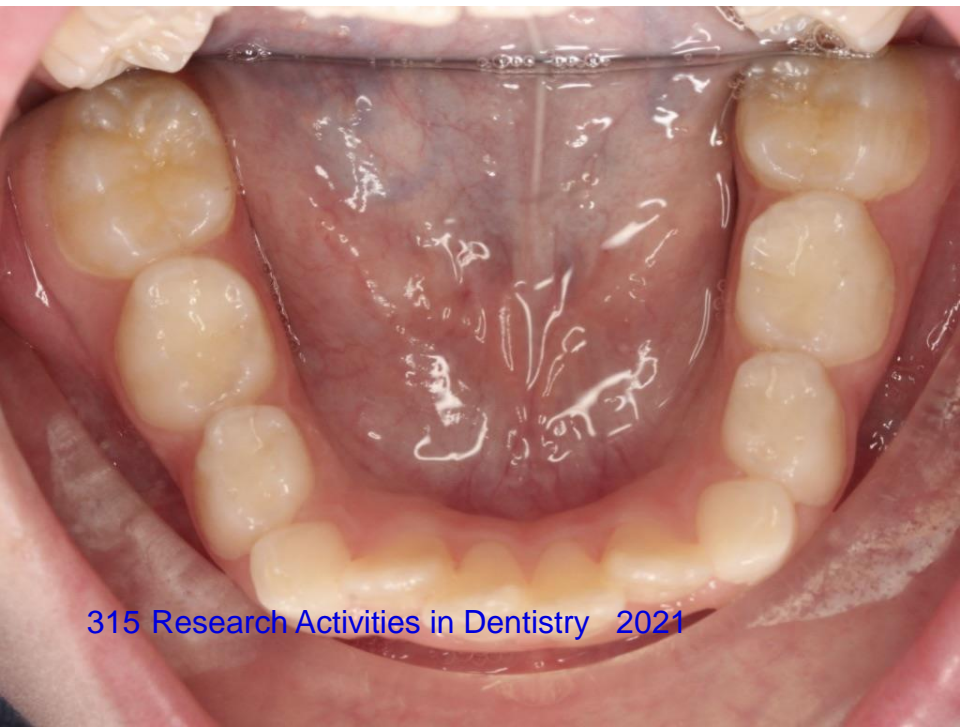
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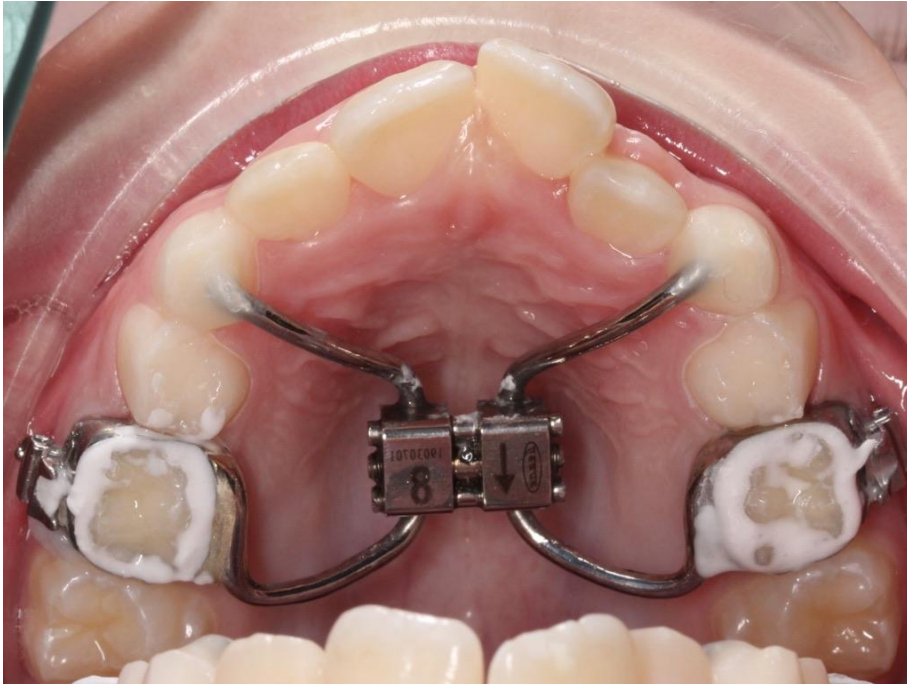
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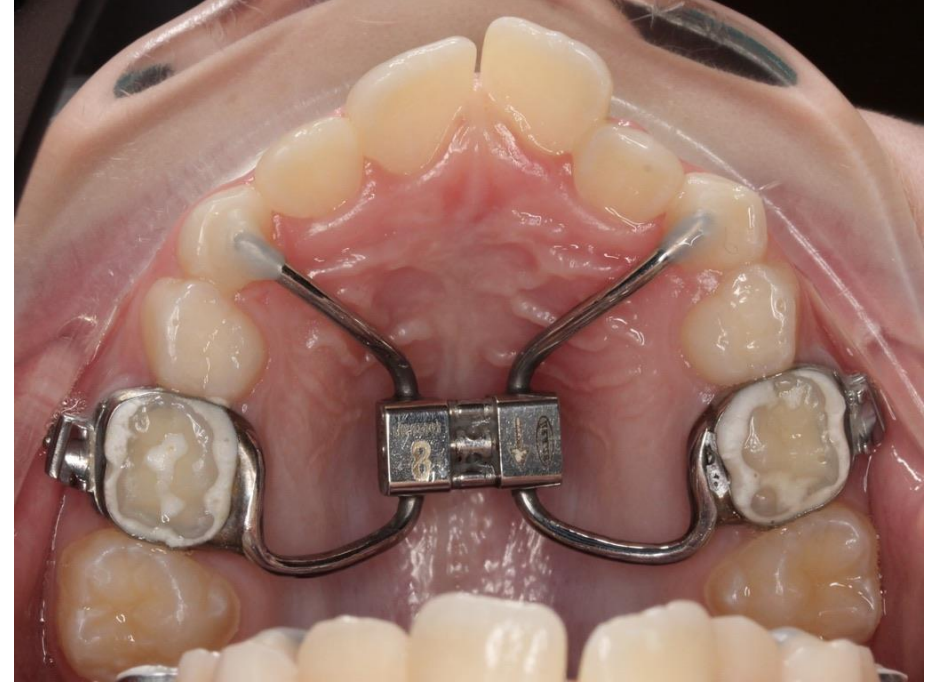
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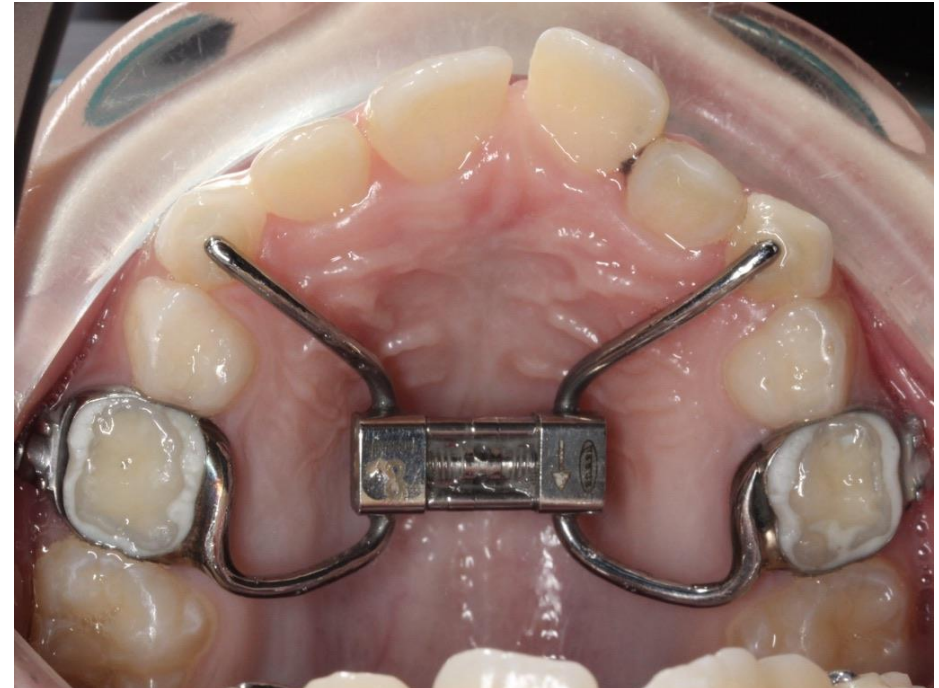
2nd phase of the therapy - TWIN BLOCK
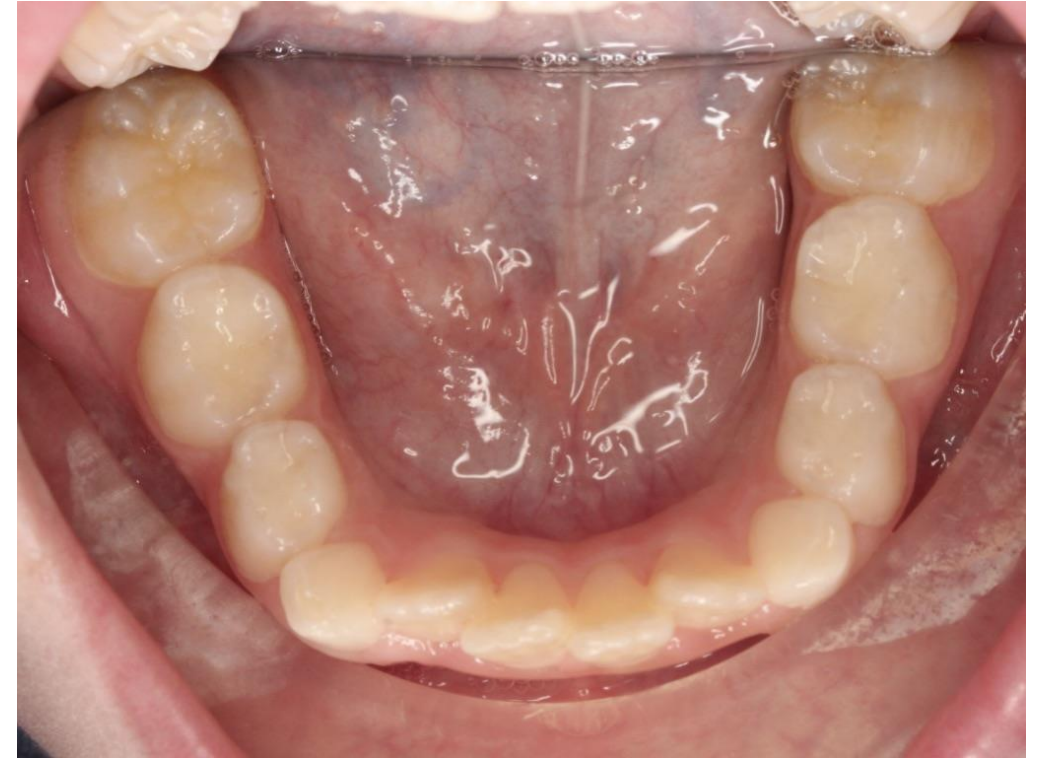
Jan, 8 years old
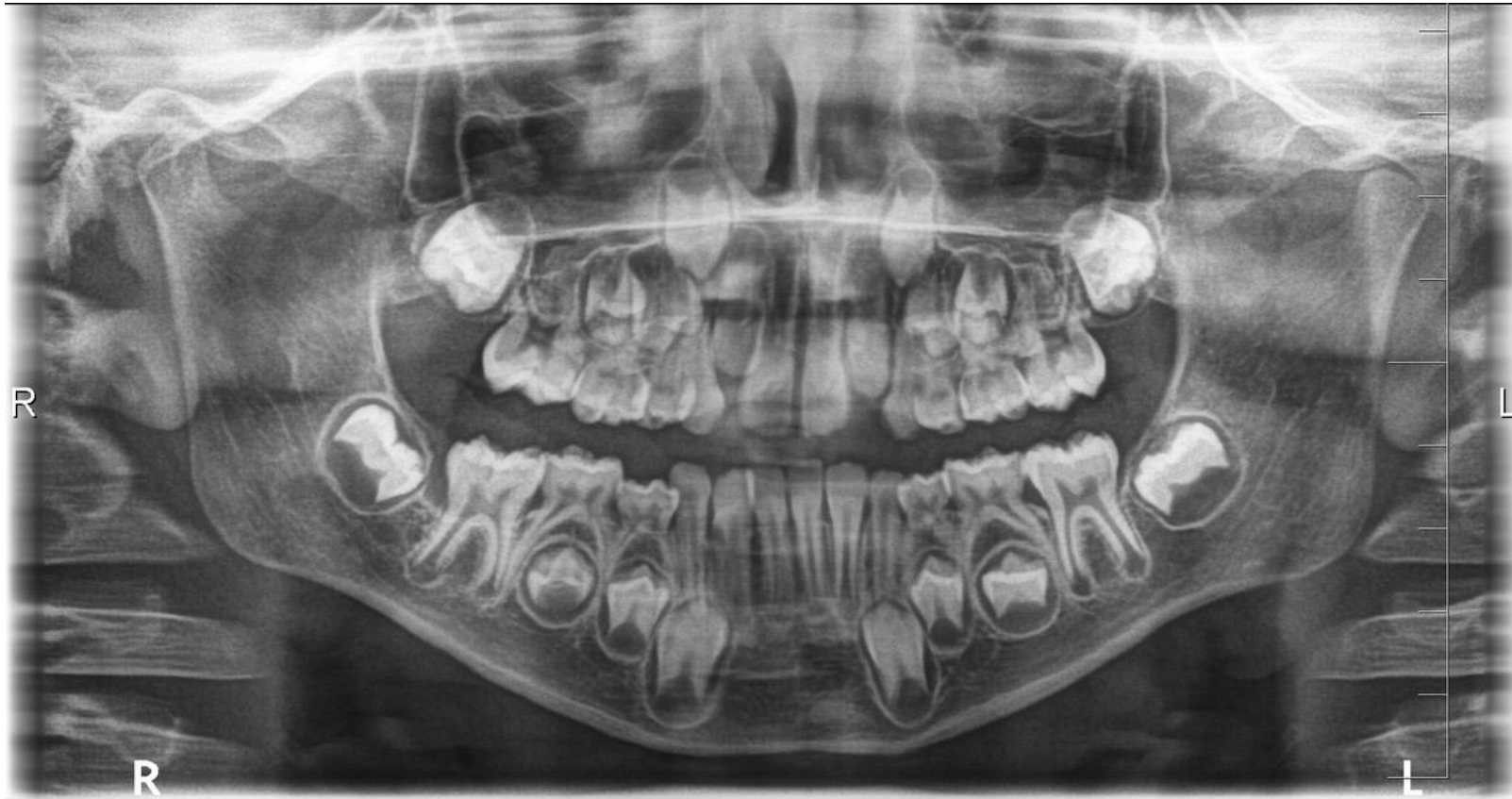
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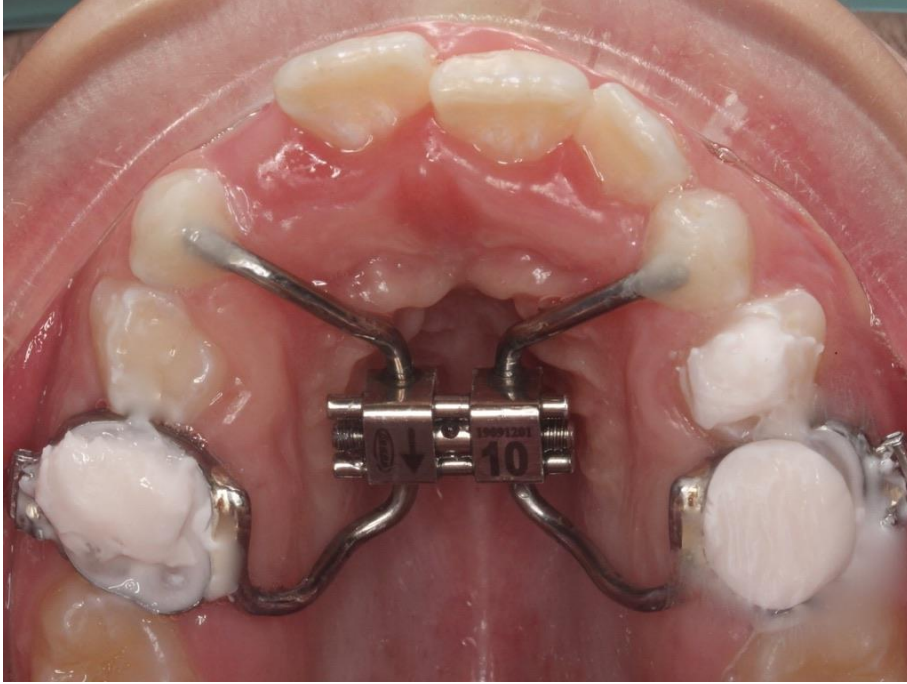
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Take home message

1. Dentists are **specialists in the orofacial area** and by precise examination they can significantly contribute to the **detection and diagnosis of OSA** in (not only) pediatric patients

2. Efforts should be made for the **earliest possible detection** (increasing the range of therapeutic options) and subsequent **appropriate choice of a causal therapy**

3. **Orthodontic treatment** can be a valuable treatment **for children with OSA who have craniofacial anomalies**

Abbreviations used

OSA – obstructive sleep apnea

SRBD - sleep-related breathing disorders

AHI index – apnea-hypopnea index

CBCT - Cone Beam computer tomography

PSG – polysomnography

MFT - myofunctional therapy

RME - rapid maxillary expansion

SME - slow maxillary expansion

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Congenital developmental anomalies of the orofacial region (cleft defects)

MUDr. Alena Bryšová, Ph.D.
Clinic of Stomatology, St. Anne's University Hospital
Faculty of Medicine, Masaryk University Brno

Content of the lecture

— Research projects of our team

— Cleft lip and palate - theory

— Cleft lip and palate - research part

— Why become a researcher

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Introduction of the researcher

MUDr. Alena Bryšová, Ph.D.

— Orthodontist at the Dental Clinic of the St. Anne's University Hospital, Brno

— Teacher of undergraduate and postgraduate students in orthodontics – MED MUNI, Brno

— Private orthodontic clinic

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Research of orthodontic brackets and adhesive materials

Team : <u>MUDr. Alena Bryšová, Ph.D. – FNUSA and MED MUNI Brno, Prof. RNDr. Bohumil Vlach, CSc. – FCE Brno University of Technology, Ing. Filip Mika, Ph.D. – ISI CAS</u>

Research objectives:

- Comparison of the strength of the binding of adhesive materials for bonding orthodontic brackets

- Morphology of the bases of orthodontic brackets

- Comparison of cracks in enamel after removal of brackets

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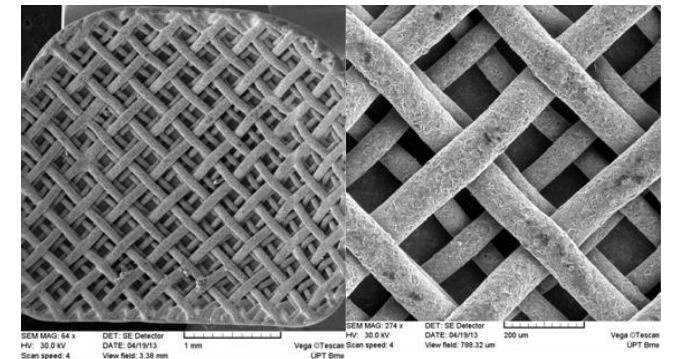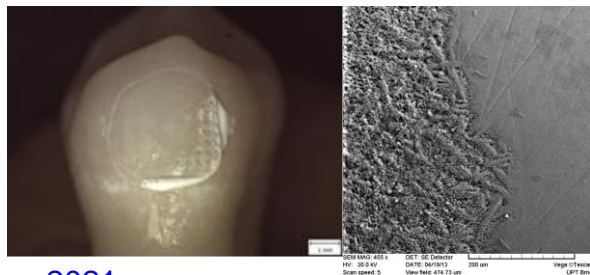
Research into the treatment of crowding with fixed lingual appliances

Team : <u>MUDr. Alena Bryšová, Ph.D. ,MDDr. Dušan Kuric, MDDr. Mariana Raszková –</u>

<u>FNUSA a MED MUNI Brno</u>

Research objectives:

-Comparison of the final inclination of lower incisors in patients with crowding treated with the lingual appliances and conventional fixed appliances

-Comparison of the change of the intercanine distance in patients with crowding treated with the lingual appliances

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Current research
Evaluation of upper jaw growth in cleft patients after neonatal lip surgery

Team : MDDr. Margarita Rousi, MUDr. Alena Bryšová Ph.D., prof. MUDr. Lydie Izakovičová Hollá, Ph.D. - Clinic of Stomatology, St. Anne's University Hospital, Faculty of Medicine, Masaryk University Brno

MUDr. Jitka Vokurková, Ph.D., MUDr. Olga Košková, Ph.D. – Department of Plastic Surgery, Children's Hospital FN Brno

— Research objectives:

— Evaluation of the five-year-old's index of patients with a unilateral cleft lip and palate after lip surgery neonatally at 5 years

— Evaluation of the Goslon yardstick index of patients with unilateral cleft lip and palate after lip surgery neonatally at 9-10 years

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Lip and palate clefts

- One of the most common congenital developmental defects of the orofacial system

- localised in the visible area of the face

- this anomaly affects the patients morphologically, functionally and socially

- can be with or without association with a syndrome

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Prevalence of clefts

— 1 cleft per 500 - 800 live births (in the Czech Republic 1.87: 1000)

 - significantly higher incidence of clefts in aborted fetuses (1 cleft per 50 aborted fetuses)

— Differences in the incidences according to :
—   sex   boys : girls 3 : 1
—   affected side   left : right 2 : 1
—   ethnicity   Asian population 1: 500
—   African-American population 1 : 2000
—   seasonal incidence

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Etiology

– The cleft defect is caused by the non-fusion of the facial parts during embryogenesis

– **75% unclear etiology**

 - it was experimentally demonstrated that cleft can be triggered both by several subliminal impulses

 acting together and by a single strong factor

– **Genetic influences - 10%**

 - defect of one or more genes, the defect has a demonstrably genetic dependence

Burdi A.R., 2006

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Classification of clefts - Burian classification 1954

A. TYPICAL CLEFTS:

Group I: (cleft always present)

        1. Cleft lip   -unilateral
                           - bilateral

        2. Cleft lip and jaw - unilateral
                                 - bilateral

        3. Cleft lip, jaw and palate (total cleft)
                                - unilateral
                                - bilateral

Group II: 1. isolated cleft palate
                 2. submucous cleft palate
                 3. insufficient palate

B. ATYPICAL CLEFTS:

I. transverse clefts
II. upper middle clefts
III. lower middle clefts
IV. oblique clefts

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Classification of clefts

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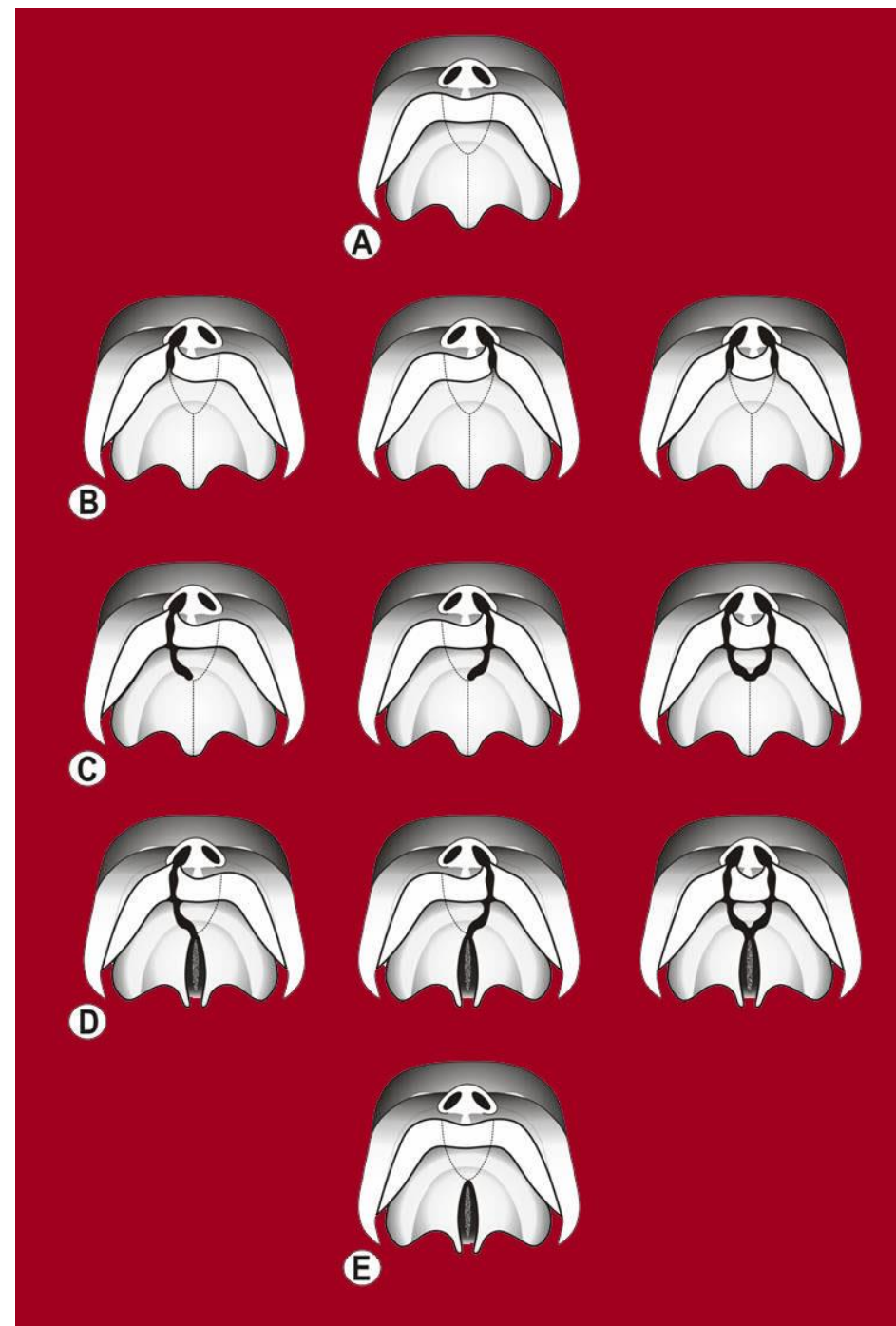
Facial clefts are associated with :

– Aesthetically adverse impact on the appearance of the child

– Swallowing problems

– Problems with food intake

– Negative influence on speech development

– Frequent inflammations of the middle ear

– Inflammations of the upper respiratory tract

– **Dentition**:

– Numerous anomalies in dentition (hypodontia, hyperodontia)

– **Compared to the general population, 8 times more common:**

– Morphological anomalies of denition

– Upper jaw underdevelopment - crossbite, anterior crossbite, midline shift

– retention of incisors, canines

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Multidisciplinary care for a cleft patient

– Cleft centres - Prague, Brno

– Care for cleft patients is multidisciplinary, long-term and systematic

AGE	OPERATION TYPE
0-3 months	Primary recronstruction of the lip
7-12 months	Primary recronstruction of the palate
3-8 years	Secondary recronstruction of the lip, palate and nose
8-12 years	Implantation of the secondary bone graft into the alveol
From 17 years	Orthognathic surgery, secondary reconstruction of the lip and nose

AGE	ORTHODONTIC THERAPY
newborn	OBTURATORS (for simplified breastfeeding), MOLDING (preparation for the lip operation, to bring the cleft segments closer)
3-6 years	OBTURATORS (for word practice), REMOVABLE APPLIANCES
6-9 years	REMOVABLE and FIXED APPLIANCES, PROTRACION MASK (for the therapy of narrow maxilla and frontal crossbite)
9-13 years	FIXED APPLIANCES – definitive solution of the shape of dental arches, teeth position and bite
14 and older	FIXED APPLIANCES – long-term stabilization

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Five-year old's index by Atack (Atack et al., 1997)

Atack et al., 1997

— 1997 Atack

— Evaluation of dental arch relationships at the age of five

— It is divided into 5 groups - 1 is the best and 5 is the worst

— The main criterion for classification into the five groups is according to the sagittal, transverse and vertical relationship of dental arches

— Evaluation can be performed on plaster diagnostic models, 3D digital models or on photographs of five-year-old children with unilateral cleft lip and palate

— Prognosis of further development of the relationship of arches and future orthodontic or orthodontic-surgical treatment

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Group	General Features of the Five-Year Old's Index	Long-term results	
1	Physiological overjet No crossbite/openbite Physiological growth of the upper jaw and palate	Excellent	
2	Physiological overjet Unilateral crossbite, tendency to crossbite +/- tendency to open bite in the area of cleft	Good	
3	Bite edge to edge or retroclined incisors Unilateral crossbite +/- tendency to open bite in the area of cleft	Satisfactory	
4	Inverted bite with retroclines or proclines of incisors Unilateral crossbite, tendency to bilateral crossbite +/- tendency to open bite in the area of cleft	Unsatisfactory	
5	Inverted bite with proclined incisors Bilateral crossbite Underdevelopment of the upper jaw and palate	Unsatisfactory	

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Methodology of measuring in our research

- Study models of 46 patients born between 2009 and 2013

- Unilateral cleft lip and palate

- All patients were operated by the same surgeon, with the same protocol - neonatal lip surgery and closure of the soft and hard palate between the 7th and 12th month

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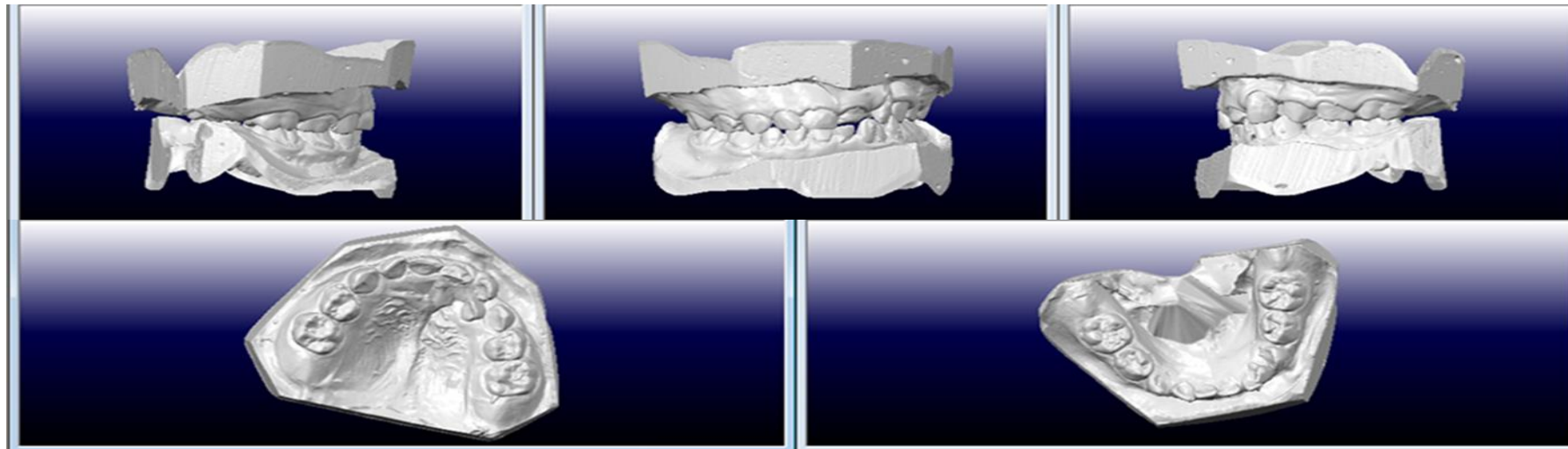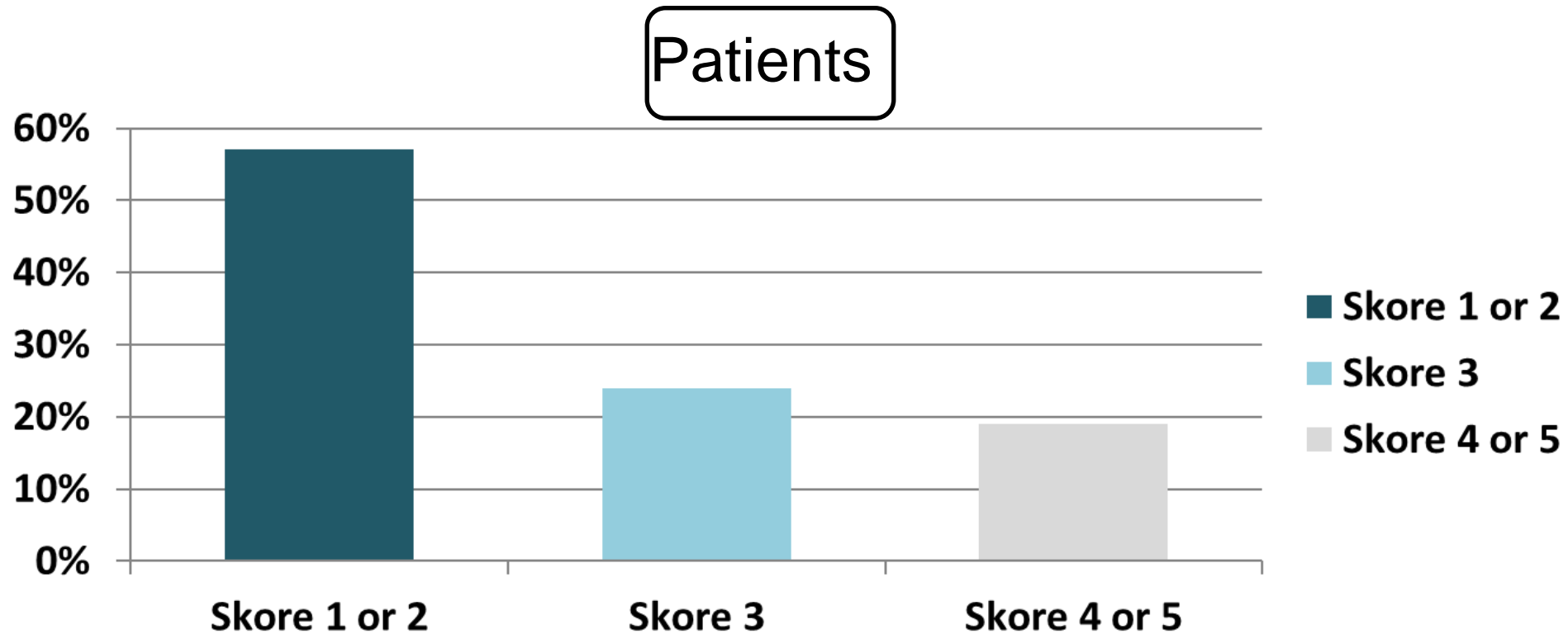
Results

Comparison of results with similar studies

These comparisons are possible thanks to the implementation of Eurocleft and Eurocran

Shaw et al., 2001 , European Collaboration on Craniofacial Anomalies (EUROCRAN), 2003

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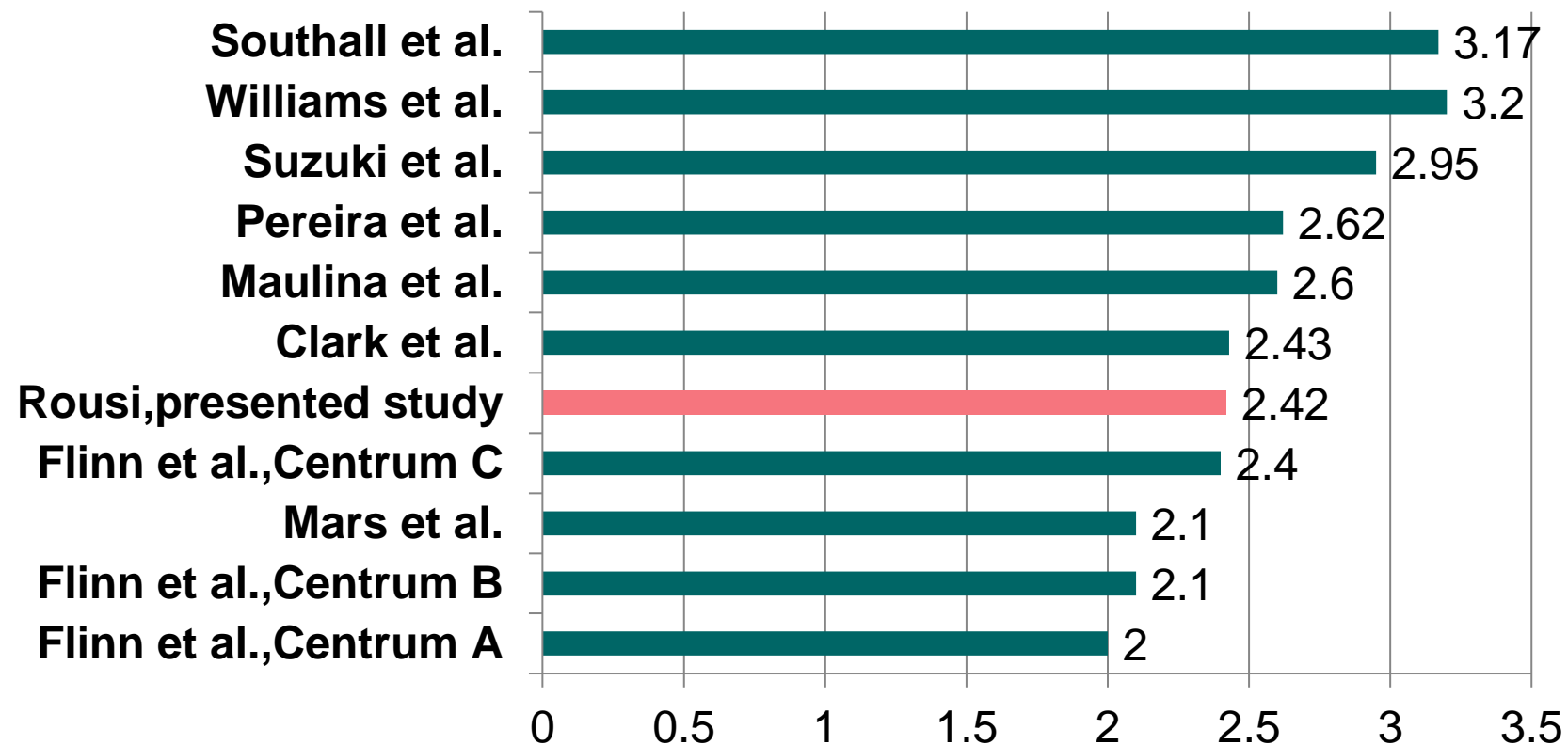
Conclusion of the research

Surgery of patients with cleft lip and palate always affects the growth of the upper jaw; however, the timing (neonatally or in the 3rd month of life) does not affect the size of the growth of the upper jaw

The results of our study showed that the correction of the cleft lip neonatally and closure of the soft and hard palate from 7 to 12 months lead to a satisfactory result of the upper jaw growth and intermaxillary relations and are comparable to the results of centers with different surgical protocols.

Schweckendiek et Doz., 1978

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Research activities – why become a researcher

— opportunities to acquire new and expand already acquired knowledge

— the path to your further professional growth

— opportunities to establish interdisciplinary cooperation with top Czech and foreign workplaces

— opportunity to participate in major Czech and foreign projects

— application of research into practice, completion of international internships, conferences…

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Research and development of modern oral dosage forms

Assoc. Prof. PharmDr. Jan Gajdziok, Ph.D.

Department of Pharmaceutical Technology,
Faculty of Pharmacy, Masaryk University Brno

gajdziokj@pharm.muni.cz

Introduction of the researcher and his team

Assoc. Prof. Dr. Jan Gajdziok, Ph.D. + Dr. Jan Elbl + Ph.D. Students

Department of Pharmaceutical Technology, Faculty of Pharmacy, MUNI

Development, formulation and evaluation of application/medical/dosage forms and microforms focused on drugs with limited solubility and bioavailability; innovative dosage forms; DDS for individualized therapy, etc.

Innovation and optimization of technological processes - the last step in drug development

Use of innovative excipients

Printing technologies for individualized therapy

Microparticle systems for pulmonary application

In situ forming gel systems
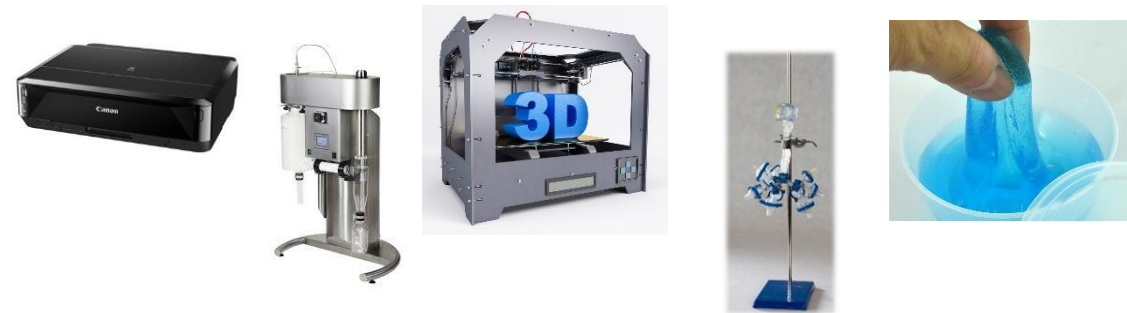
TEAM - HYPOTHESIS - INTERDISCIPLINARITY - PREPARATION - ROUTES - ERRORS (rabbits, rosuvastatin, clinical research, motivation)

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Research and development of modern oral dosage forms

Content of the lecture

– Oral cavity as the application site for the drug

– Used oral dosage/application forms

– Innovative drug delivery systems and their research and development

– Oral films

– Printing technologies in the formulation of dosage forms
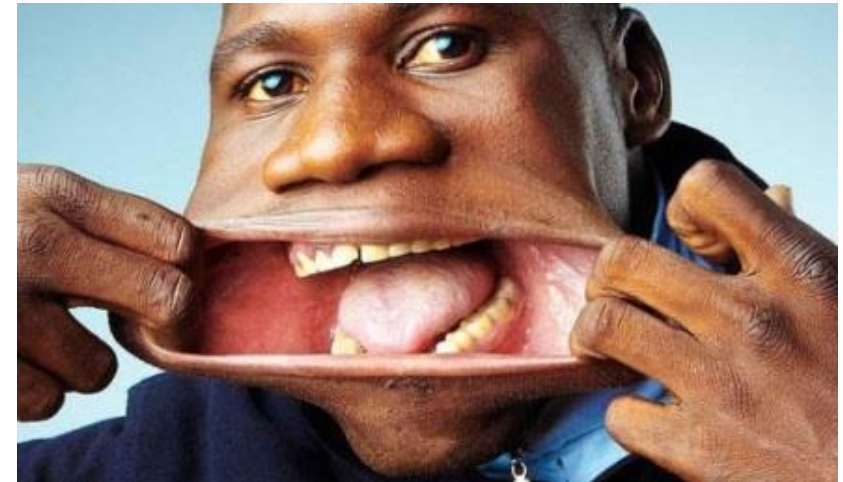
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Oral cavity as the application site for the drug

OC (buccal and sublingual mucosa) - a convenient area for the application of a number of drugs with local/systemic effects

Advantages of oral application:

— sufficient area, accessibility

— rich blood supply

— good permeability of non-keratinized areas

— bypass the first-pass effect

— high patient acceptance

— easy and quick removal of the drug if necessary

— rapid regeneration of the mucosa

— presence of saliva - sufficient amount of aqueous medium to dissolve API

— low enzymatic activity and non-aggressive environment

— possibility to use mucoadhesion

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Oral cavity as the application site for the drug

Disadvantages and limitations:

— small absorption area compared to the intestine (100-200 cm2)

— strong barrier properties of keratinized areas of the oral mucosa

— a mucus layer capable of binding the drug

— continuous saliva secretion leading to dilution and flushing of API into the lower parts of the GIT

— aggressive effects of drinking, eating, chewing and talking

— potential pathological condition of the OC

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Used oral dosage/application forms

Oral preparations (Oromucosalia)

– solid, semi-solid or liquid preparations containing one or more APIs, intended to be administered into the oral cavity and/or the oral part of the pharynx to achieve local or systemic effects

According to the pharmacopoeia, oral preparations can be divided into two basic categories:

- pharmaceutical forms *with mucoadhesive* properties

- formulations for which *mucoadhesive properties are not required* (orodispersible)

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Used oral dosage/application forms

Pharmacotherapeutic group	API	Pharmaceutical forms
Local antiseptics	Cetylpyridinium Chlorhexidine Triclosan	**Gargles**
NSAIDs	Benzydamine Flurbiprofen Cholin-salicylate	**Mouthwash** **Solutions on the gums** **Oral solutions, suspensions, drops, sprays**
Corticosteroids	Triamcinolone Dexamethasone	**Glazes** **Oral gels, pastes** **Gum gels, pastes**
ATBs	Hexetidine Fusafungin	**Lozenges** **Sublingual tablets** **Buccal tablets**
Antimycotics	Clotrimazole Nystatin Natamycine	**Oral capsules** **Chewable tablets**
Drugs for the therapy of salivary glands	Atropine Pilocarpine Neostigmine Acetylcysteine	**MA films, discs, tablets**

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Innovative drug delivery systems - research and development

Buccal films

– longer stay on the oral mucosa – mucoadhesion

– cover – pain reducing, increasing the effectiveness of topical treatment

Films dispersible in the mouth

– disseminated lesions of the upper respiratory tract and swallowing tract

– rapid dissolving/disintegration

Manufacturer	Trade name	API	Indication
Novartis	Theraflu	dextrometorfane/ difenhydramine	cold
	Gas-X	simethicone	flatulence
Pfizer	Listerine	volatile oils	oral hygiene
	Sudafed	fenylefrine HCl	runny nose
InnoZen	Chloraseptic	benzocaine/ menthol	sore throat
	Suppress	menthol	cough
Glaxo	**NiQuitin**	nicotine	smoking cessation
Sandoz	Sildenafil Sandoz	sildenafile	erectile dysfunction
Orajel	Ultra Canker Film	benzocaine/ menthol	sore throat
MonoSol Rx	Zuplenz	ondansetrone	nausea, vomiting
	Suboxone	buprenorfine/ naloxone	opioid addiction
Tesa Labtec	Zolmitriptan Rapidfilm	zolmitriptane	migraine
Hexal AG	Risperidon HEXAL	risperidone	schizophrenia
Meda	**Breakyl** buccalfilm	fentanyl	breakthrough pain

Research and development of modern oral dosage forms

Oral films – properties and composition

- comfortable, discreet DF

- more beneficial properties than buccal
  and ODT

- instability in humid environments

- limited size and weight

Film-forming polymers

- ensure mechanical resistance of the film and appropriate application behavior
- determine the type of OF (BMF: derivatives of cellulose, chitosan, carbomers; ODF: pullulan, starch derivatives)

Plasticizers

- reduce the glass transition temperature of the polymers, thereby increasing the flexibility and reducing the fragility of films (e.g. glycerol, propylene glycol, sorbitol)

Other

- solvents, fillers, dyes, taste-masking agents, absorption enhancers, salivation stimulators, etc.

Bőhová Linhartová et al., 2020

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Oral films - methods of preparation and production
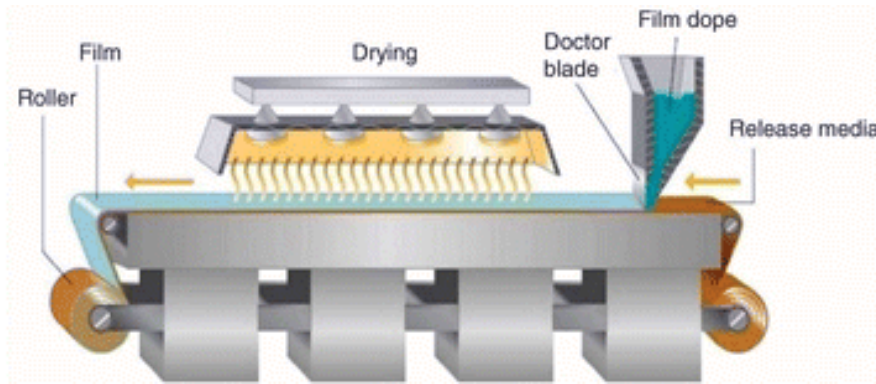
solvent casting
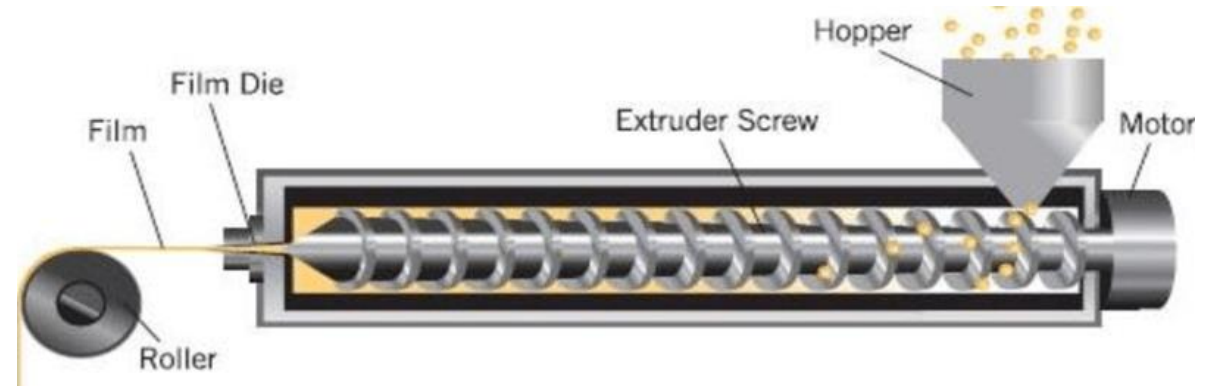
hot-melt extrusion
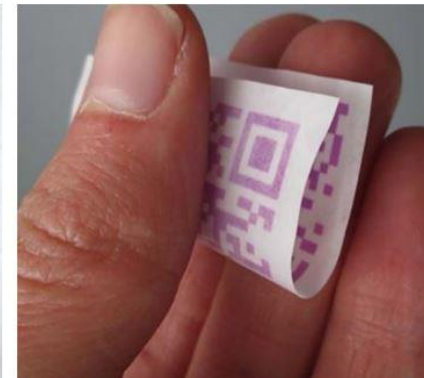
printing methods

- electrospinning
- spraying
- impregnation

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Oral films – solvent casting method

MAF

– mucoadhesive layer – NaCMC, POE, hyaluronan, chitosan x backing layer (EC, bone wax)

– nystatine, CPX, chlorhexidin, nanocomposites - modification of release (direction, time)

ODF

– film-forming maltodextrin, plasticizers, disintegrants

– BZD, bisulepine, moxastine

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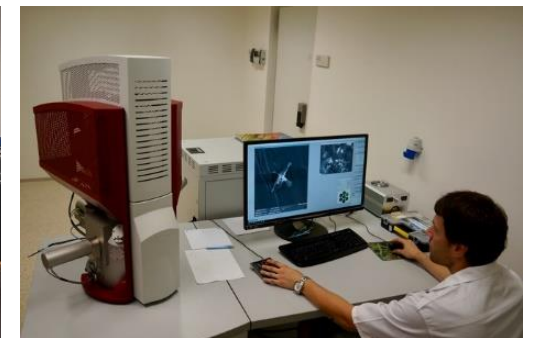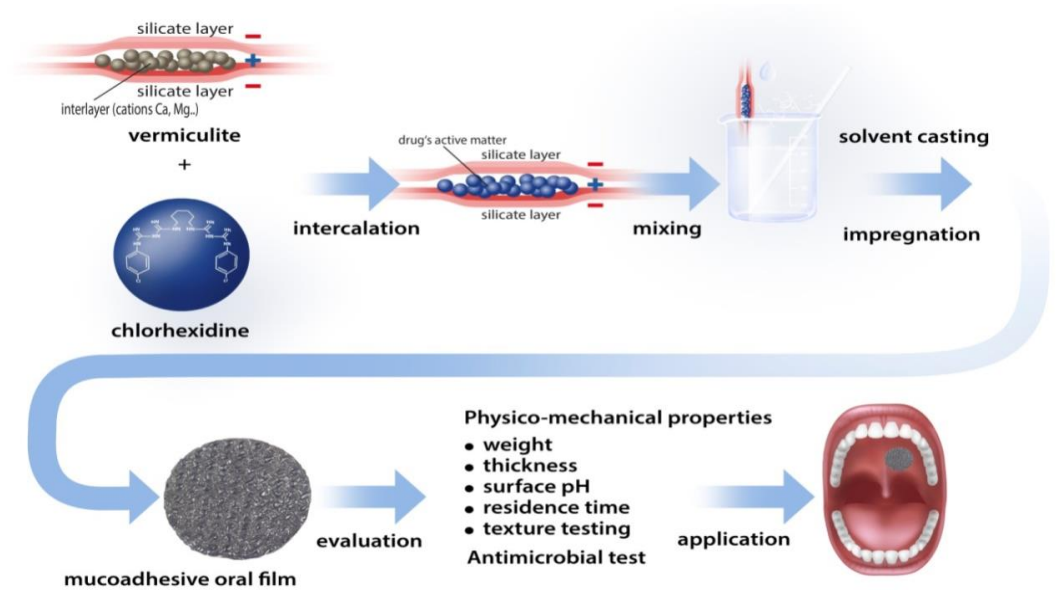
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Printing technologies in the formulation of dosage forms

– 2D (inkjet) and 3D (mSSE)

– relative simplicity of the process

– practical application perspective - GAMU 2020 - UI and legal framework solution

– **individualization** of the dose, combination of drugs, precise deposition, design flexibility (size, shape, form)

– new DDS features – smart, taste masking, patient compliance and adherence
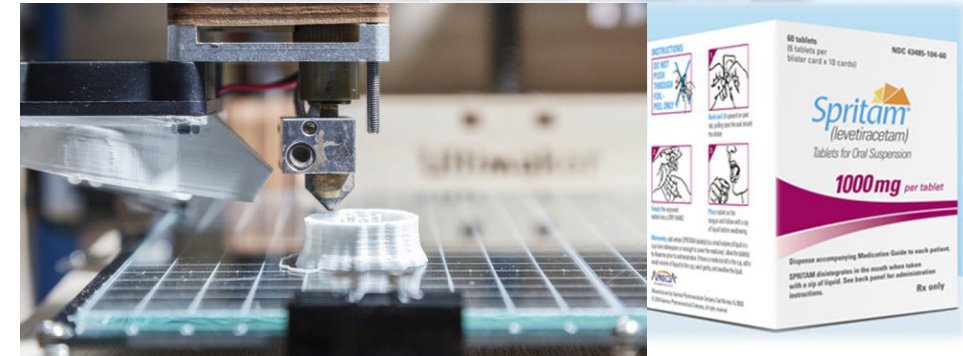
ZipDose® technologie - patent Aprecia

Hippocrates: "Treat man, not disease!"

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Oral films - printing technology

— Fast and low-cost modification for production in small batches

— Possibility to define the dose, release kinetics, shape, size, color, etc.

— DF with properties unattainable by common production (compartmentalization, complex shapes)

— Pre/clinical phase of new drug research

— DF tailored to the patient - dose, form, stability (preparation at the time of need)

— On-site preparation / production - developing countries (outbreak)

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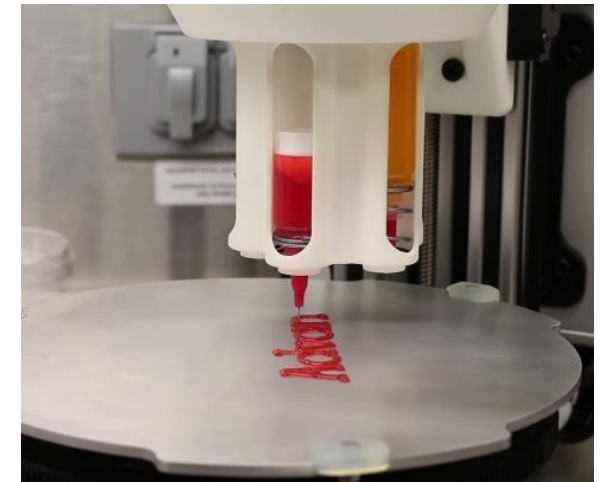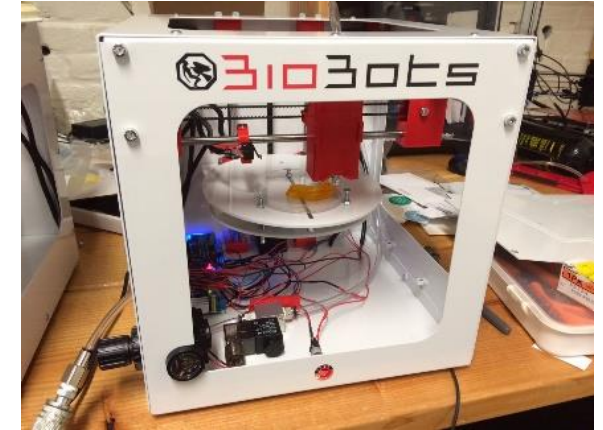
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Oral Films - 2D printing

- Suitable for low-dose, sensitive APIs

- Deposition accuracy in multi-layer printing

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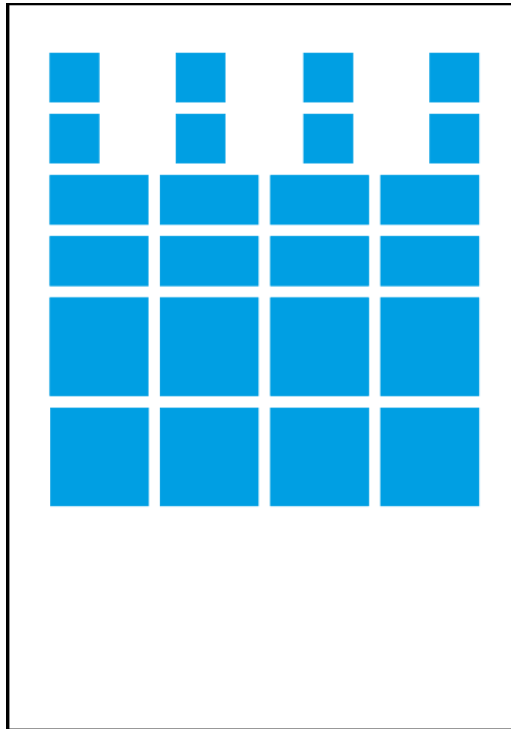
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Oral Films - 3D bioprinting

- Application of principles and techniques of 3D printing for biomaterials formulated to both dosage forms and biomedical objects (implants, tissues, organs, etc.) replacing their natural equivalents

- Printing of bioinks (bioinks = mixtures of cells and intercellular mass)

3 phases:

Pre-processing (tissue mapping and the 3D model creation)

Processing (printing objects from bioink using a 3D printer)

Post-processing (graduating printed objects in a bioreactor)

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Oral Films - 3D bioprinting

– Laser-based bioprinting

• uses laser energy to deposit bioinks in a three-dimensional arrangement

• laser - energy - heat - liquid evaporation - ejection of bioink into a given position

– Droplet-based bioprinting

• application of bioink to the substrate in the form of drop

– Microextrusion-based bioprinting

• **extrusion of bioink from the reservoir through the nozzle by air pressure or mechanical force on the substrate**

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Oral Films - 3D bioprinting

— Preparation of ODF by 3D printing through combining film-forming polymers and plasticizers in order to prepare the optimal matrix for API incorporation

— Use of 3D printer and optimization of its settings for ODF printing with various compositions

— Evaluation of physical and mechanical properties of prepared ODF

— type: HyperCube Evolution (according to Scott3D, USA)

— 3D printing principle: microextrusion piston printing

— firmware: Marlin (Open Source)

— control principle: transmission belt system

— 3D motion orientation: extruder = X (left / right) and Y (forward / reverse)

— printing plate = Z axis (up / down)
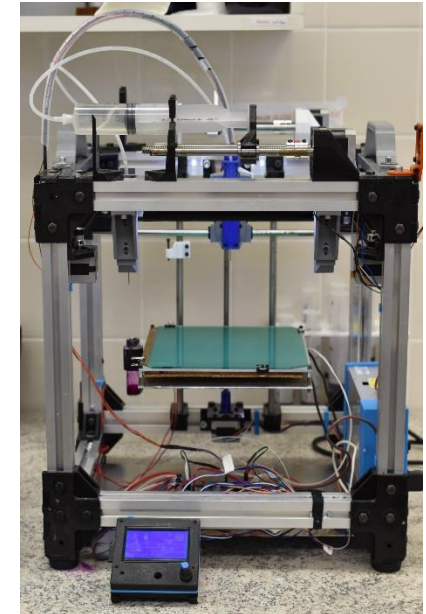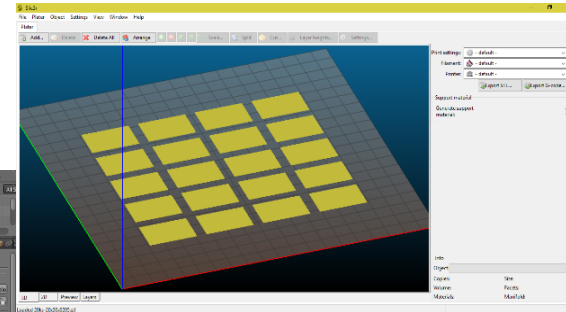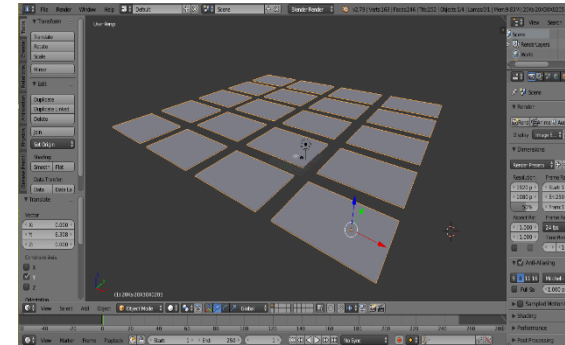
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Oral Films - 3D bioprinting

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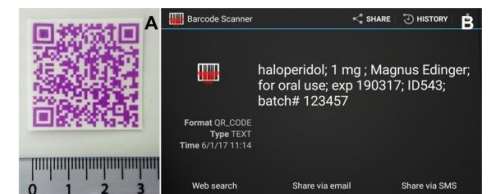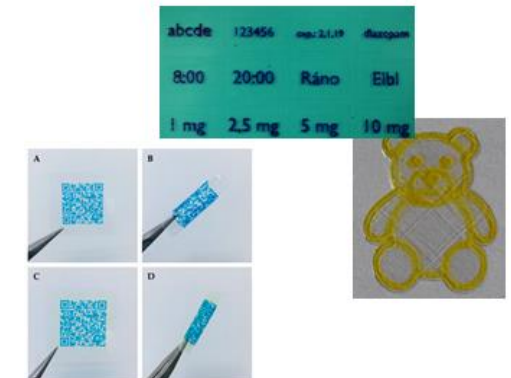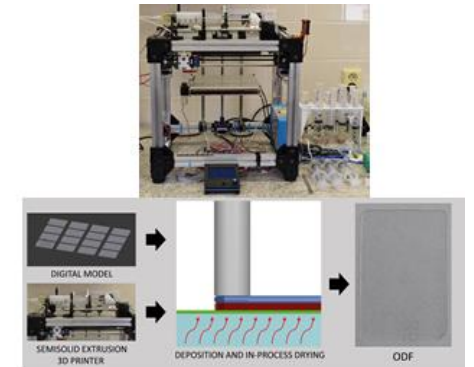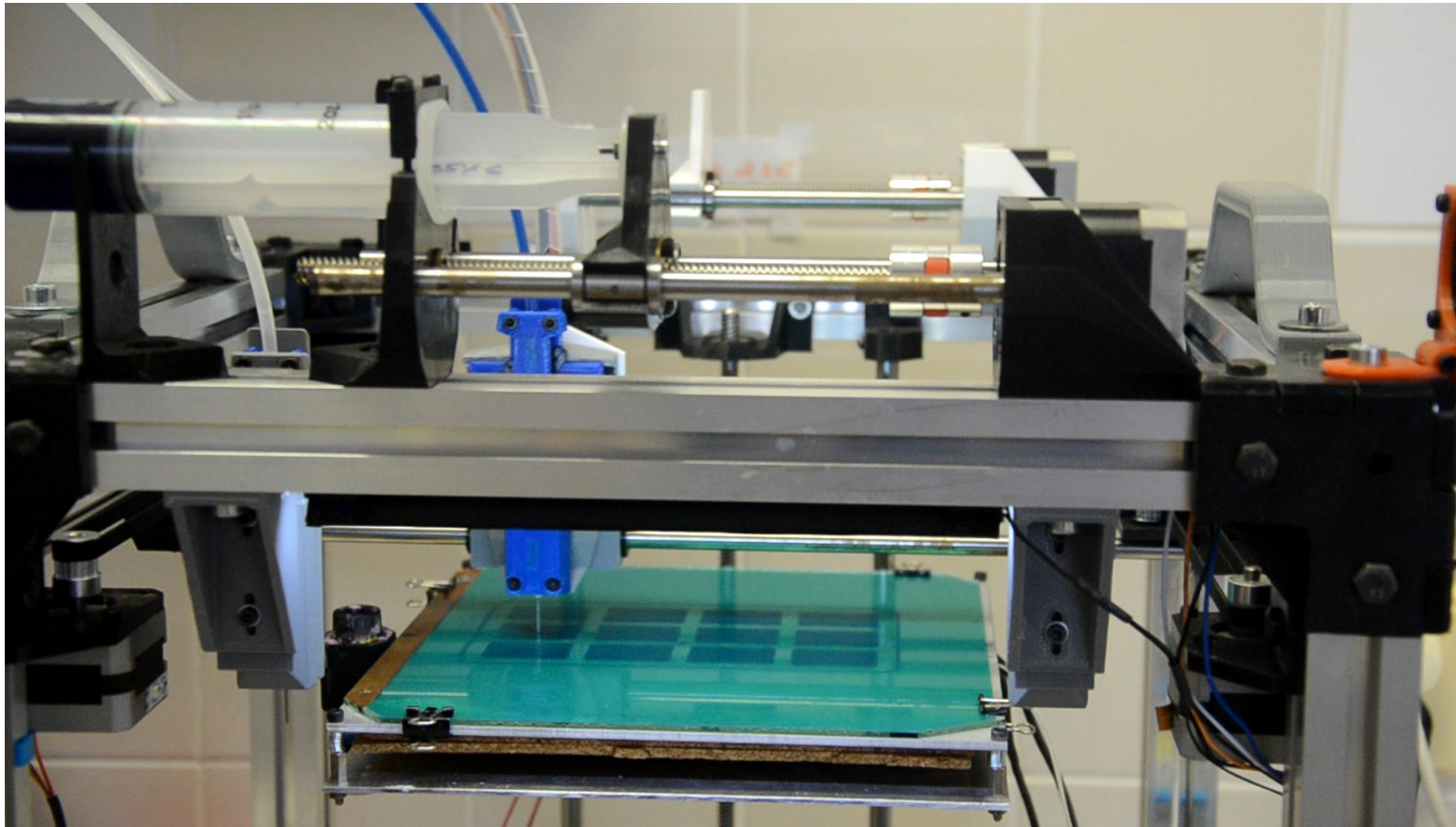
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Oral films - publications

GAJDOŠOVÁ, M., VETCHÝ, D., MUSELÍK, J., GAJDZIOK, J., JUŘICA, J., VETCHÁ, M., HAUPTMAN, K., JEKL, V.: Bilayer mucoadhesive buccal films with prolonged release of ciclopirox olamine for the treatment of oral candidiasis: In vitro development, ex vivo permeation testing, pharmacokinetic and efficacy study in rabbits, International Journal of Pharmaceutics, in press.

ELBL, J., GAJDZIOK, J., KOLARCZYK, J.: 3D printing of multilayered orodispersible films with in-process drying, International Journal of Pharmaceutics, 2020, 575, 118883.

PECHOVÁ, V., GAJDZIOK, J., MUSELÍK, J., VETCHÝ D.: Development of orodispersible films containing benzydamine hydrochloride using modified solvent casting method. AAPS PharmSciTech, 2018, 19 (6), 2509-2518.

DANĚK, Z., GAJDZIOK, J., DOLEŽEL, P., LANDOVÁ, H., VETCHÝ, D., ŠTEMBÍREK, J.: Buccal films as a dressing for the treatment of aphthous lesions. Journal of Oral Pathology and Medicine, 2017, 46 (4), 301-306.

LUKÁŠOVÁ, I., MUSELÍK, J., VETCHÝ, D., GAJDZIOK, J., GAJDOŠOVÁ, M., JUŘICA, J., KNOTEK, Z., HAUPTMAN, K., JEKL, V.: Pharmacokinetics of ciclopirox olamine after buccal administration in rabbits. Current Drug Delivery, 2017, 14 (1), 99-108.

WALICOVÁ, V., GAJDZIOK, J., PAVLOKOVÁ, S., VETCHÝ, D.: Design and evaluation of mucoadhesive oral films containing sodium hyaluronate using multivariate data analysis. Pharmaceutical Development and Technology, 2017, 22 (2), 229-236.

WALICOVÁ, V., GAJDZIOK, J., VETCHÝ, D.: Orodispergovatelné filmy, technologie jejich výroby a specifické pomocné látky pro přípravu. Chemické listy, 2016, 110 (6), 424-429.

JEKL, V., HAUPTMAN, K., MINARIKOVA, A., KOHUTOVA, S., KNOTEK, Z., GAJDZIOK, J., MUSELIK, J., SEDLAK, L., VETCHÝ, D.: Pharmacokinetic study of benzylpenicillin potassium after intramuscular administration in rabbits. Veterinary Record, 2016; 179 (1), 18-U59.

GAJDOŠOVÁ, M., VETCHÝ, D., DOLEŽEL, P., GAJDZIOK, J., LANDOVÁ, H., MUSELÍK, J., ZEMAN, J., KNOTEK, Z., HAUPTMAN, K., JEKL, V.: Evaluation of mucoadhesive buccal films containing nystatin. Journal of Applied Biomedicine, 2016, 14, 247-256.

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Research and development of modern oral dosage forms

Oral films - projects

Projekt programu výzkumu a vývoje Ministerstva zdravotnictví "Moderní léková forma pro terapii orálních kandidóz", identifikační kód NT14477, zahájení 2013-05-01, ukončení 2015-12-31

Projekt programu výzkumu a vývoje Ministerstva zdravotnictví " Mukoadhezivní filmy určené ke krytí slizničních defektů dutiny ústní ", identifikační kód NT11396, zahájení 2011-01-01, ukončení 2013-12-31

Projekt programu ALFA "Modifikované materiály pro léčbu chronických a akutních ran a prevenci chirurgických infekcí ve zdravotnictví" č. TA 01010244, zahájení 2011-01-01, ukončení 2013-12-31

Projekt programu "Trvalá prosperita" "Nové farmaceutické produkty na bázi polysacharidů", evidenční číslo 2A-1TP1/073, zahájení 2006-01-01, ukončení 2011-12-31 (člen řešitelského týmu) RICHTER, J.: Nové farmaceutické produkty na bázi polysacharidů. MPO 2A-1TP1/073.

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HOŘAVOVÁ, H., BLAHÁČKOVÁ, D., GAJDZIOK, J.: Bioprinting - návod a instruktážní video pro předměty "Lékové formy vyšších generací" a "Cvičení diplomantů". IVA VFU Brno No. 2019FaF/3130/77.

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Abbreviations used

- OC – oral cavity

- API – active pharmaceutical ingredient

- NSAIDs – non-steroidal anti-inflammatory drugs

- ATB – antibiotics

- MA – mucoadhesion/mucoadhesive

- DF – dosage form

- ODT – orodispersible tablet

- OF – oral film

- BMF – buccal mucoadhesive film

- ODF – orodispersible film

- MAF – mucoadhesive film

- NaCMC – carmellose natrium

- POE – polyoxyethylene

- CPX – cyklopiroxolamine

- EC - ethylcelulose

- BZD – benzydamine

- mSSE – micro-semisolid extrusion

- DDS – drug delivery system

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Muco-adhesive films designed to cover defects of the oral mucosa

MUDr. et MUDr. Zdeněk Daněk, Ph.D.
Clinic of Maxillofacial Surgery, University Hospital Brno

Muco-adhesive films designed to cover defects of the oral mucosa

Research objective

Stomatitis aphtosa – aphtosis minor

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Muco-adhesive films designed to cover defects of the oral mucosa

Team members

Institute of Drug Technology
doc. PharmDr. Jan Gajdziok, Ph.D.
doc. PharmDr. Mgr. David Vetchý, Ph.D.

Preparation and development of mucoadhesive drug forms

Department of Oral, Jaw and Facial Surgery, Brno University Hospital
MUDr. et MUDr. Zdeněk Daněk, Ph.D.

Clinical testing

Department of Oral, Jaw and Facial Surgery of the Ostrava Hospital
MUDr. et MUDr. Jan Štembírek, Ph.D.

Clinical testing

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Muco-adhesive films

— Cover layer and mucoadhesive layer - mucoadhesive polymers

— ability to form adhesive interactions with biological surfaces (mucus layer)

— a suitable polymer must meet criteria of non-toxicity, non-irritancy, biodegradability

— resistant in the environment of physiological pH in the oral cavity

— viscoelastic properties

— rapid adhesion to the mucosa

— stability

M U N I
M E D

Preparation of muco-adhesive films
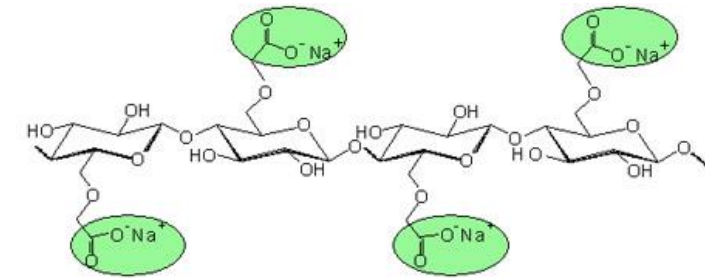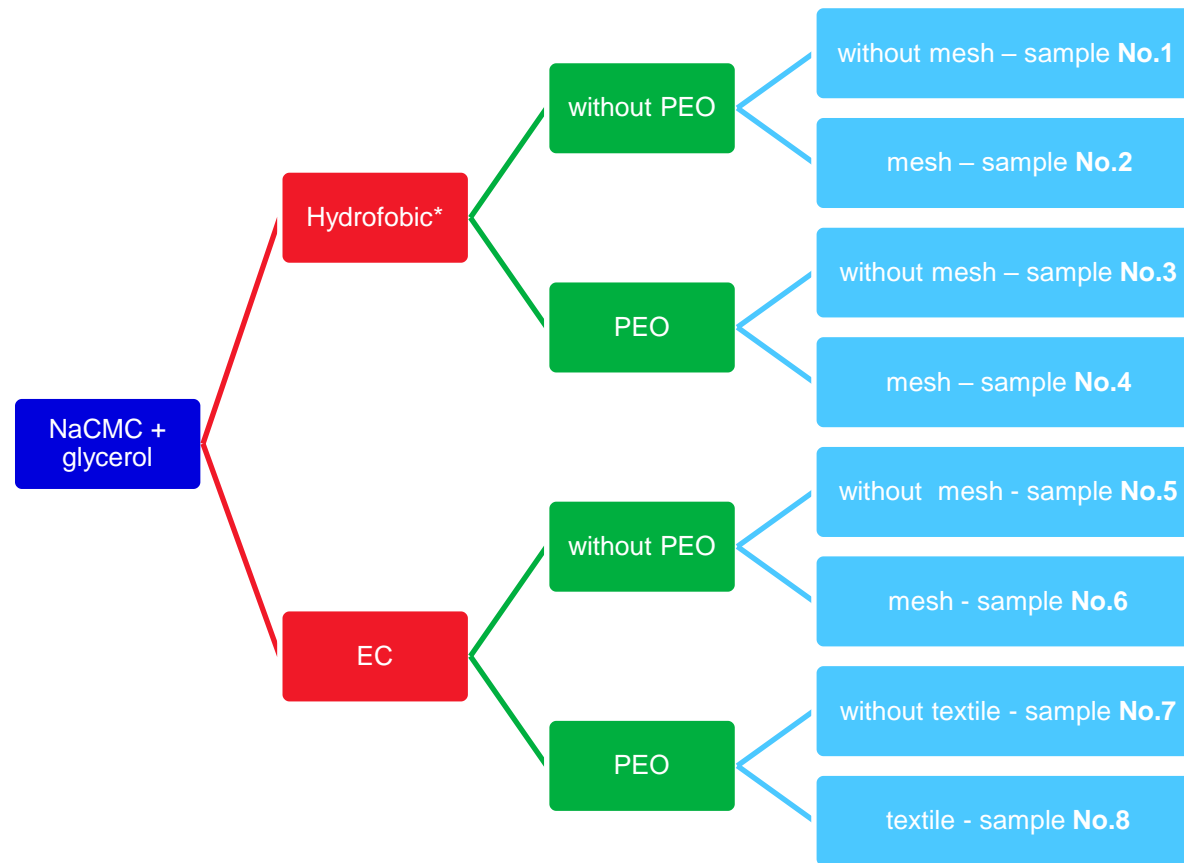
sodium salt carboxymethylcellulose (Na-CMC)

EC – ethyl cellulose

PEO – polyethylene oxide

NaCMC - sodium salt carboxymethylcellulose

* mixture of white beeswax and white Vaseline

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Pilot study

— **Clinical test on 22 volunteers (11 women, 11 men)**

— Approved by the EC FNO (No. 152/2011)

— Evaluated using an evaluation questionnaire, informed consent

— 3 films from each sample

— **Monitored parameters:**

— Film duration

— Cause of film release

— How to release the film

— Subjective feelings during application

— The appearance of the film

Vzorek 1		1	2	3
Doba setrvání filmu na sliznici (min)		110	40	85
Pocit překážení v ústech (ano/ne)		ne	ne	ne
Pocit tlaku (ano/ne)		ne	ne	ne
Pocit zvýšeného slinění (ano/ne)		ne	ne	ne
Chuť/pachuť (ano-jaká/ne)		ne	ne	ne
Příčina uvolnění filmu	Spontánně	x	x	
	Mluvením			
	Při pití			x
Mechanismus uvolnění filmu	Odloupnutí celé krycí vrstvy			
	Postupné olupování krycí vrstvy	x		x
	Odlepení celého filmu		x	
Bolest (ano/ne)		ne	ne	ne
Otok (ano/ne)		ne	ne	ne
Svědění (ano/ne)		ne	ne	ne
Pálení (ano/ne)		ne	ne	ne
Celková reakce (nevolnost, zvracení)		žádná	žádná	žádná
Problémy při aplikaci (jaké)		žádné	žádné	žádné
Hodnocení vzhledu filmu (1-nejlepší až 5-nejhorší)		2	1	1
Hodnocení vzhledu filmu (slovně – deformace filmu, změna barvy, polámaní)		Mírně olámané okraje		
Jiné postřehy				Způsobeno teplým nápojem

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Pilot study

Clinical test:

22 volunteers

(11 women, 11 men)

selected film No.7

– NaCMC + PEO + EC

Sample	Average [min])	SD [min]	min [min]	max [min]
1	74.18	5.13	68.27	77.55
2	65.29	2.61	62.27	66.86
3	87.26	6.06	81.64	93.68
4	71.05	3.15	68.05	74.32
5	-	-	-	-
6	53.24	5.38	47.41	58.00
7	98.12	1.75	96.18	99.59
8	81.00	8.47	73.86	90.36

No. 1
No. 2
No. 3
No. 4
No. 6
No. 7
No. 8

Vetchý et al., 2014

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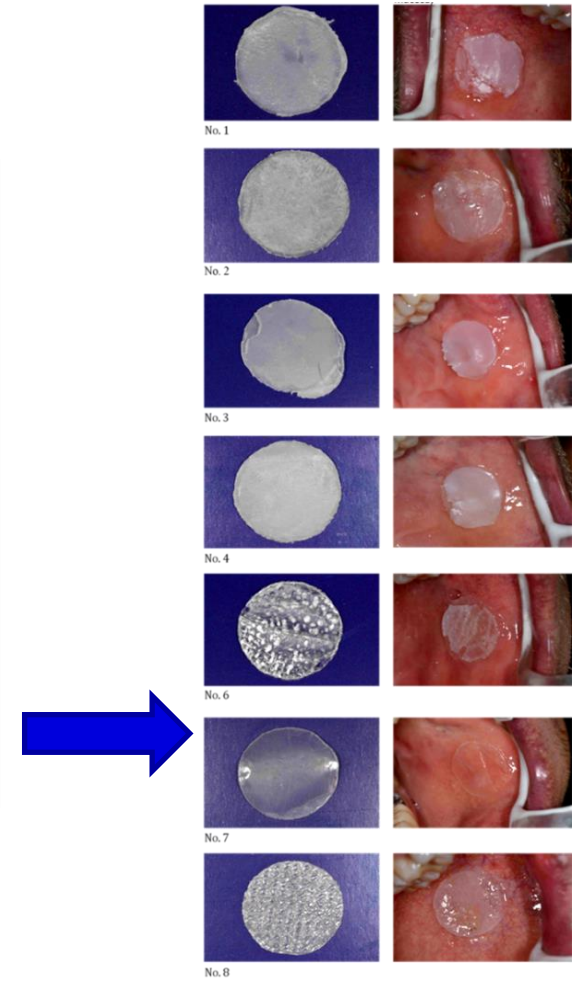
Clinical study - therapy of the aphthosis

— Selected patients with **aphtosis minor**

— Multifactorial cause, affected population estimate 5-60%

— The exact mechanism of development is not yet fully known

— Circumscribed epithelial necrosis to ulcers with circular erythema

— Occurrence on non-keratinizing mucosa

— Defects up to 1cm

— There is no causal treatment

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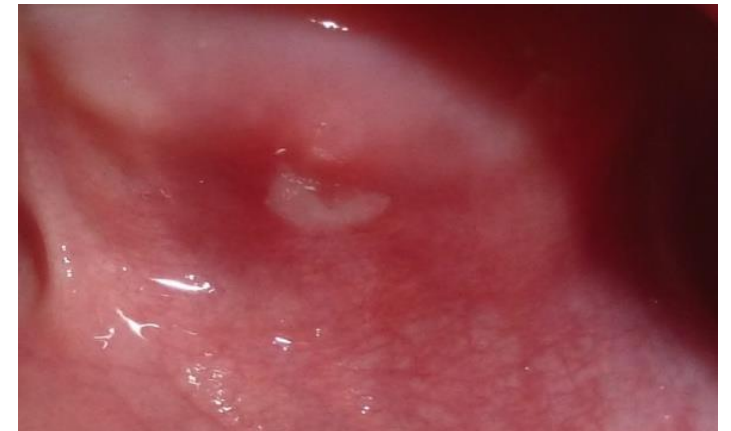
Clinical study - therapy of aphthosis

- Approved by the EC FNO (No. 25/2013)
- Evaluated using an evaluation questionnaire, informed consent
- 36 patients control group
- 18 - treated with Mundisal gel experimental group
- 18 - treated with mucoadhesive film + Mundisal gel

- **Subjective:**
- Pain assessment – VAS
  - Cure criterion VAS ≤ 2

- **Objective:**
- duration of the drug vs. film
  - measuring the size of the lesion

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Clinical study - therapy of aphthosis
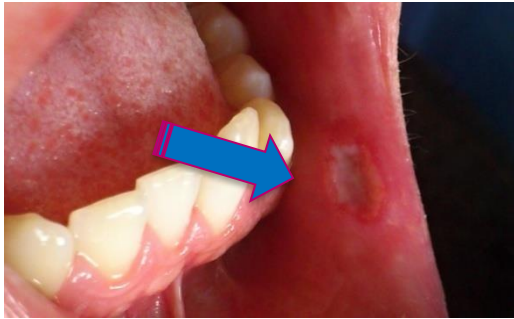
Control group

Day 1   Day 3   Day 5
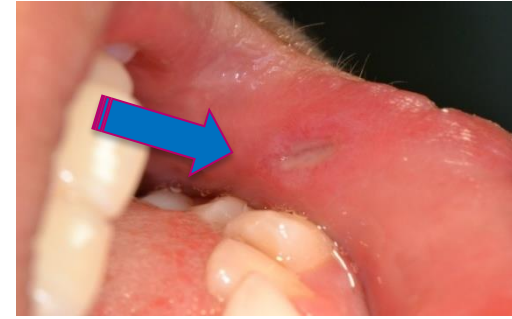
Experimental group
with muco-adhesive film

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Clinical study - therapy of aphthosis
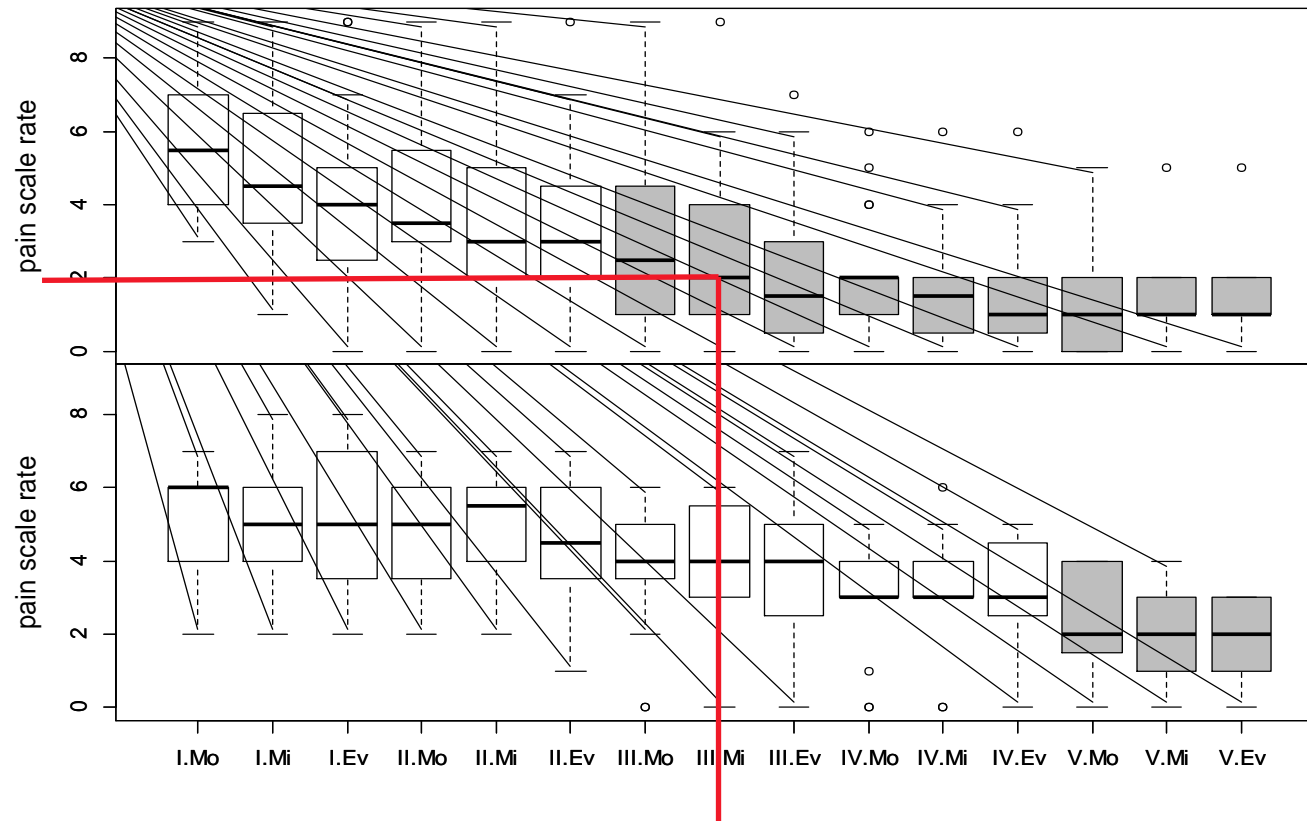
Box charts recording PAIN at individual time points

experimental group (top)

control group (bottom)

(Tukey-Kramer test with Nemenyi modification, p<0.05)
(Mo - morning, Mi - noon, Eve - evening)

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Clinical study - therapy of aphthosis
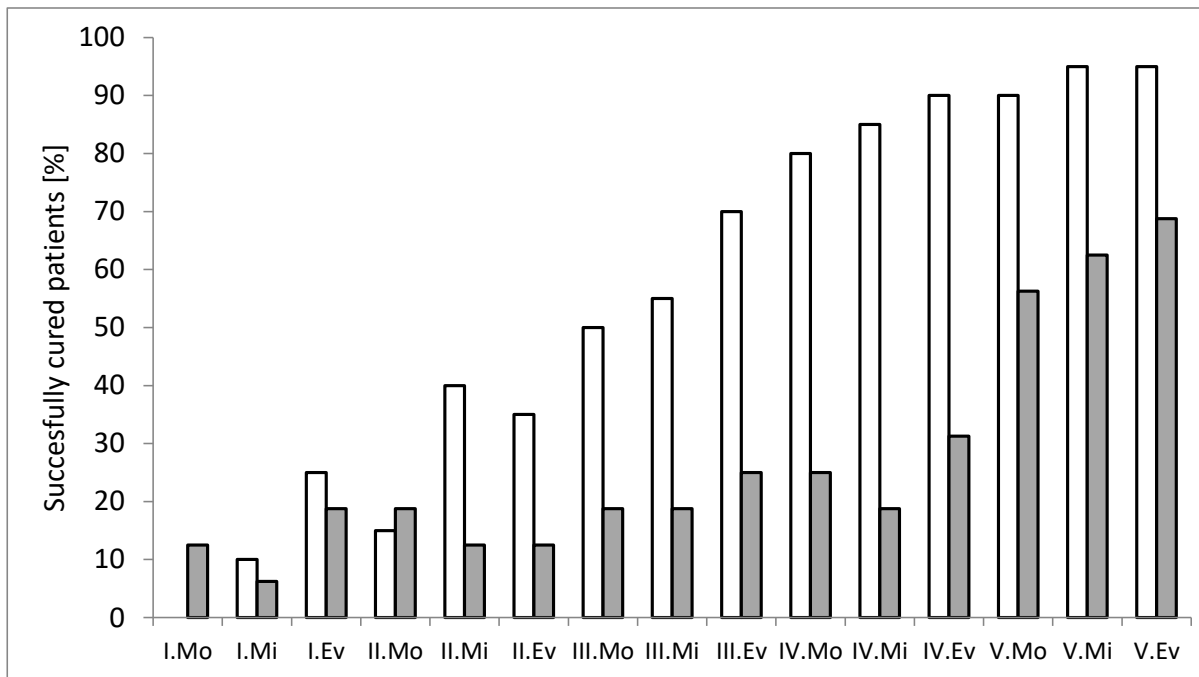
Relative number (%) of successfully cured patients at individual time points

experimental group (white columns)

control group (grey columns)

(Mo - morning, Mi - noon, Ev - evening)

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Clinical study - therapy of aphthosis

Size of lesions

in experimental (top)
and control (bottom) group

1st, 3rd and 5th day of therapy.

Boxes highlighted in grey show the time points statistically significantly different from the first record

(Tukey-Kramer test with Nemenyi modification, $p < 0.05$)

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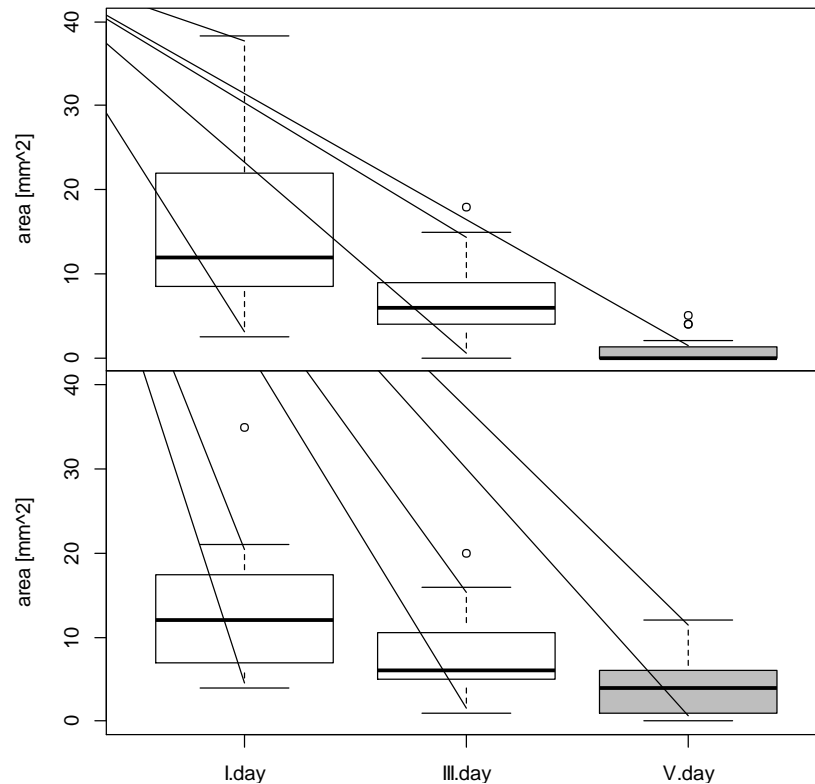
Clinical study - therapy of aphthosis
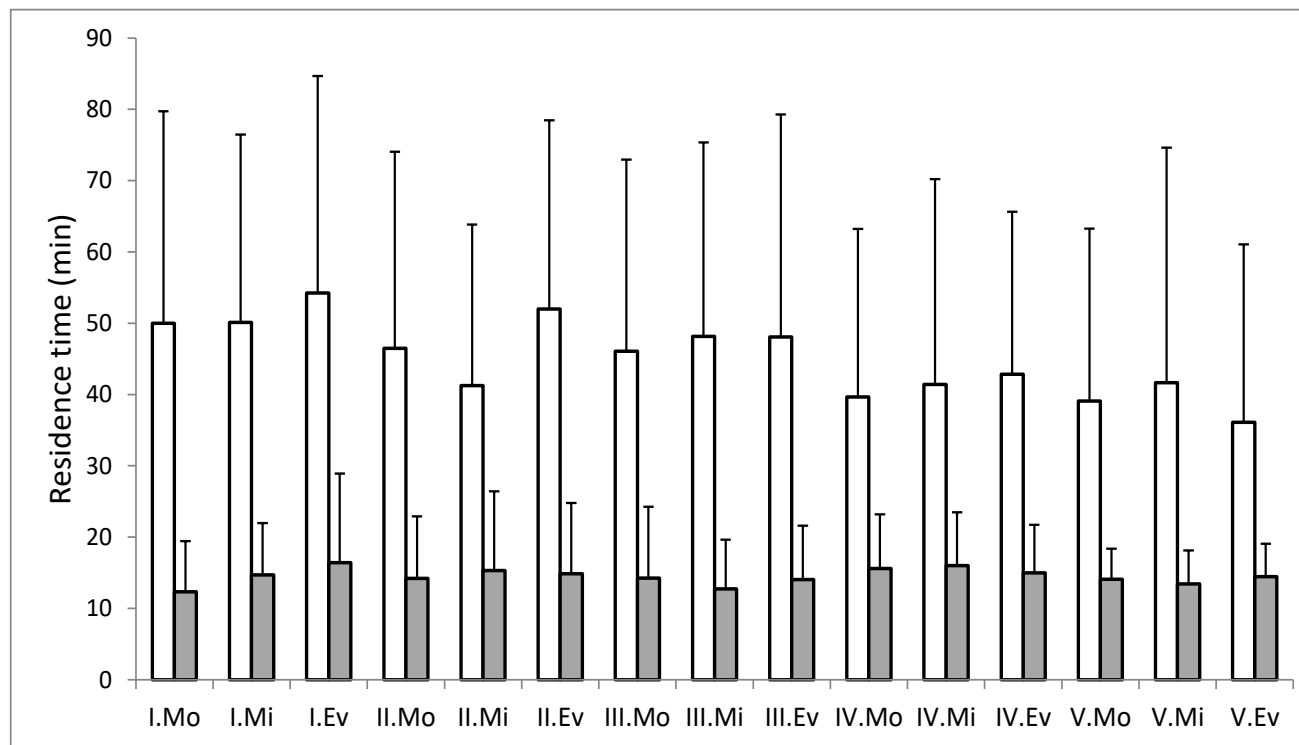
Duration

of the film on the mucous membrane (white columns) is 3.17 times longer than the duration of the gel remaining on the mucous membrane in the control group (grey columns).

(t-test, p<0.001)
(Mo - morning, Mi - noon, Ev - evening)

Daněk Z, Gajdziok J, Doležel P, Landová H, Vetchý D, Štembírek J. Buccal Films as a Dressing for the Treatment of Recurrent Aphthous Stomatitis. J Oral Pathol 2017, Apr;46(4):301- 306

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Clinical study - therapy of aphthosis

Results

Reduction of pain

Experimental group - Day 3

Control group – Day 5

Experimental group - **3.17 times longer drug contact** with lesions than control group

Lesion overlap

Faster healing

Prolonging the period of contact of the drug

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Muco-adhesive films designed to cover defects of the oral mucosa

Vision

Incorporation of nanocomposite carrier substances into a mucoadhesive film (patent)

Improving the properties of muco-adhesive films, extending the duration of film adhesion on the mucous membrane

Clinical trials

Future ➡️ **transfer into practice**

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Oral microbiome in patients with head and neck cancer

MUDr. et MUDr. Zdeněk Daněk, Ph.D.
Clinic of Maxillofacial Surgery, University Hospital Brno
doc. RNDr. Petra Bořilová Linhartová, Ph.D., MBA
Dental Clinic, Department of Pathological Physiology, Institute of Medical Genetics and Genomics, Faculty of Medicine, Masaryk University Brno

383 Research Activities in Dentistry   2021

Oral microbiome in patients with head and neck cancer

MICROBIOME

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Oral microbiome in patients with head and neck cancer

Factors influencing the oral microbiome - alcohol

– Association with the total composition of the oral microbiome and the number of specific taxa

– Increased number of *Neisseria (*metabolization of ethanol to acetaldehyde!!!)

– Exhaustion of comensals, space for potential pathogens

Excessive use of alcohol

•2 and more drinks/day

•3 and more drinks/day

Head and neck cancer
GIT Cancer
Periodontitis

Fan X. et al., 2018

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Oral microbiome in patients with head and neck cancer

Factors affecting the oral microbiome - smoking

– Differences between smokers and
non-smokers

– There was no difference between ex-
smokers and non-smokers

– The changes are not permanent

– It can support the anaerobic oral
environment and bacterial community
with an altered ability of xenobiotic
degradation
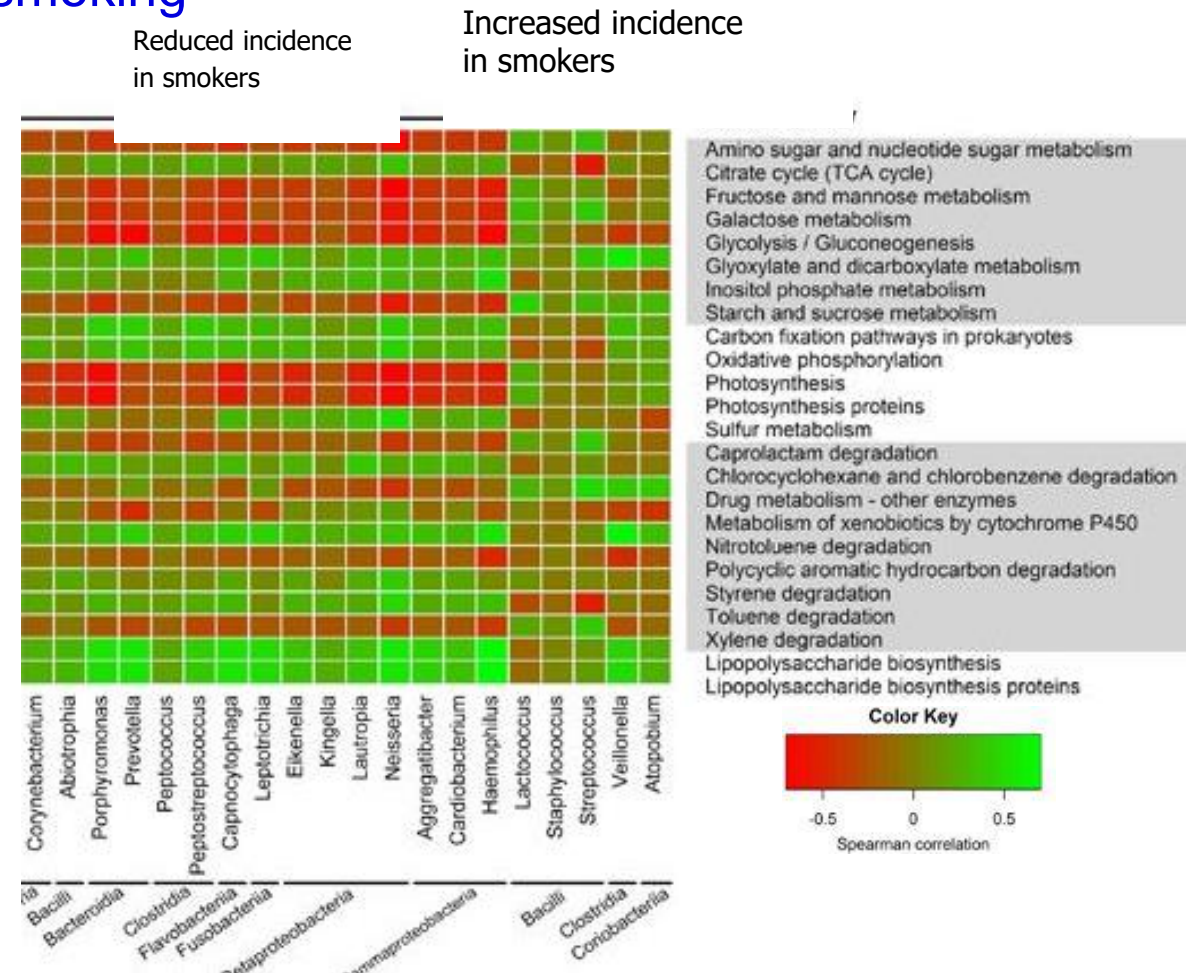
Reduced incidence
in smokers

Increased incidence
in smokers

Wu J. et al., 2016

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Oral microbiome in patients with head and neck cancer

Squamous cell carcinoma of the oral cavity (OSCC)

— most commonly occurring malignancy in the orofacial

region

— incidence of 4.2/100,000 inhabitants

— mortality of 2.2/100,000 inhabitants

— National Oncology Register of the Czech Republic
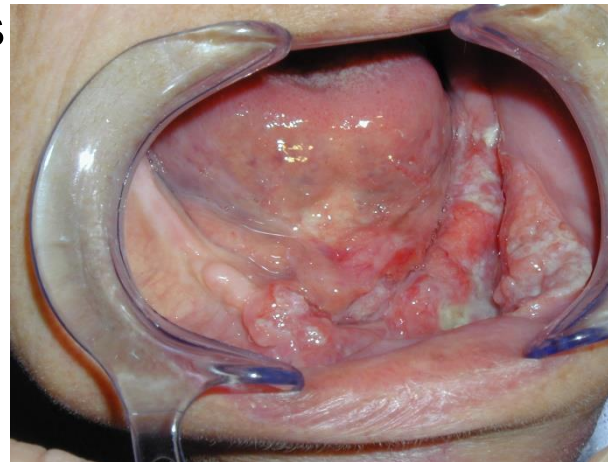
Squamous cell carcinoma of the edge of the tongue

Squamous cell carcinoma of the scum of the oral cavity

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Oral microbiome in patients with head and neck cancer

Squamous cell carcinoma of the oral cavity (OSCC)

— Frequent detection in the advanced stadium

— Poor prognosis

— Search for biomarkers for early diagnosis

— Prognostic biomarkers

Advanced stadium of squamous cell carcinoma of the lower jaw alveolus

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Oral microbiome in patients with head and neck cancer

Relationship between oral microbiota and squamous cell carcinoma of the oral cavity
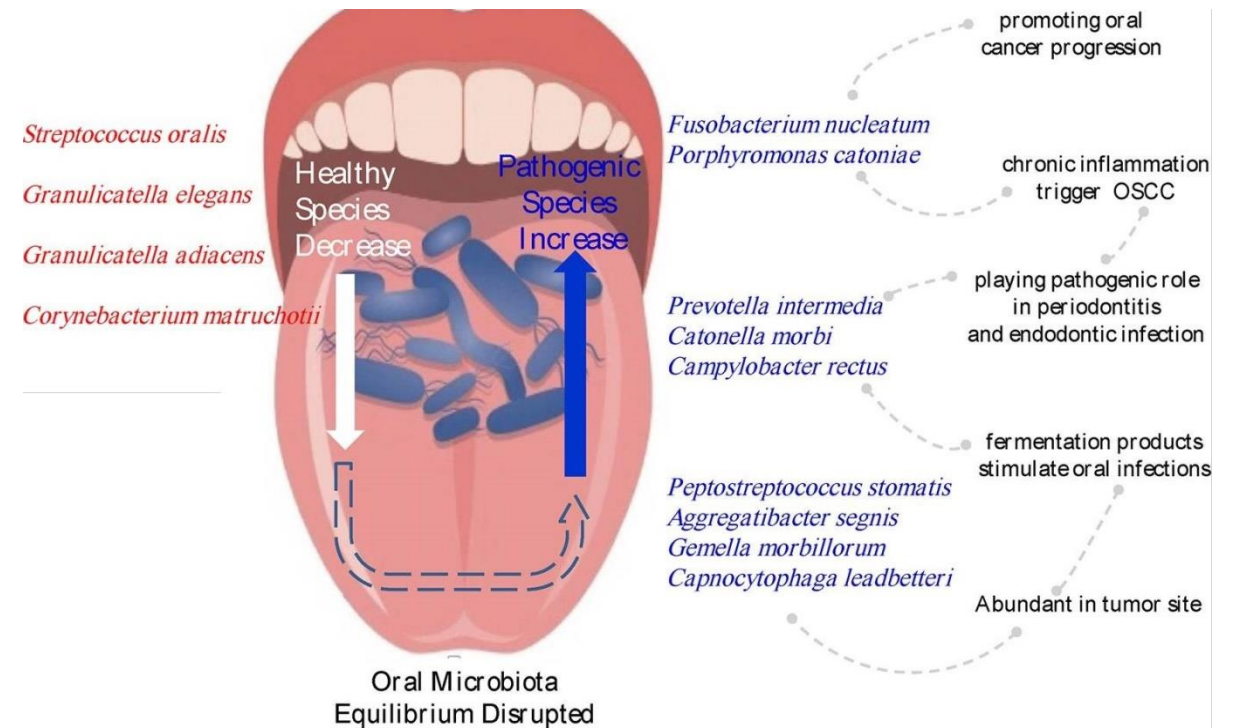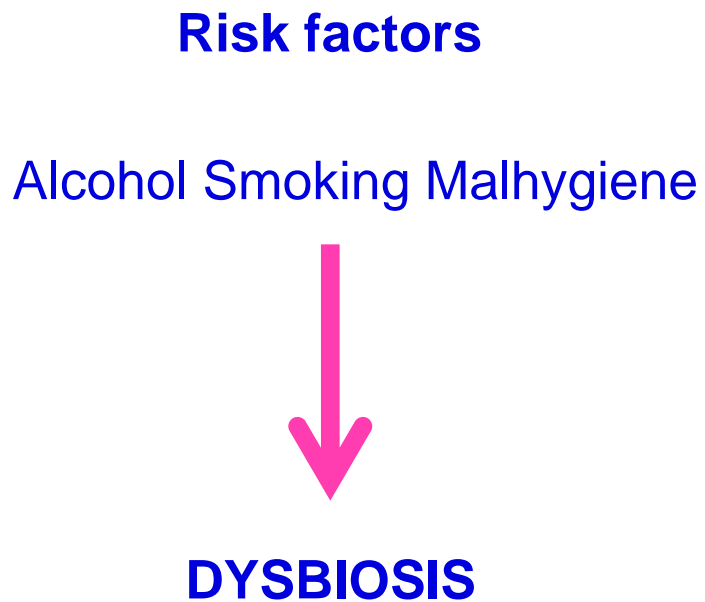
Risk factors

Alcohol Smoking Malhygiene

DYSBIOSIS

Zhang et al., 2020

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Oral microbiome in patients with head and neck cancer

Squamous cell carcinoma of the oral cavity
Oral dysbiosis

– Higher bacterial diversity in tumour

   samples than in the normal oral mucosa

– Several operational taxonomic units associated

   with *Fusobacterium* – good diagnostic potential

   for OSCC
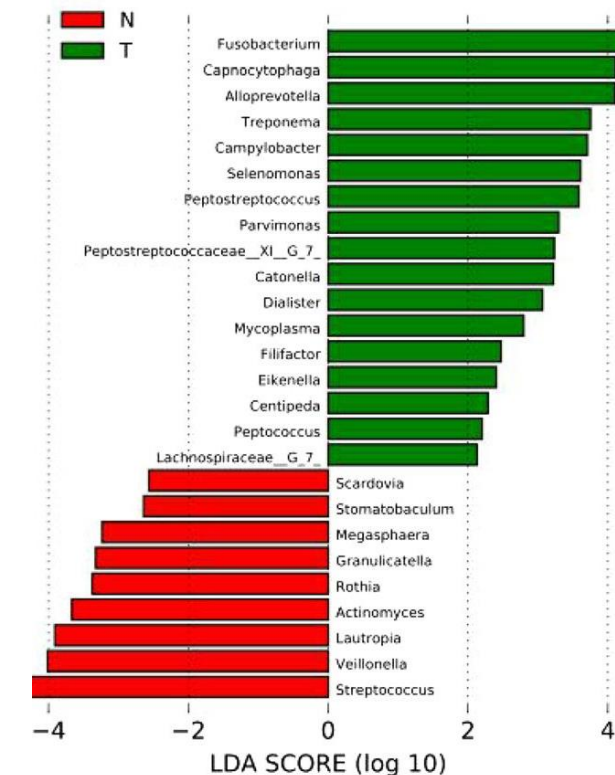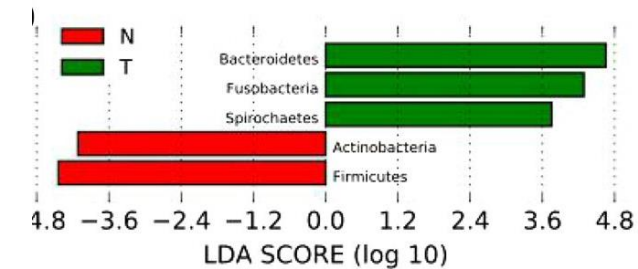
Zhao et al., 2017

Oral microbiome in patients with head and neck cancer

Squamous cell carcinoma of the oral cavity
Oral dysbiosis

— *P. gingivalis a F. nucleatum* associated with the OSCC, but the etiology is unclear

— impaired function of the originally "passive" microbiome in the microenvironment of the tumor

— does it contribute to the progression of the tumor by maintaining chronic inflammation?

| Production of cancerogenic metabolites | Inhibition of apoptosis |

Alkohol → Acetaldehyd

Buňky vrozené imunity

Oral epithelium

Mutations
DNA adducts
Mutations
→ STAT3
→ NF-kB

⊣ ATP/P2X7
→ PI3K /Akt
→ JAK1/STAT3 ⊣ p53
↑ miR-203   → β-catenin
→ NF-kB ↑ Cyclins
→ p38
→ PAR2/4

Activation of cellular proliferation

ROS, RNI, cytokiny

Pro-malignant microenvironment

MMPs
EMT
Stemness

| Induction of a chronic inflammation | Promotion of cellular invasion |

✿ Porphyromonas gingivalis   ⬭ Fusobacterium nucleatum   ◯ Streptococcus spp.   ◯ Neisseria spp.

Al-Hebshi et al., 2019

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Oral microbiome in patients with head and neck cancer

Al-Hebshi et al., 2019

Oral microbiome in patients with head and neck cancer

Study objectives

— Determination of risk factors (oral microbiome) for the tumor development and growth

— Analysis of interactions between microbiota and host in the context of oral squamous

cell carcinoma

— Demonstration or exclusion of the presence of bacterial DNA in

metastatic lymph nodes

Comparison of oral microbiota in patients with oral squamous cell carcinoma

depending on the condition of their dentition

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Oral microbiome in patients with head and neck cancer

Originality of the research project

— Oral microbiome as a predictive marker

— Species diversity of intratumorous microbiota

— Presence of microbiota in the metastatic tissue

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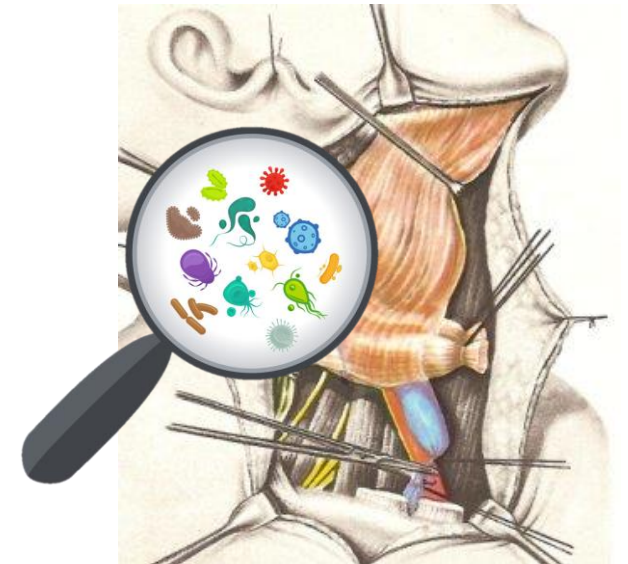
3D Virtual Planning and Reconstruction of the Lower Jaw

Project of cooperation with the commercial sphere

MUDr. et MUDr. Zdeněk Daněk, Ph.D.
Clinic of Maxillofacial Surgery, University Hospital Brno

3D virtual planning and reconstruction of the lower jaw

Reconstruction of the lower jaw with a free flap
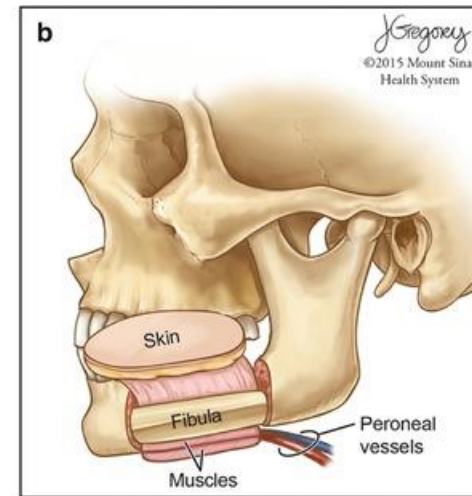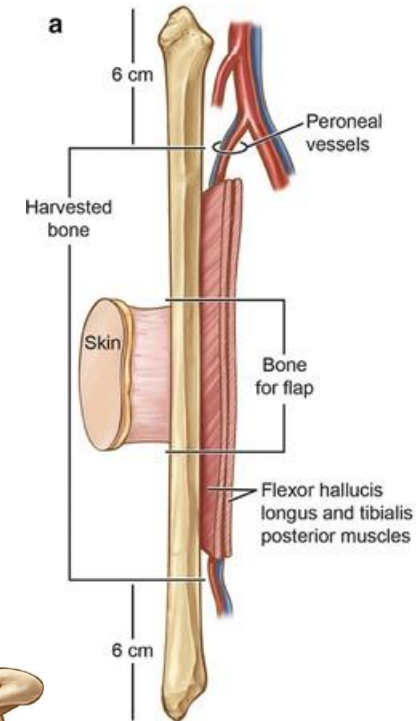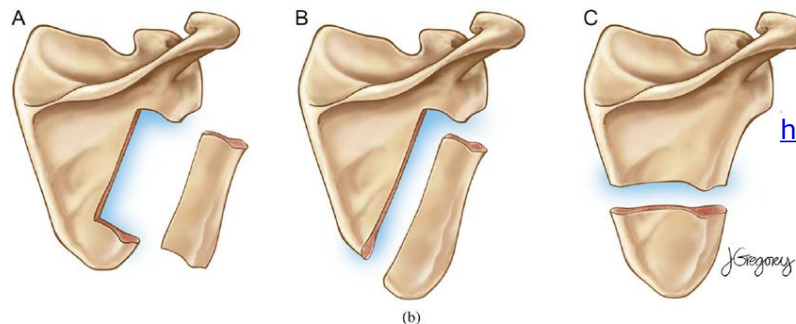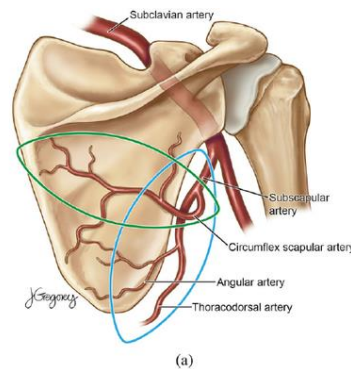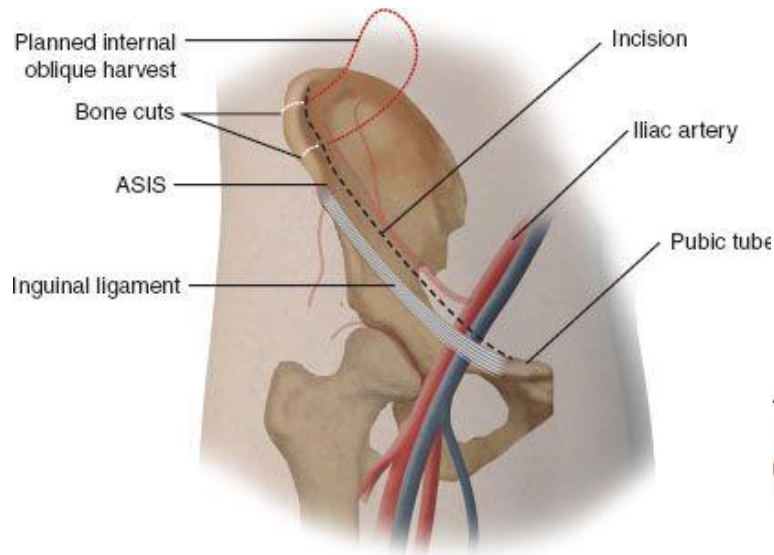
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3D virtual planning and reconstruction of the lower jaw

Reconstruction of the mandible with vascularized fibula flap - case report

- Squamous cell carcinoma of alveolus mandibule l.sin – G1, T4aN0M0

- Jaw resection with continuity break

- The need for reconstruction to restore functionality

  - (without reconstruction of the patient's disability permanent tracheostomy and gastrostomy)
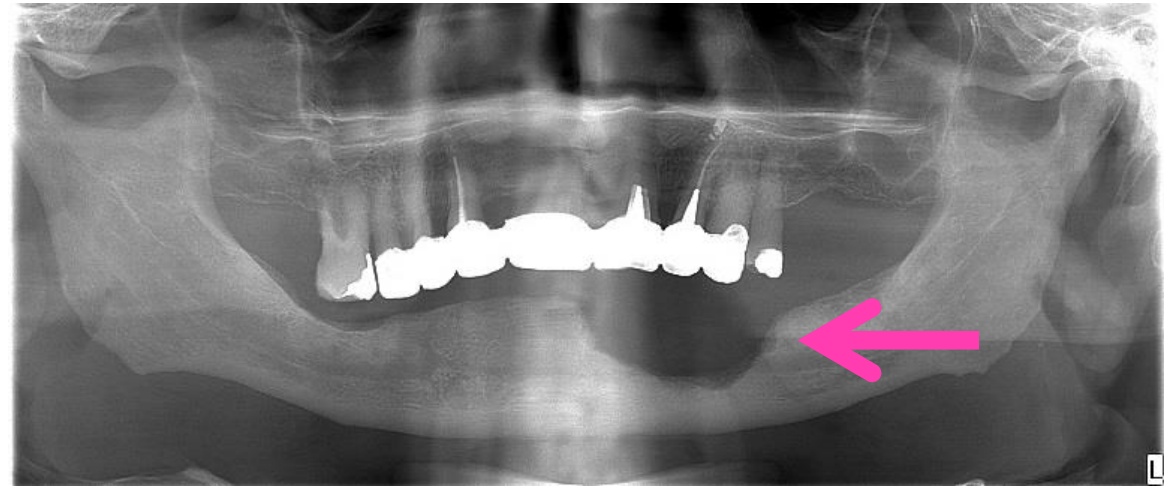
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3D virtual planning and reconstruction of the lower jaw

3D planning of lower jaw reconstruction
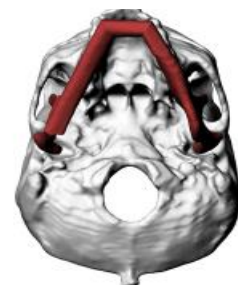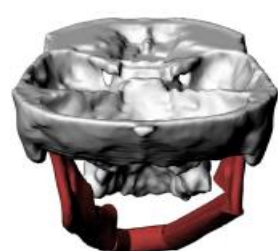
Virtual and printed model of the jaw with transferred fibular flap
(compared to the original mandibular shape)

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3D virtual planning and reconstruction of the lower jaw

3D planning of reconstruction of the lower jaw
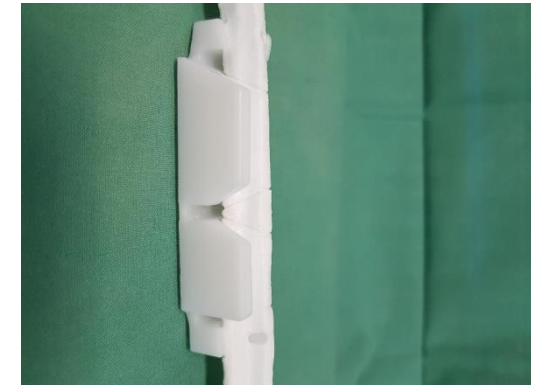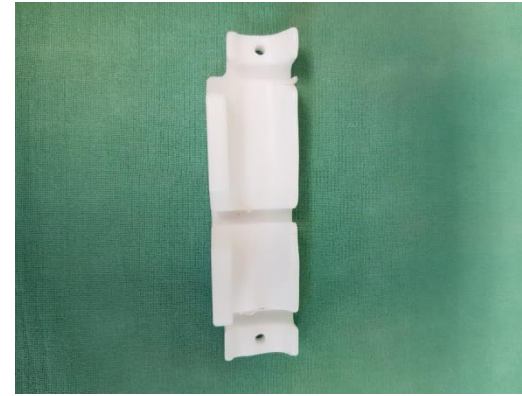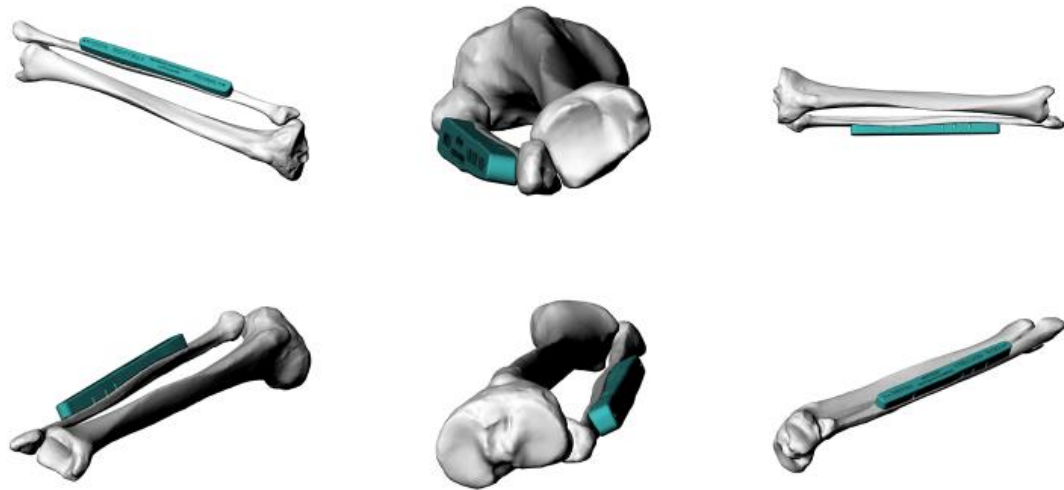
Virtual and printed fibula model with cutting guide

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3D virtual planning and reconstruction of the lower jaw

3D planning of lower jaw reconstruction

Printed mandible model with cutting guides

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3D virtual planning and reconstruction of the lower jaw

3D planning of lower jaw reconstruction
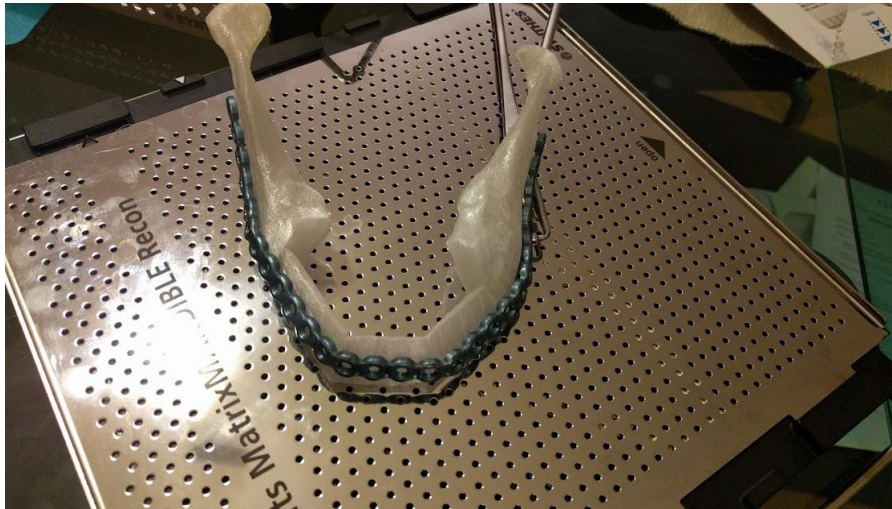
Preoperative preparation of the osteosynthetic titanium plate

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3D virtual planning and reconstruction of the lower jaw

The reconstruction of the lower jaw – flap harvesting
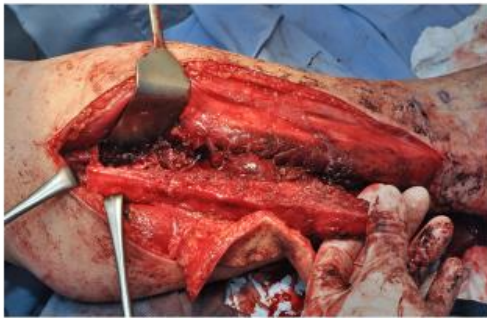
Harvesting of fibula flap, application of cutting guides on fibula and positioning of the flap into the reconstruction plate

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3D virtual planning and reconstruction of the lower jaw

Perioperation phase of lower jaw reconstruction
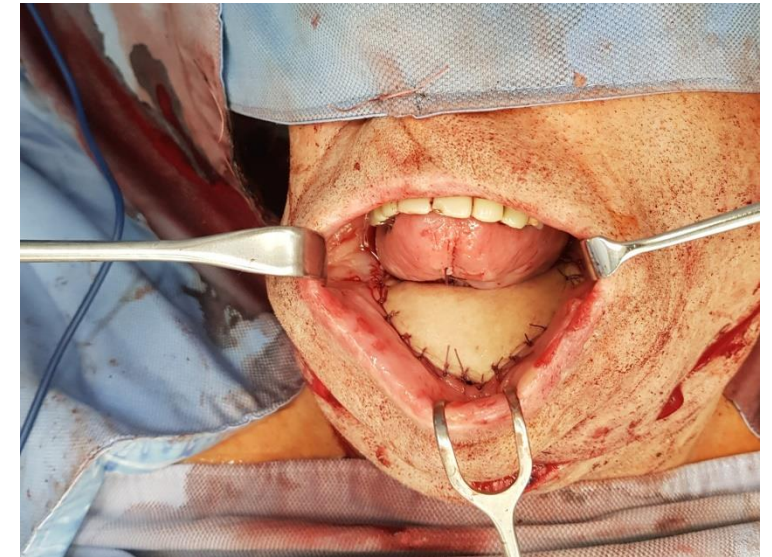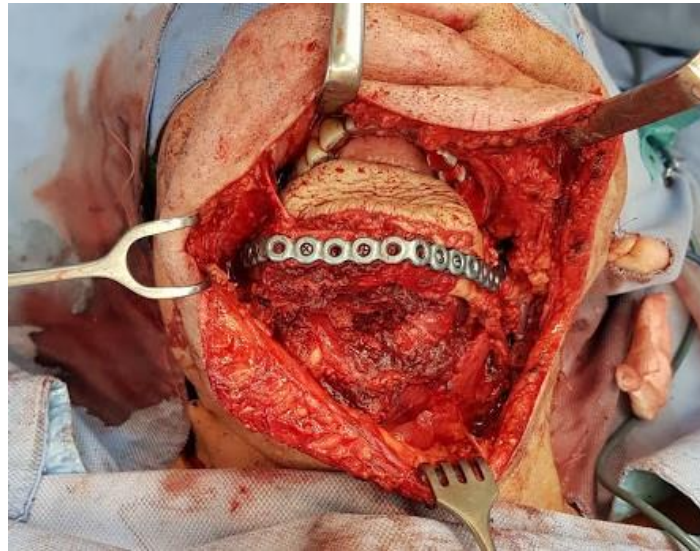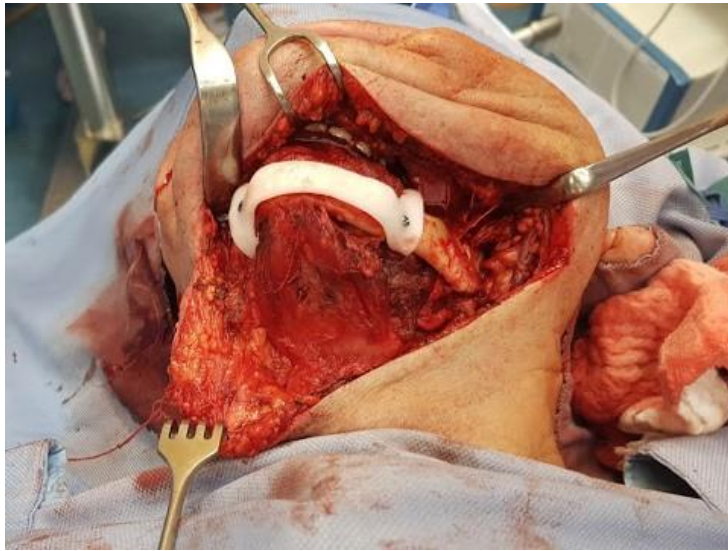
Tumor resection, flap positioning and final reconstruction of soft tissues of the oral cavity

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3D virtual planning and reconstruction of the lower jaw

Postoperative situation of the reconstruction of the lower jaw
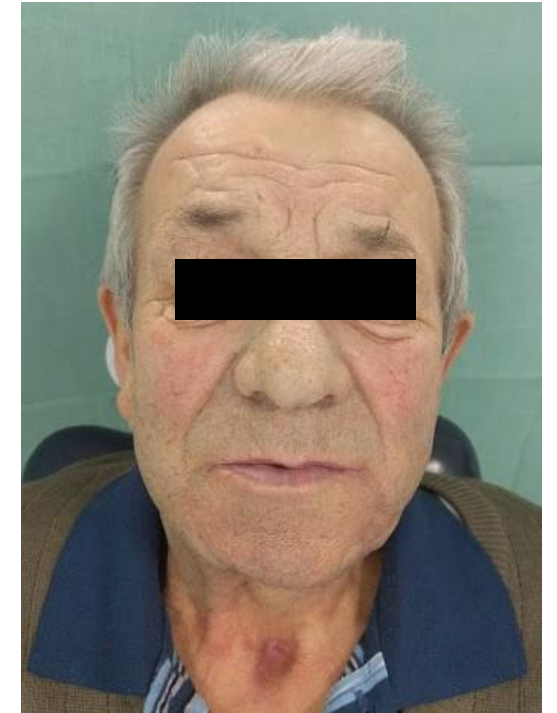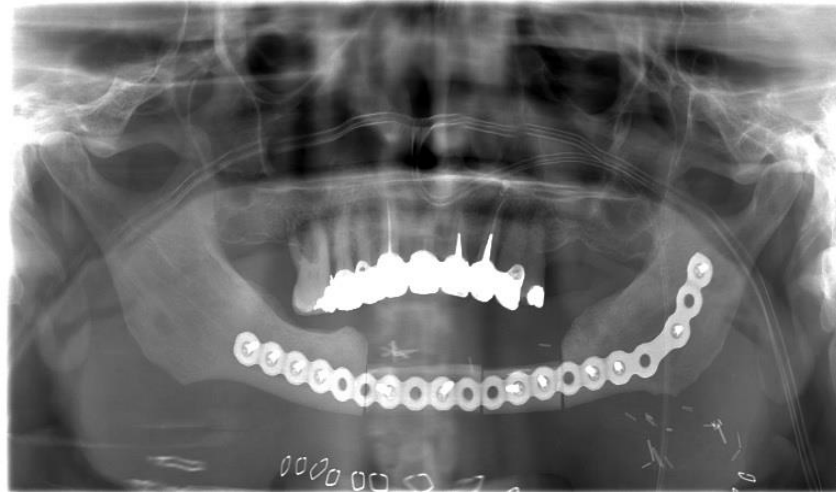
Postoperative soft tissues healing situation after six months, the situation on the X-ray, the resulting outcome of the patient

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3D virtual planning and reconstruction of the lower jaw

Economy of 3D planning reconstruction of the lower jaw

stryker®	**150.000,- Kč** • planning • cutting guides • reconstructive printed individual splint • is not in the Czech Republic
DePuy Synthes COMPANIES OF Johnson&Johnson	**100.000,- Kč** • planning • cutting guides • reconstructive printed individual splint
Česká cesta Laboratory 3D Digital Imaging and Modeling Laboratory	**50.000,- Kč** • planning • cutting guides • reconstruction plate Synthes + ZP

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3D virtual planning and reconstruction of the lower jaw
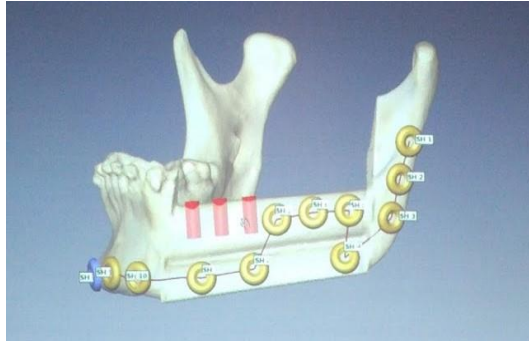
Biological accuracy

Do we need it?

Custom printed osteosynthetic plates

vs.

Conventional osteosynthetic plates

Is the pursuit of absolute precision in the planning of reconstruction operations the right way?

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Abbreviations used

- EC – ethylcellulose
- PEO – polyethylen oxid
- NaCMC – sodium carboxymethylcellulose
- VAS – visual analog pain scale
- IS – immune system
- NO – nitrous oxide
- MZ – monozygotic twins
- DZ – dizygotic twins
- GIT – gastrointestinal tract
- OSCC – squamous cell carcinoma of the oral cavity
- CR – Czech republic
- G1 – well differentiated cancer

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Modern methods in the study of the development and maintenance of the vitality of the tooth

Mgr. Jan Křivánek, Ph.D.
Department of Embryology and Histology
Faculty of Medicine, Masaryk University, Brno

jan.krivanek@med.muni.cz

Introduction of the researcher and his team

Jan Křivánek, Department of Histology and Embryology, LF, MU

– Our research group is primarily concerned with the study of **tooth development**, its **regeneration**, **reparative processes, physiology,** and the study of **functional microstructure**.

– We study the processes that lead to the development of complex organ systems from initial **stem cells to fully differentiated functional systems**.

– The vast majority of our research takes place **in vivo**. As a model system, we use a number of **various genetically modified mouse** strains and the latest methods of genetic labelling, visualization methods or **transcriptomics** at the unicellular level (scRNA-seq).

– We combine **some results obtained from the research of developmental and regenerative processes** in model systems with the knowledge of human medicine and thus try to reveal the potential of human regenerative medicine.

https://www2.med.muni.cz/histology/jan-krivanek/

🐦 @KrivanekLab

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Modern methods in the study of the development and maintenance of the vitality of the tooth

Content of the lecture

— Tooth = living organ

— Continuously growing teeth as a model system for studying development – why a mouse?

— Possibilities of genetic mapping of in vivo development Single-cell RNA-seq (Single-cell transcriptomics)

— Modern 3D imaging methods (lightsheet and confocal microscopy, live-imaging, μCT, FIB-SEM)

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Tooth = living organ

- **Primary function of teeth:** Food intake and processing

- **Secondary functions of teeth:** Speech articulation, nonverbal communication, sensory properties

- **In an adult tooth, only enamel (**avascular, no nerves, no cells**)** is inanimate, the rest of the tooth is alive, innervated, vascularly supplied and metabolically active tissue

- **The tooth reacts to stimuli from the surrounding area –** tertiary dentin formation, inflammation, activation of stem cells, changes in anchoring to the dental bed (principle of orthodontics)

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Continuously growing incisors – why a mouse?

Incisor

Molar

Man

Mouse

2-1-0-2
2-1-2-3

1-0-0-3

Continuously growing incisors – why a mouse?

Continuously growing mouse incisor

Incisor

Dentin   Molars

Enamel

Pulp

Growth area with stem cells

LiCL

LaCL
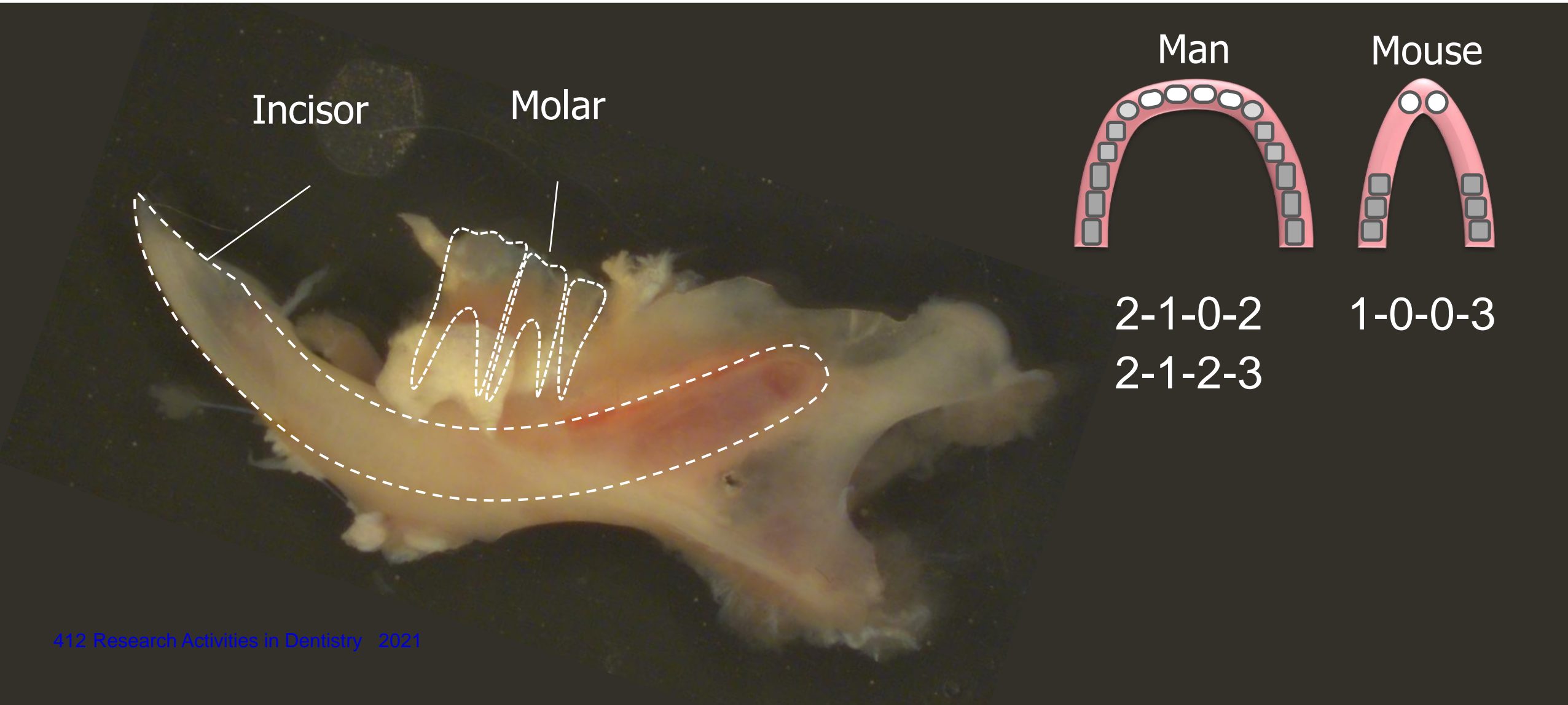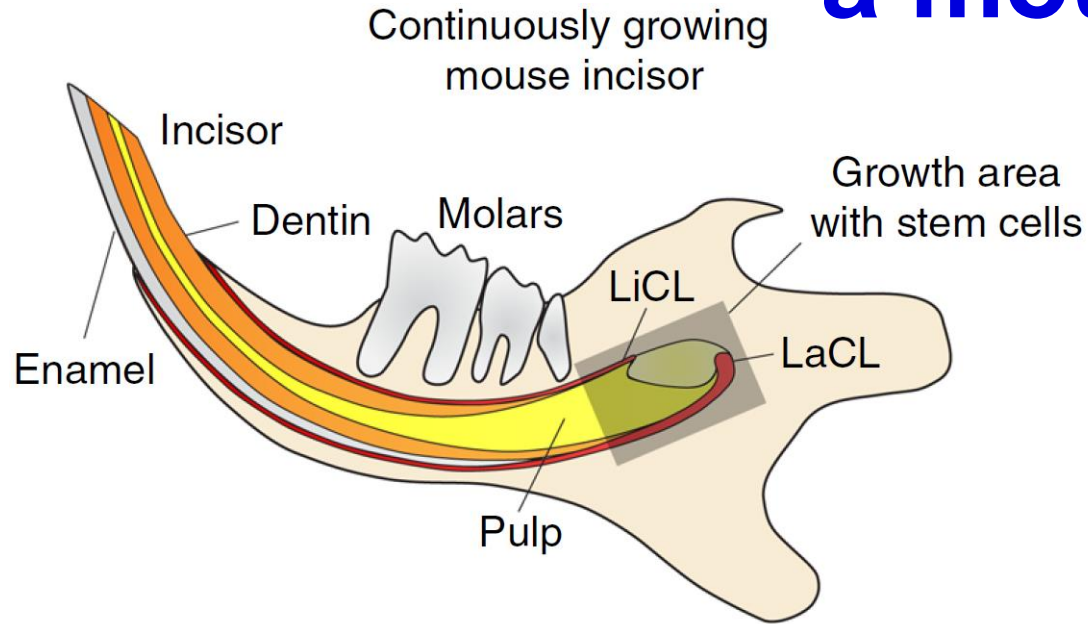
- Characteristics of mouse dentition

- Dental formula 1-0-0-3

- Monophyodont, heterodontous dentition

- Hypselodont incisors

- Brachyodont molars

µCT mouse head scan with enamel highlighted

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Continuously growing incisors – why a mouse?
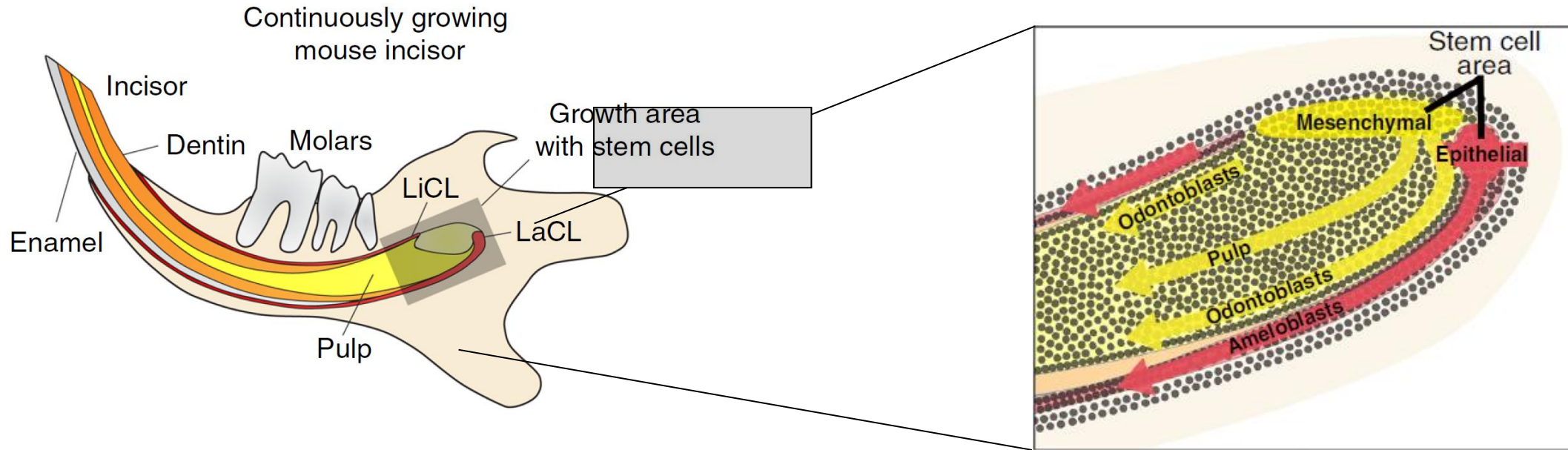
Continuously growing complex organ - the time of complete tooth restoration is about 4 weeks
Precisely controlled growth and its control - after pinching the end of the tooth, growth accelerates
Continuously active dual-type stem cell niche (mesenchymal and epithelial)
Possibility to study epithelio-mesenchymal interactions and differentiation processes
Model system for the study of amelogenesis and dentinogenesis and general odontogenesis

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Why a mouse?

- **The mouse is a model organism of mammals**

- Relatively easy care and handling

- Biological resemblance to humans

- Short generation time, short pregnancy time

- Higher number of offspring in litter (5-15)

- Inbred strains available

- Genome sequenced and studied in detail

- Tens of thousands of genetic modifications available

Wikimedia Commons

The Jackson Laboratory

>11500 kmenů geneticky definovaných myších kmenů

IMPC
International Mouse Phenotyping Consortium

6440 ofenotypovaných mutací různých genů

(1. 10. 2020)

ALLEN BRAIN ATLAS
DATA PORTAL

Expresní databáze během vývoje i v dospělosti

a další...

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Single-cell RNA-seq

Analysis of gene expression at the level of individual cells

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Continuously growing mouse incisor

Growth area with stem cells

Stem cell area

Mesenchymal

Epithelial

Odontoblasts

Pulp

Odontoblasts

Ameloblasts

In vivo validation

FACS

Bioinformatic analysis

Enzymatic digestion
Single cells preparation

Single-cell RNA-seq
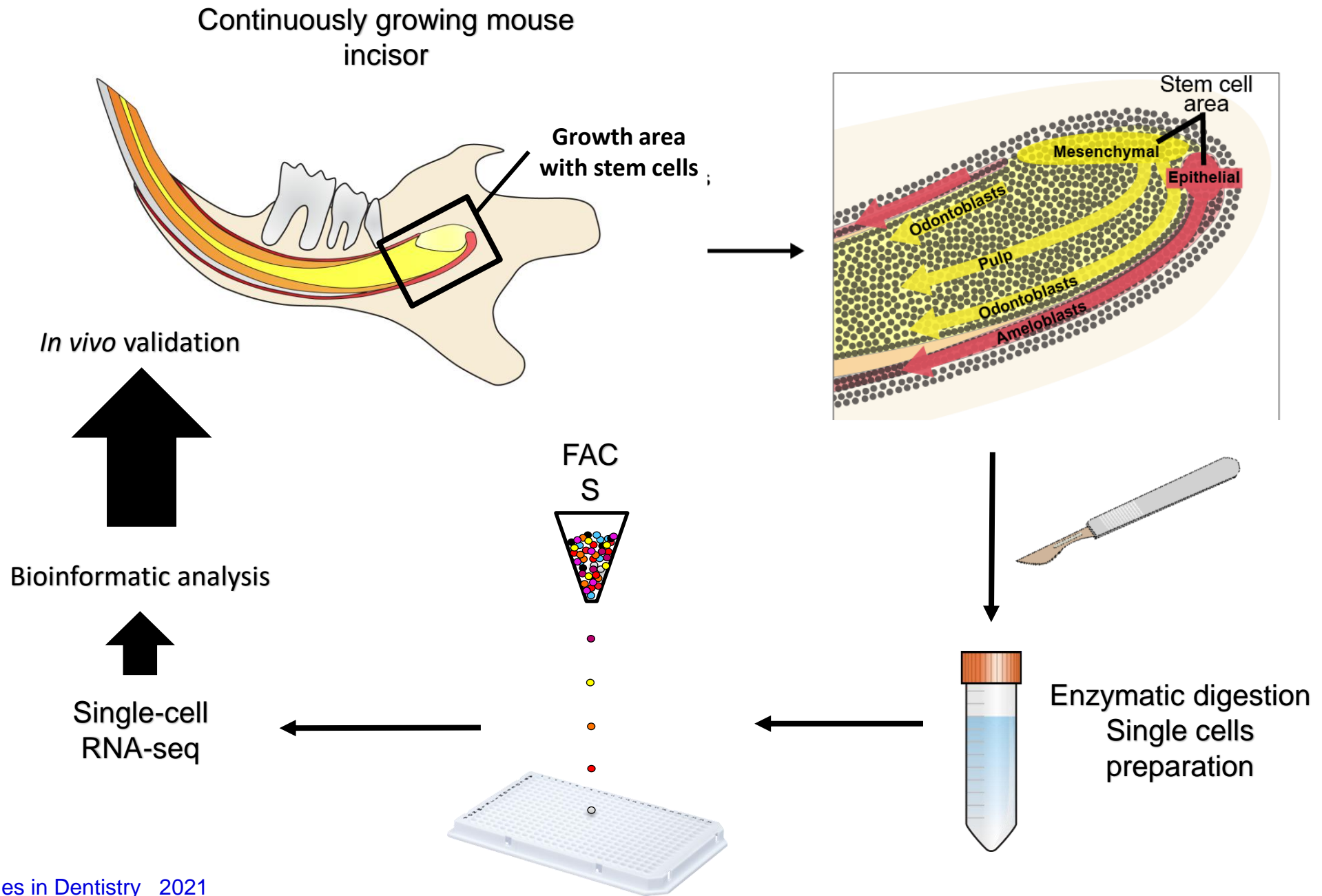
Why is scRNA-seq such a revolutionary method?

Northern blot — **Tens of thousands of cells needed to analyze expression of 1 gene**

qRT-PCR — **Thousands of cells needed to analyze expression of 1 gene**

Chip array — **Thousands of cells needed to analyze the expression of tens to hundreds of genes**

Single cell RNA-seq — **Analysis of the expression of thousands of genes from each individual cell**

1 Cell
(each cell)

→
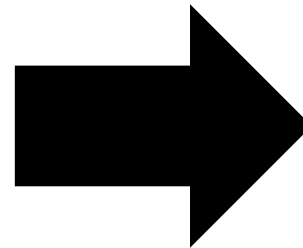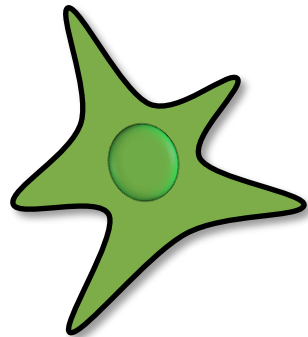
"COMPLETE"
transcriptome

Coding genes
   Transcription factors
   Ligands
   Receptors
   Structural genes
   …
miRNA's
Spliced variants
RIK's
…

What type of information do we get?

- **Qualitative information on gene expression**

- **Quantitative information on gene expression**

"Hardcore bioinformatics analysis"

- Clustering of cells according to similarities based on their gene expression

- Defining major and minor cell populations

- Identification of unknown cell populations

- Detection of expression of new genes in known or unknown populations

- Determination of the function of unknown cell populations; Mapping of the "Gene Regulatory Network" → monitoring of development paths

- Creating an interaction scheme - mutual interaction of various cell types (ligands and receptors)

Isolated cells
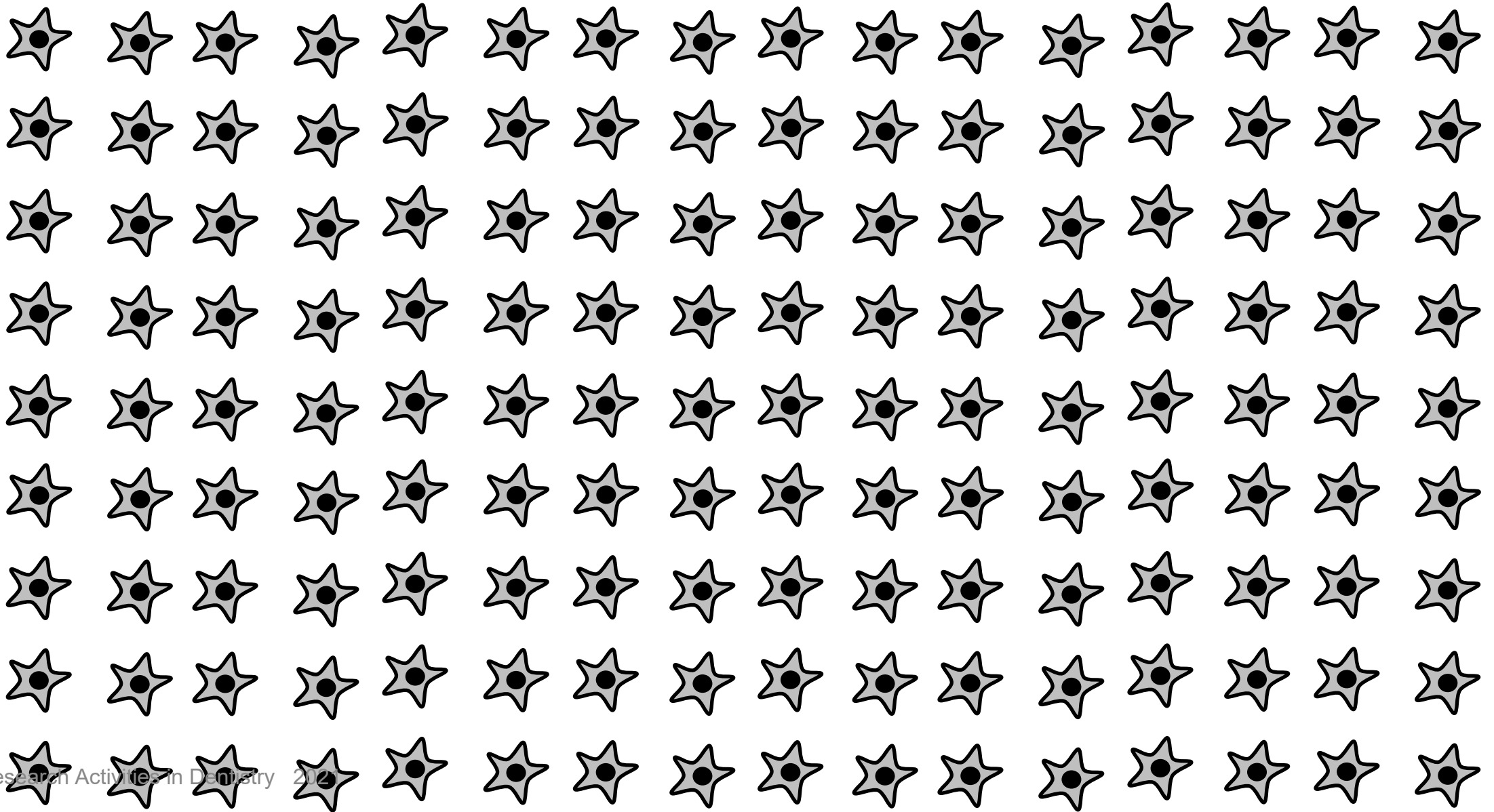
Analyzed cells

Clustering according to selected parameters (gene expression)

https://www.polygon.com/
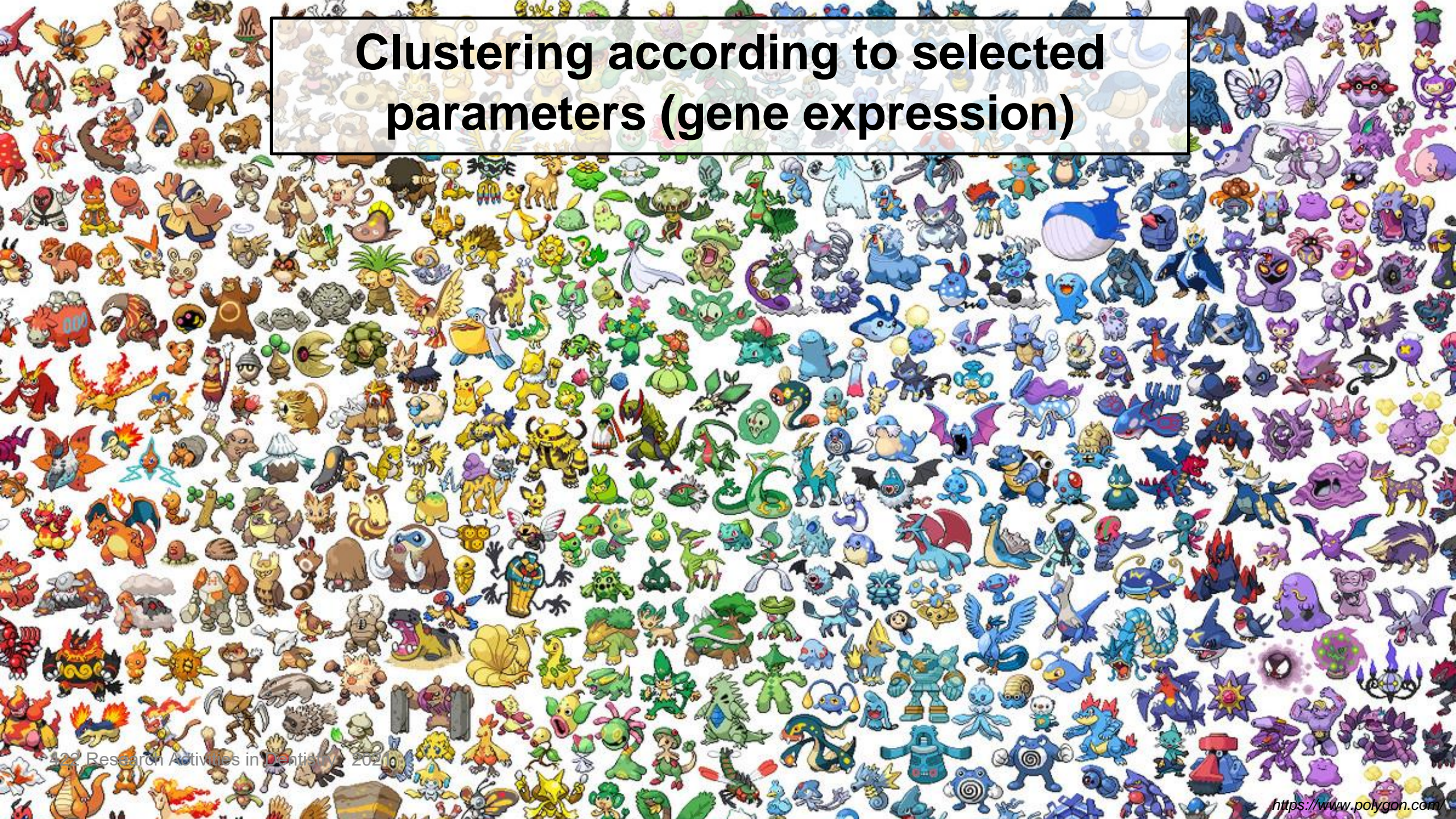
Features and interactions

By analyzing the properties (gene expression values) of individual cells, we can predict how these cells will mutually interact (expression of different combinations of ligands and receptors) and how they are prepared for these interactions (expression of effector genes of different signaling pathways).
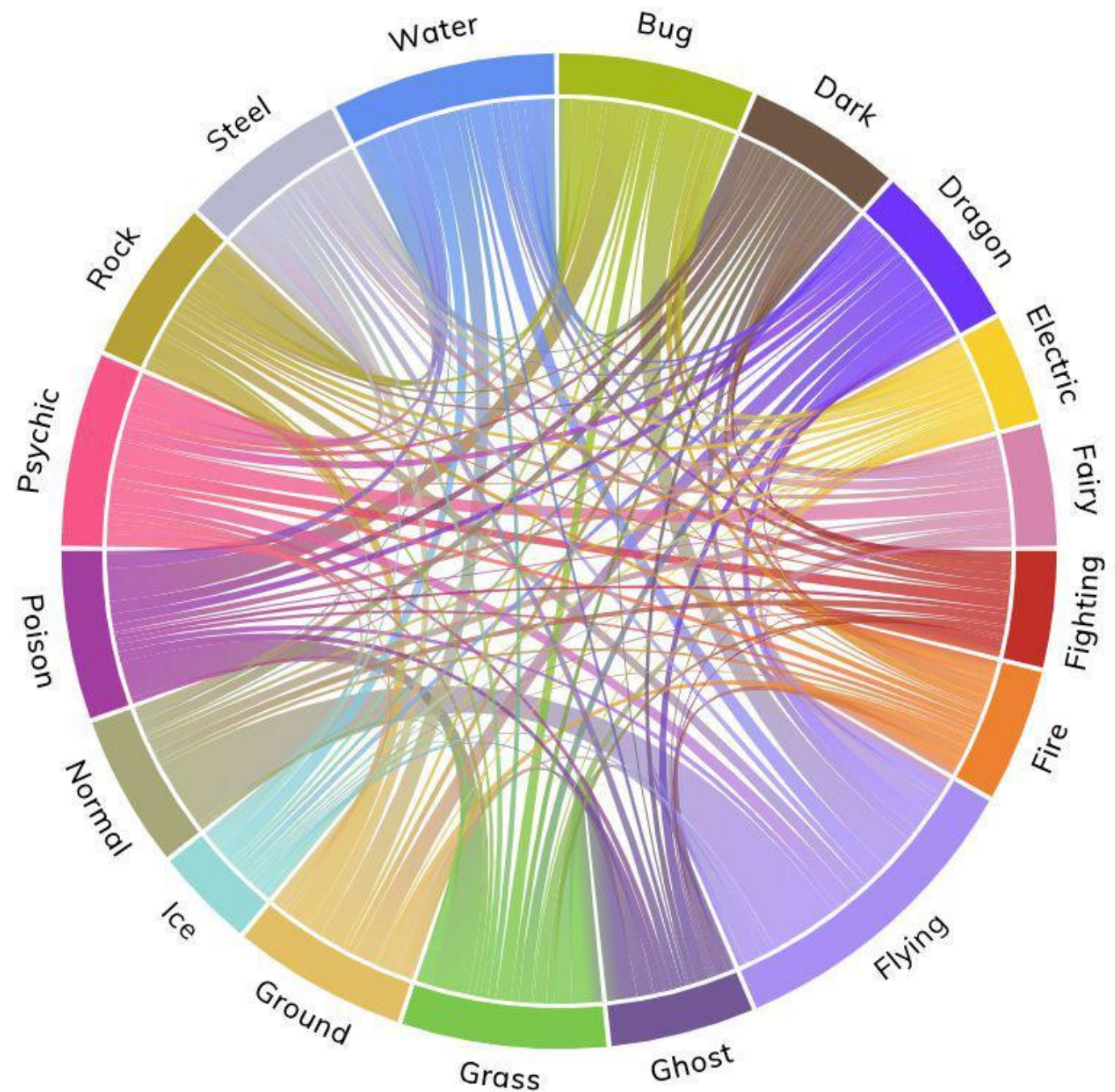
Development

By analyzing the similarities in the gene expression of individual cells, we are able to predict not only which cells are more similar or different, but also to **determine which cell is developmentally younger (more "stem") or developmentally differentiated ("adult").**
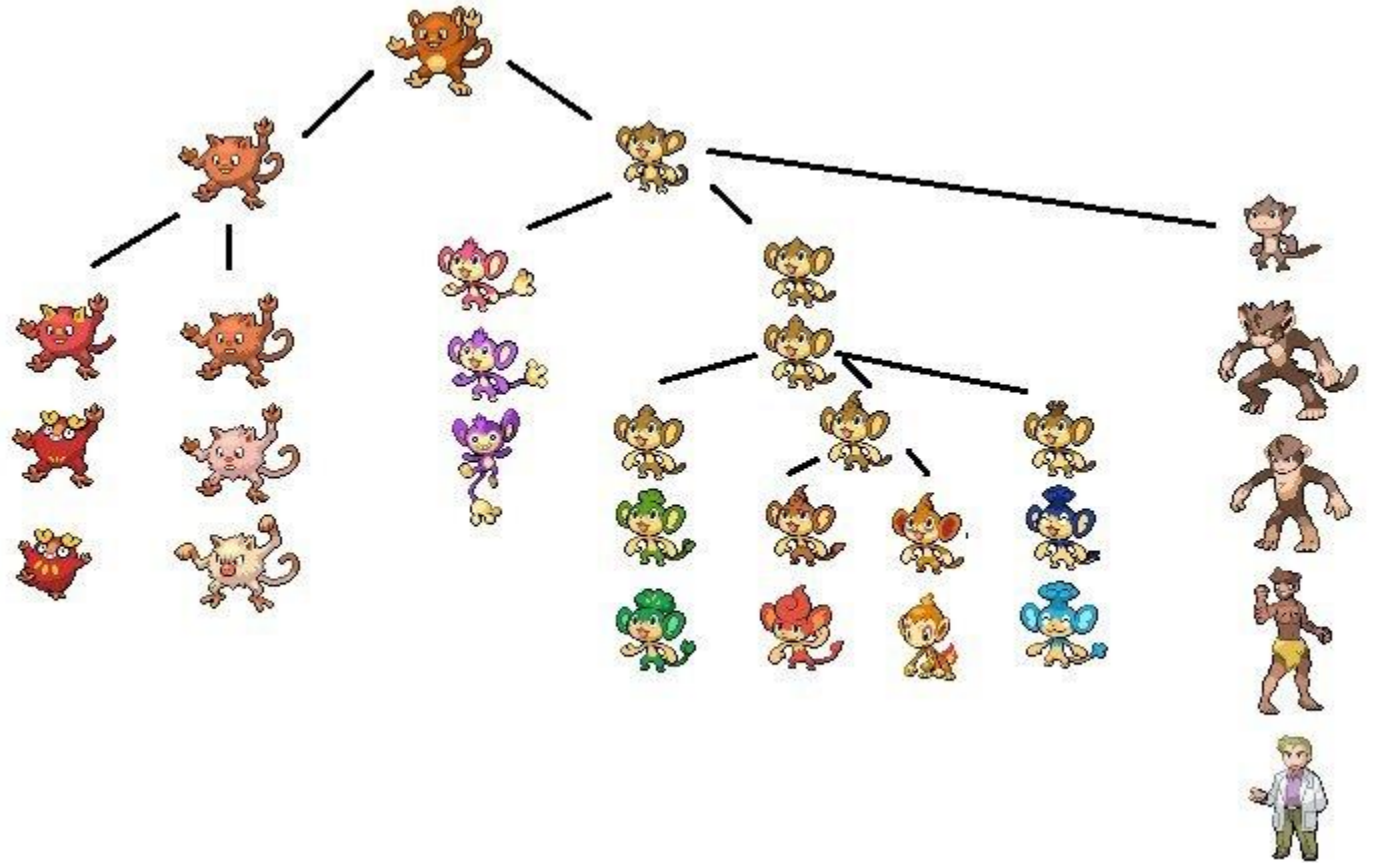
Creation of a "Dental Cell Type Atlas"

Complex organ in the living organism

scRNA-seq

Analysis
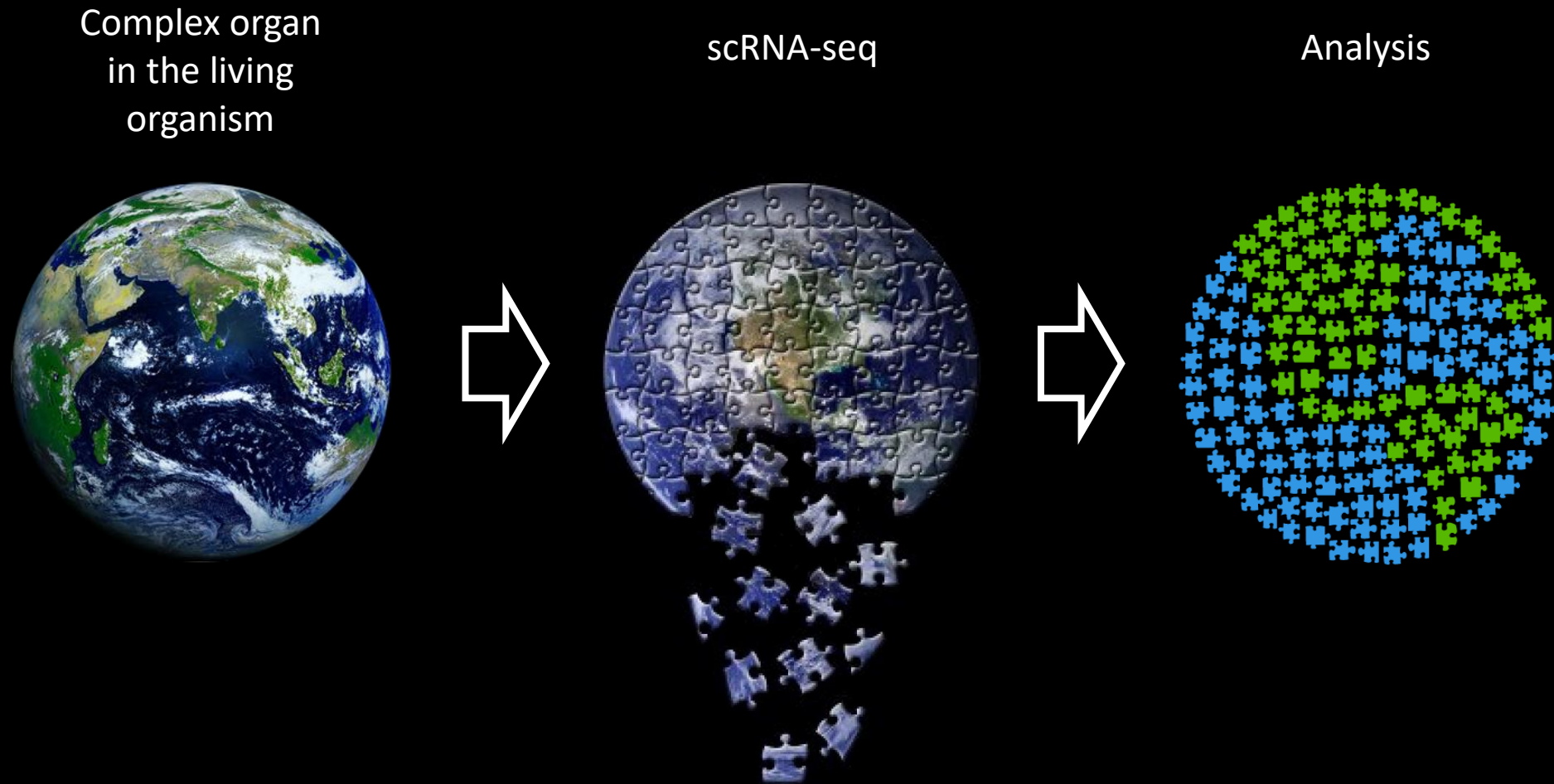
Cdh5
Plvap
Tie1
Ptprb
Eltd1
Ecscr
Robo4
Ushbp1
Myct1
Podxl
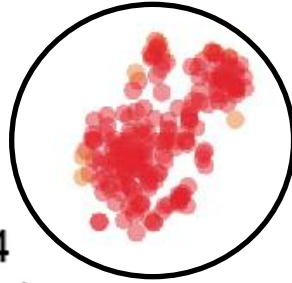
t-SNE
(T-distributed Stochastic Neighbor Embedding)
Machine learning algorithm for visualization
Nonlinear dimensionality reduction technique

Krivanek et al. 2020
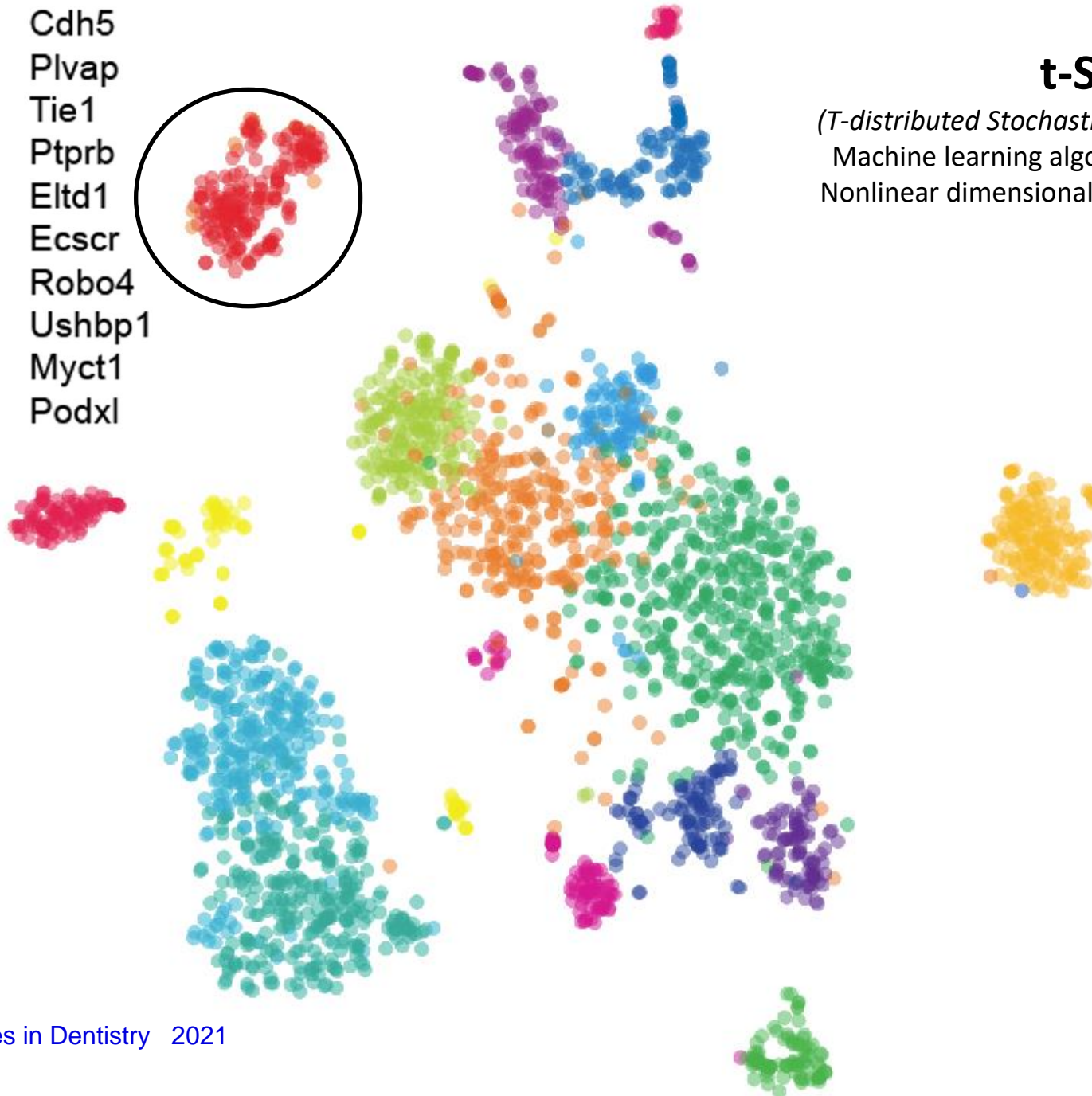
Endothelial cells

Cdh5
Plvap
Tie1
Ptprb
Eltd1
Ecscr
Robo4
Ushbp1
Myct1
Podxl

COL4
CDH1
DAPI

Pulp

Blood
vessels

Ameloblasts

Endothelial cells

OEE

SI + SR

Innate
leukocytes

Distal
pulp

Preodontoblasts

Glia

Lymphocytes

Dental pulp cells

Apical
pulp

Dental follicle
cells

Macrophages

Lyve1+
macrophages

Alveolar
osteocytes

Perivascular cells
(Pericytes and
smooth muscle cells)

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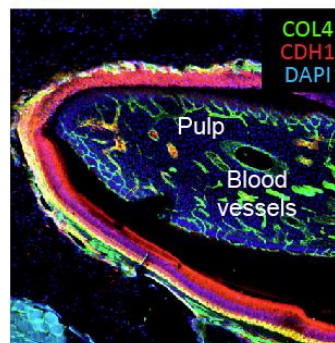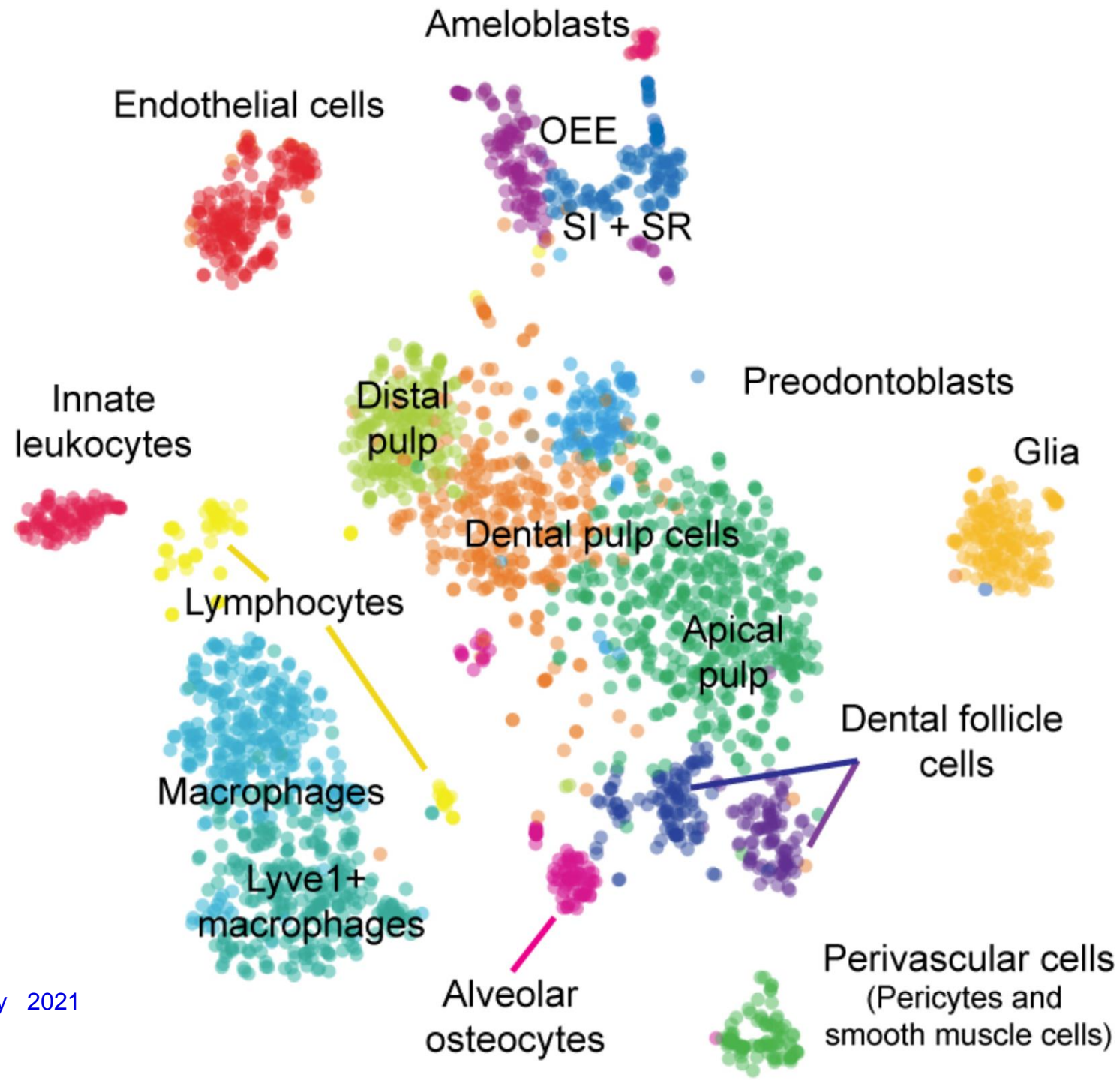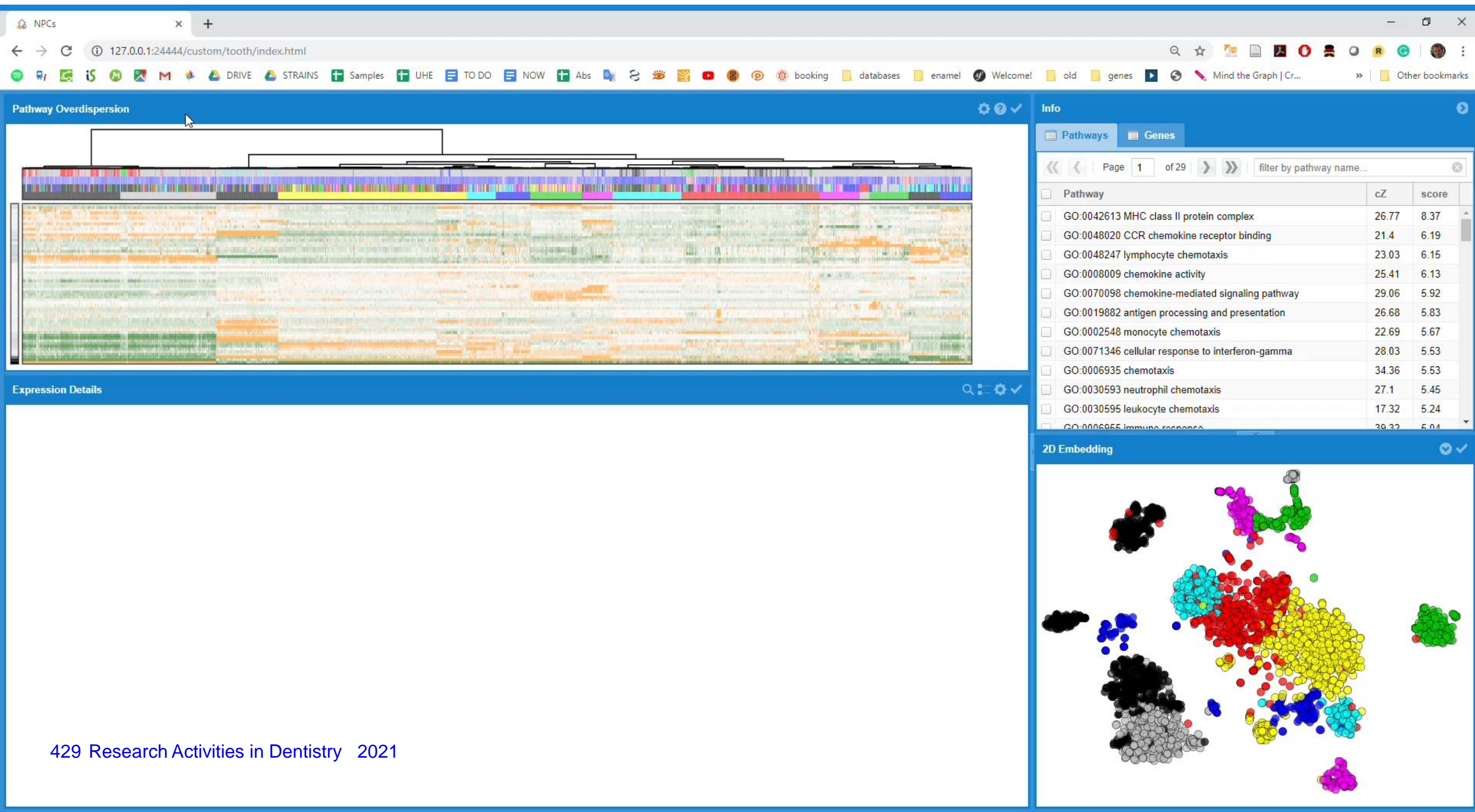
Detailed analysis of dental EPITHELIUM

Dental cell types of epithelial origin

Ameloblasts
(Inner enamel epithelium)

Outer enamel epithelium

Stellate reticulum

Stratum intermedium

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Epithelial cells

Ameloblasts
OEE
SI + SR

Endothelial cells

Innate leukocytes

Distal pulp

Dental pulp cells

Preodontoblasts

Glia

Lymphocytes

Apical pulp

Dental follicle cells

Macrophages

Lyve1+ macrophages

Alveolar osteocytes

Perivascular cells
(Pericytes and smooth muscle cells)

8 **Stratum intermedium**
Rab3il1
Pmch
Cyp2s1

4 **Outer enamel epithelium**
Slco4a1
Th
Amer1

13 SFRP5+ progenitors
Sfrp5
Grp
Rhoc

9 **Stratum intermedium**
Psmb10
C1qb
Ibsp

1 **Cuboid layer of SI**
Thbd
Gnrh1
Jph4

7 OEE progenitors
Fos
Egr1
Vrtn

6 **Am. Protectory**
Gm17660
Slc5a8
Ptpn22

3 Am. Ryr2+
Ryr2
Mylk
Sox5

12 SI progenitors
Cdh6
Lrp11
Cpne5

10 **Am. Maturation**
Klk4
Gpr155
Slc34a2

5 **Am. Secretory**
Enam
Amelx
Ctnna2

11 Preameloblasts
Col22a1
Vwde
Kif5c

2 Stellate reticulum
Vat1l
Fam19a4
Hey2

Differentiation state

Krivanek et al. 2020

Differentiation trajectories of ameloblasts
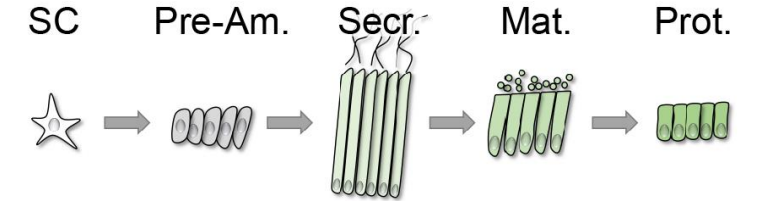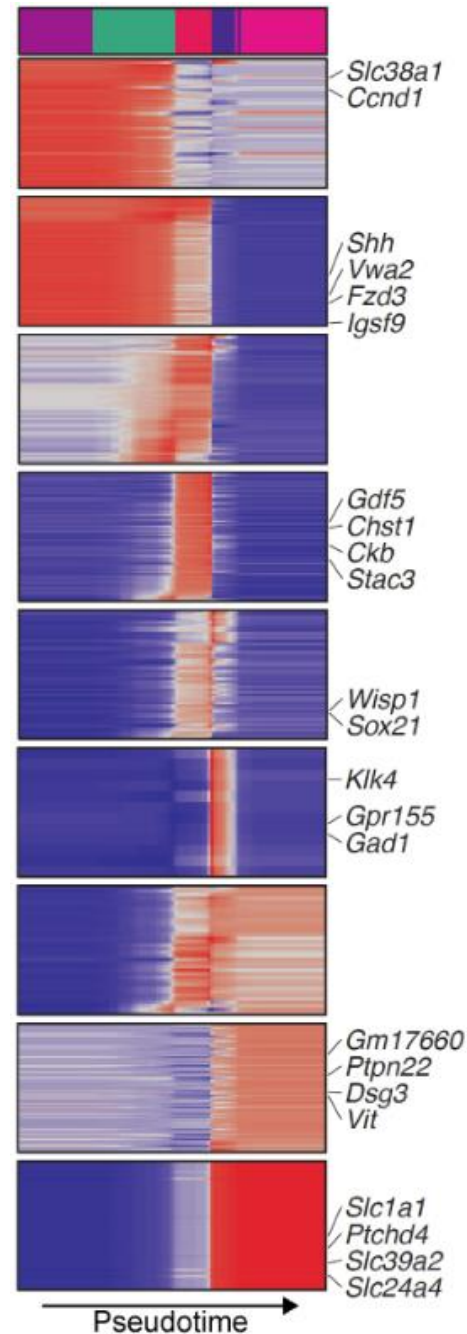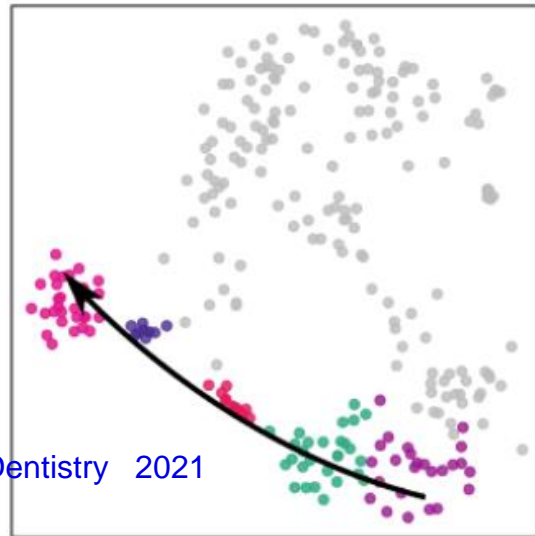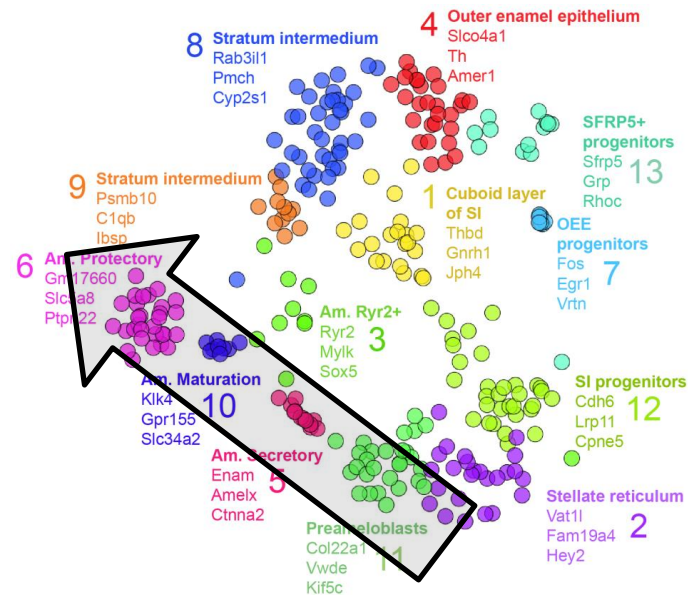
Transition from preameloblasts into all ameloblast stages mapped

Krivanek et al. 2020

Cuboidal layer
newly described cell type in the tooth

8 **Stratum intermedium**
Rab3il1
Pmch
Cyp2s1

4 **Outer enamel epithelium**
Slco4a1
Th
Amer1

SFRP5+ progenitors
Sfrp5
Grp 13
Rhoc

9 **Stratum intermedium**
Psmb10
C1qb
Ibsp

1 **Cuboid layer of SI**
Thbd
Gnrh1
Jph4

OEE progenitors
Fos
Egr1 7
Vrtn

6 **Am. Protectory**
Gm17660
Slc5a8
Ptpn22

Am. Ryr2+
Ryr2
Mylk 3
Sox5

Am. Maturation
Klk4
Gpr155 10
Slc34a2

SI progenitors
Cdh6
Lrp11 12
Cpne5

Am. Secretory
Enam
Amelx 5
Ctnna2

Preameloblasts
Col22a1
Vwde 11
Kif5c

Stellate reticulum
Vat1l
Fam19a4 2
Hey2

Oxygen and ion transportation   Cygb, Nphs

Backbone - junctional complexes   Kirrel2, Jph4

Supportive role in ameloblasts differentiation   Gnrh1, Paqr5

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https://pocketdentistry.com/7-enamel-composition-formation-and-structure/

Krivanek et al. 2020

RYR2⁺ ameloblasts

8 Stratum intermedium
Rab3il1
Pmch
Cyp2s1

4 Outer enamel epithelium
Slco4a1
Th
Amer1

SFRP5+ progenitors
Sfrp5
Grp
Rhoc **13**

9 Stratum intermedium
Psmb10
C1qb
Ihhp

1 Cuboid layer of SI
Thbd
Gnrh1
Jph4

OEE progenitors
Fos
Egr1
Vrtn **7**

6 Am. Protectory
Gm17660
Slc5a8
Ptpn22

Am. Ryr2+
Ryr2
Mylk
Sox5 **3**

Am. Maturation
Klk4
Gpr155
Slc34a2 **10**

SI progenitors
Cdh6
Lrp11
Cpne5 **12**

Am. Secretory
Enam
Amelx
Ctnna2 **5**

Stellate reticulum
Vat1l
Fam19a4
Hey2 **2**

Preameloblasts
Col22a1
Vwde
Kif5c **11**

Cation channels   Piezo2,   Trpm2,   Trpm3, Trpm6

Calcium-dependent genes   Itpr1, Ryr1, Ryr2

Krivanek et al. 2020

OEE progenitors

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Krivanek et al. 2020

Detailed analysis of the dental PULP

RNA-velocity analysis

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Krivanek et al. 2020

Detailed analysis of the dental PULP

Foxd1 Sox9

Mki67 Gli1

Fgf10 Igfbp3

Fgf3 Thy1

Ameloblasts
Endothelial cells
OEE
SI + SR
Innate leukocytes
Distal pulp
Preodontoblasts
Dental pulp cells
Glia
Apical pulp
Lymphocytes
Macrophages
Dental follicle cells
Lyve1+ macrophages
Alveolar osteocytes
Perivascular cells (Pericytes and smooth muscle cells)

Apical pulp
Odontoblasts
Distal pulp

Preodontoblasts

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Krivanek et al. 2020

Odontoblast branch analysis

Late odontoblasts
Dspp
Dmp1

Odontoblasts differentiation

Near-CL area

Pre-odontoblasts
Sall1
Notum
Dkk1

Early odontoblasts
Wisp1
Col24a2
Slc8a3

Dkk1 Col1a1
Notum Sall1
Wisp1
Dmp1 Dspp

Odontoblasts branch differentiation

Tnc
Postn
Moxd1
Twist2
Alx3
Etv4
Tgfbr2
Klf4
Bmp4
Msx2
Fam20a
Wnt10a
Notum
Sall1
Fgf10
Wnt6
Wisp1
Col1a1
Ifitm5

Pseudotime
Odontoblast precursors → Odontoblasts

Validation of identified odontoblast branch

od. pulp LaCL

b a

SALL1
DAPI

pulp

NOTUM
DAPI

od.

a

pre-od.

am.

pulp

b

od.

am.

HUMAN
MOLAR

SALL1
DAPI

pulp

od.
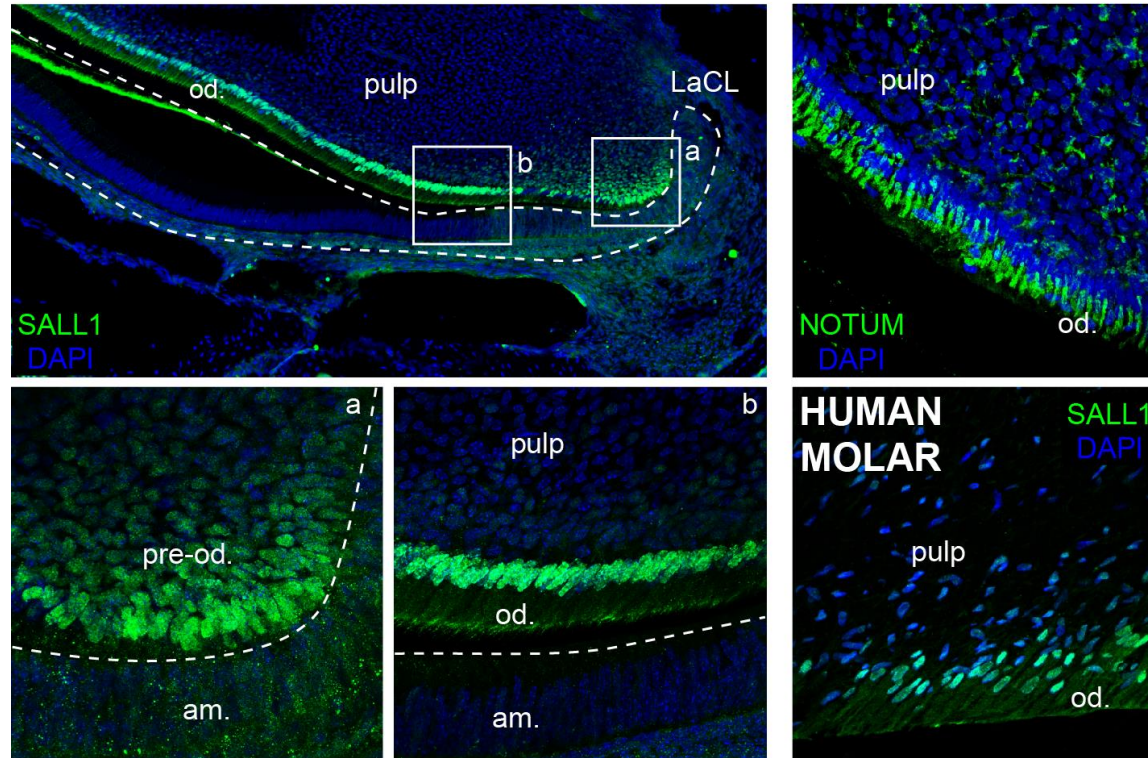
Building the map leading into **ODONTOBLAST** specification

Near-CL Pre-Od. Early Od. Late Od.

Krivanek et al. 2020
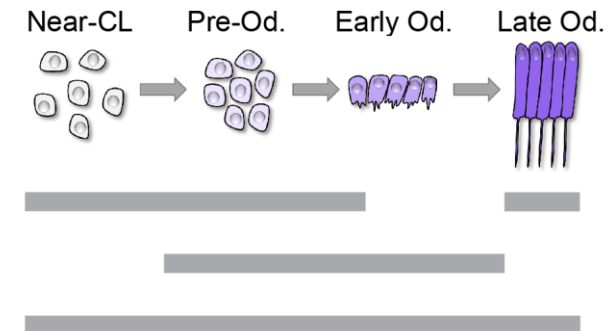
Analysis of the developmental dynamics of the dental pulp

Apical pulp

Odontoblasts

Distal pulp

RNA-velocity analysis
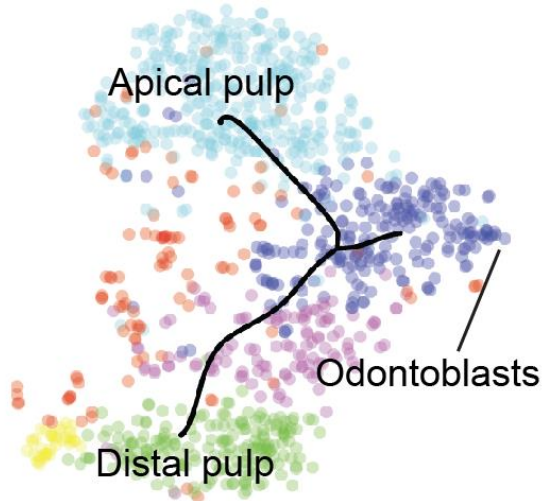
Non-mature population selection

non-mature pulp cluster

commited to preodontoblasts

progenitor population

likely biologically driven ICs

shuffled expression matrix

stability

IC
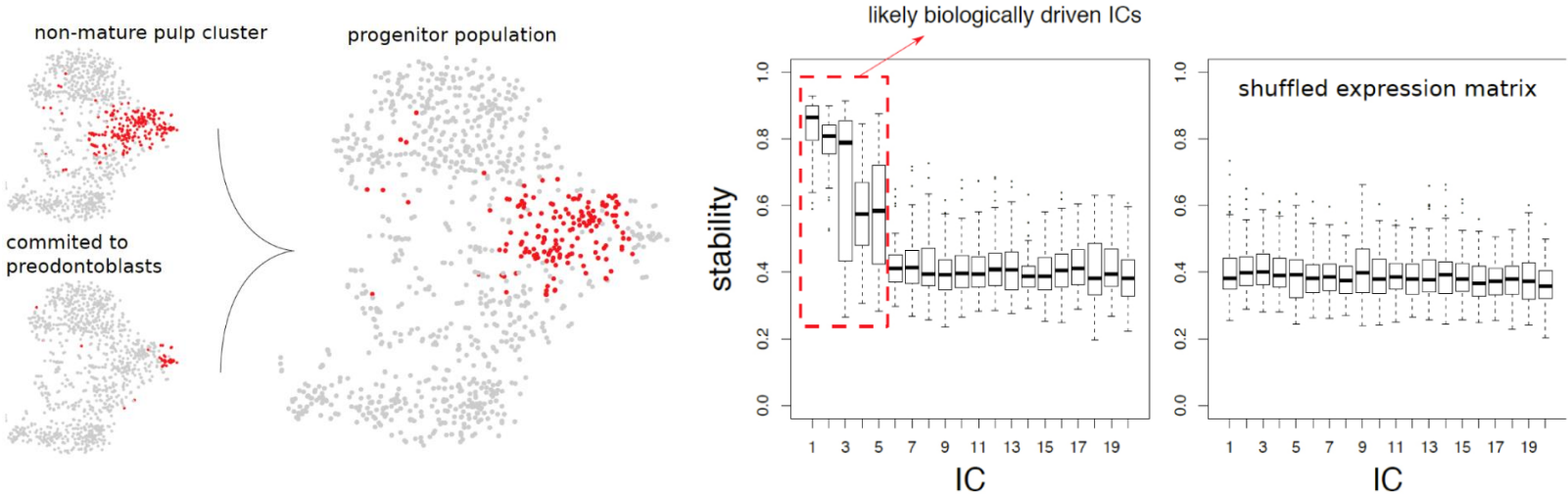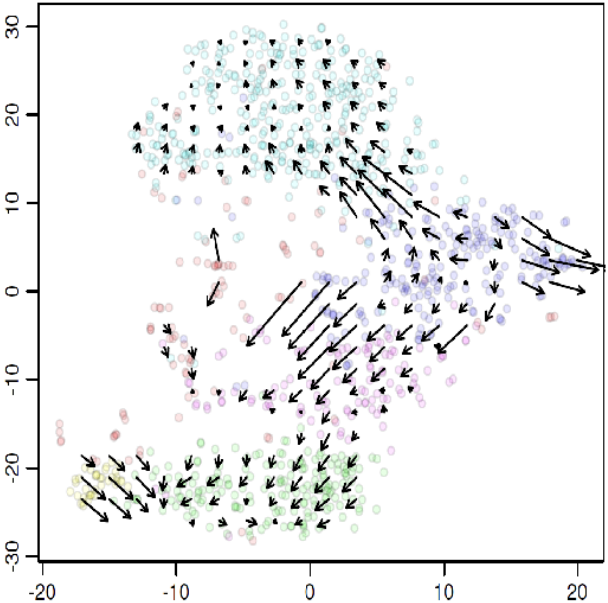
Individual components analysis

IC 1	IC 2	IC 3	IC 4	IC 5
Igfbp3	Mcm5	Nts	Xist	Lypd1
Bhlhe41	Fam111a	Wif1	Trrap	Hsd11b2
Thbs4	Mcm10	Lef1	Col6a3	Hhip
Gpx3	Chaf1b	Nrp2	Arhgef17	Sostdc1
H19	Cdc6	Dock10	Mll1	Foxd1
Dio3	Cdk1	Nr4a2	Usp34	Shisa2
Omd	Rdh10	Gfra2	Ifngr1	Pcp4
Lum	Sox9	Ecel1	Csf1r	Dcbld1
Rspo4	Nell1	Fgf3	Cxcl12	Tnc
Col12a1	Cpm	Nell1	Ano6	Perp
Vit	Prss35	Arpc5	Scoc	S100a10
Cthrc1	Enpp2	Lypd1	Dkk3	Postn

Krivanek et al. 2020

Identification of FoxD1⁺ stem cells

IC5

Lypd1
Hsd11b2
Hhip
Sostdc1
Foxd1
Shisa2
Pcp4
Dcbld1
Tnc
Perp
S100a10
Postn

Foxd1
DAPI

Newly discovered population of mesenchymal cells in close proximity to the labial cervical tinge

How to determine the function of newly described Foxd1+ cells in a continuously growing mouse incisor?

In vivo development monitoring
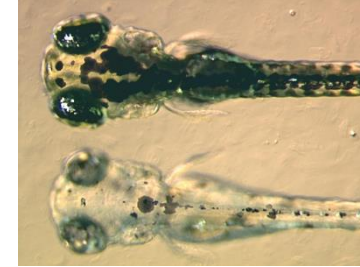
- **Direct observation**

- Simplicity, accuracy

- The method can only be used in transparent organisms – often used in invertebrates, fish or amphibians.

  Classical model organisms: *D. rerio* nebo *C. elegans*

- **Use of cell-based dyes or extracellular matrix (e.g. DiI, Calcein)**

  - Relatively simple application

  - Non-specificity, need for precise handling, short-term marking (clearing, instability)

    Marking with thymidine analogues (BrdU, EdU)

  - Monitor the activity of dividing cells

  - The exact method can be used universally regardless of the model organism or the duration of application

  - (limited to cells that currently synthetize DNA)

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In vivo development monitoring

- **Genetic methods**

- Precise targeting, high specificity, very wide application possibilities, diversity, availability of many already prepared systems, variable use

- Disadvantages: Demanding and long-term preparation of new constructs

-

- Various methods count among the genetic methods. Among the most widely used are:

- Cre/CreERT2

- Floxed

- TetON/TetOFF

- Cell barcoding

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Lineage tracing!

Method allowing performing of controlled genetic changes in specific cells

Where did GFP come from??

- Genetic code is universal across species

- Possibility to form hybrid organisms – a basic prerequisite for genetic engineering

-

Saler, 2019

KGEELFTGVVPILVELDGDVNGHKFSVSGEGEGDATYGKLTLKFICTTGK
VPWPTLVTTFSYGVQCFSRYPDHMKQHDFFKSAMPEGYVQERTIFFKDD
YKTRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYIMADK
NGIKVNFKIRHNIEDGSVQLADHYQQNTPIGDGPVLLPDNHYLSTQSALSK
NEKRDHMVLLEFVTAAGITHGMDELYK

1

Expression

DNA
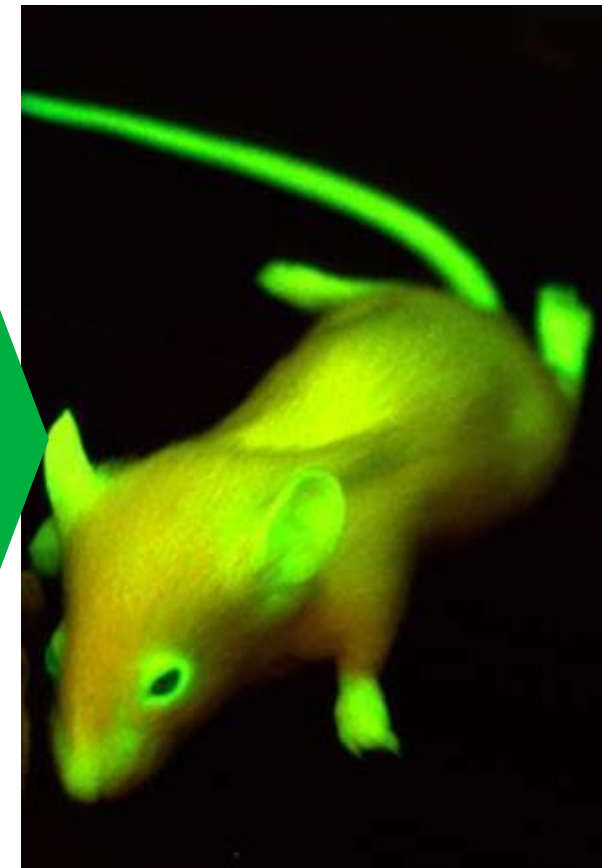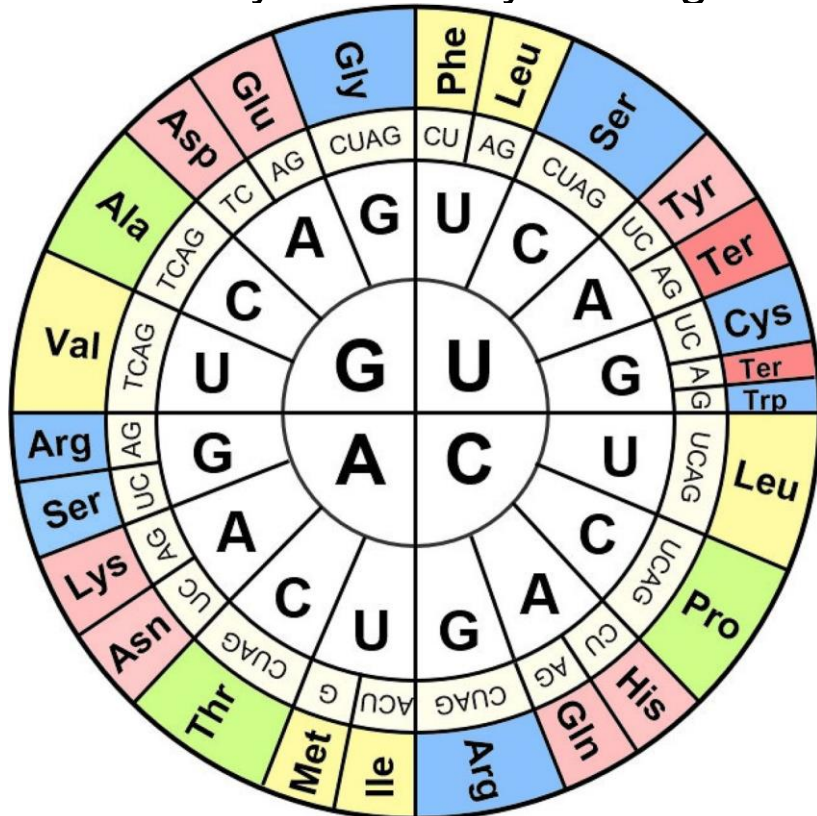
2 Cut out the (trans)gene bounded by LoxP in places

loxP TransgeneX loxP

Cre recombinase ✂
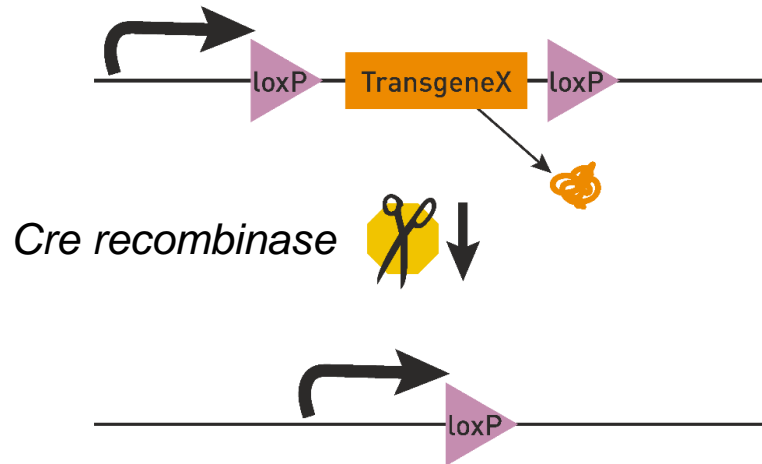
loxP

3 Cut out STOP sequences bounded by LoxP locations

Promoter loxP STOP loxP GFP ✗

Cre recombinasa ✂

Promoter loxP GFP
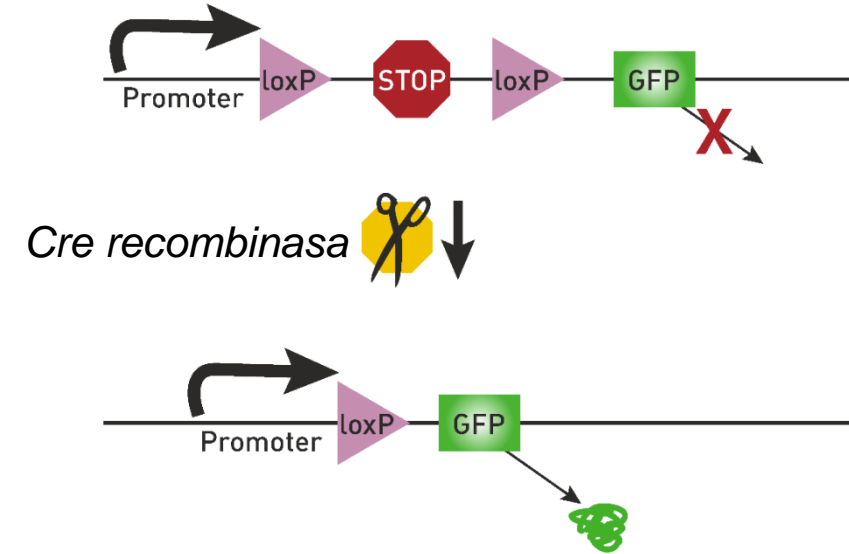
4 Tissue-specific expression of GFP

Myh6 cre

loxP STOP loxP GFP

Cre reporter strain

X

1 Generation

loxP STOP loxP GFP
Myh6 cre

loxP GFP
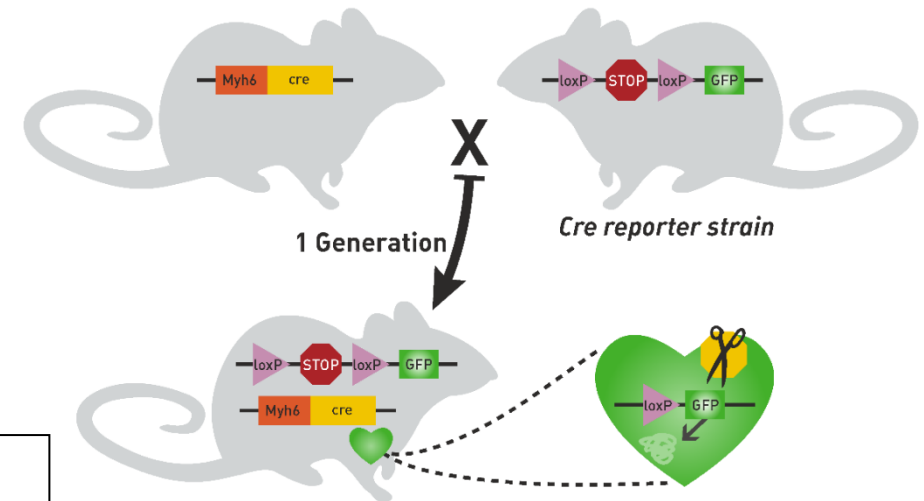
GFP fluorescence confirms Cre activity in expected tissues

Knockout in a heterozygous form
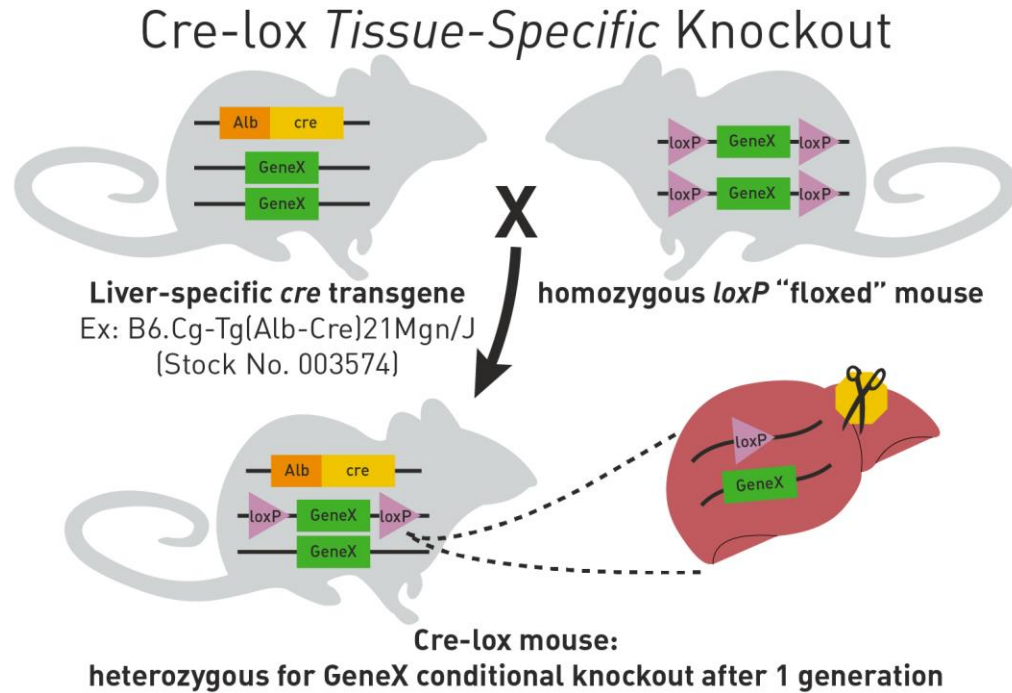
Knockout in a homozygous form

Cre-lox *Tissue-Specific* Knockout

Liver-specific *cre* transgene
Ex: B6.Cg-Tg(Alb-Cre)21Mgn/J
(Stock No. 003574)

homozygous *loxP* "floxed" mouse

Cre-lox mouse:
heterozygous for GeneX conditional knockout after 1 generation

Cre-lox Tissue-Specific Knockout, cont.

hemizygous cre
heterozygous "floxed" gene

homozygous "floxed" mouse
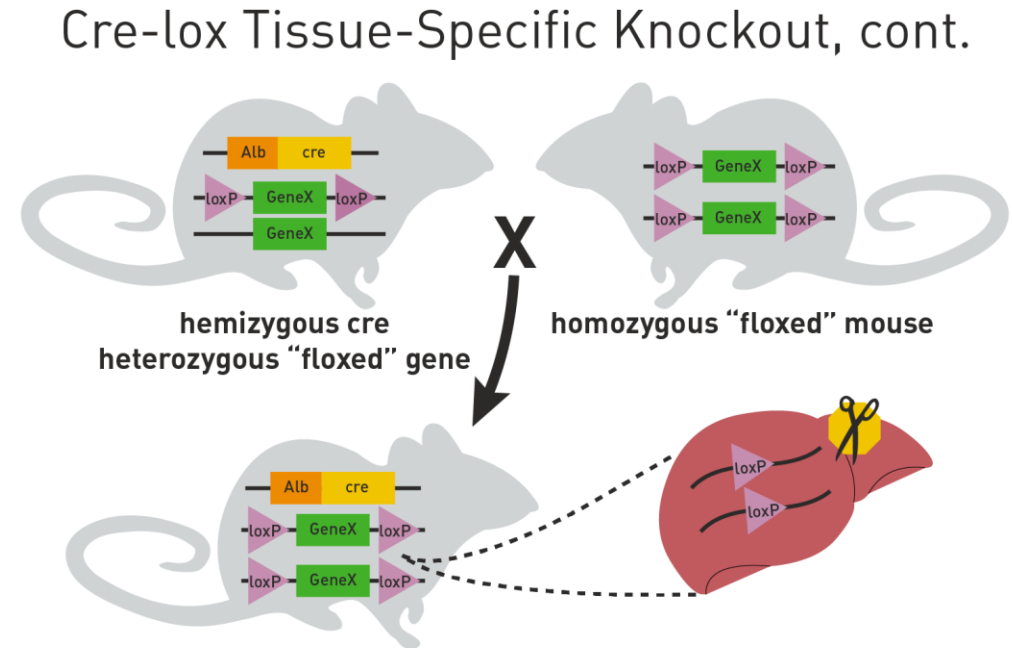
- Other transgenic organisms are obtained in the same way

Mendelian inheritance

Constitutional vs. Inducible System

In the constitutional system, stable genetic changes occur without the possibility of influencing them
In an inducible system, we can initiate genetic change - time

Inducible

Constitutional

Osório et al., 2016
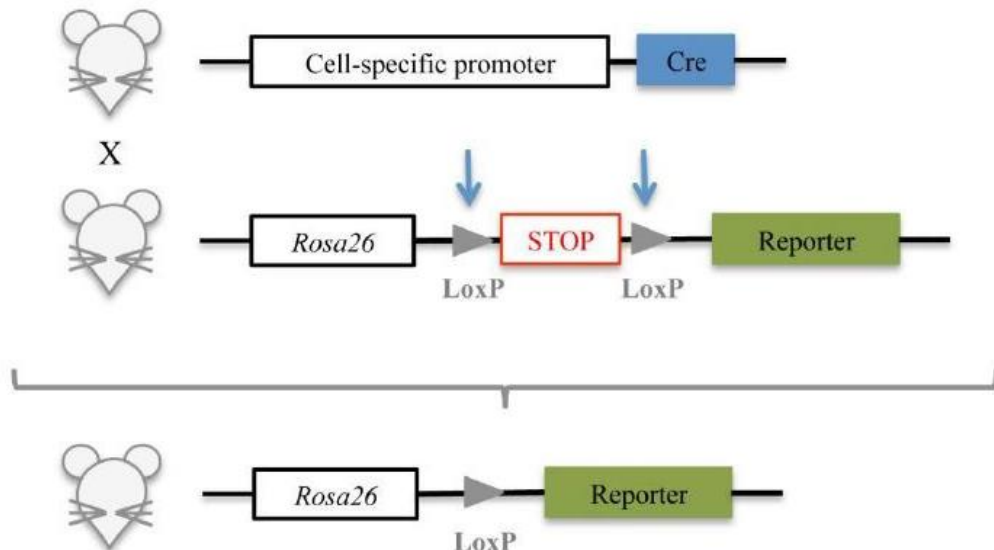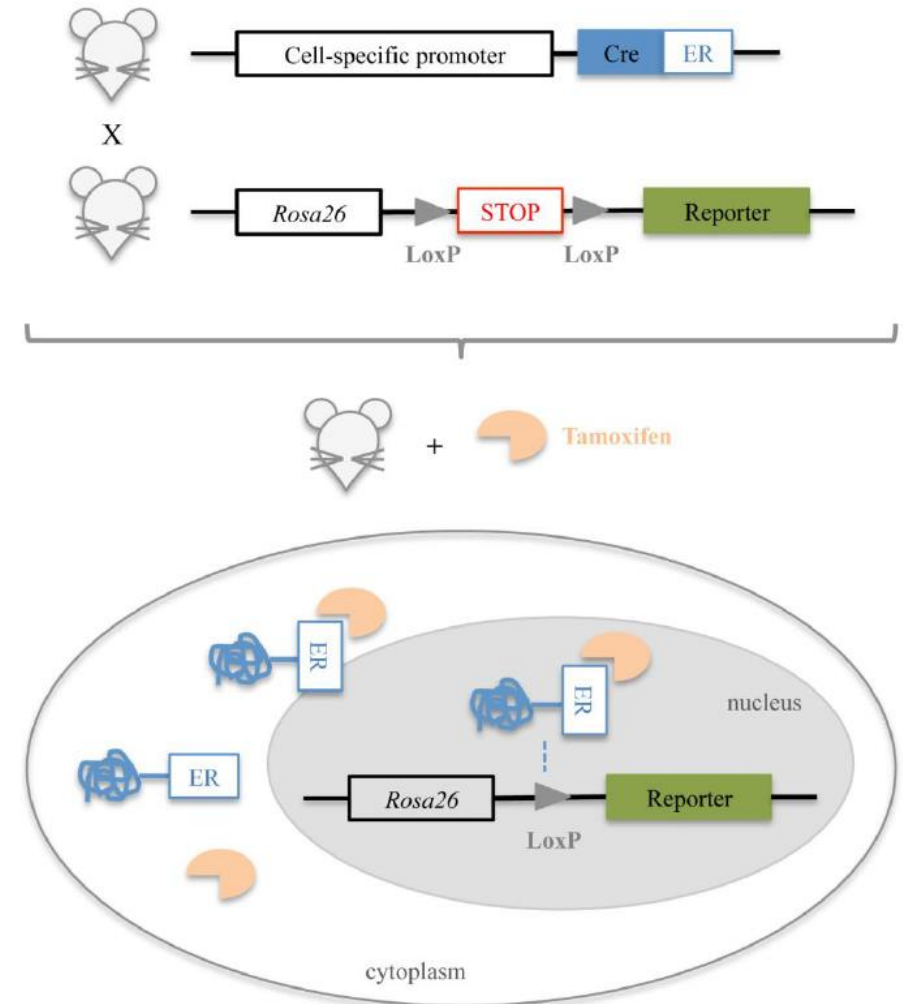
Multi-color systems

- The transgene contains multiple genes encoding different fluorescent proteins
- These fluorescent proteins are randomly expressed
- Possibility of clonal genetic tracing

Snippert et al., 2010

Confetti (Brainbow 2.1)

Nature Reviews | Cancer

Ellenbroek, van Rheenen, 2014

Livet et al., 2007
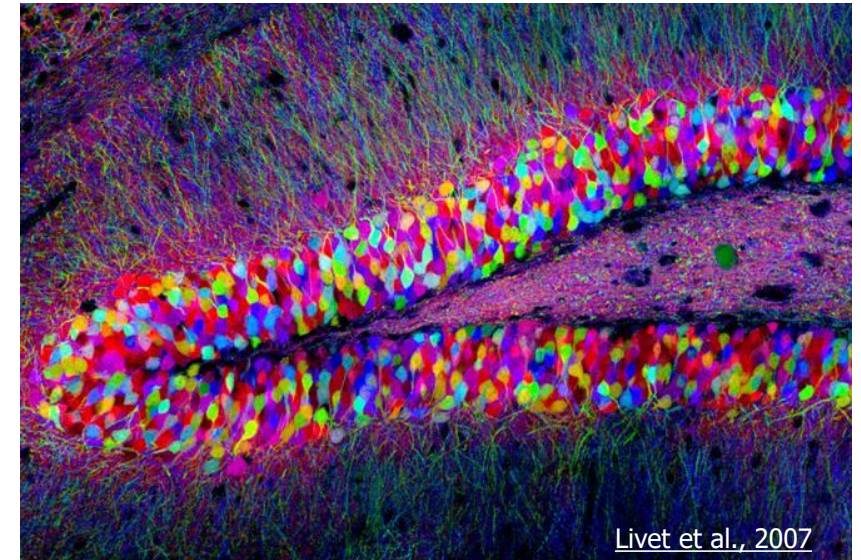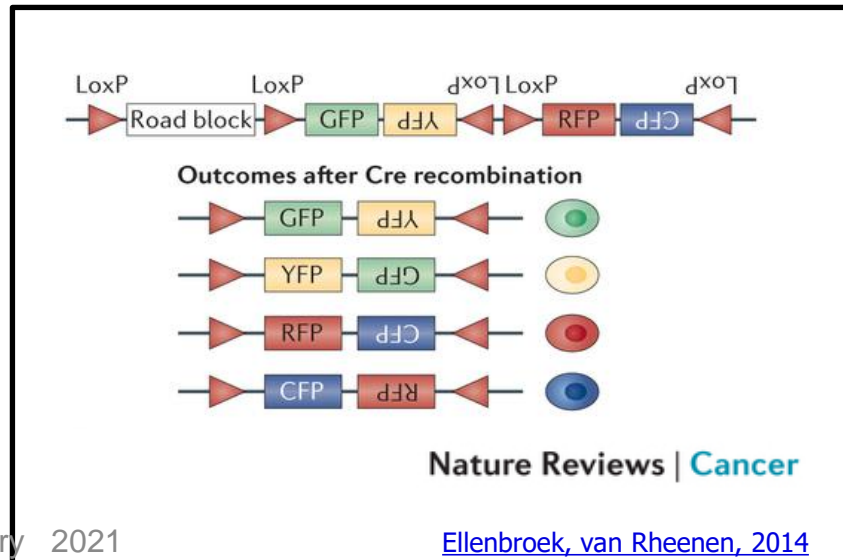
Practical use

Identification of FoxD1$^+$ stem cells
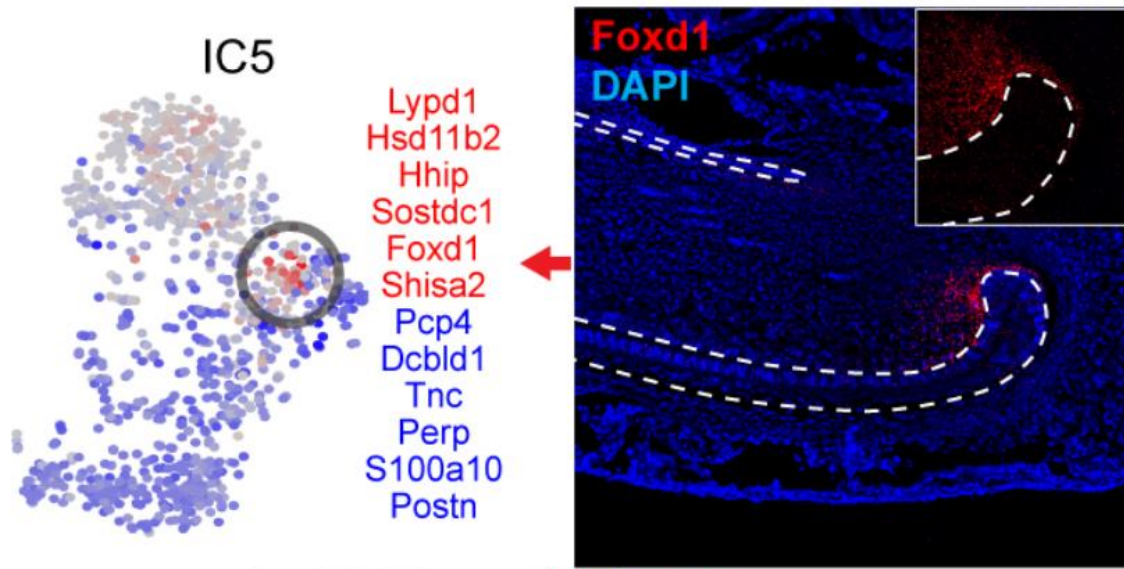
Use of *Foxd1^{CreERT2}* mouse strain to map Foxd1$^+$ cells *in vivo*

Identification of a new type of dental mesenchymal stem cells!

Krivanek et al. 2020

Modern 3D/4D imaging methods

Fluorescence-based display

- Confocal microscopy, Lightsheet microscopy, Live-imaging (time-lapse)

- Display using 3D electron microscopy

- FIB-SEM (Focused Ion Beam Scanning Electron Microscopy)

o SEM + sectioning

o TEM + tilt series

- Methods of computed tomography (CT)

o microCT

o nanoCT

3D/4D fluorescence-based display

Confocal microscopy

- Use of lasers for excitation of fluorophores

- Detection of reflected light of a defined wavelength after passing through a pinhole - detection of light from only one "focused" plane

- Increasing the resolution of classical fluorescence microscopy

- Possibility to combine individual images into a 3D image

- Combination of different colors / fluorophores

- The limit of resolution depends on the wavelength of light itself (maximum resolution is half the wavelength)
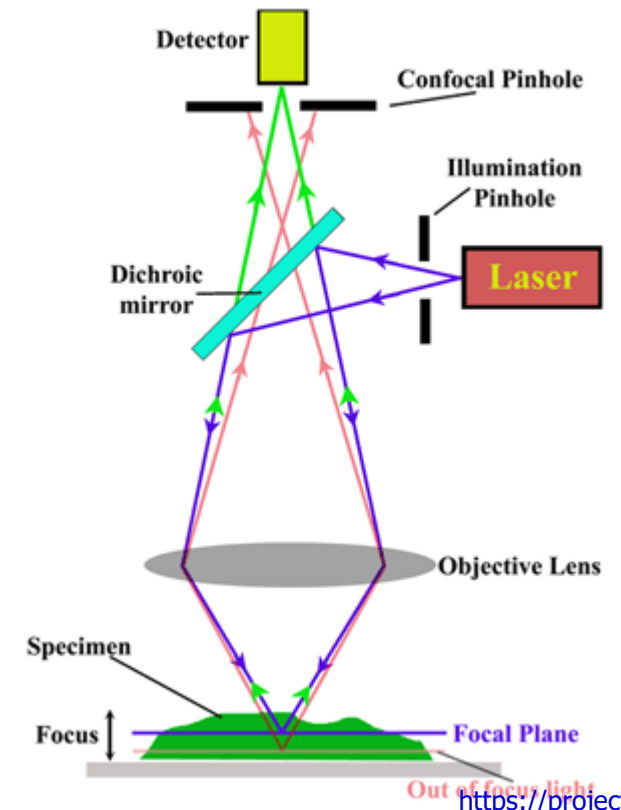
3D/4D fluorescence-based display

▪ **Lightsheet micrscopy**

- Same principles as for confocal microscopy
- The excitation laser (s) illuminate the sample from the side at an angle of 90 °
- Possibility to use two lasers - increased speed (reduced phototoxicity)
- Possibility to rotate the sample during scanning

Both confocal and Lightsheet microscopy

enable time-lapse scanning - a **necessity**

for monitoring developments over time.

Confocal microscopy | Light-sheet microscopy

A — Illumination: Objective lens, Laser, Scan, Evenly illuminated, Plane of interest
C — Laser, Unaffected, Selectively illuminated
B — Detection: Scan, Pinhole rejects out-of-focus light and confines the detection plane
D — Widefield detection with a CCD camera
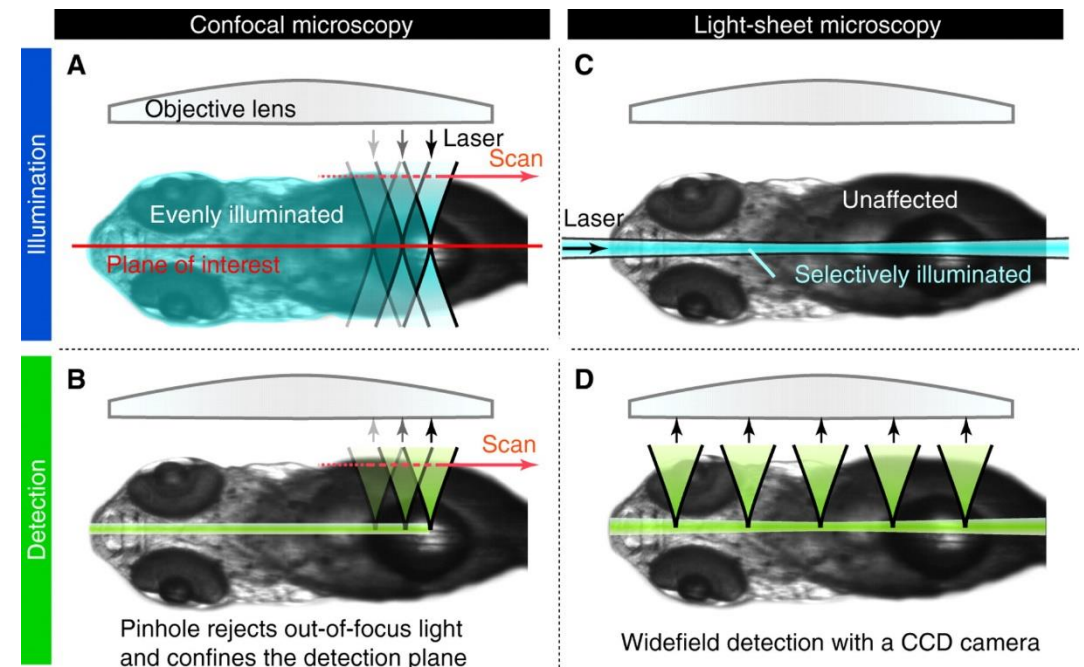
3D/4D fluorescence-based display

Examples of the use of Lightsheet microscope:

- Calcium release in brain: https://youtu.be/IppAwkek6DI

- Zebrafish development: https://youtu.be/-WaseO2Vw5Q

- Zebrafish development: https://youtu.be/yk7TWOtrphM

- Beating heart: https://youtu.be/bQ4GWJ7F7eU

- Adult mouse brain: https://youtu.be/-QJlBQkDH00

Plunger
Syringe
Agarose
Fry
Egg water
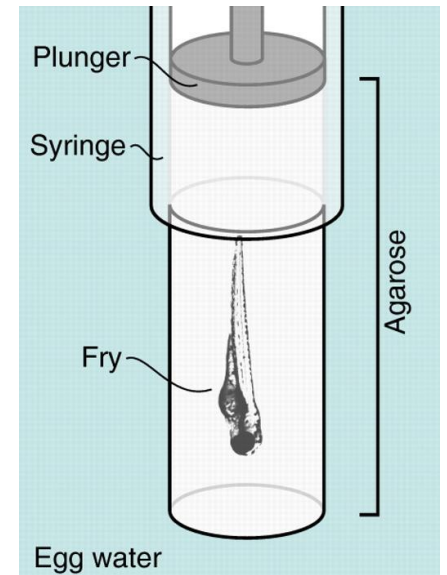
Huisken et Stainer, 2009

Lightsheet Microscopy (live imaging)
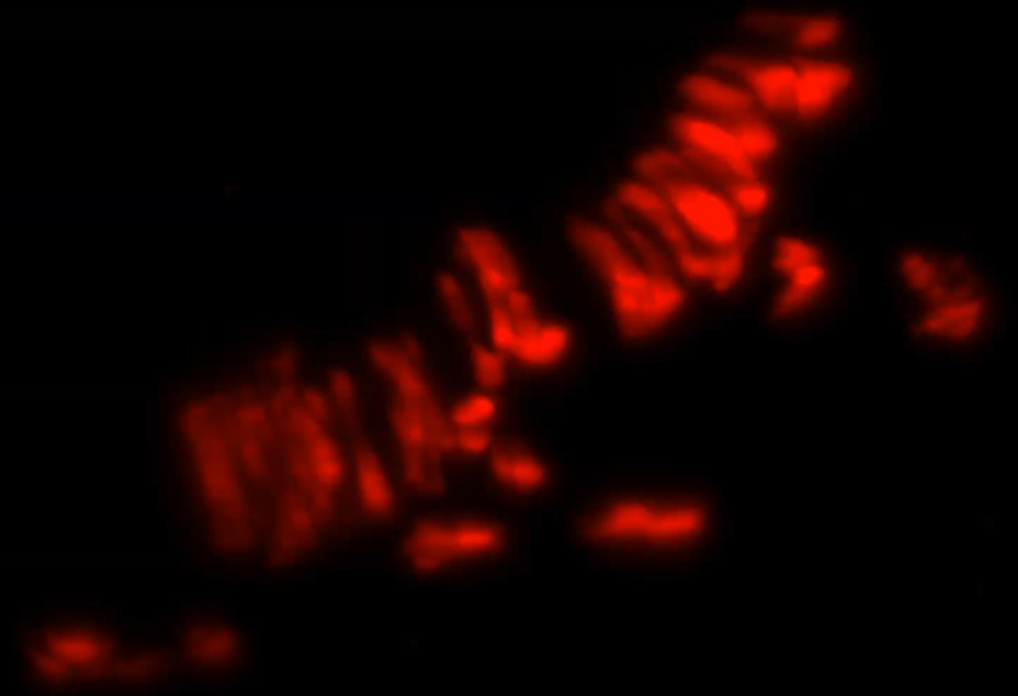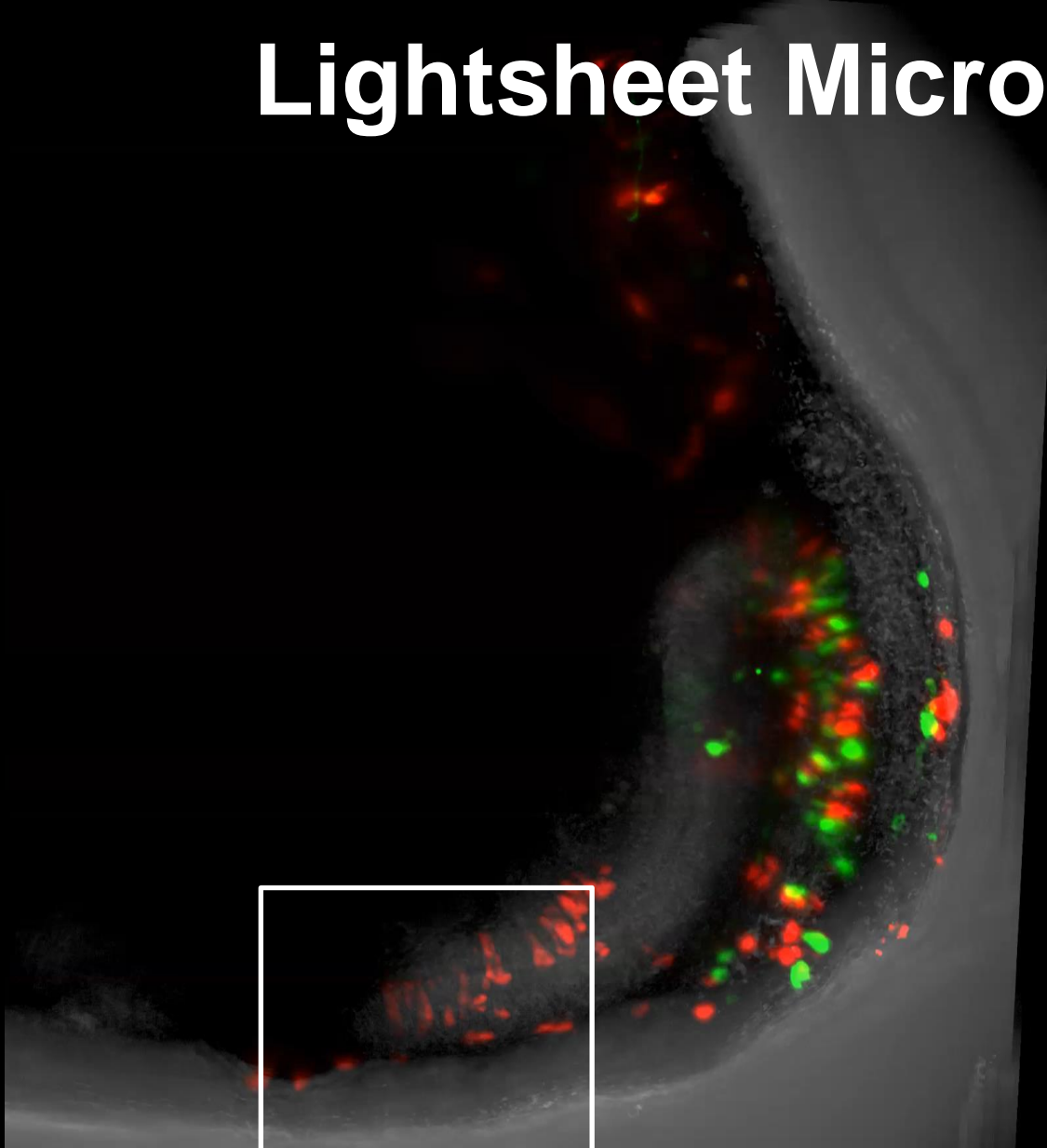
Research Activities in Dentistry   2021

3D electron microscopy

- **FIB-SEM** (Focused Ion Beam Scanning Electron Microscopy)

  o Use of focused ion (Gallium, Xenon) beam for precise cutting of thin slices of the sample in combination with scanning electron microscopy

  o It enables high resolution at the SEM level, including subsequent 3D reconstruction

  o Demanding sample preparation, long scanning time, high cost, technically demanding process

  o The sample is completely destroyed during scanning, tracking objects over time is impossible

FIB-SEM

a — Image acquisition / 2D image-stack registration / 3D reconstruction

SEM — FIB z progression — Patterned protective Pt pad — Trench — Imaging target (cell)

b   c   d
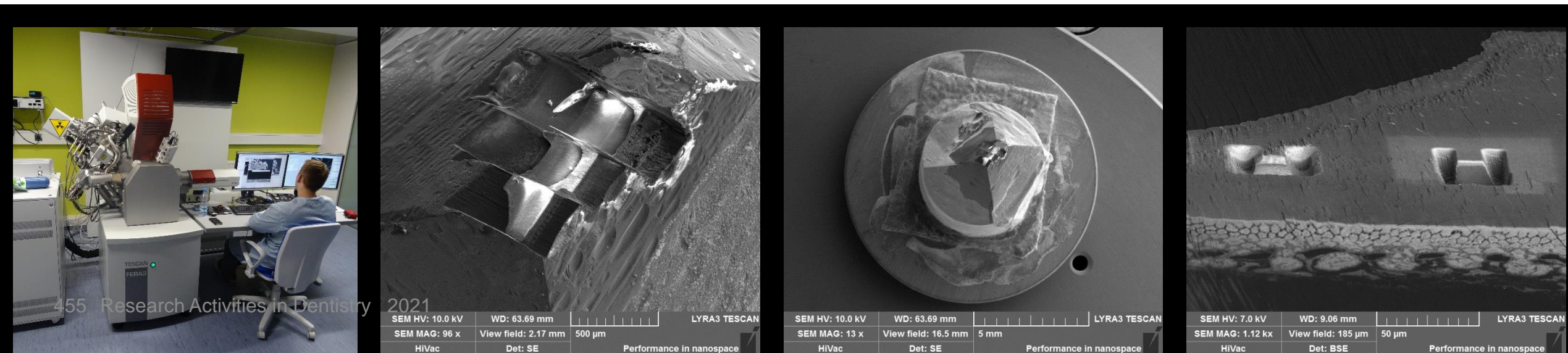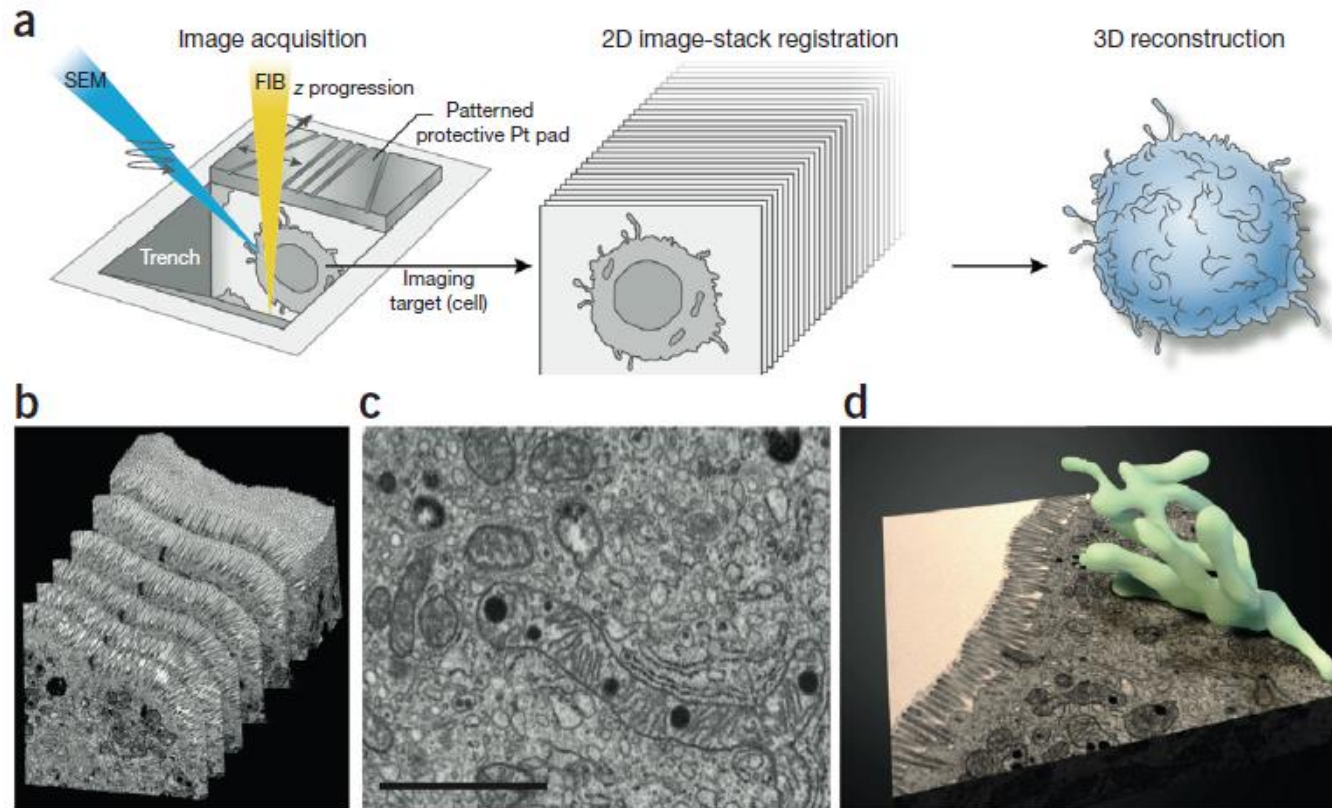
Narayan et Subramaniam, 2015

Credit: Jonty Hurwitz

10 microns — human hair — www.art.ninja
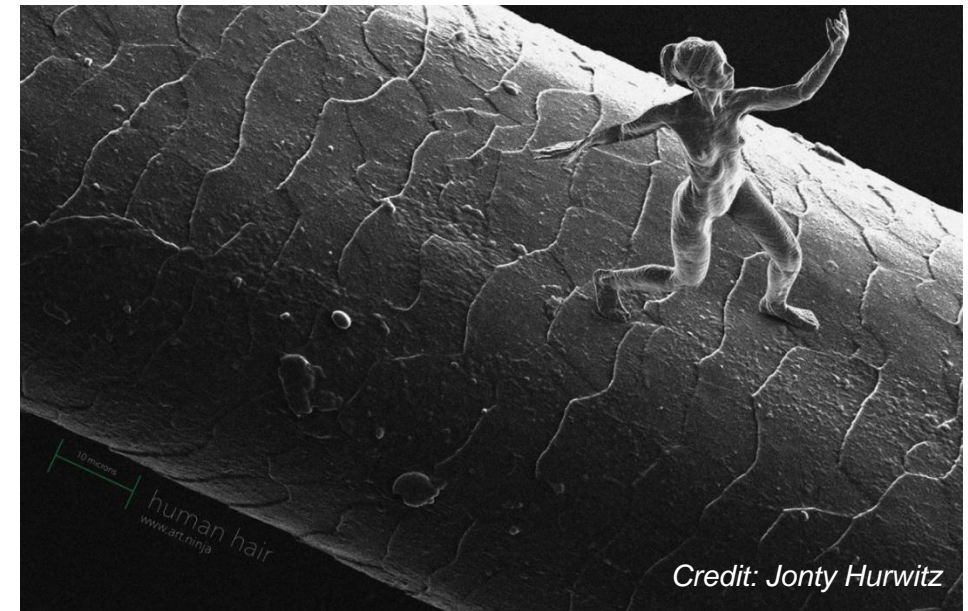
FIB-SEM
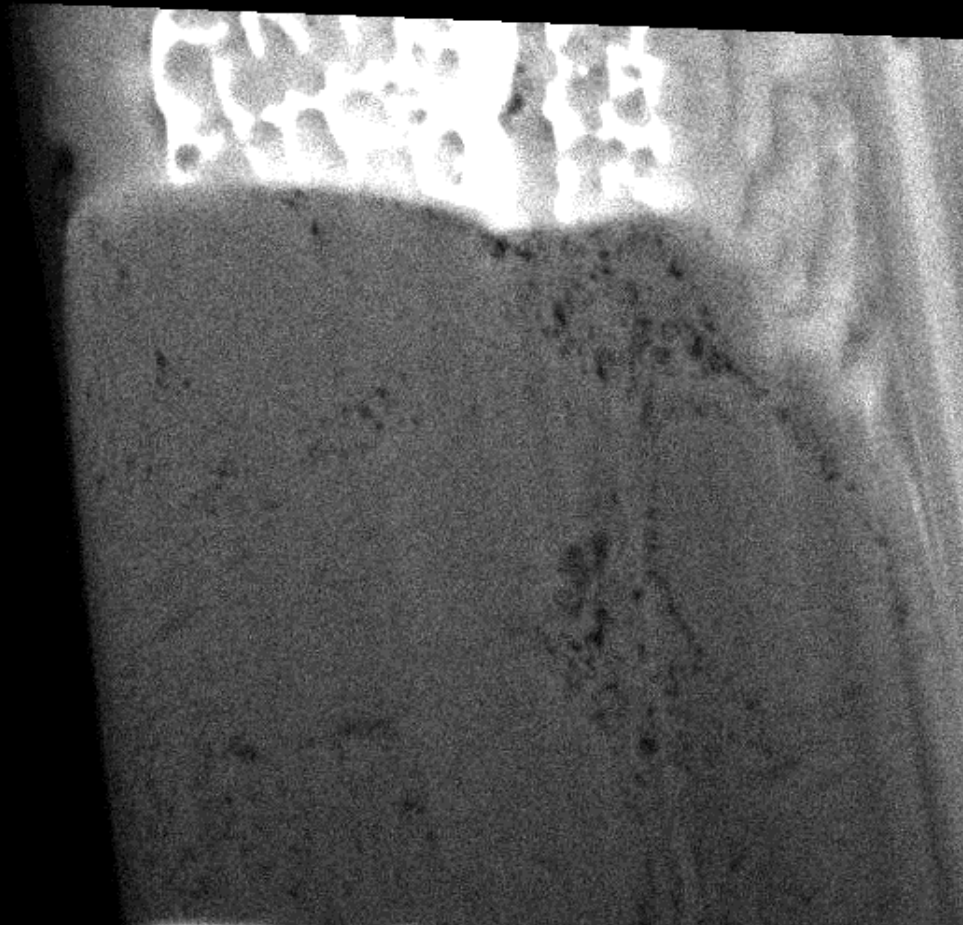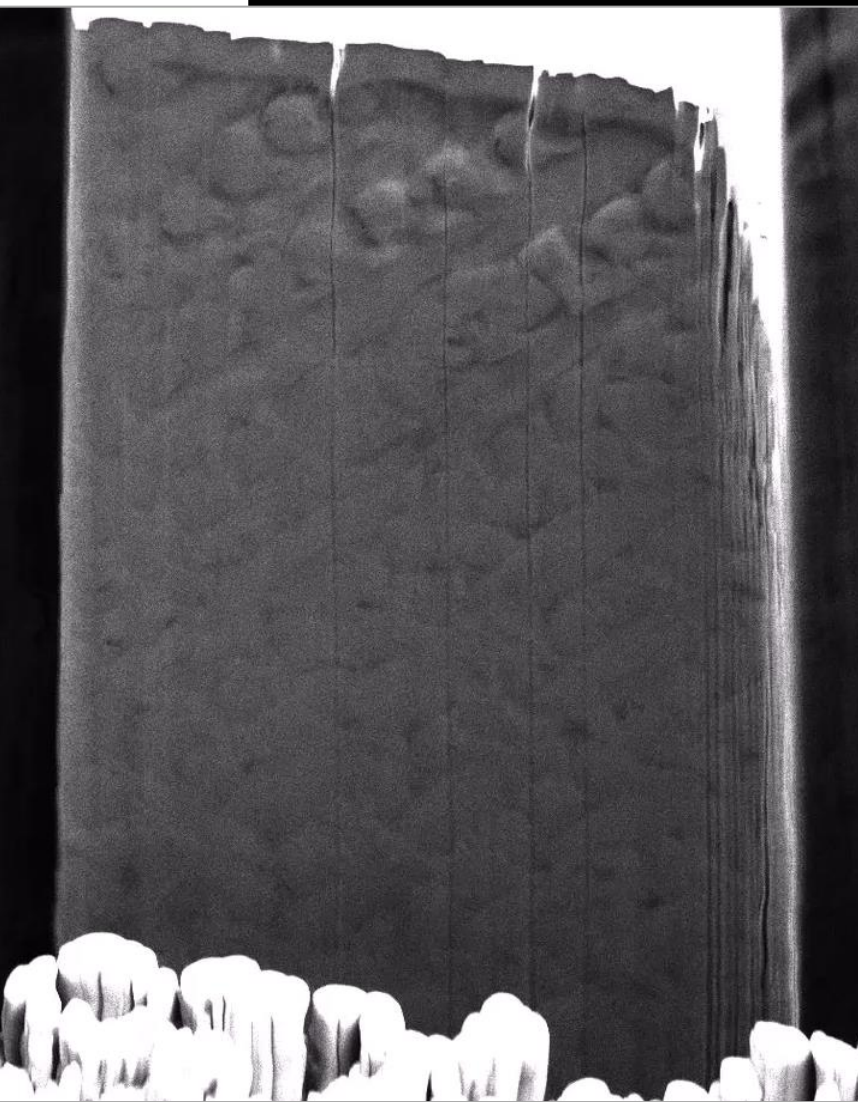
Methods of computed tomography (CT)

- Use of computed tomography technique with adaptation to small samples (microCT / nanoCT)

- X-rays in combination with sample rotation

- Displaying 3D sample structure

- A routinely used technique for hard tissue imaging

- Various contrast techniques must be used when soft tissue imaging is required

- Inability to track objects over time, samples are fixed

μCT

https://www.microphotonics.com/what-is-micro-ct-an-introduction/
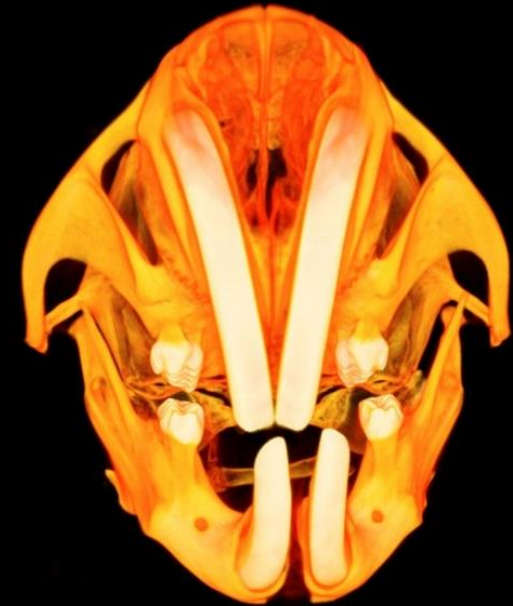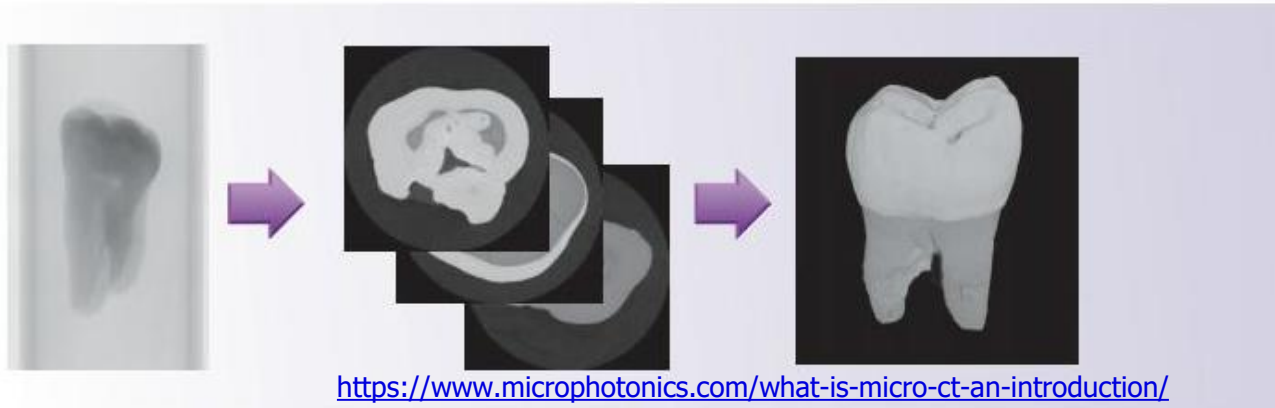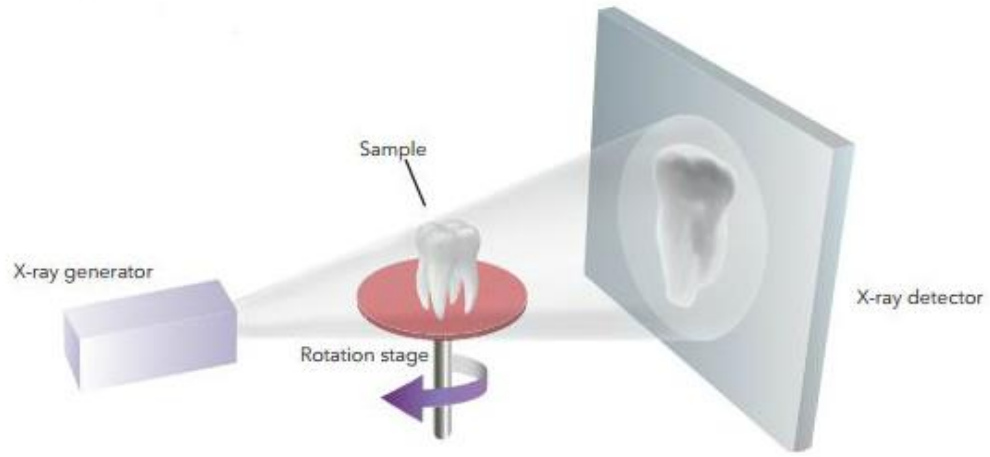
Abbreviations used

- FACS — Fluorescence-activated cell sorting, Fluorescenčně-řízené třídění buněk

- LaCL — Labial Cervical Loop, Labiální cervikální klička

- LiCL — Lingual Cervical Loop, Linguální cervikální klička

- scRNA-seq — single-cell RNA-sequencing, sekvenování mRNA na úrovni jednotlivých buněk

- tSNE — T-distributed Stochastic Neighbor Embedding

- OEE — Outer Enamel Epithelium, vnější sklovinný epitel

- (BrdU, EdU) — Bromodeoxyuridine, 5-ethynyl-2'-deoxyuridine

- GFP — Green Fluorescent Protein, Zelený Fluorescenční protein

- FIB-SEM — Focused Ion Beam – Scanning Electron Microscopy

- TEM — Transmisní Elektronová Mikroskopie

- CT — Computed Tomography (µCT, micro computed tomography)

- Pokemon — Poketto Monsutá – kapesní příšerky

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Odonogenesis - use of alternative model organisms

doc. RNDr. Marcela Buchtová, Ph.D.
Institute of Experimental Biology
Faculty of Science, Masaryk University Brno

Laboratory of Molecular Morphogenesis

Marcela Buchtová (IAPG CAS/Section of Animal Physiology and Immunology, MU)

- Development of craniofacial structures and causes of related malformations

- Comparative odontogenesis

- The role of stem cells in the development of teeth

- Pathogenesis of squamous cell carcinoma, odontogenic tumors and cysts in the oral cavity

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Odontogenesis

Content

— Comparative odontogenesis (traditional vs. non-traditional model organisms, their advantages and disadvantages)

— Morphogenesis and regulation of the development of replacement generations of teeth

— Causes of reduction of the development of surrogate generations in some model organisms with emphasis on cellular and molecular processes

Borilova Linhartova et al., 2020

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Number of generations of teeth

- Monophyodont dentition

- Diphyodont dentition

- Polyphyodont dentition

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What is the basis for the formation of replacement generations of teeth?

– dental lamina - species-specific differences in size, duration

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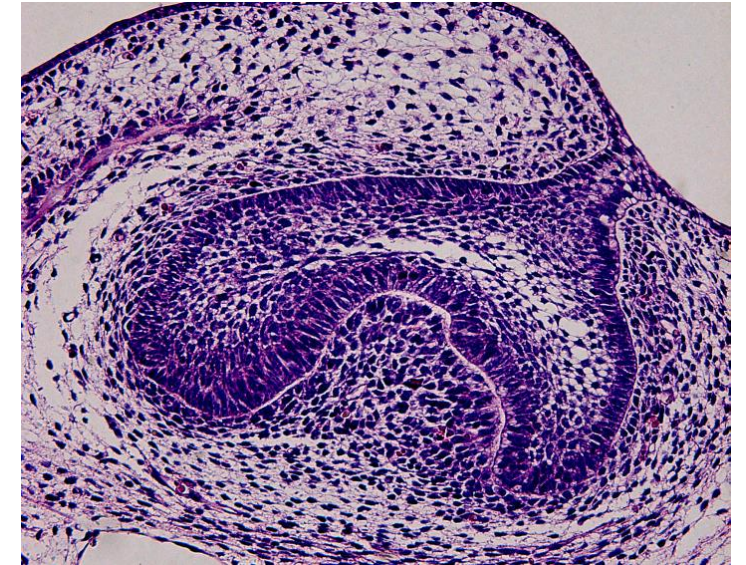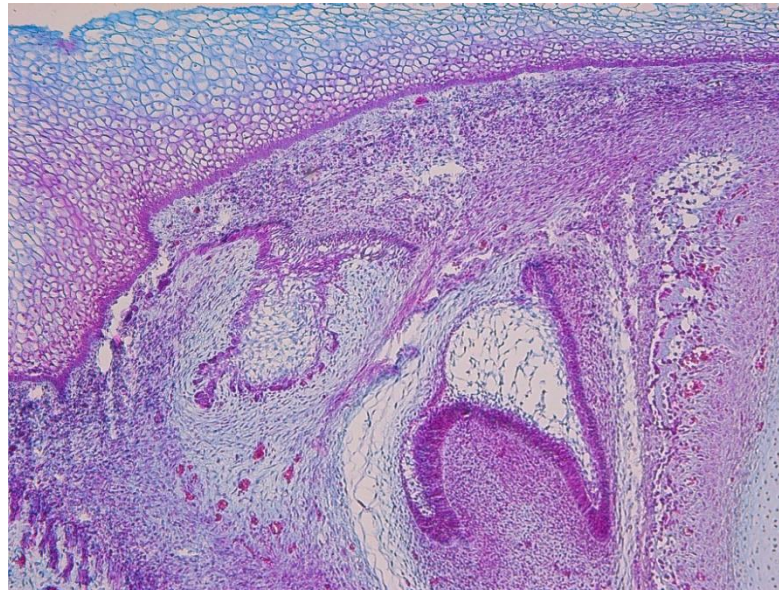
Dental lamina is formed of thickened epithelum
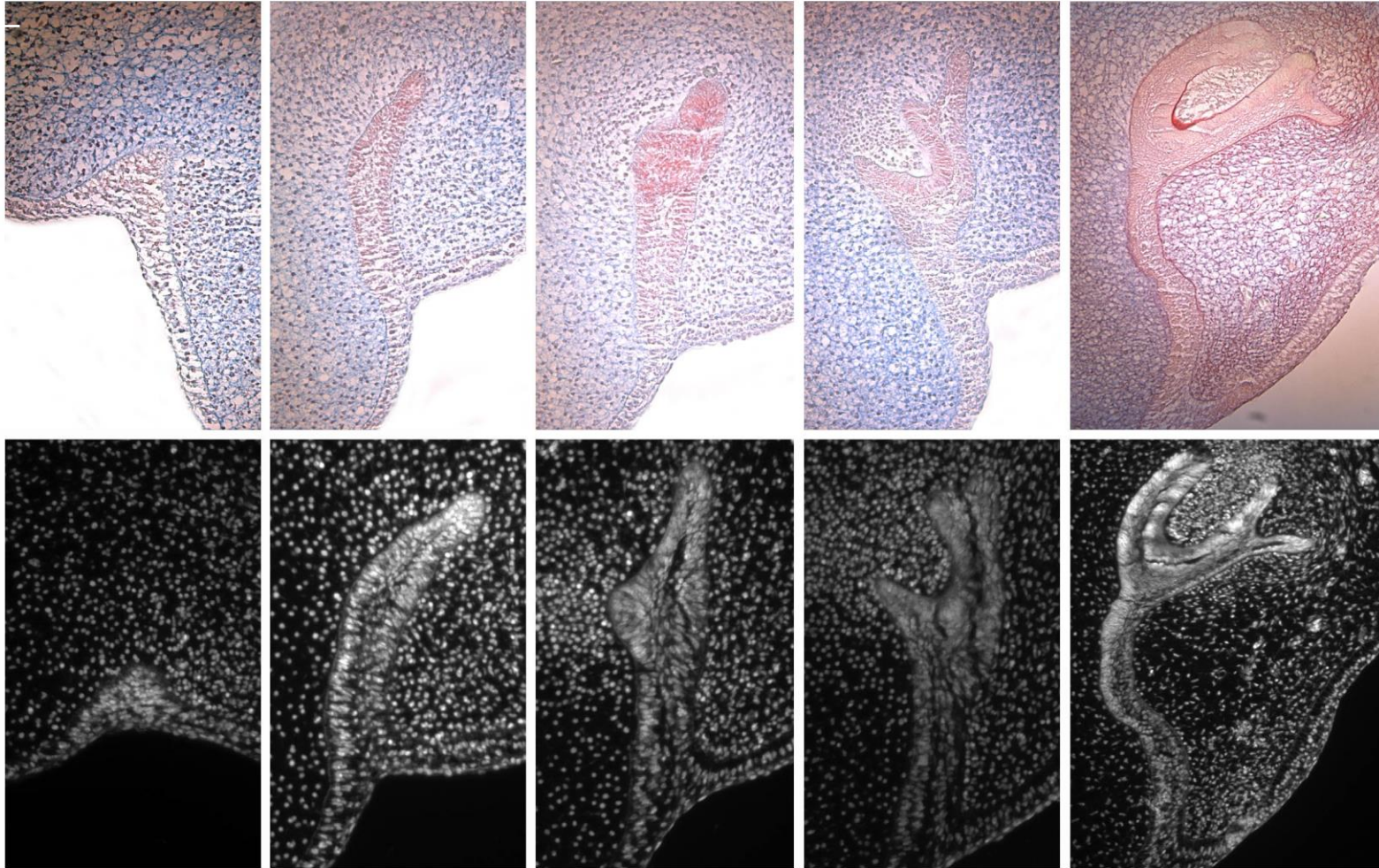
Polyphyodontic dentition

– Homodont dentition

Buchtova et al. 2008

premaxilla ⎫
maxilla   ⎬ Outer row

pterygoid ⎫
palatine ⎬ Inner row

dentary

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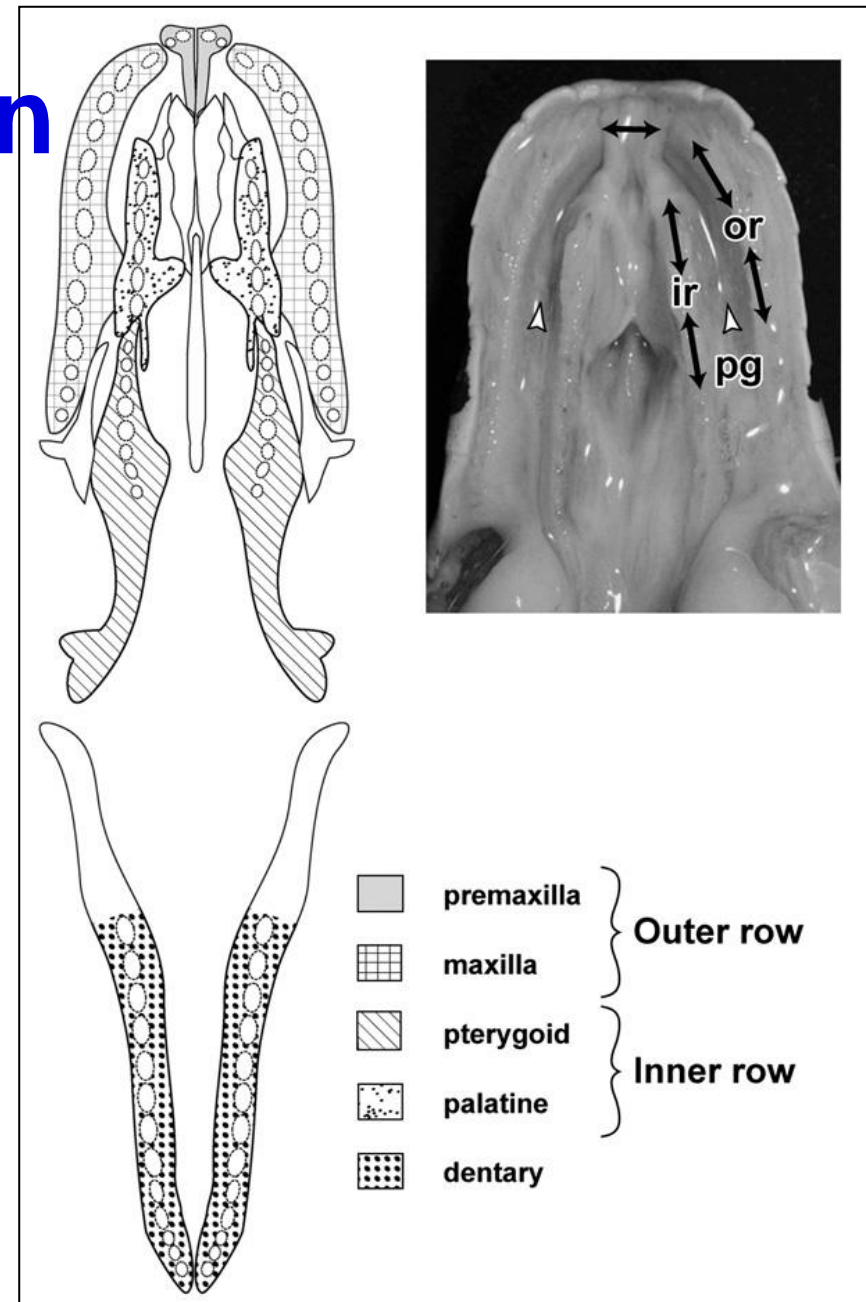
Dental lamina – continuous structure along the jaw

Stage 3   Stage 4   Stage 6

Buchtova et al. 2008
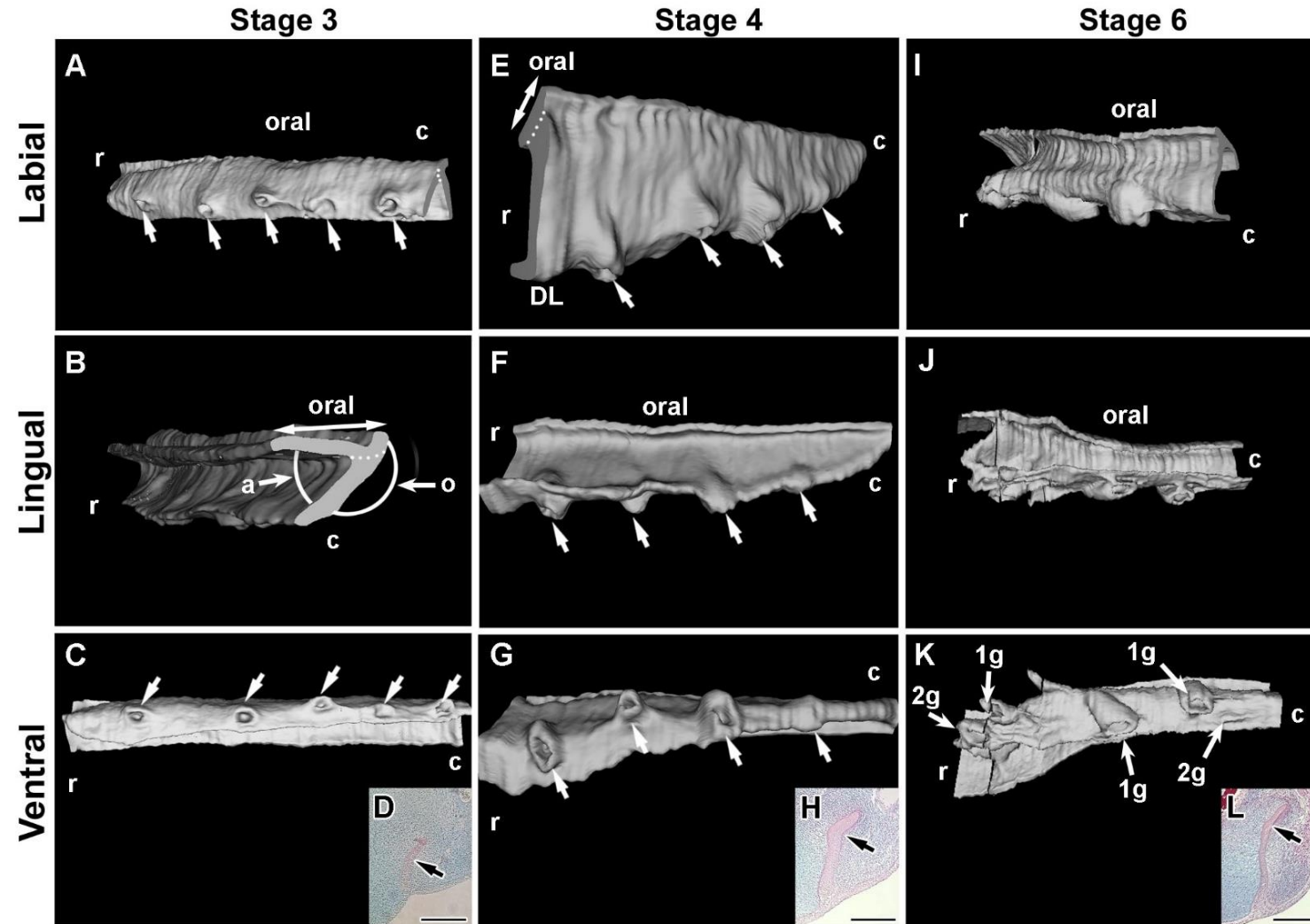
External vs. internal dental line

Buchtova et al. 2007

Frazzetta 1966
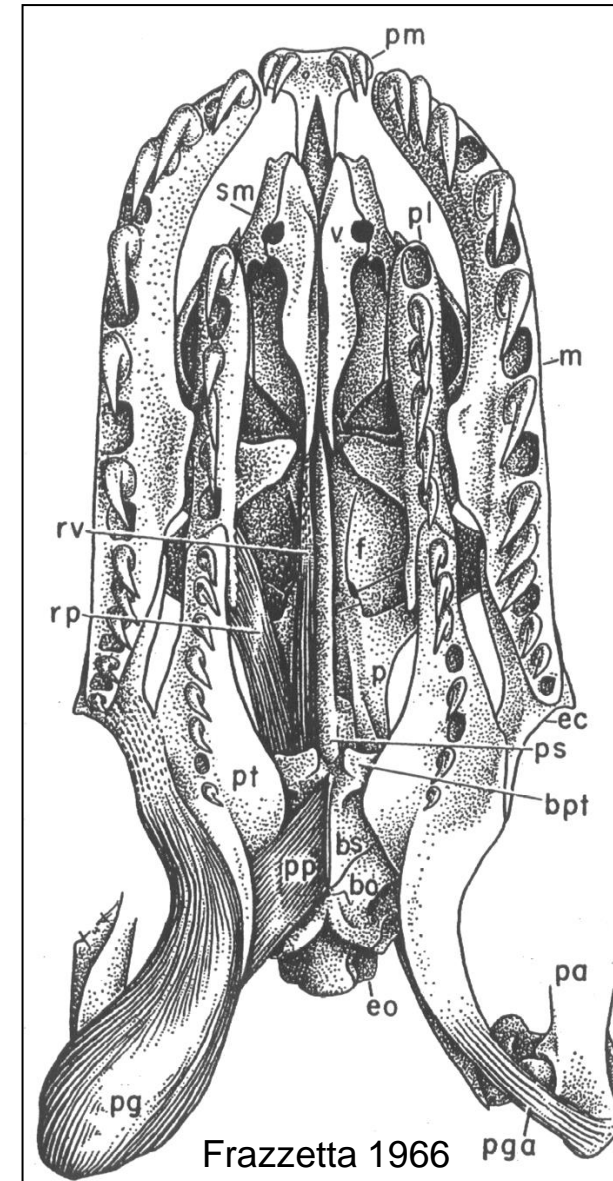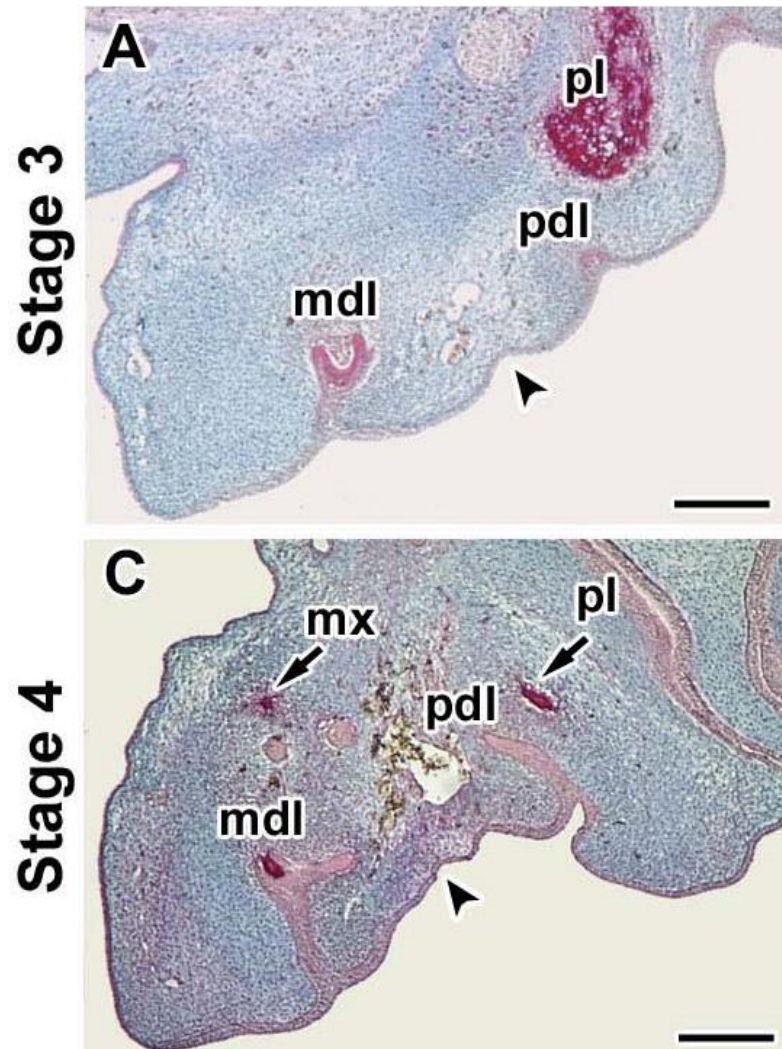
Mirrored inverted dental lamina in the upper jaw

Buchtova et al. 2007
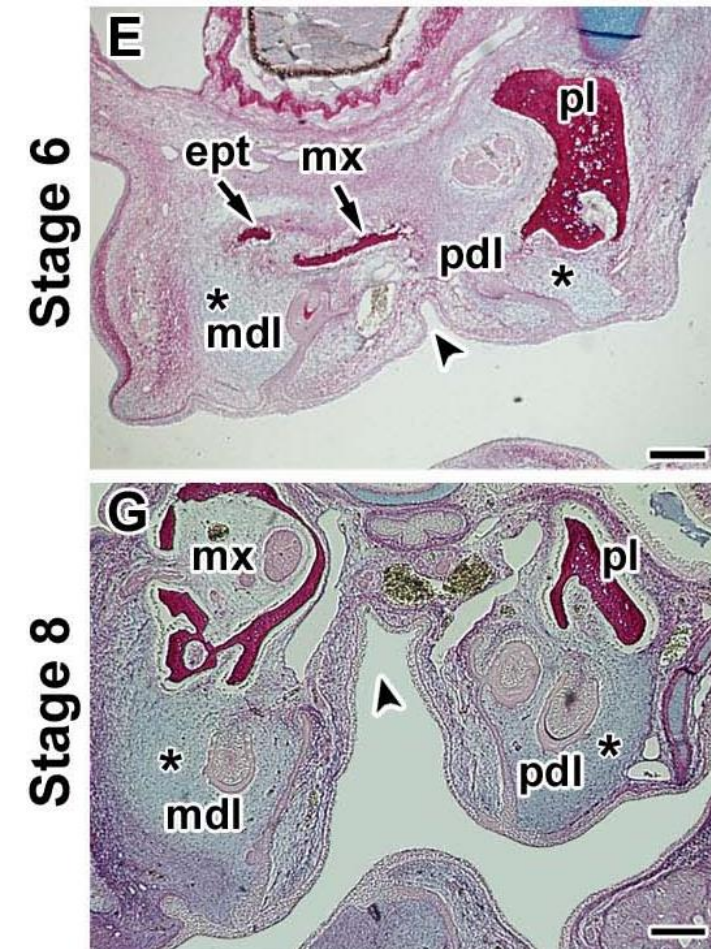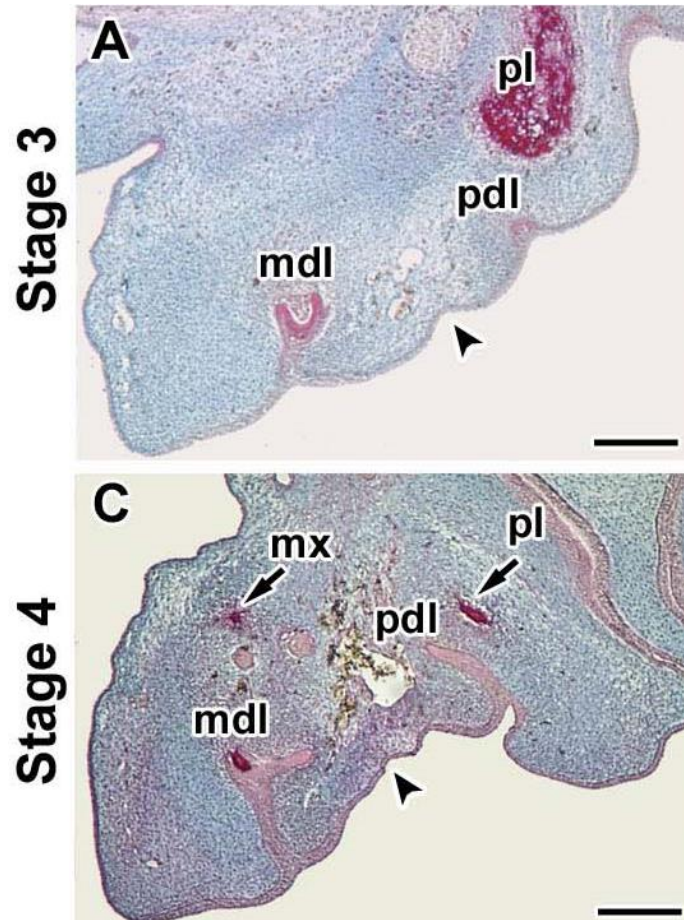
Shh expression in the dental lamina

Buchtova et al. 2008

MUNI
MED
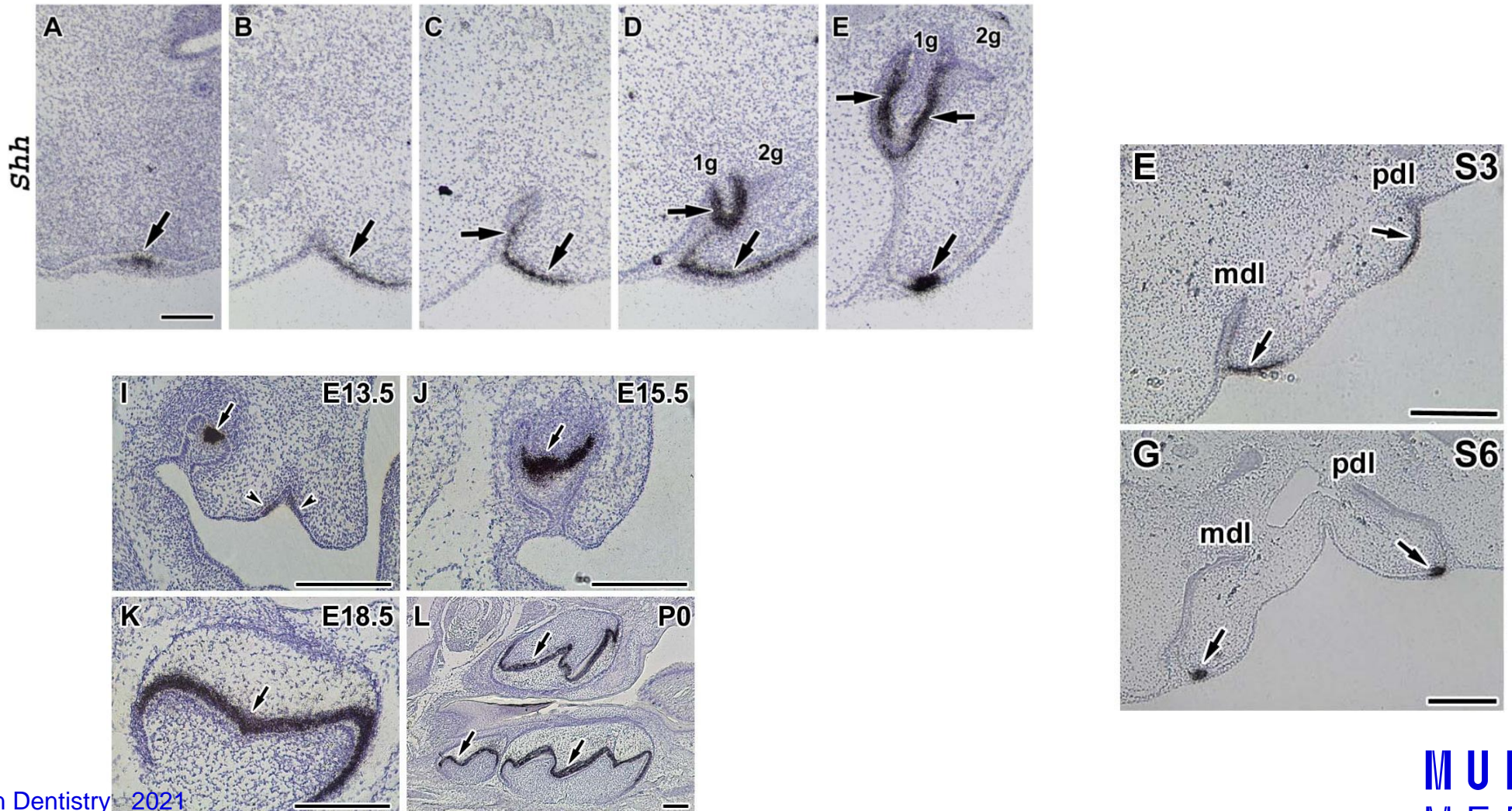
SHH regulates the asymmetric growth of the dental lamina

Buchtova et al. 2008

MUNI
MED
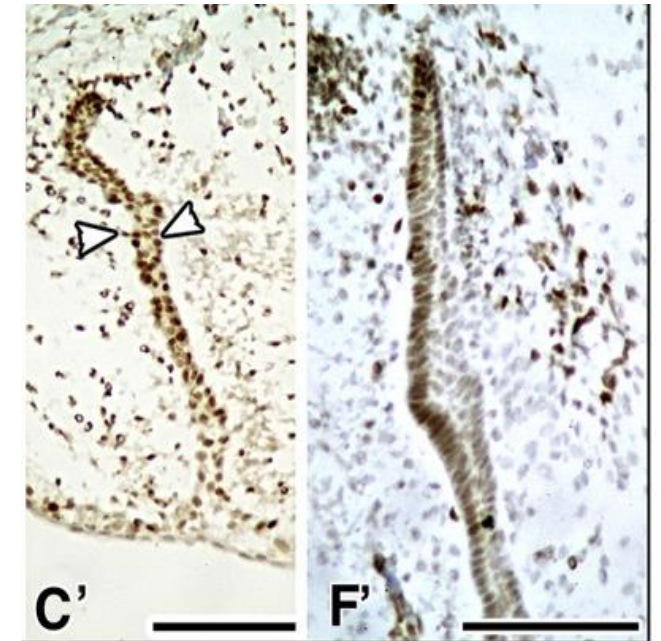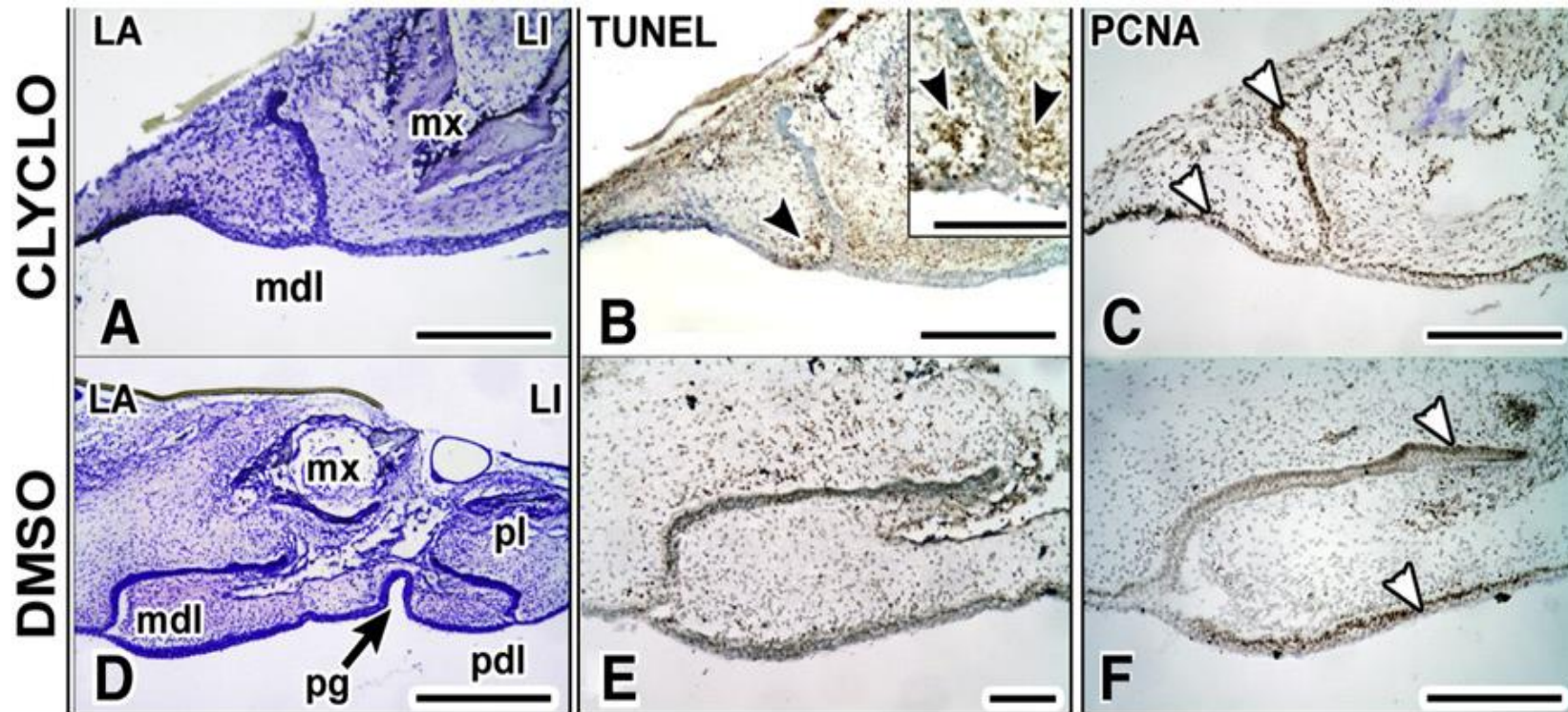
Do poison teeth develop from the dental lamina?

Trimeresurus

b

Edmund, 1960

MUNI
MED

Dental lamina of Cobra

SOX2/cytokeratin

O. Zahradníček

MUNI
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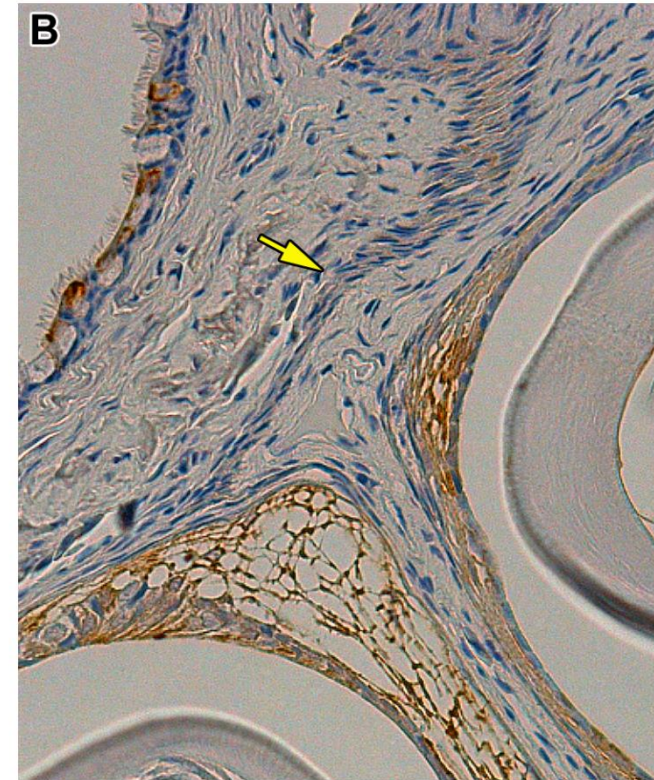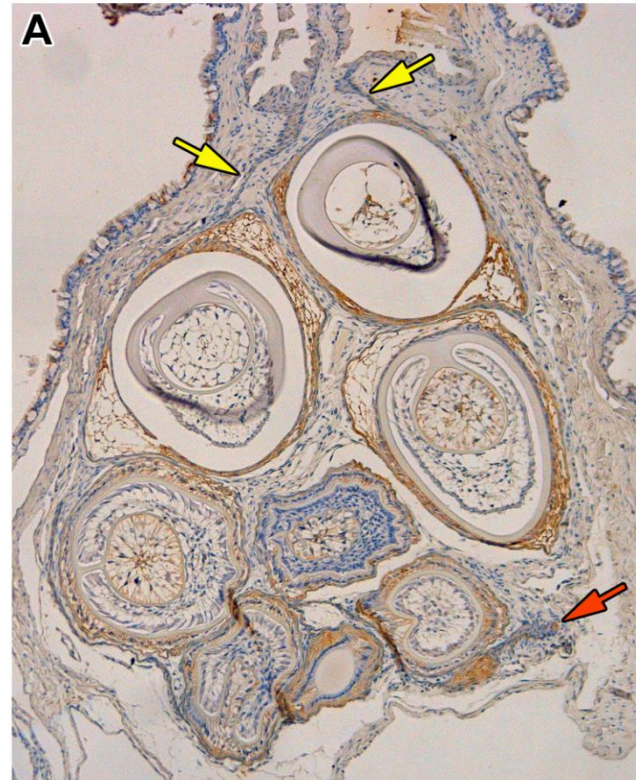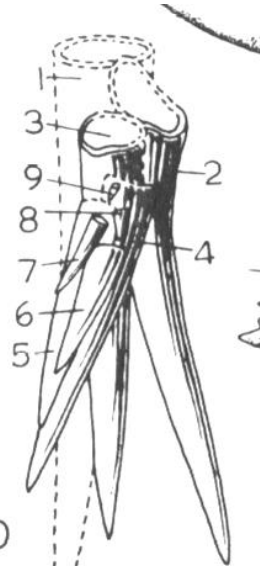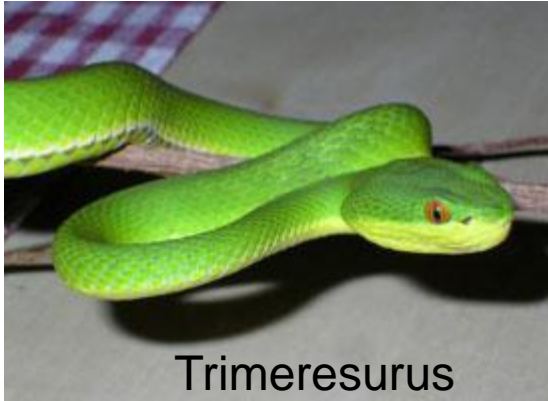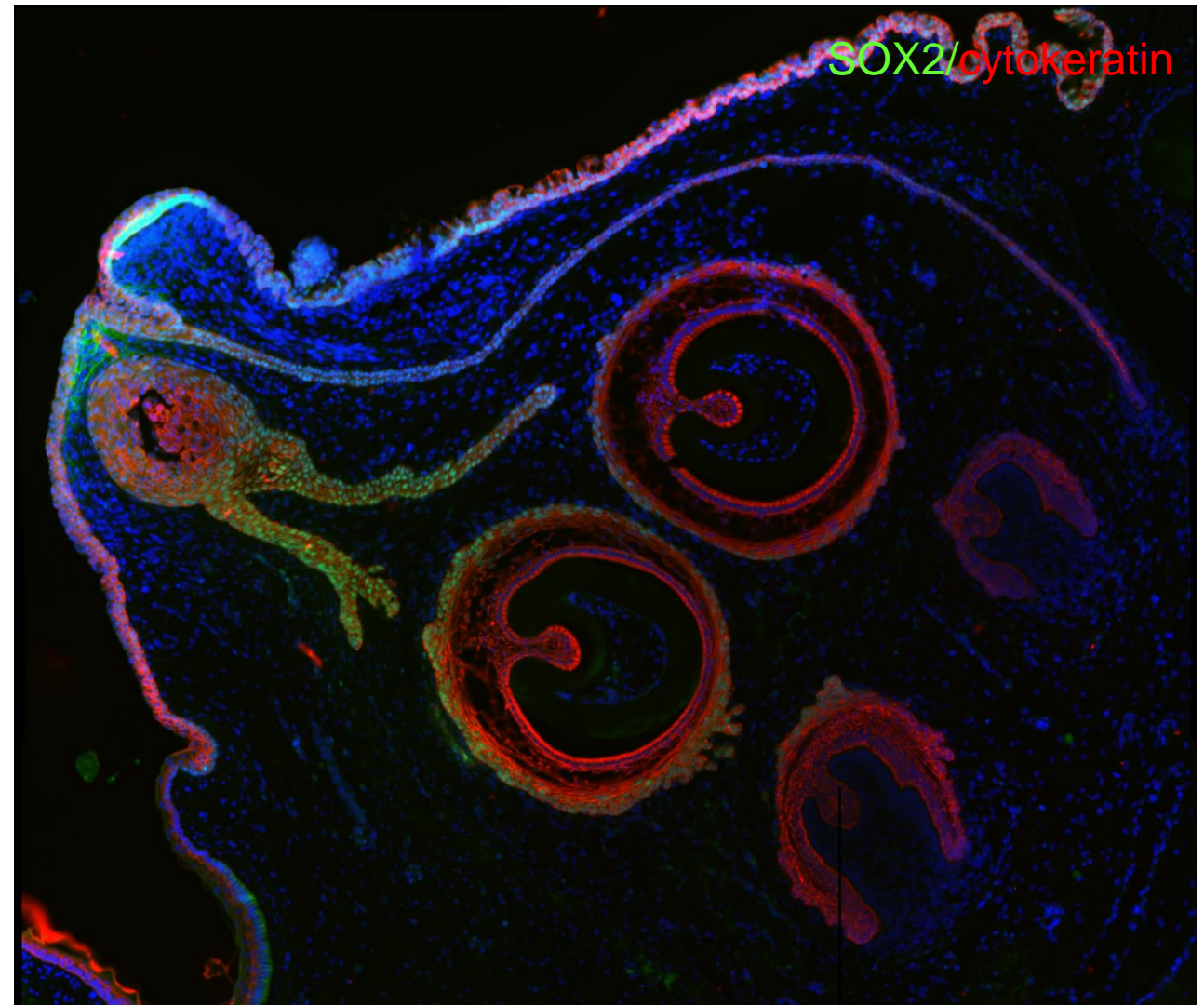
Advantages of using reptiles for the study of odontogenesis

Research into polyphyodont dentics may answer:

— How is a replacement dental lamina initiated?

— How is the dental lamina maintained?

— How are the spacings between the tooth germs observed?

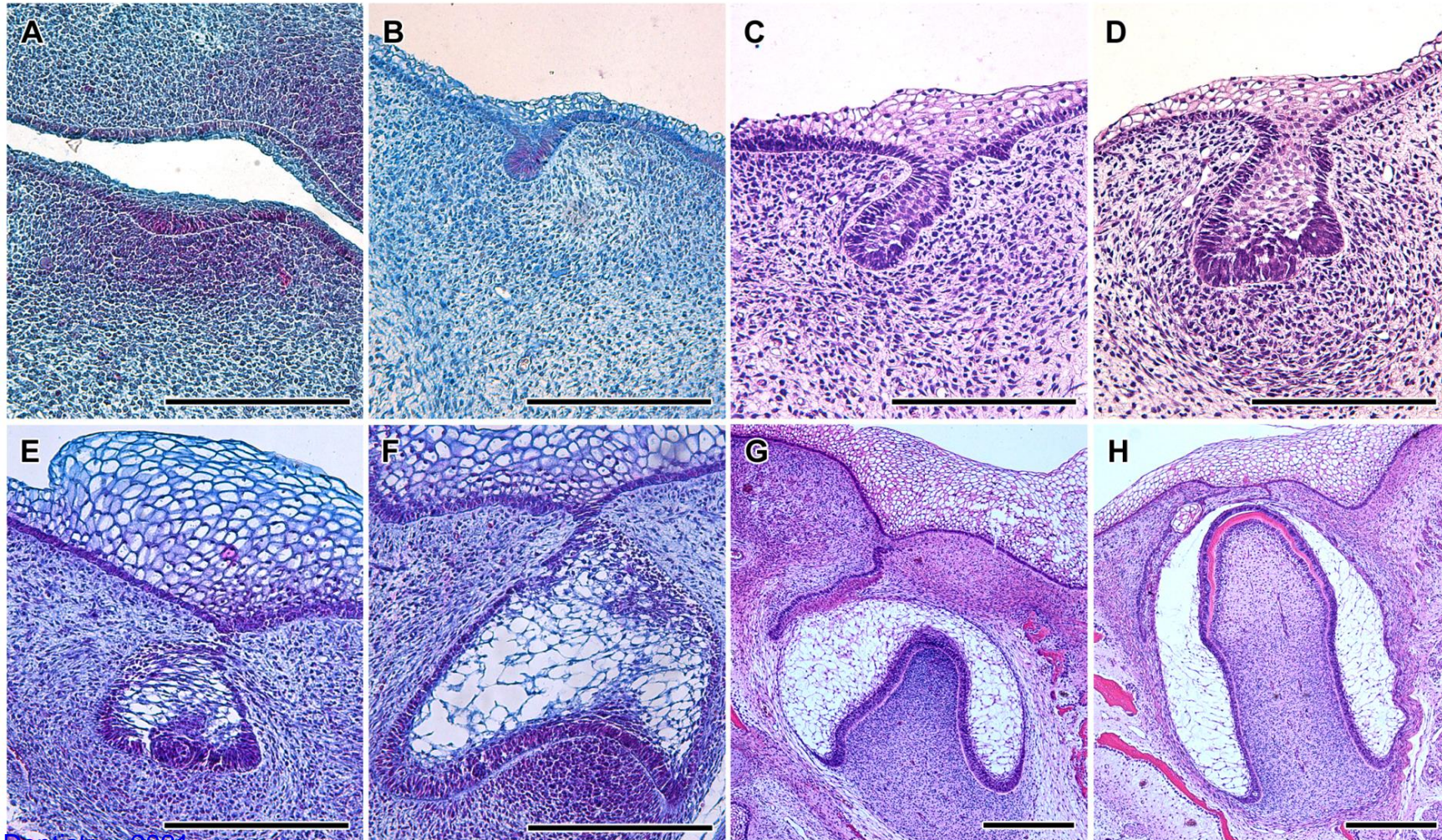— How are stem cells regulated in the dental bar?
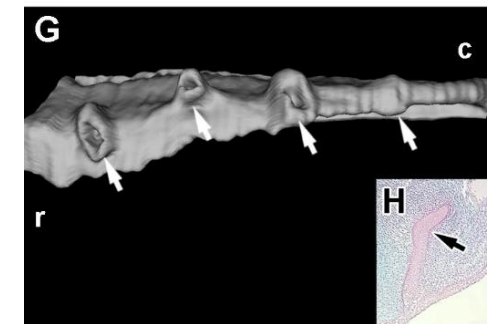
MUNI
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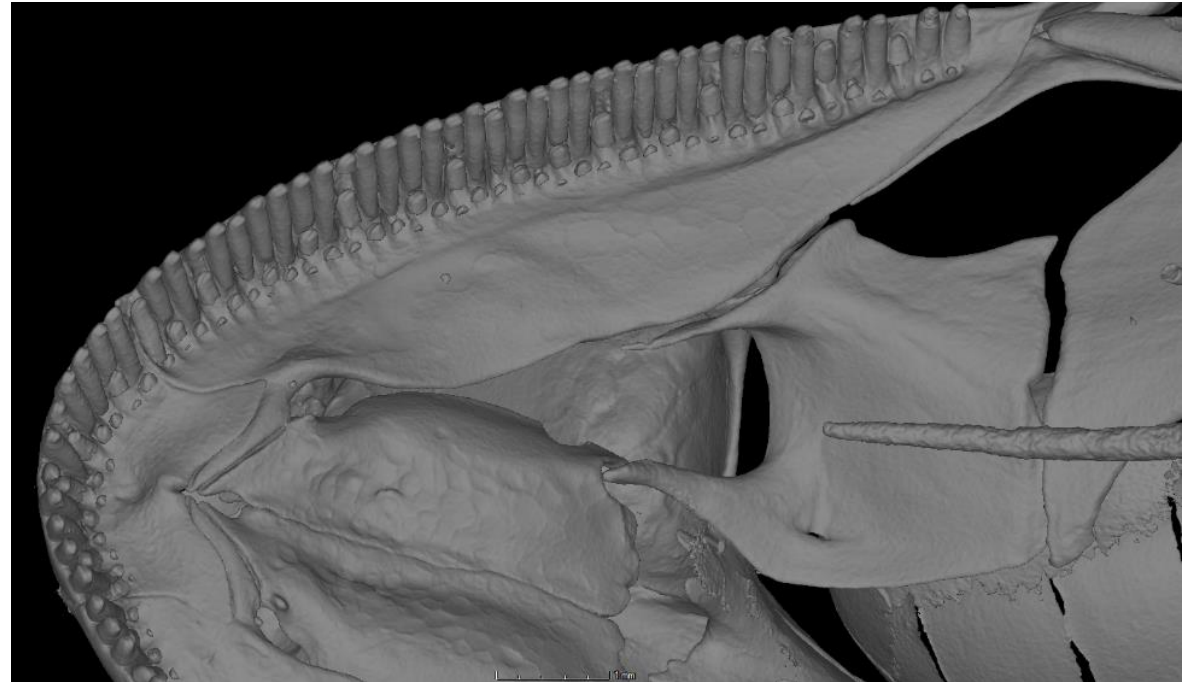
Diphyodont dentition
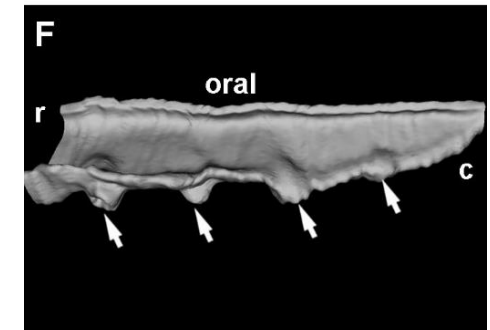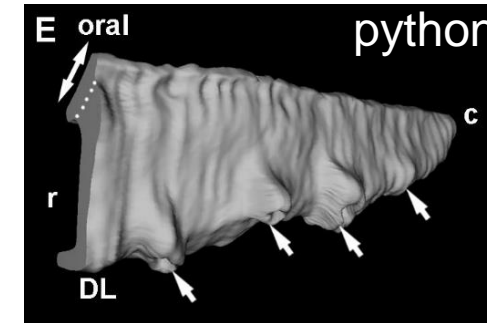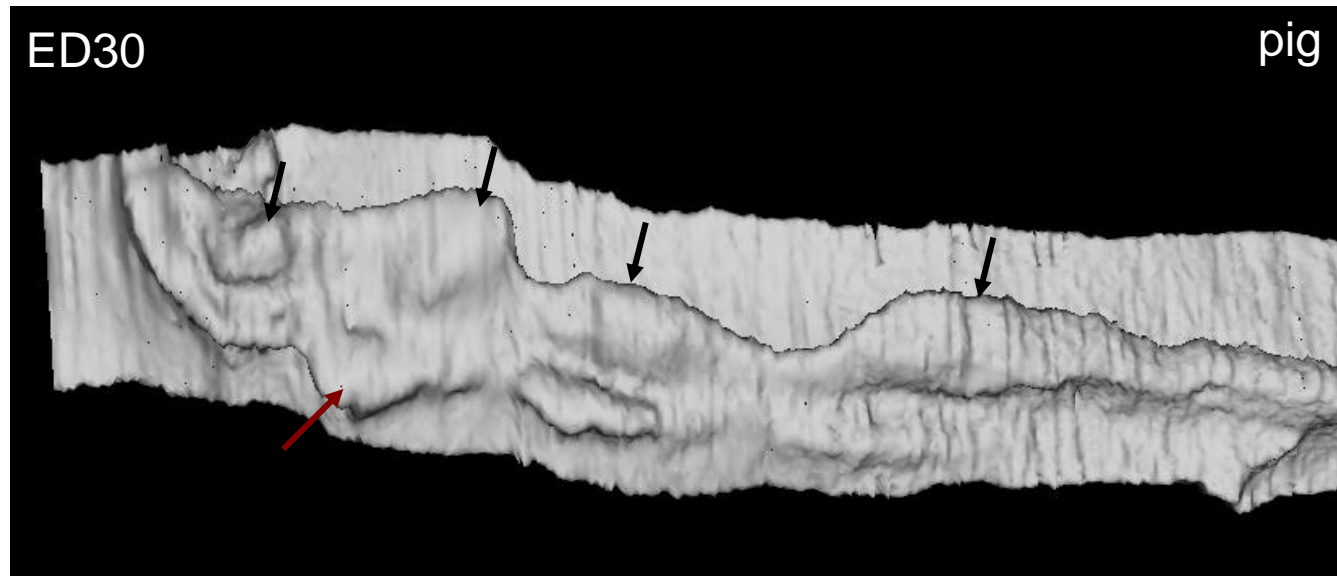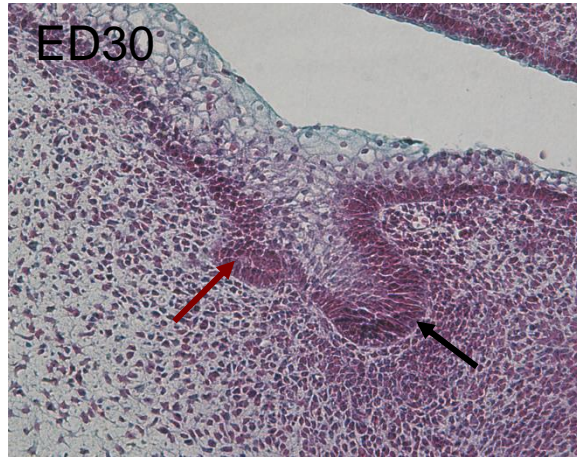

First generation - i3/3 c1/1 p1/1 m3/3 = 32
Second generation - I3/3 C1/1 P4/4 M3/3 = 44

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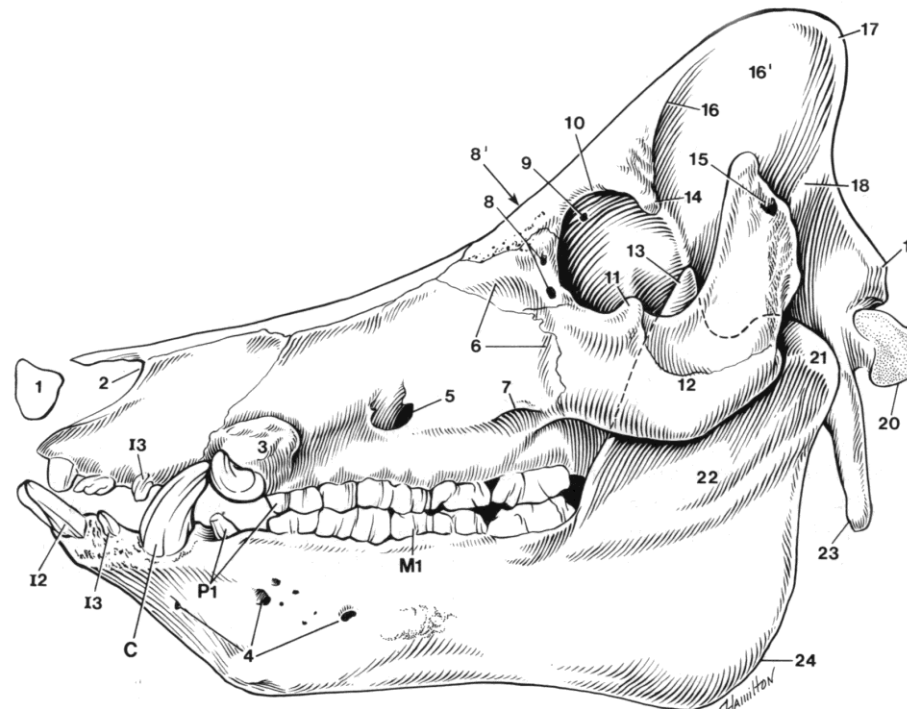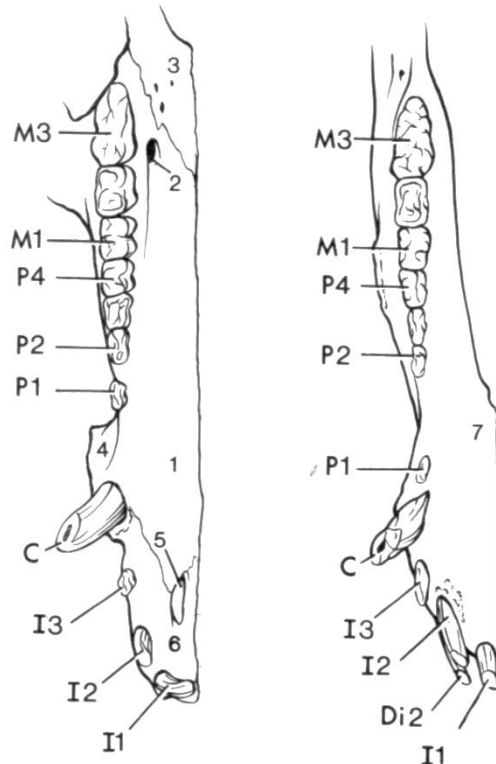
Initiation of the replacement dental lamina

Stembirek et al., 2010

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MED

The interdental lamina is delayed in development

ED30

ED30 pig

python

Stembirek et al., 2010

MUNI
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Early regression of the dental lamina in diphyodont dentition
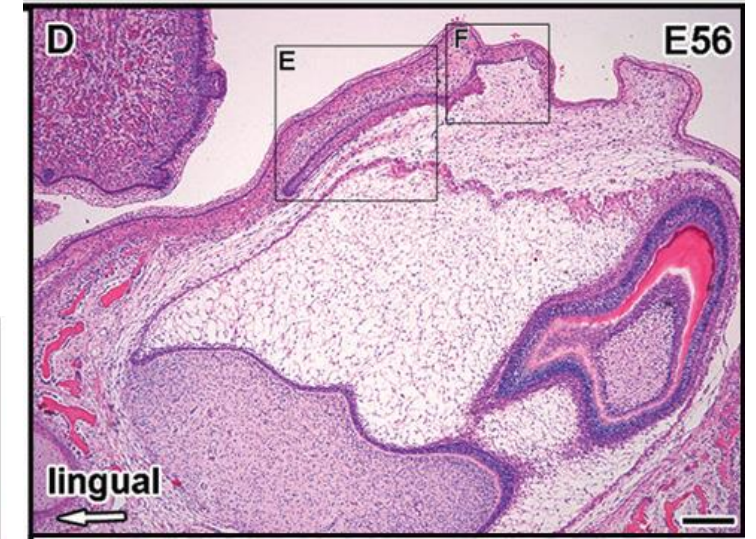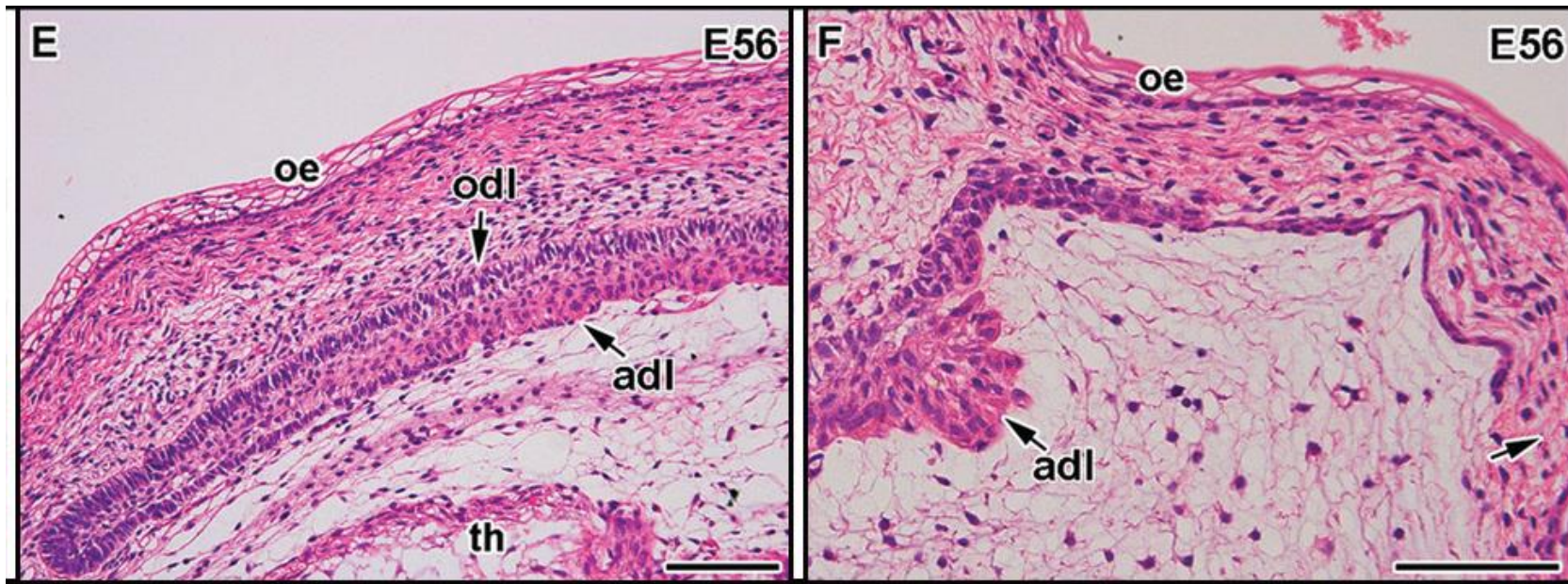
Buchtova et al. 2012

Fragmentation of the dental lamina in the embryonic period

Buchtova et al. 2012

MUNI
MED
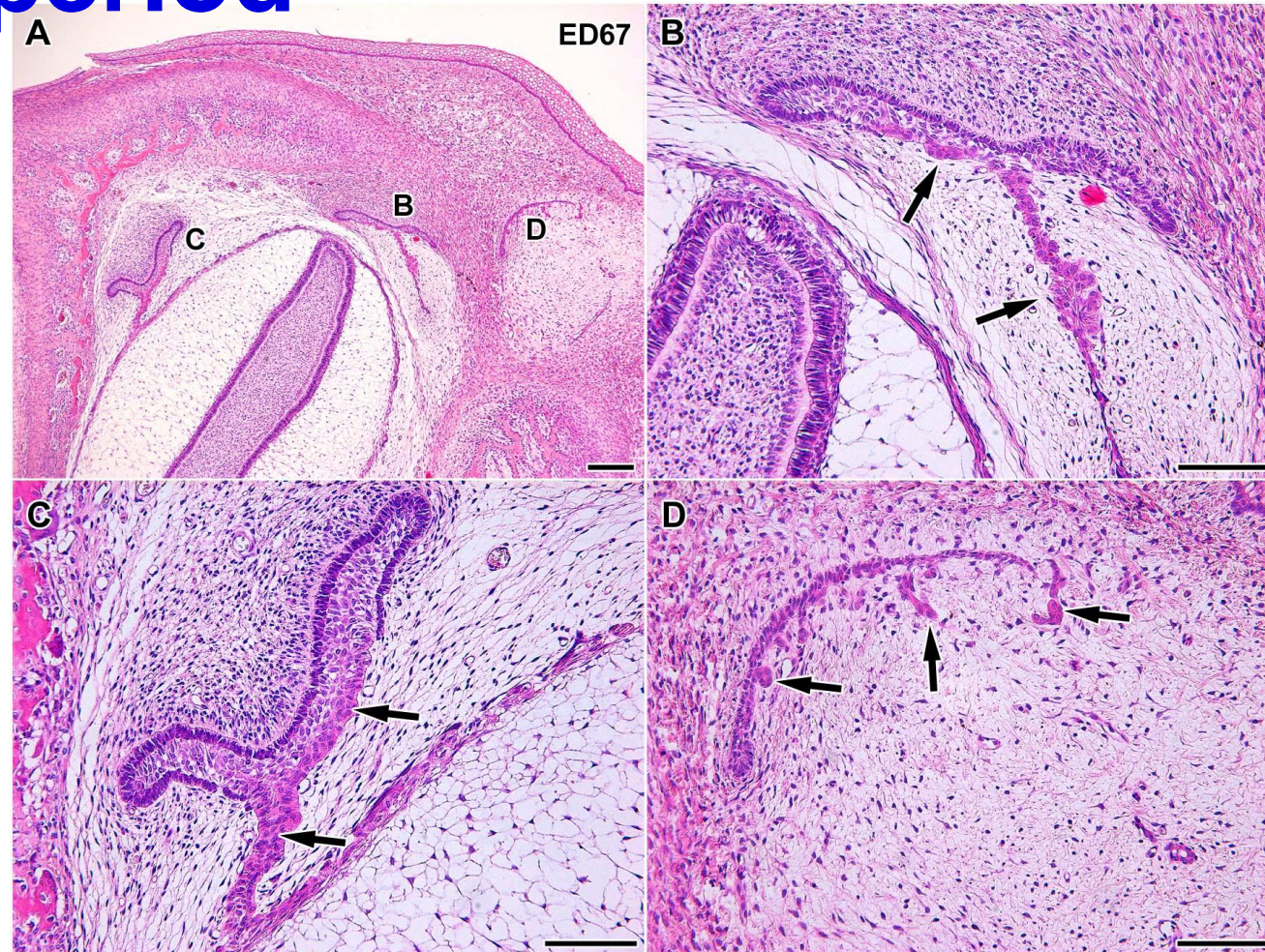
The role of cell death during fragmentation of the dental lamina
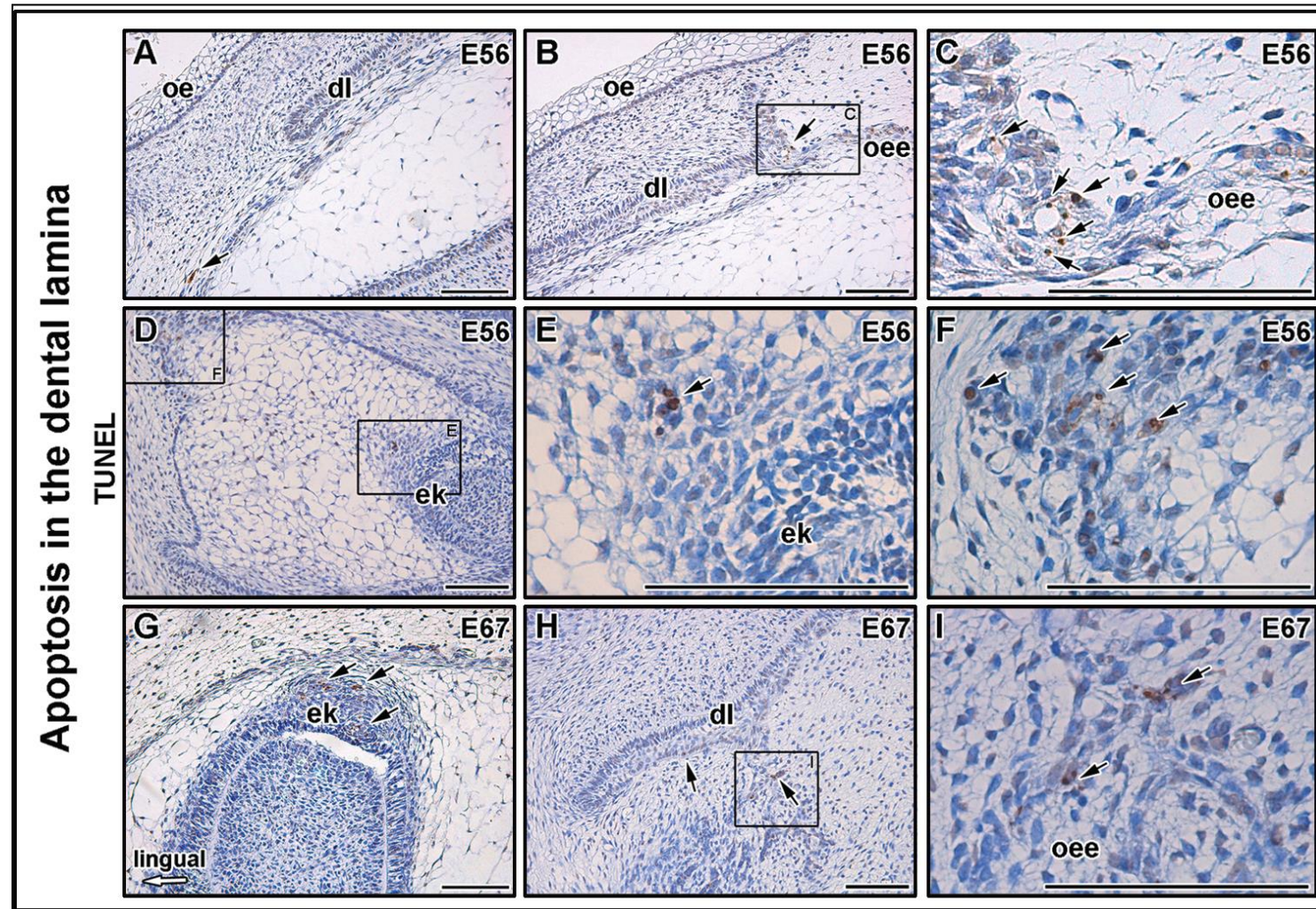
Buchtova et al. 2012

Fragmentation of the dental lamina begins with disruption of the basement membrane

Buchtova et al. 2012

MUNI
MED
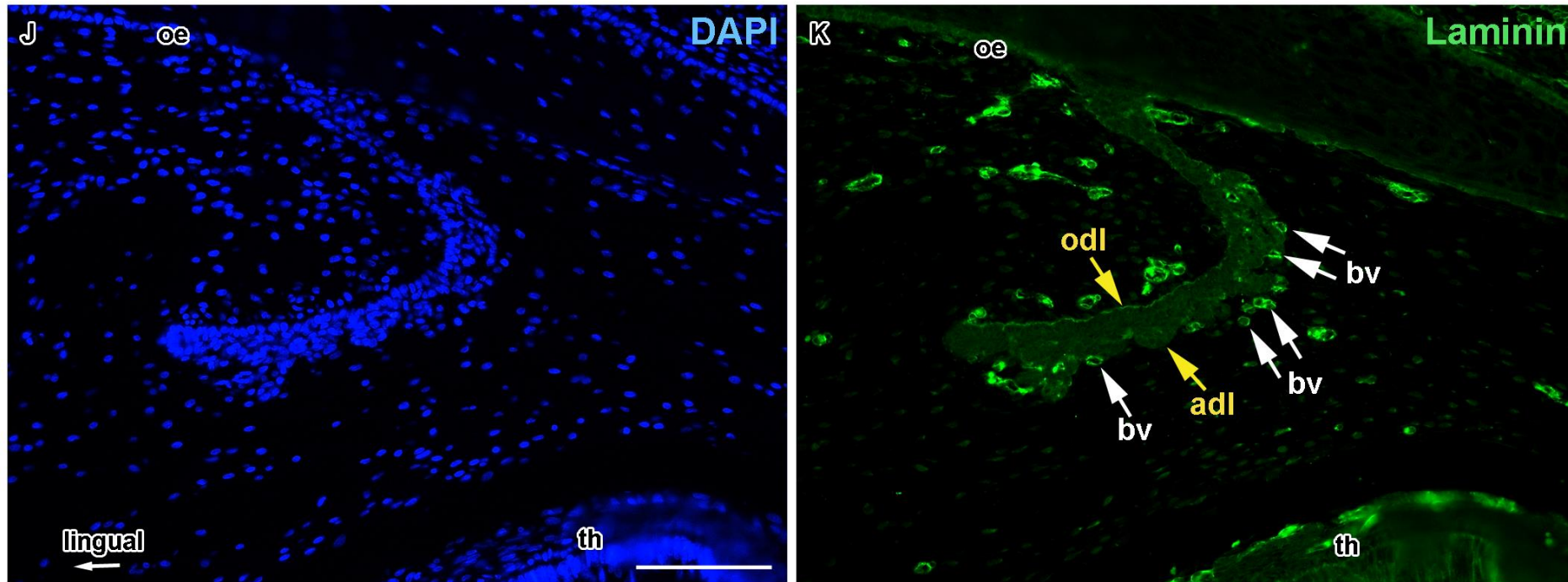
Migration of dental lamina cells
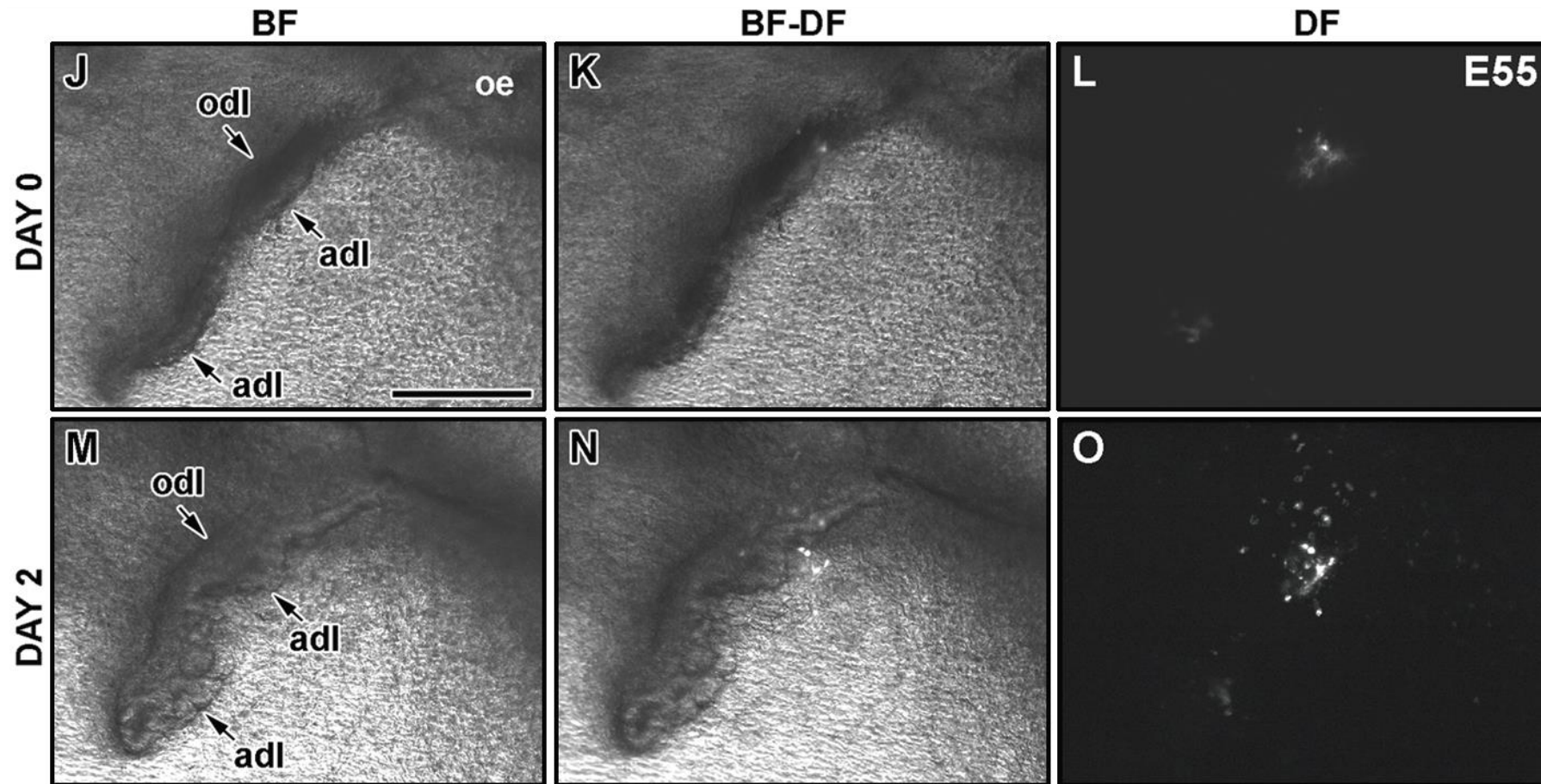
DiI Marking

Buchtova et al. 2012

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Transformation of epithelial cells of the dental lamina into mesenchymal cells
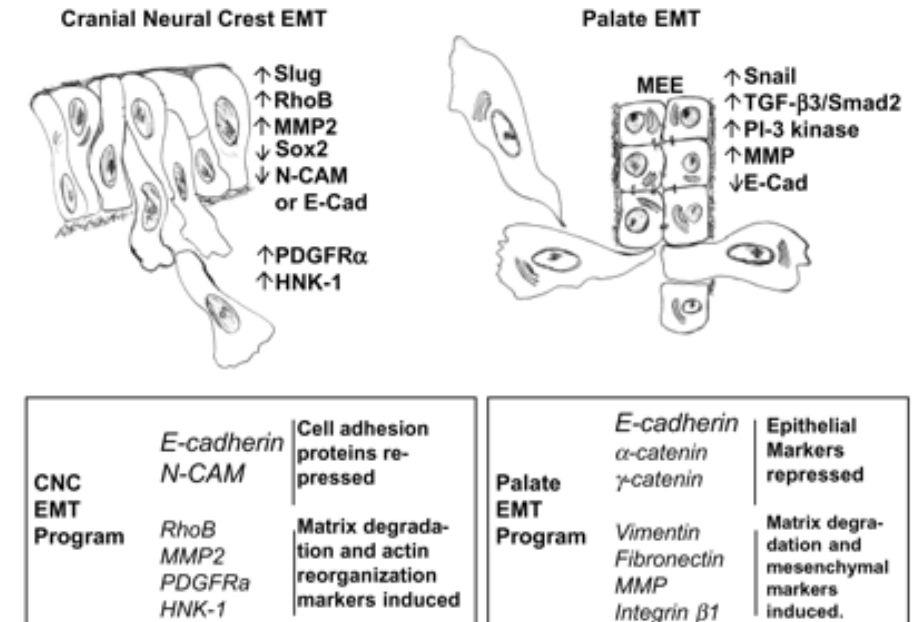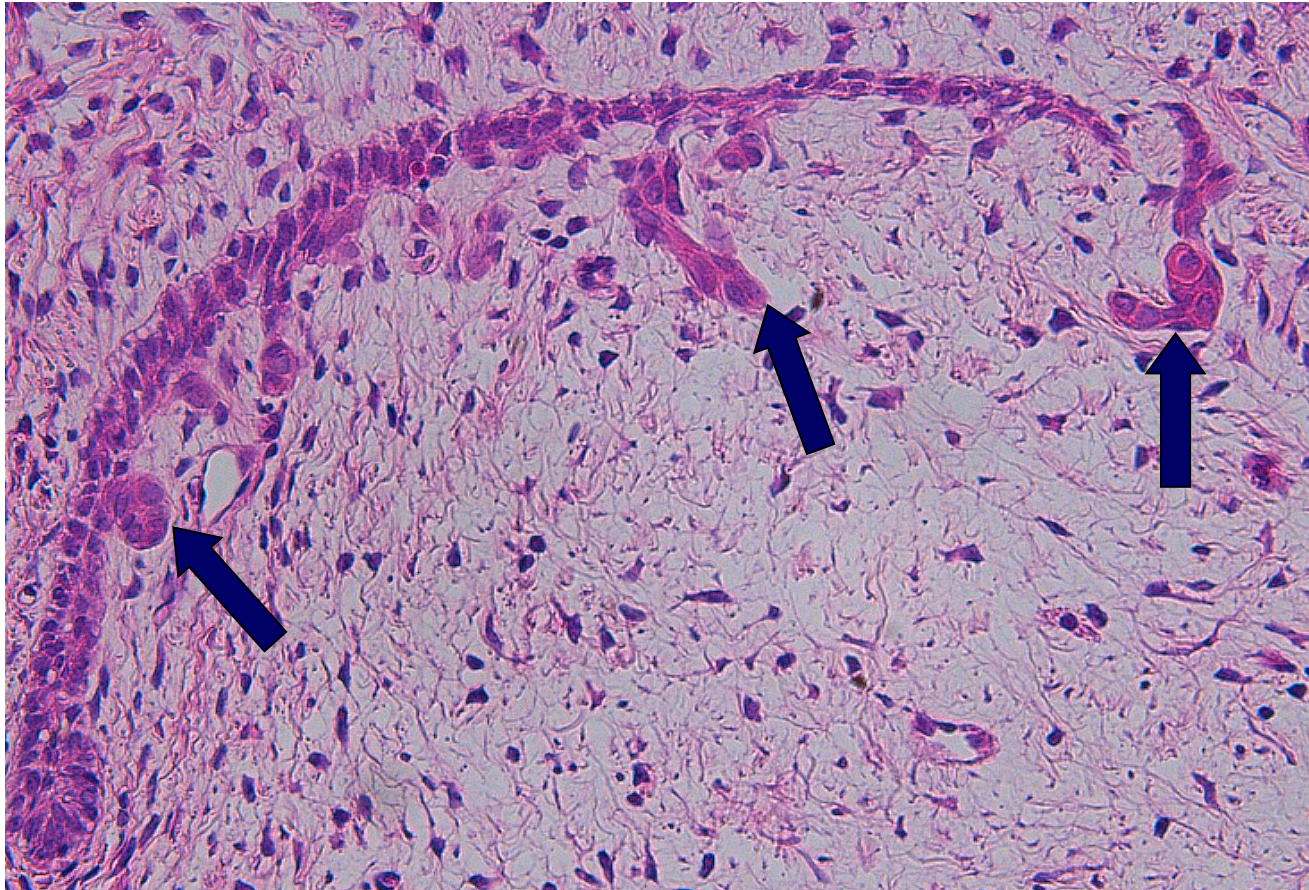
Transformation of epithelial cells of the dental lamina into mesenchymal cells

Buchtova et al. 2012

MUNI
MED
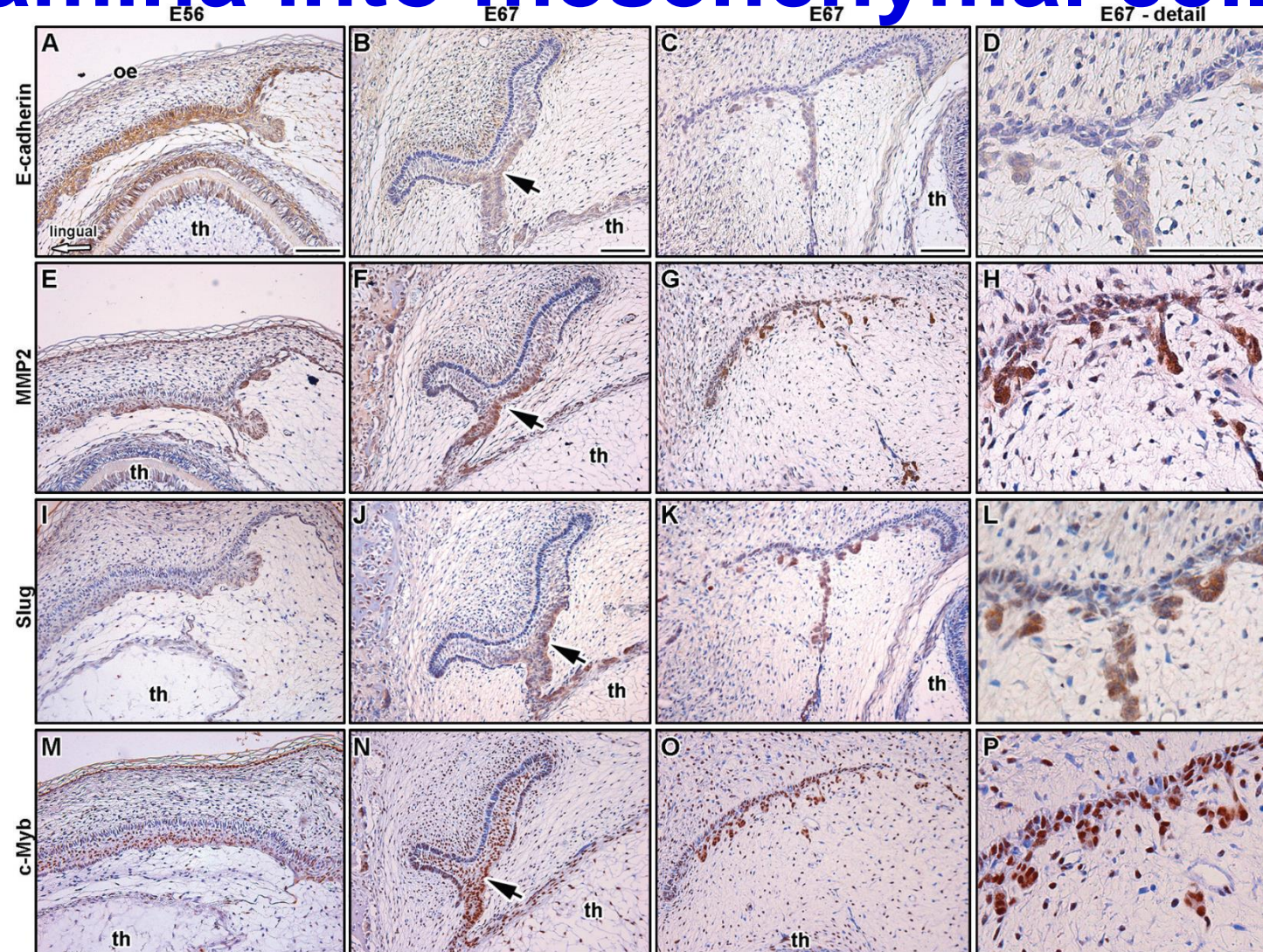
Cells located in the direction of migration show an overlap of cytokeratin and vimentin expression

Buchtova et al. 2012
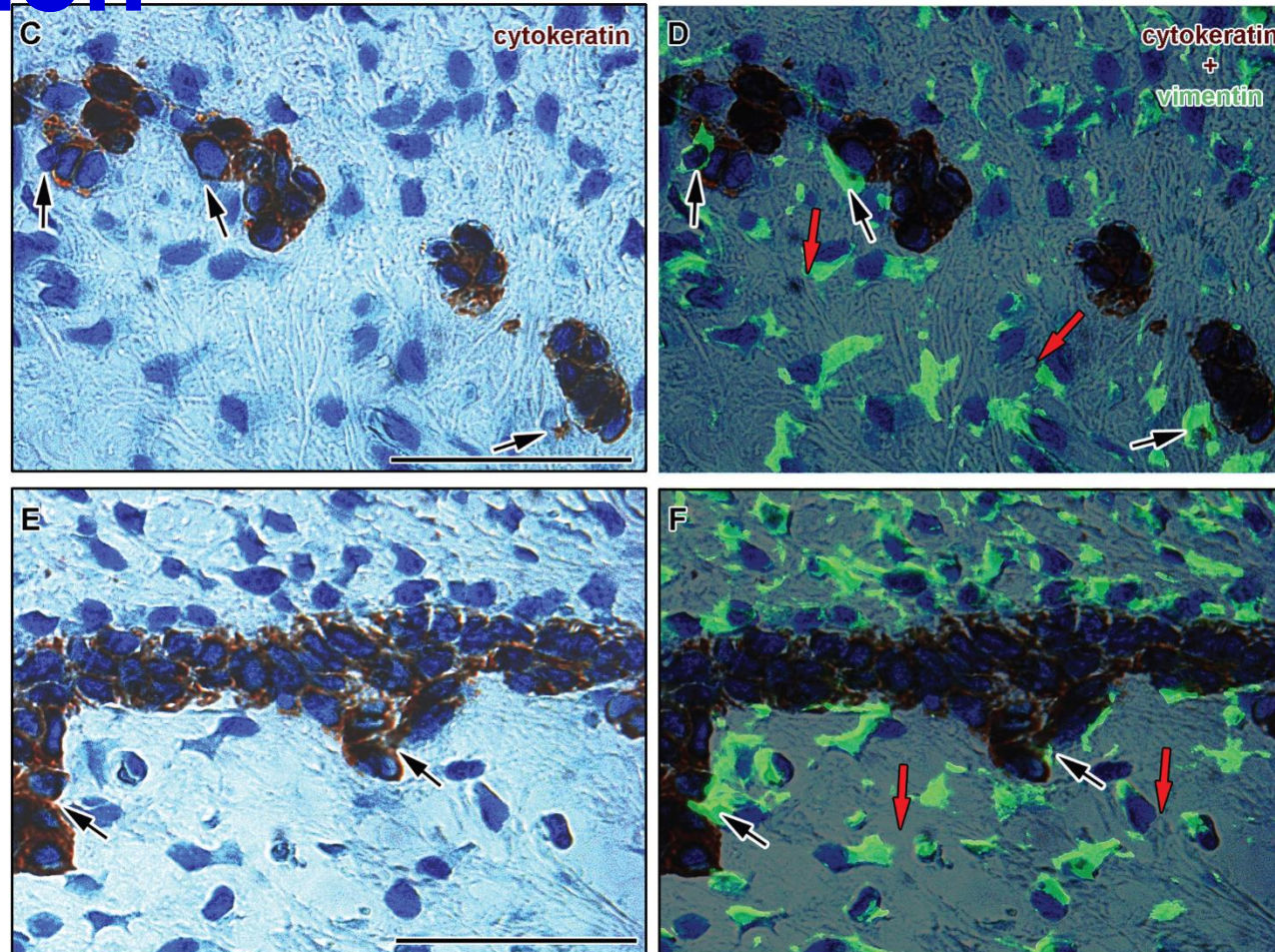
Why does a human not form a third generation of teeth?

— fragmentation and degradation of the dental lamina

— the dental lamina loses the ability to form replacement

dental generations during the prenatal period

WorldPress.com

MUNI
MED

Is the replacement dental lamina also initiated in monophyodont species?

MUNI
MED

Why is there no change of teeth in mice?

Dental formula: 1-0-0-1
Monophyodont
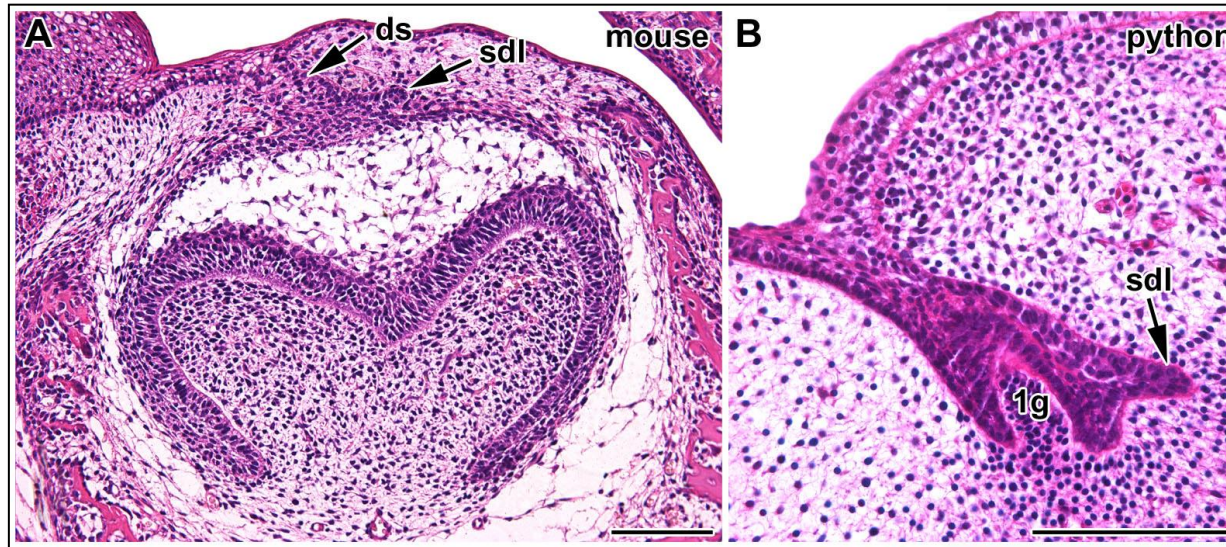
replacement dental lamina (sdl)
dental stem (ds)

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MED
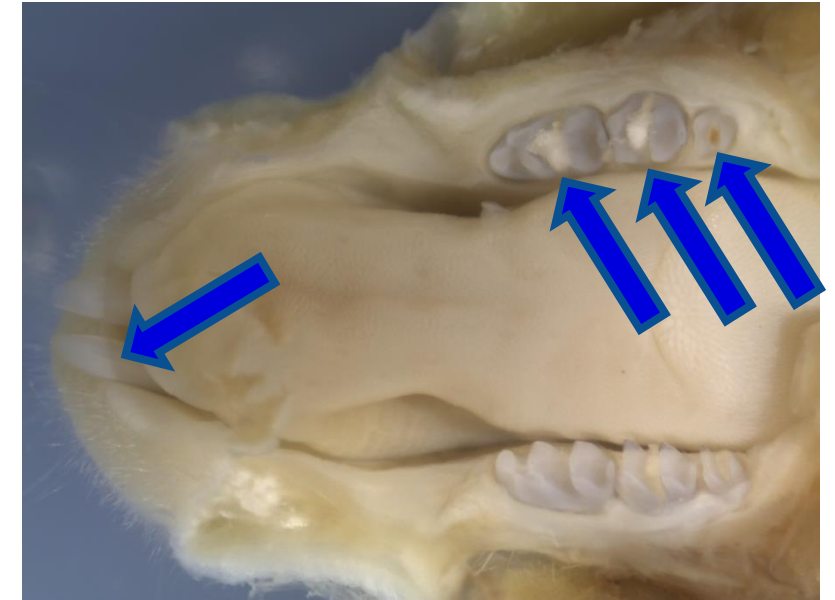
Replacement dental lamina develops at a late stage of embryonic development

Dosedelova et al. 2015
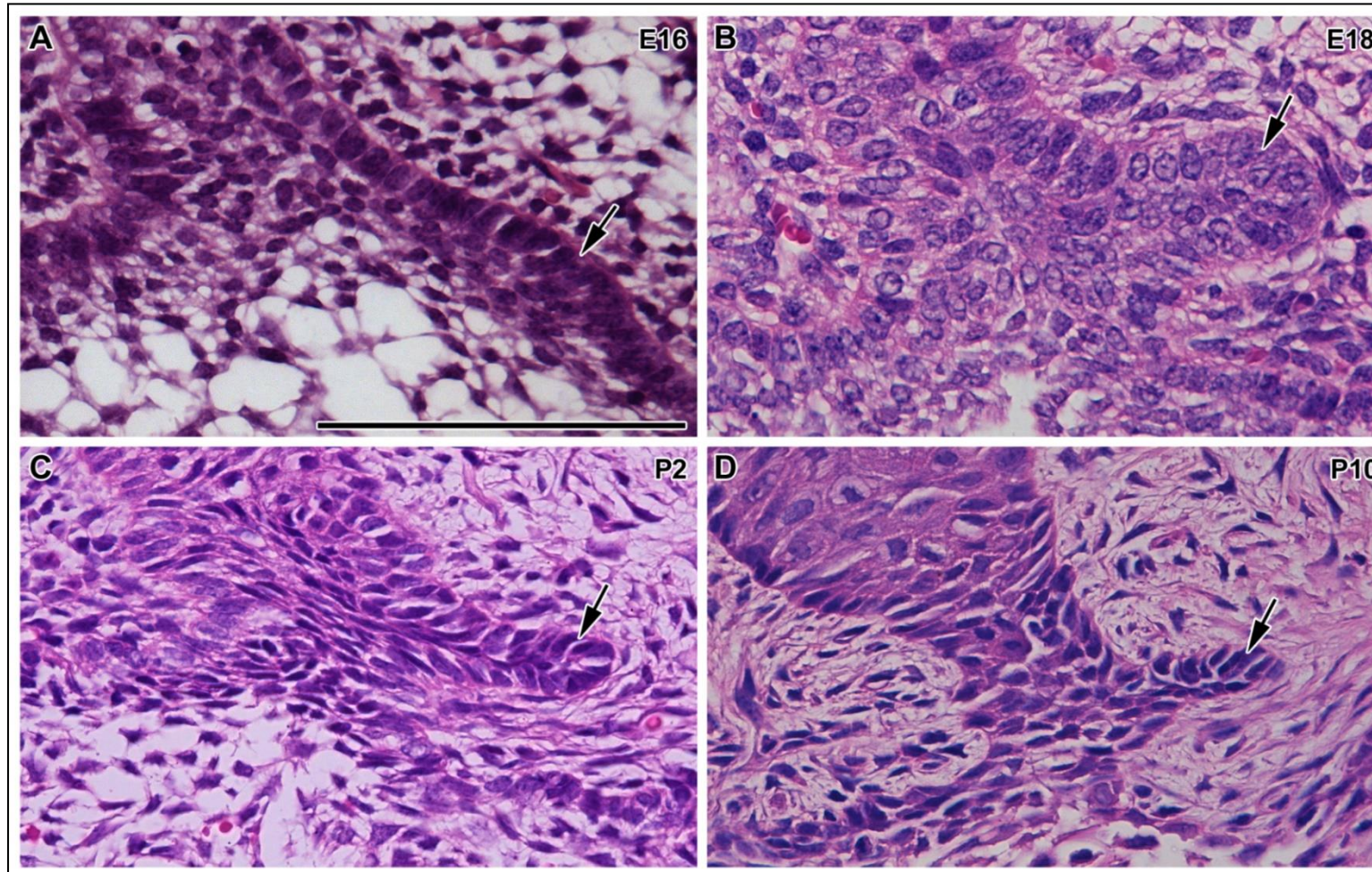
Degradation of the dental lamina occurs in the mouse during the postnatal stages

— reduced proliferation

— cytokeratin expression is increased

— formation of epithelial folds with numerous lysosomes

— nuclear degradation

Dosedelova et al. 2015
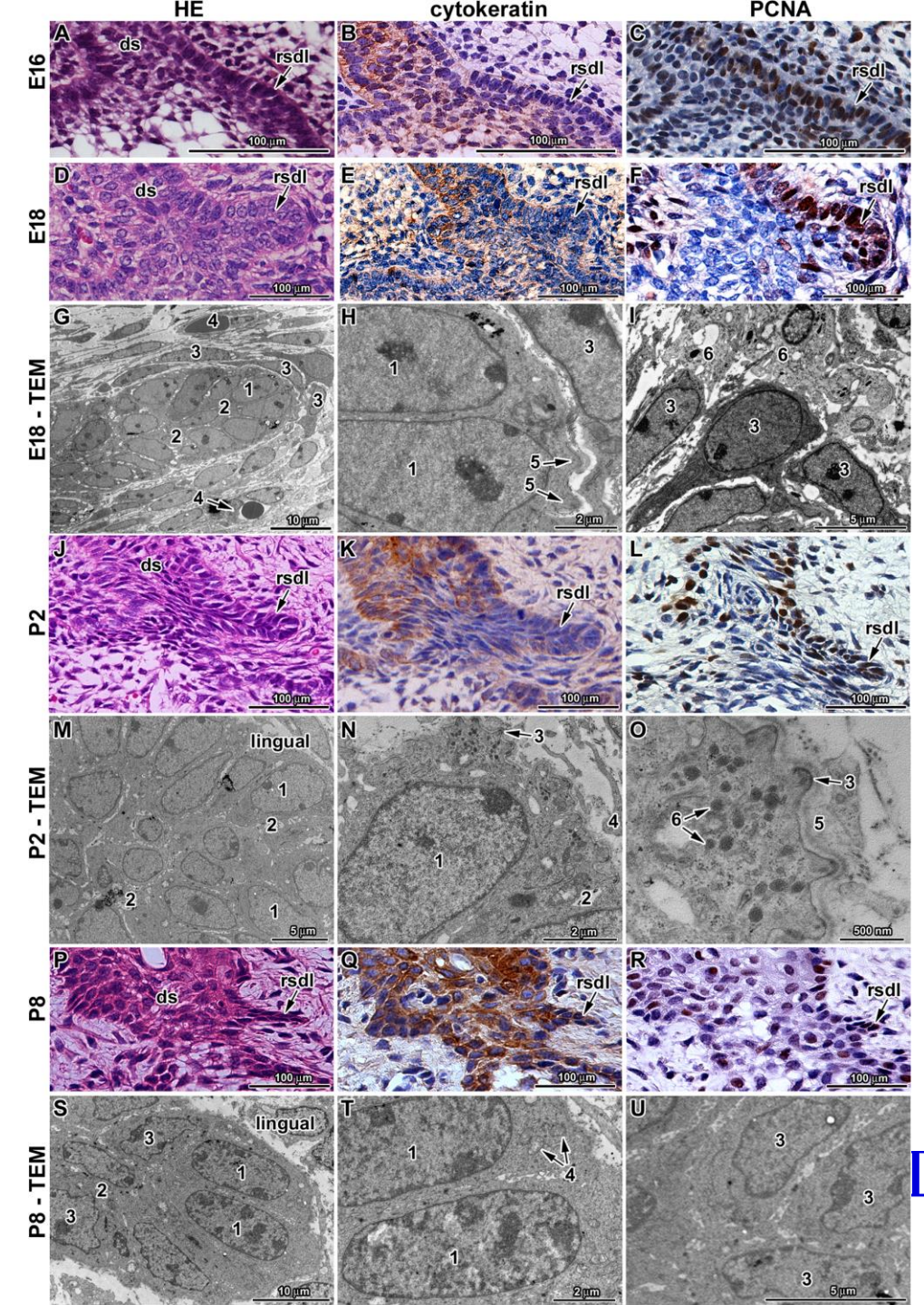
Are there localized progenitor cells in the rudimentary dental lamina?

Hypothesis:
Sox2/Lgr5 can play a role in the restriction of replacement dental lamina

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Where are the progenitor cells localized during odontogenesis?

HE (M1)	LGR5 (M1)	HE (M2)	LGR5 (M2)

Lgr5-EGFP-CreERT2 mice

K. Olbertová

MUNI
MED
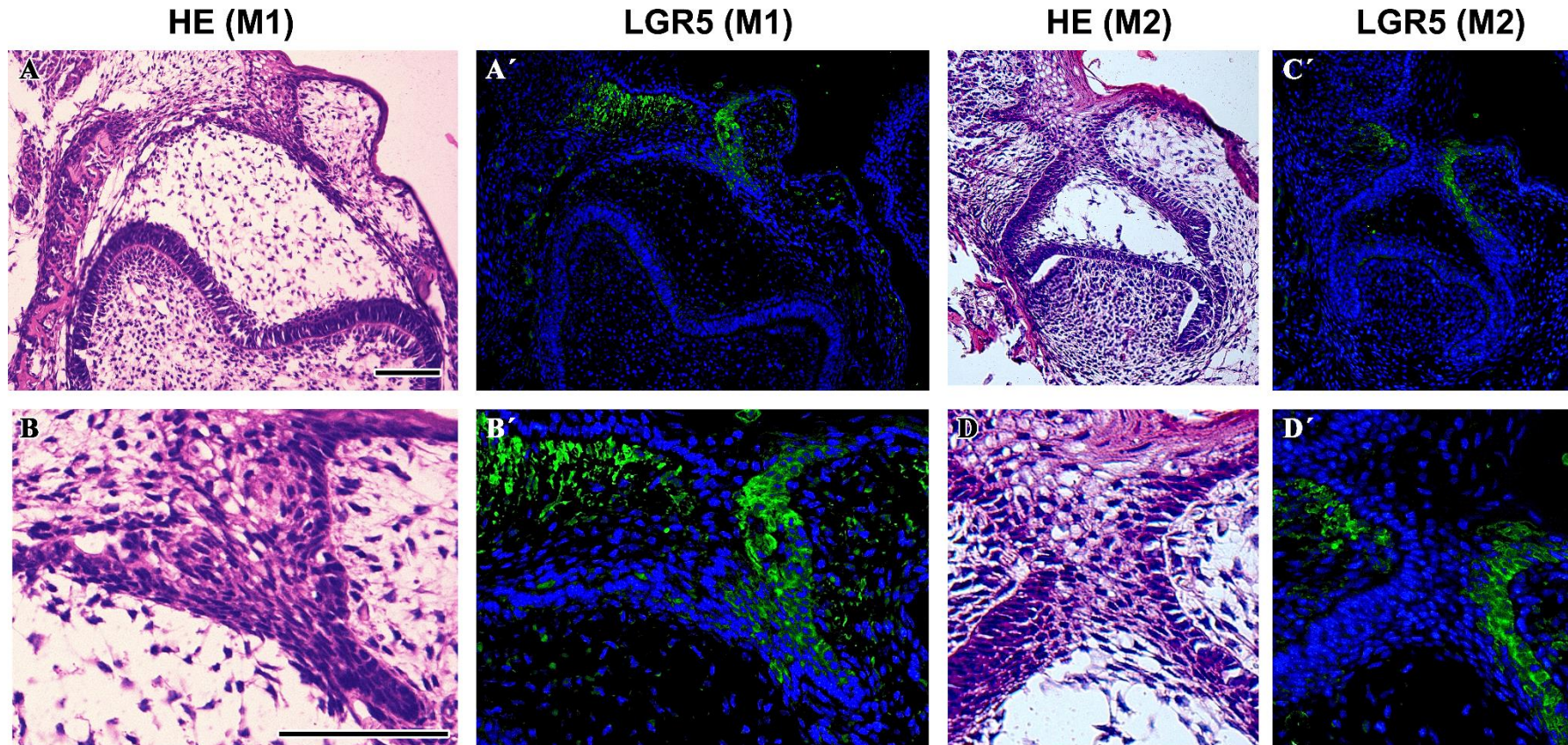
Progenitor marker SOX2 is expressed in the dental stem and in the rudimentary dental lamina

Research Activities in Dentistry   2021

Dosedelova et al. 2015

MUNI
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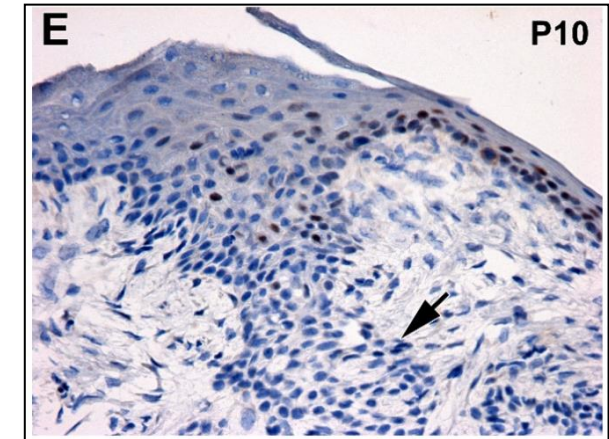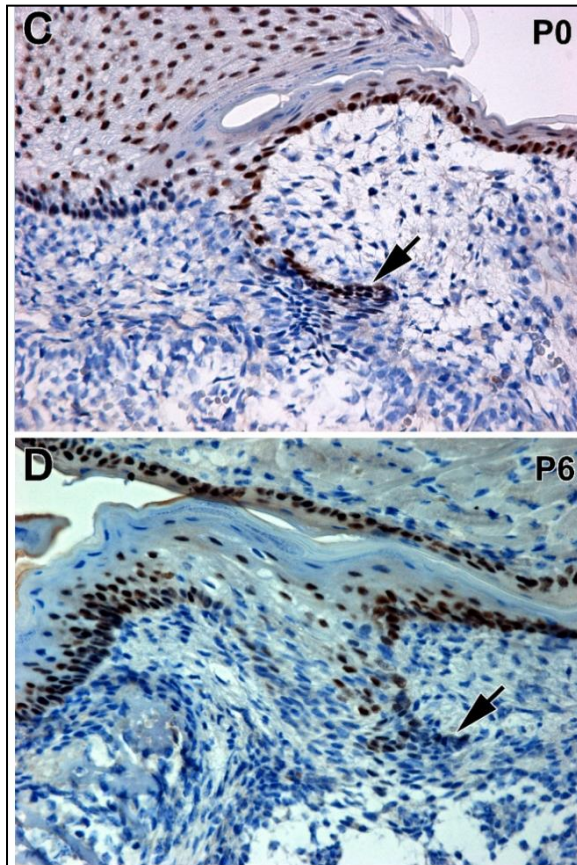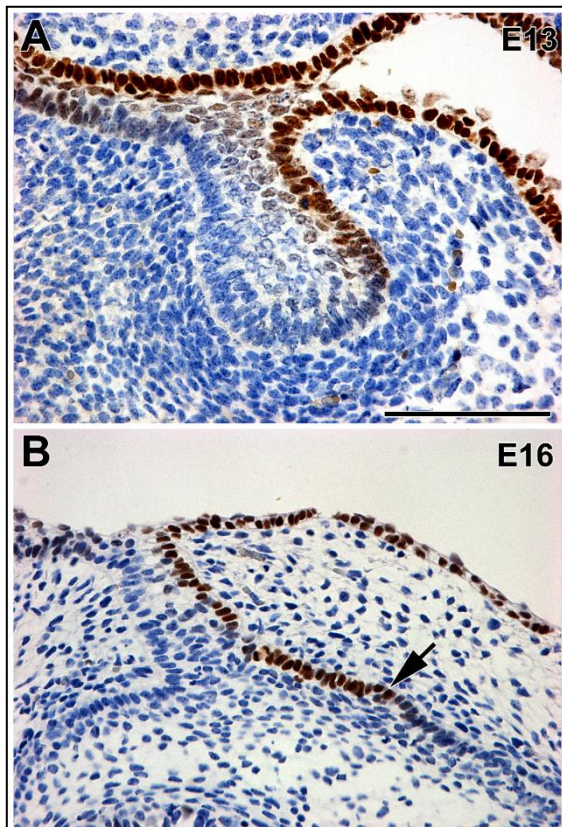
Do SOX2-positive cells overlap the LGR5-positive domain?

K. Olbertová

Lgr5-EGFP-CreERT2 mice

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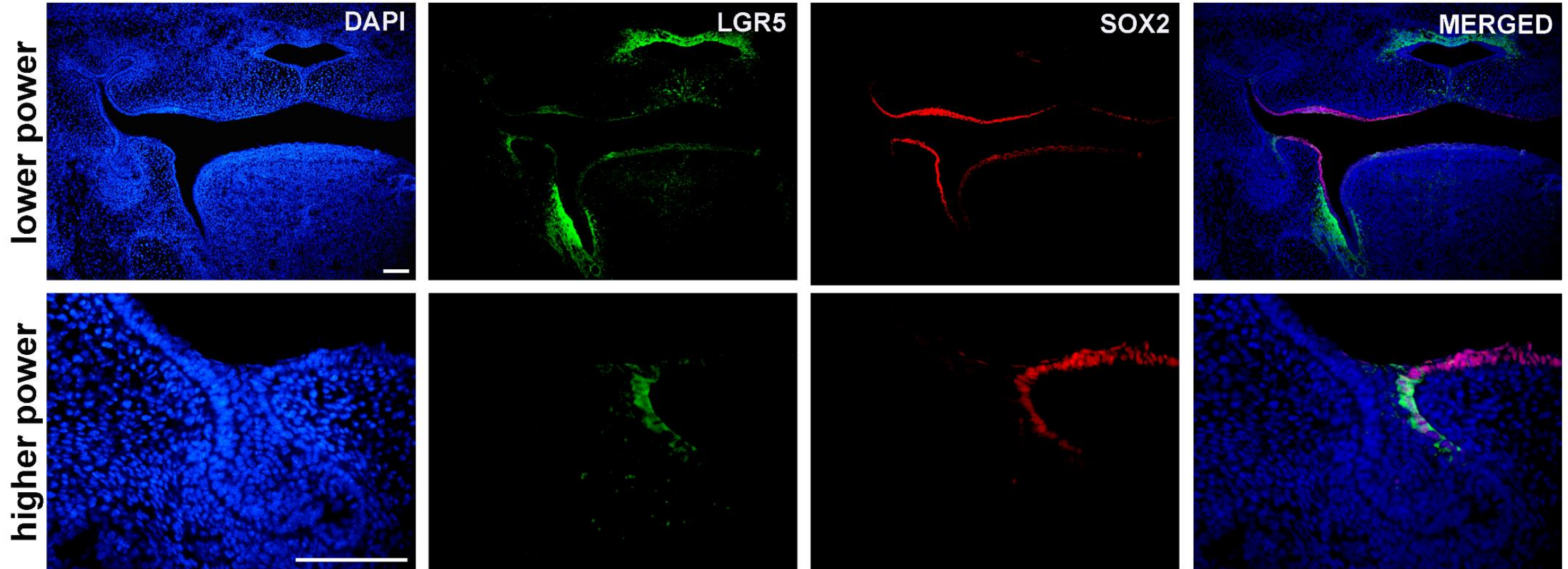
Differences in the signalling of the replacement dental lamina

Pig E67 DL | Pig E67 IDL | Mouse E18.5 | Mouse P2

Popa et al. 2019

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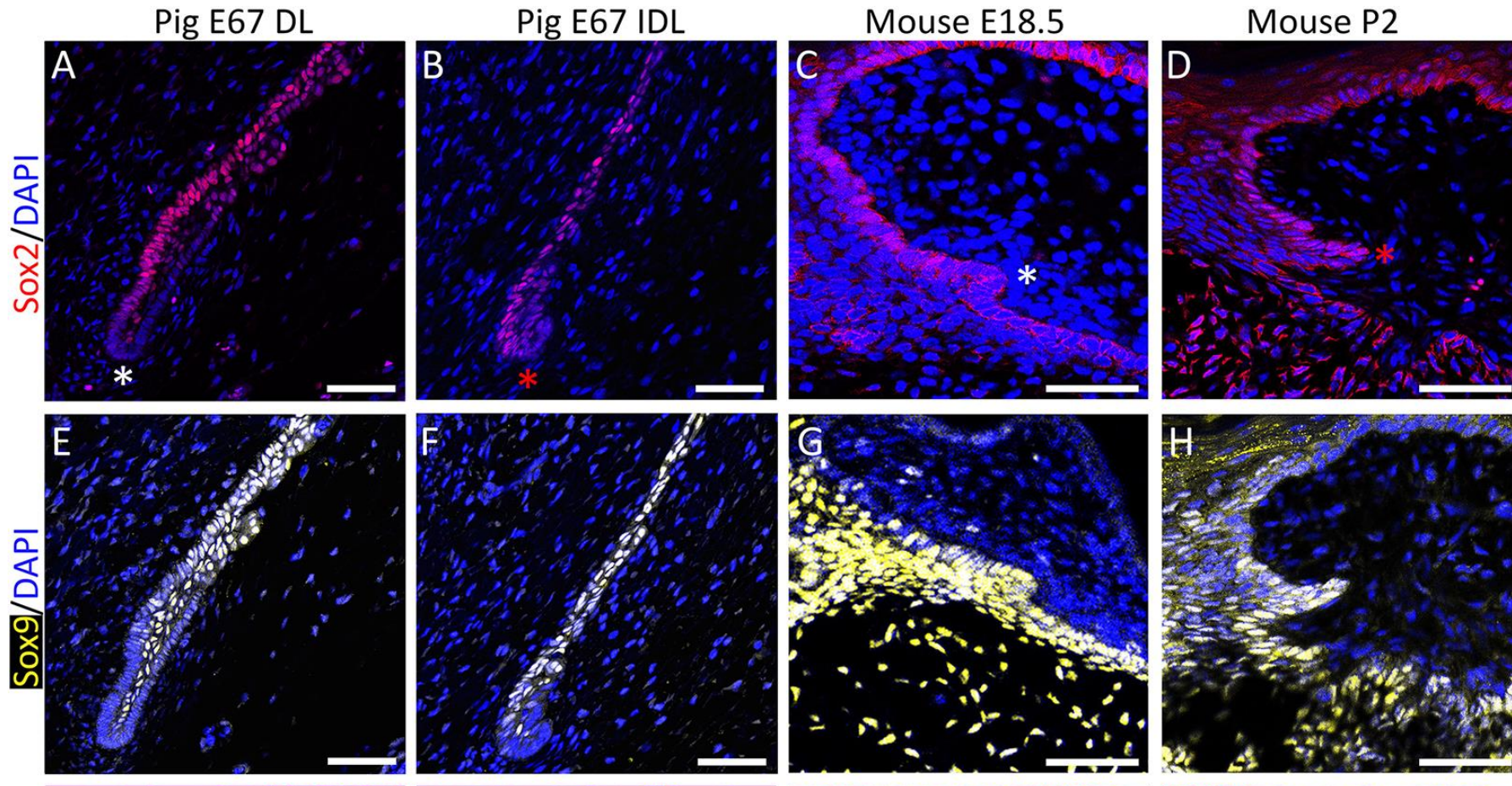
Can we initiate the formation of a replacement dental lamina in monophyodont species?

— Sox2 role in inhibition of canonical WNT signal activation

Sox2$^{CreERT2/+}$;Ctnnb1$^{lox(ex3)}$

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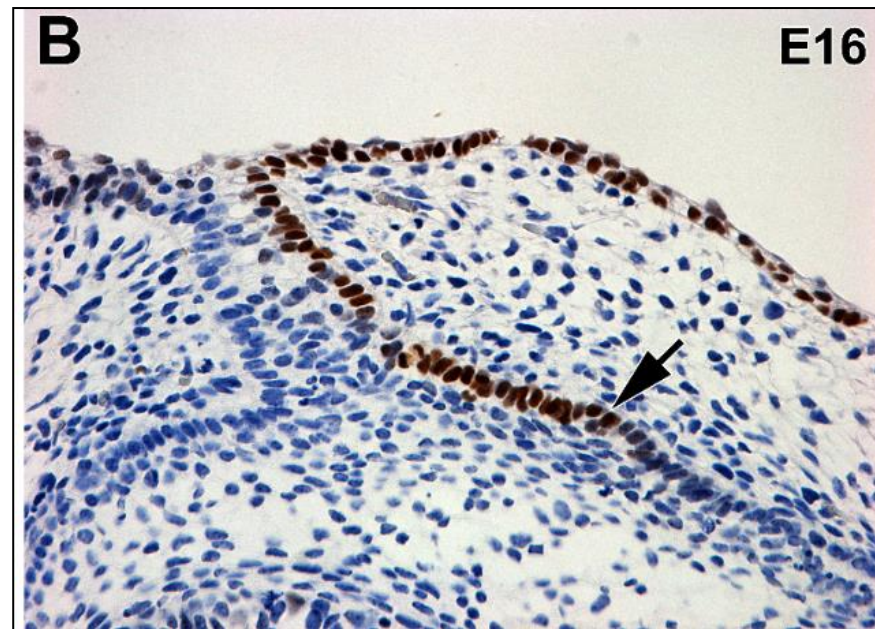
Initiation of a replacement dental lamina

– localized proliferation

and vascularization

Sox2$^{CreERT2/+}$;Ctnnb1$^{lox(ex3)}$

E16→E18.5

RSDL Control   RSDL mutant

BRDU

DAPI

Laminin

Popa et al. 2019

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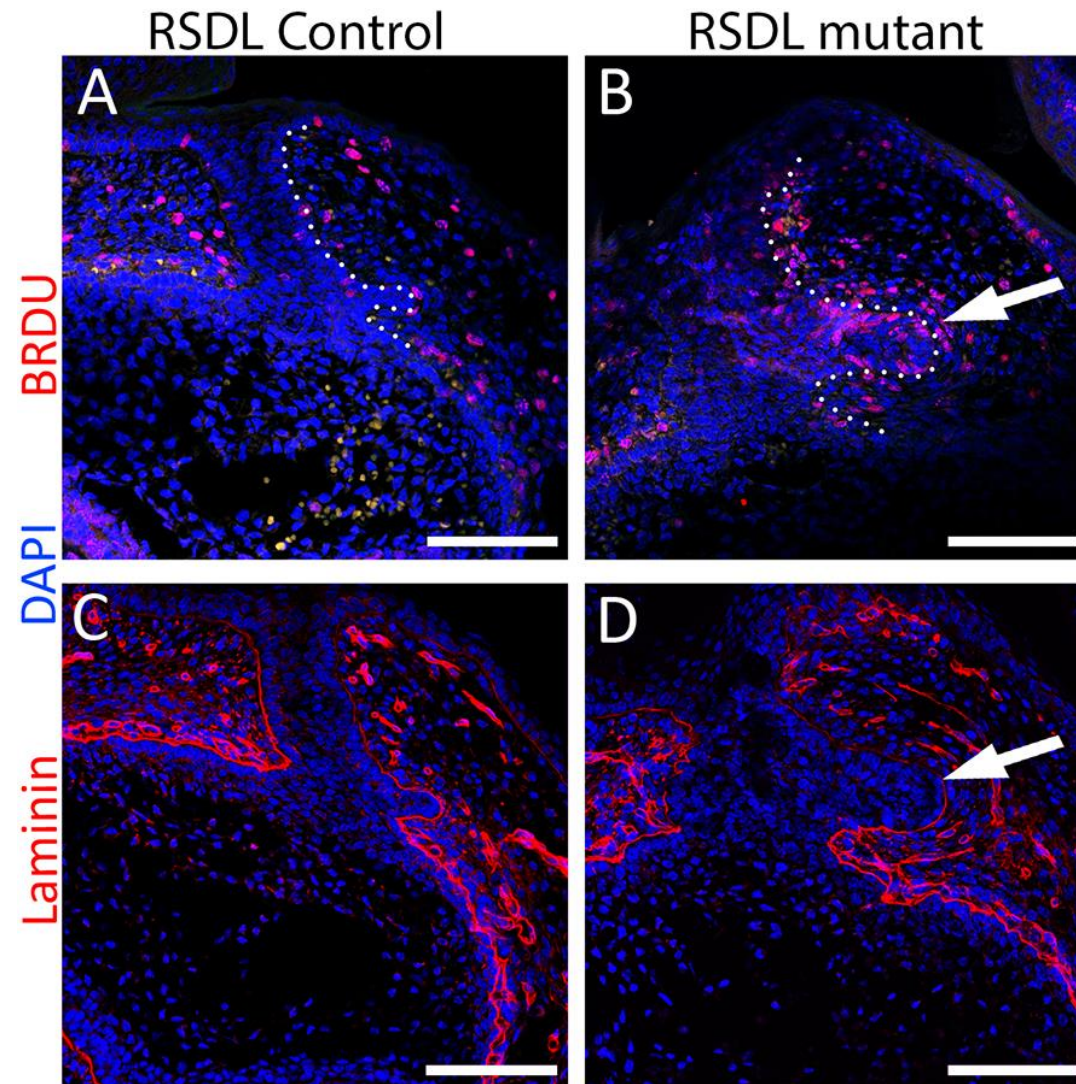
Stabilization of Wnt/β-catenin signalling

Sox2$^{CreERT2/+}$;Ctnnb1$^{lox(ex3)}$

E15.5→E18.5

Popa et al. 2019

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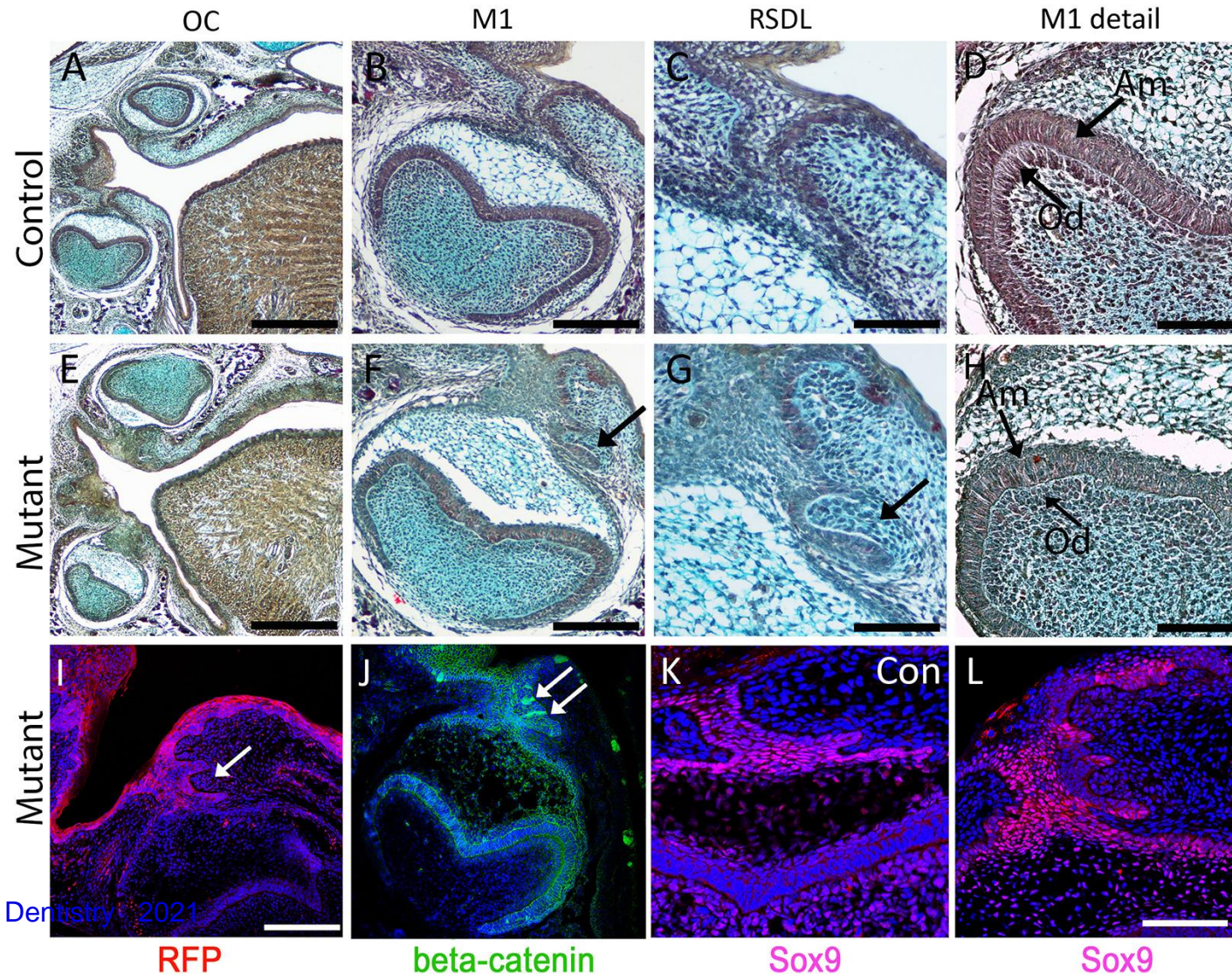
Expression of odontogenic markers in supernumerary dental bases

Shh | Fgf4 | Fgf3 | Bmp4 | Ectodin

Control: A, B, C, D, E
Mutant: F, G, H, I, J

Sox2$^{CreERT2/+}$;Ctnnb1$^{lox(ex3)}$ E15.5→E18.5

Popa et al. 2019

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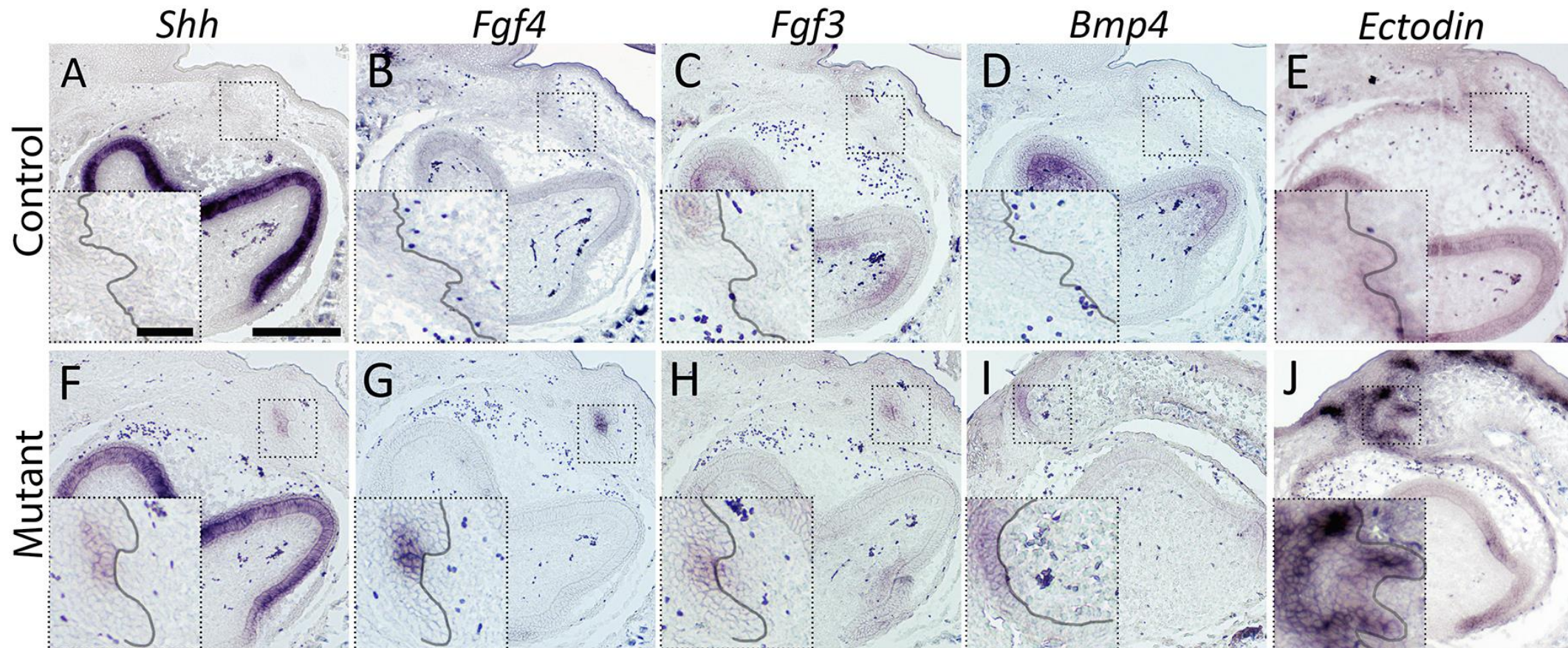
Supernumerary dental bases mineralize and form dental roots
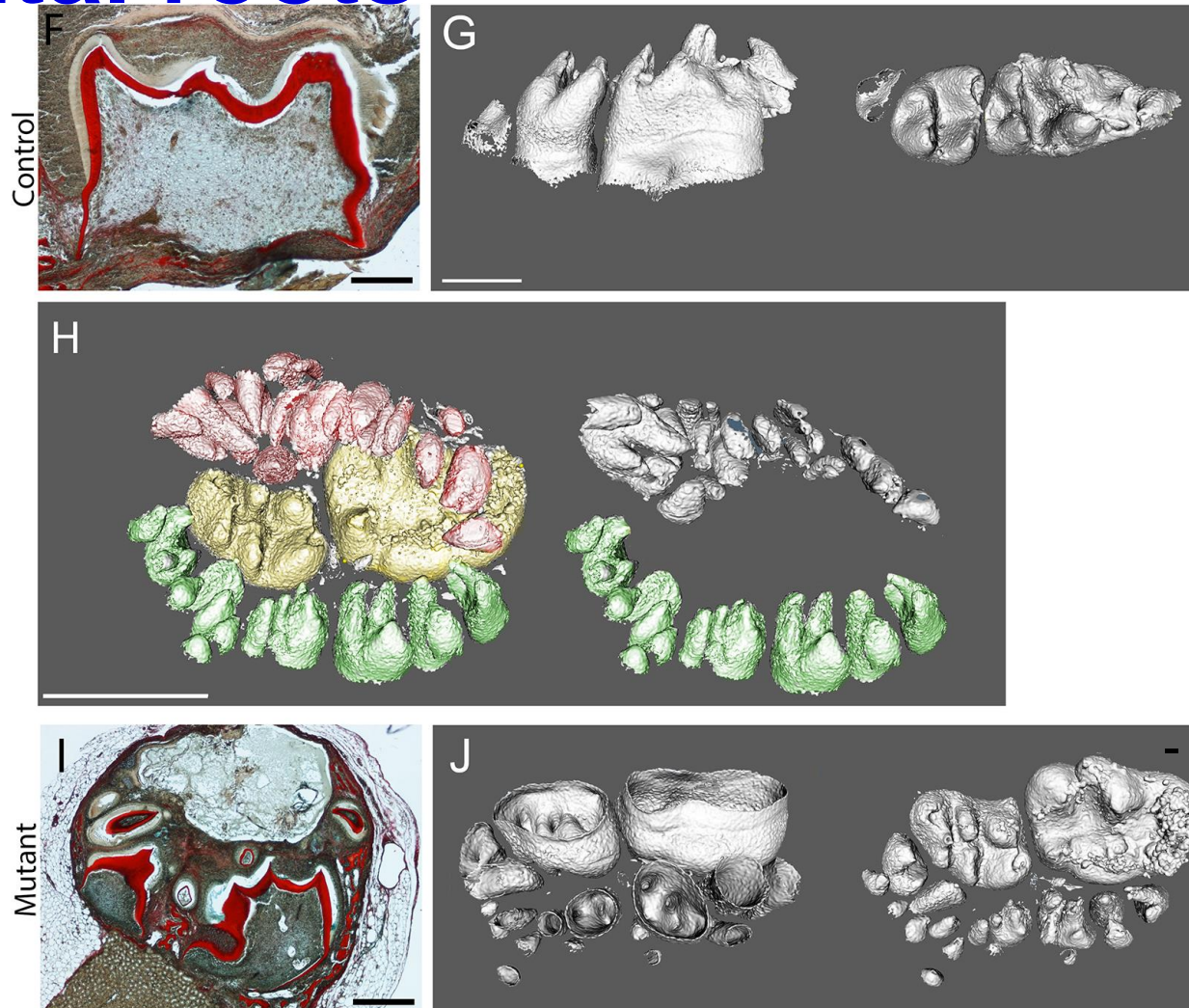
Sox2$^{CreERT2/+}$;Ctnnb1$^{lox(ex3)}$

E17.5
- 2 weeks in the kidney capsule

Popa et al. 2019

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Wnt/β-catenin activation leads to a decrease in SOX2 expression

Popa et al. 2019

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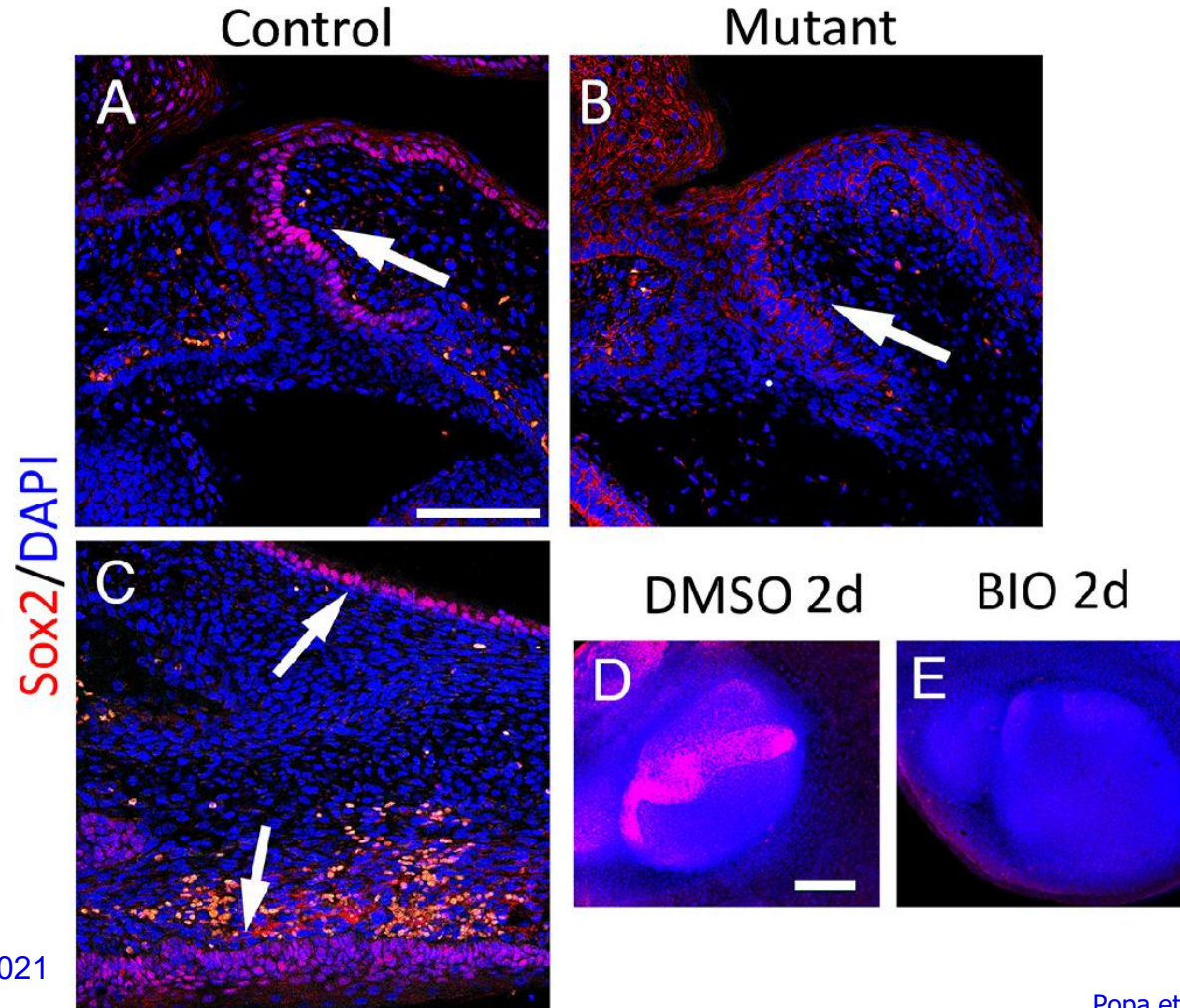
What is the destiny of LGR5-positive cells?
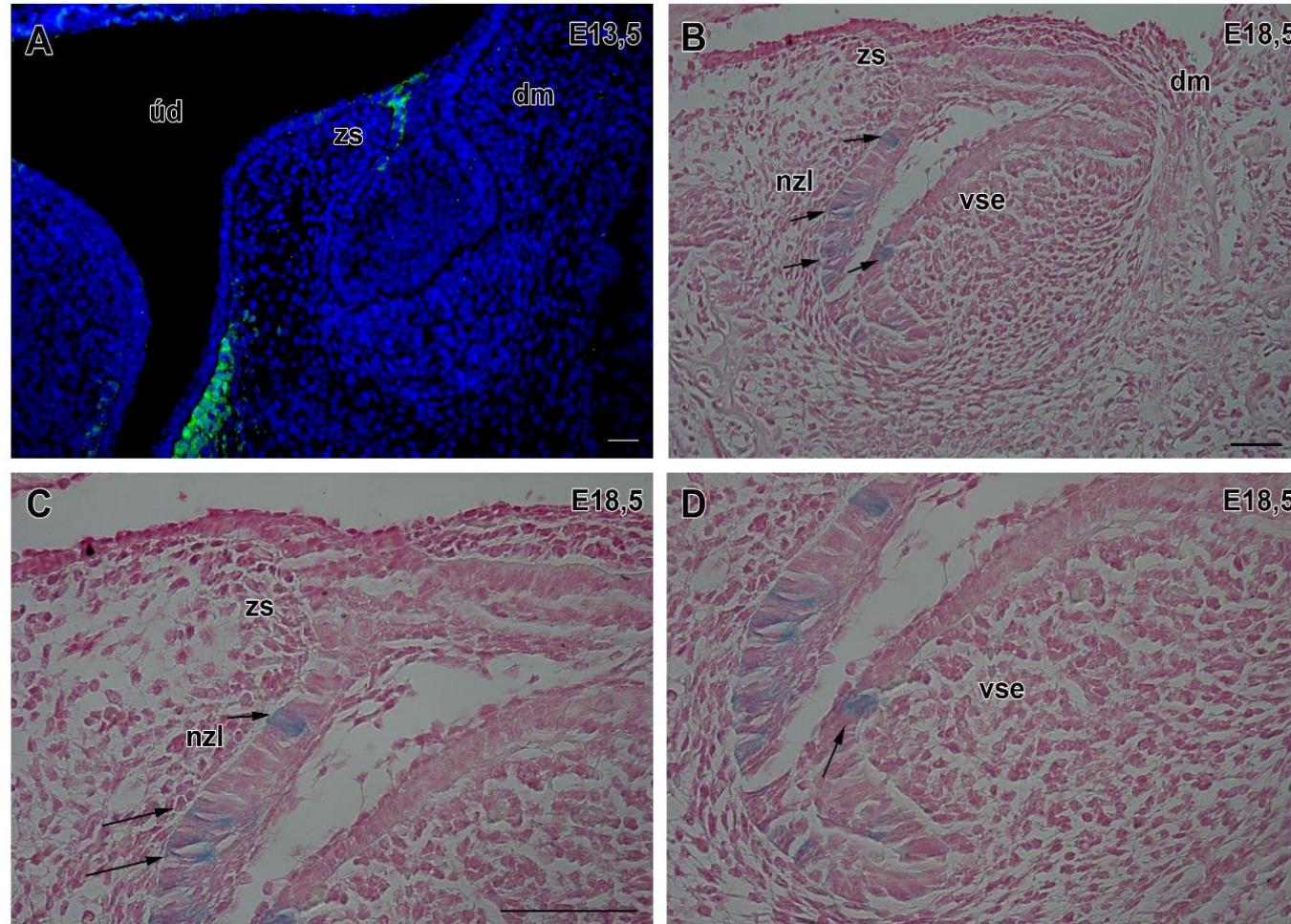
E13.5→E18.5   Lgr5-EGFP-CreERT2 mice
Rosa26-lacZ reporter mice

K. Olbertová

Dental organoids

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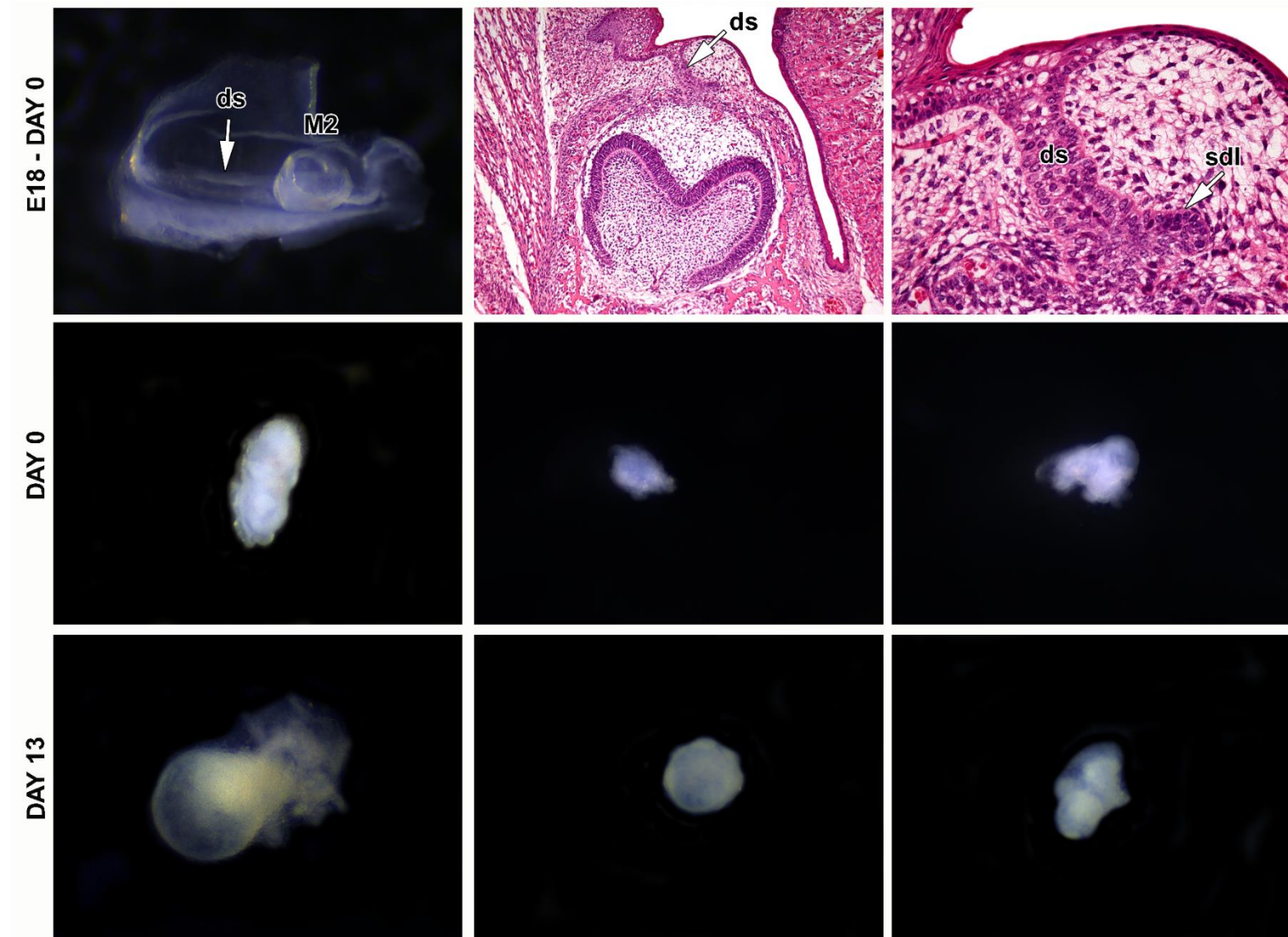
Summary

— Disruption of the dental lamina occurs in diphyodont species already during the prenatal period

— Epithelio-mesenchymal transformation contributes to the removal of epithelial cells

— We found a new stem cell niche that applied during odontogenesis

— Replacing dental lamina is also initiated in monophyodont species, but undergoes regression changes, including keratinization and stem cell loss

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Summary

— Detailed knowledge of signalling during development is necessary for understanding the differences in the growth of the dental lamina between polyphyodont and monophyodont species

— How is the transition between regression or maintenance of dental lamina regulated?

— What induces dental lamina degradation?

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Summary

– Understanding the developmental processes will help to reveal the causes of dental lamina degradation in connection with some disorders of the development of replacement generations in humans and to understand the evolutionary trends leading to diphyodontia in mammals

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Thanks

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IMG, Praha
Ladislav Kořínek
Dušan Hrčkulák

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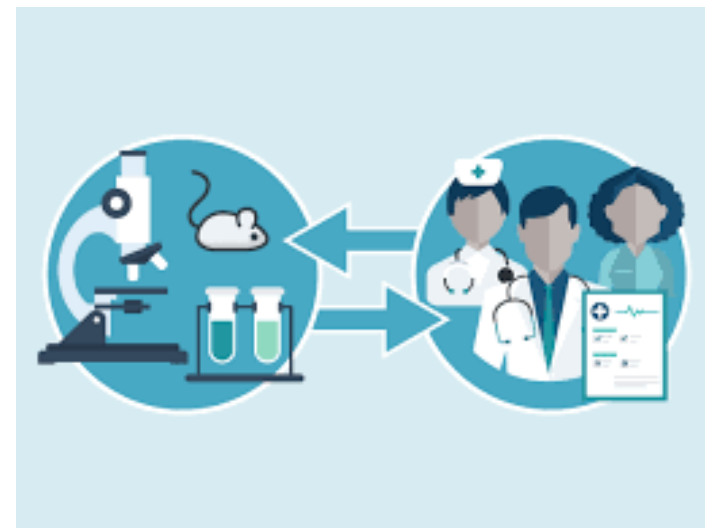
Translational research in life sciences: challenges and possibilities

MUDr. Serhiy Forostyak, PhD, CSO
Department of Burns and Plastic Surgery, Brno University Hospital
Faculty of Medicine, Masaryk University Brno
Primecell Bioscience, Ostrava

509 Research Activities in Dentistry   2021

Content

1. **A long way from basic research to the patient: types of regulatory pathways**

2. **Translational pathways toward clinical application (ATMP, Tissue transplants)**

3. **Experience of the Czech Biotechnological company Primecell**

4. **Tissue transplants: bone and bone products**

5. **Summary**

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1. A long way from basic research to the patient: types of regulatory pathways

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Clinical trials worldwide

Q2 2019 Global Regenerative Medicine
Sector Report

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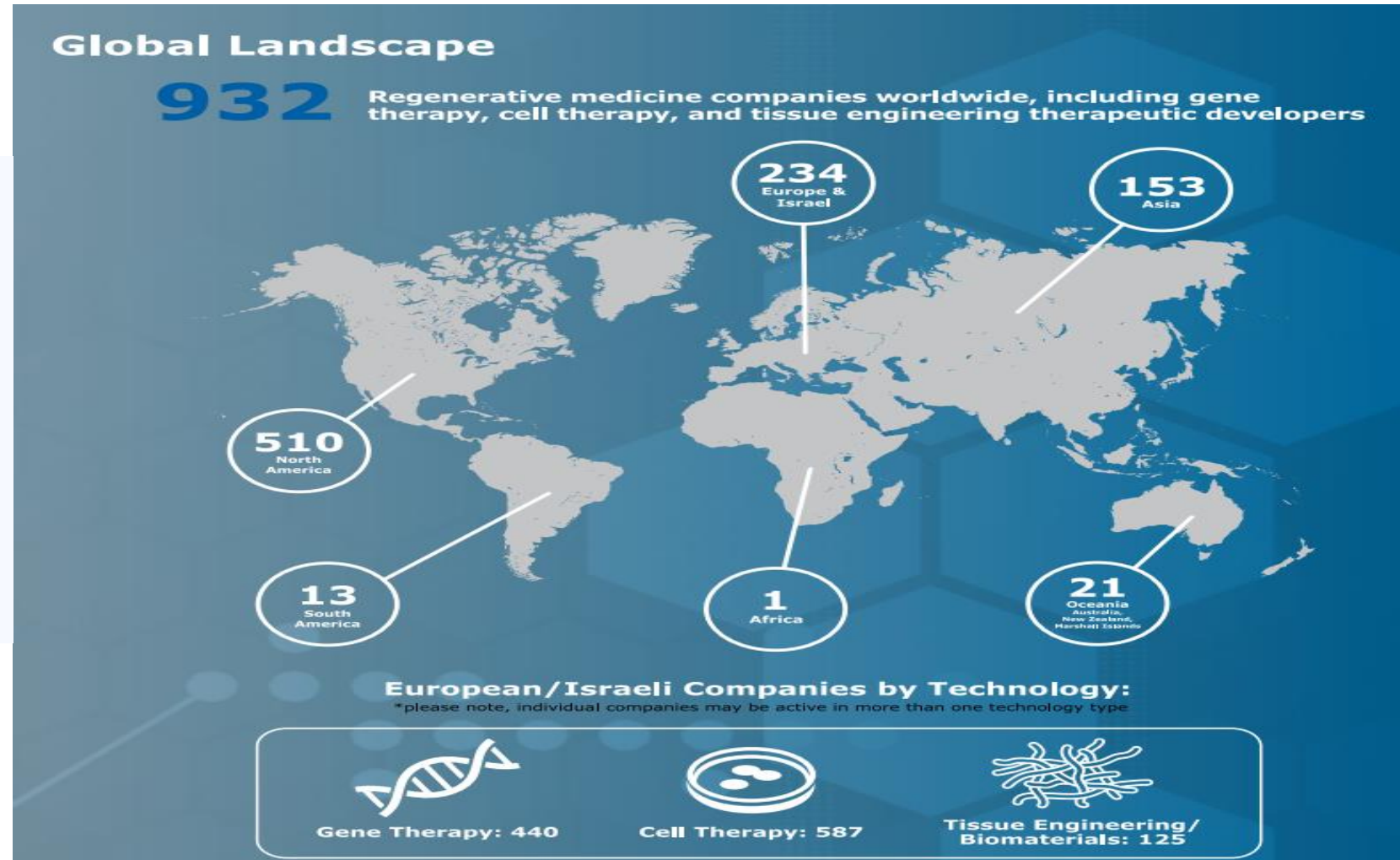
Clinical trials worldwide

1,069
Clinical Trials Underway
Worldwide by End of Q2 2019

Ph. I: 358
Ph. II: 617
Ph. III: 94

Number of Clinical Trials Utilizing Specific RM/AT Technology: Q2 2019

Gene Therapy
Total: 366
Ph. I: 117
Ph. II: 219
Ph. III: 30

Gene-Modified Cell Therapy
Total: 410
Ph. I: 187
Ph. II: 207
Ph. III: 16

Cell Therapy
Total: 249
Ph. I: 49
Ph. II: 168
Ph. III:32

Tissue Engineering
Total: 44
Ph. I: 5
Ph. II: 23
Ph. III: 16

Q2 2019 Global Regenerative Medicine
Sector Report

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Clinical trials EU

EU Market: Clinical trials in cell/gene therapy (overview)

Analysis suggests, that a company running 45 clinical trials would become a significant player in the field, attracting big pharma's and investors' attention

Current Stem Cell Trials (2017)			
...ata Therapeutics	6	SanBio	3
... Biotech	3	Celgene	60
	29	StemCells	53
	30	Genzyme (Sanofi)	60
	14	Teva	7
...tio...	27	MedImmune	8
...nium Pharmac...	51	Janssen	35
...aZeneca	7	Seattle Genetics	7
...erck	21	Baxter Healthcare	3
Chimerix	4	InCyte Corp	2
Eisai	6		

Source: Kelly Scientific

Ph.I: 42

Ph. II: 139

Ph.III: 35

European Sector Overview

European Sector Landscape

234+ Regenerative Medicine / Advanced Therapies Companies Based in Europe/Israel

- Sweden: 12
- Norway: 3
- Denmark: 3
- The Netherlands: 15
- Germany: 29
- United Kingdom: 56
- Ireland: 5
- Belgium: 11
- France: 30
- Switzerland: 15
- Portugal: 3
- Italy: 7
- Finland: 3
- Lithuania: 1
- Czech Republic: 2
- Austria: 4
- Slovenia: 1
- Greece: 1
- Israel: 21
- Spain: 12

European/Israeli Companies by Technology:
*please note, individual companies may be active in more than one technology type

- Gene Therapy: 107
- Cell Therapy: 139
- Tissue Engineering/Biomaterials: 37

Q2 2019 Global Regenerative Medicine Sector Report

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Clinical trials success rate Worldwide

a Success rates by phase
Percentage likelihood of moving to next phase, 3-year rolling average*

Phase III
Phase I
Phase II
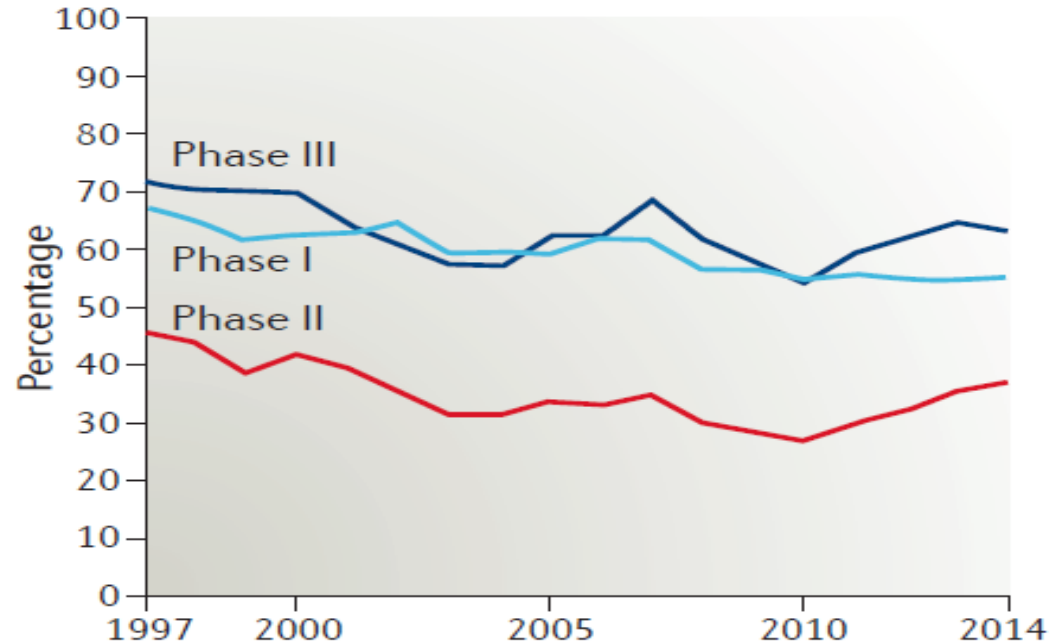
b Cumulative success rate Phase I to launch
Percentage likelihood of moving from Phase I to launch

16.4
10.8
10.0
7.5
11.6

1996–1999
2000–2003
2004–2007
2008–2011
2012–2014

Smietana K, Siatkowski M and Møller M, Nature Reviews 2016

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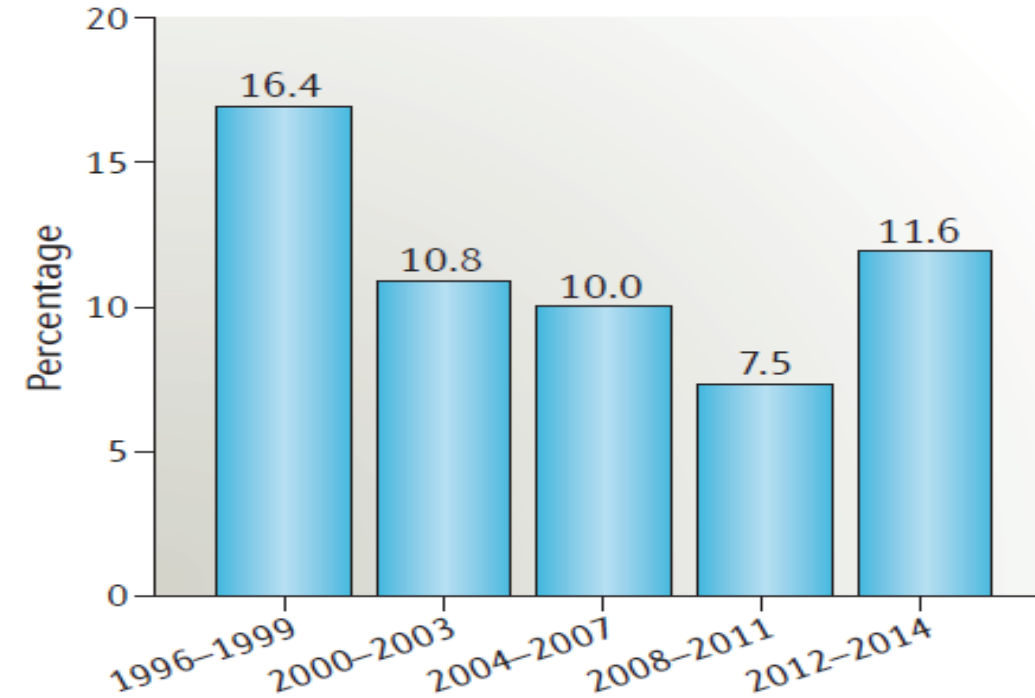
Clinical trials success rate Worldwide

a Cumulative success rates over time
Percentage likelihood of moving from Phase I to launch
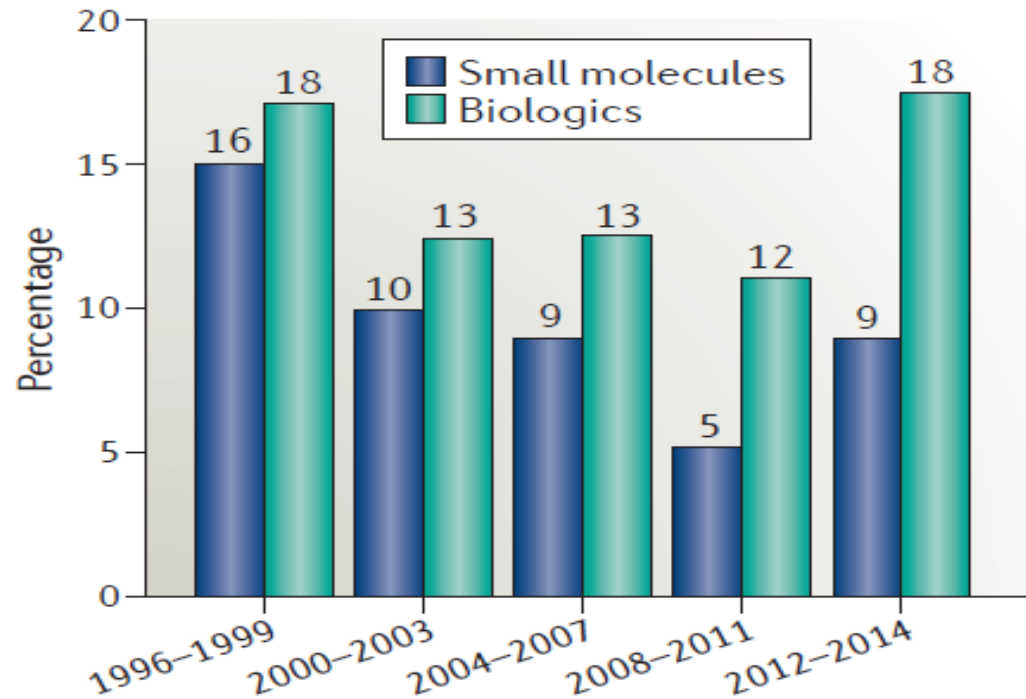
b Success rate by phase, 2012–2014
Percentage likelihood of moving to next phase

Smietana K, Siatkowski M and Møller M, Nature Reviews 2016

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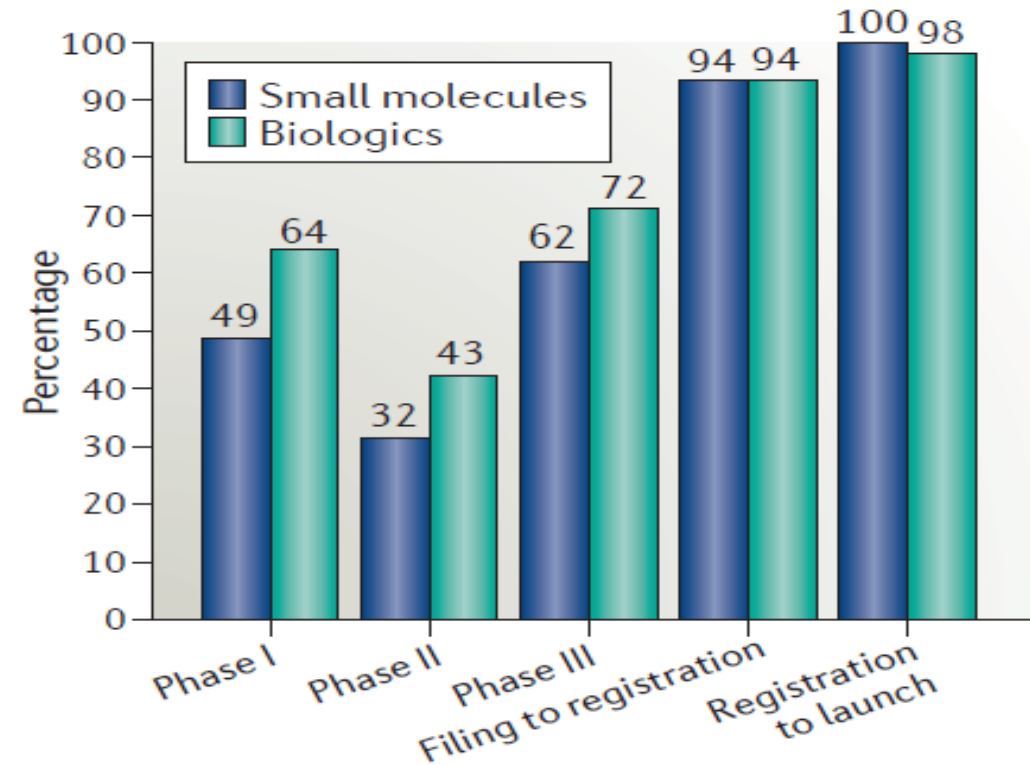
Clinical trials success rate Worldwide

Table S1 | **Number of products and phase transitions included in the calculation**

		1996-1999	2000-2003	2004-2007	2008-2011	2012-2014*	2012-2015*
Number of products included in the calculation**		2603	2965	3776	5144	4808	5558
Number of phase transitions	Phase I	755	909	1119	1893	1351	1835
	Phase II	602	600	758	1116	875	1159
	Phase III	324	275	288	309	296	400
	Filed	240	228	177	202	175	250
	Approved	246	209	160	202	160	245
	Total	2167	2221	2502	3722	2857	3889

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Worldwide way of medicinal agent to the patient, step-by-step

EU drugs approval procedures

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EU drugs approval procedures
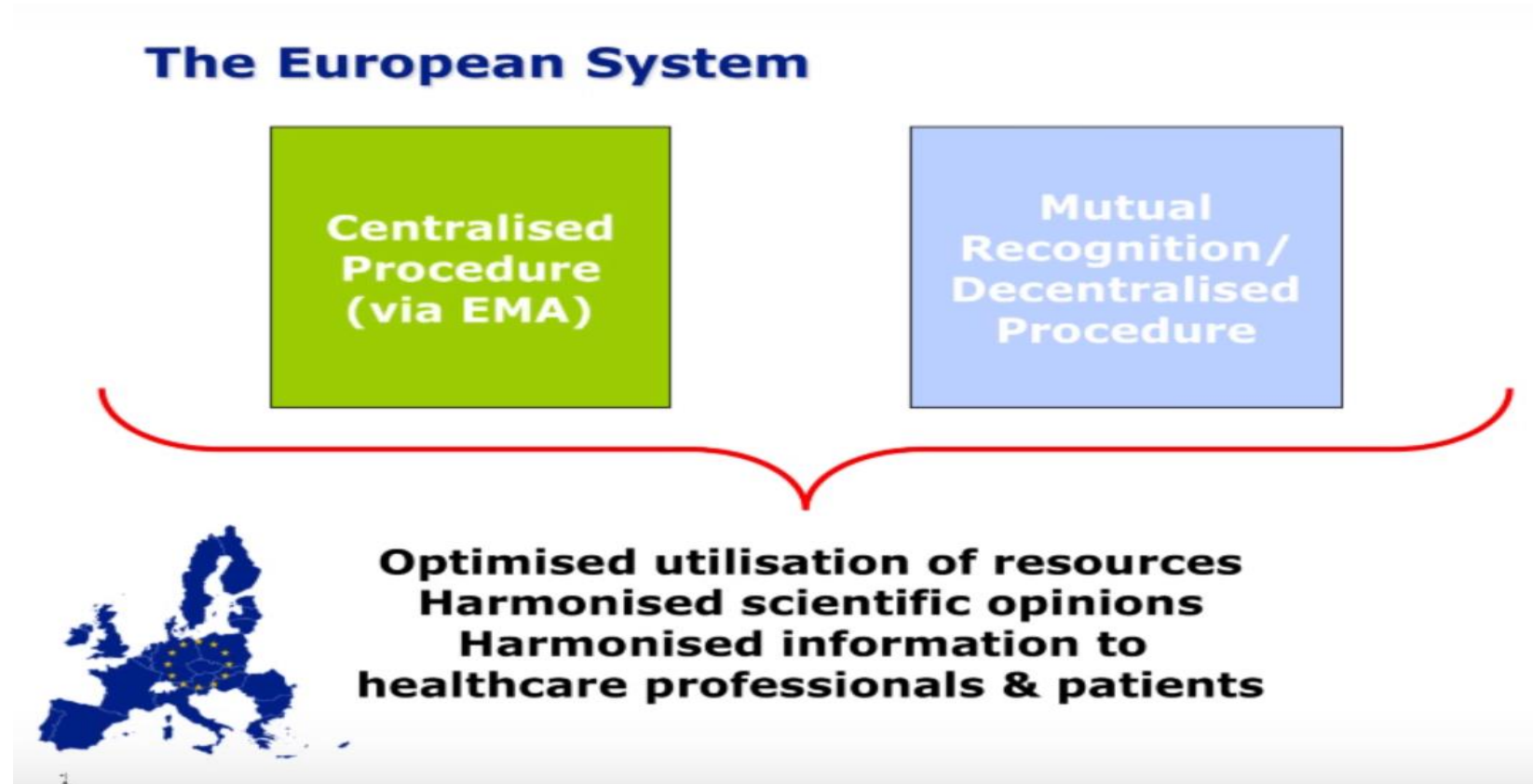
EU drugs approval procedures

Eligibility "Optional Scope"

New Active Substances

Significant Innovation (Therapeutic, &/or Scientific, &/or Technical)

OR

Interest of Patients at Community Level

Art 3(3) Generic of a product authorised via EMA

The centralised procedure attracts most innovative medicines. Decentralised and MRP mainly do generics and new indications for existing products

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The evaluation of medicines, step-by-step

1. **Initial assessment and list of questions** (by day 120)

2. Clock stop 1

3. **Further assessment and list of outstanding issues** (by day 180)

4. Clock stop 2

5. **Further consultations** (by day 210)

6. **Final discussion and adoption of opinion** (67 days)

7. **Possible re-examination**

Evaluation

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The evaluation of medicines, step-by-step

The assessment of a marketing authorisation application for a new medicine takes up to 210 'active' days. This active evaluation time is the time spent by EMA experts to evaluate the evidence provided by the applicant in support of a marketing authorisation application.

This time is interrupted by one or two **'clock-stops'** during which the applicant prepares the answers to any questions raised by the CHMP. The maximum duration of a clock-stop depend on how long the applicant thinks it will take to respond, but must be agreed by the CHMP. The first clock-stop usually lasts 3 to 6 months and the second one 1 to 3 months.

Overall, the assessment of a new medicine usually lasts **around a year**.

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EU, types of approval

Type of Approvals

Normal:
Comprehensive data

Exceptional Circumstances:
- Comprehensive data not available and cannot be provided
- Must meet criteria (rarity, medical ethics, state of scientific knowledge)

Conditional Approval:
- Comprehensive data not available; to be provided after approval
- Must fulfil scope (orphan drugs, emergency threats, serious and life-threatening diseases) Approval valid for 1 year, renewable

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2. Translational pathways towards clinical application (ATMP, Tissue transplants)

3. Experience of the Czech Biotechnological company Primecell

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PrimeCell BioScience a.s. is a biomedical/biotechnological company focusing on the development and clinical testing of biological medicinal products and technologies

https://www.primecell.eu/

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PrimeCell - a subsidiary of a major player in HealthCare in Europe with its own Hospitals Network

POLAND

CZECH REPUBLIC

SLOVAKIA

Regenerative medicine
Internal Medicine
Oncology
Ophthalmology
Psychiatry
Orthopaedics
Neurology
Surgery

527 Research Activities in Dentistry   2021

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PrimeCell focus and practice

Cooperative Research
ecosystem with academic institutions

Clinical trials

Phase I/IIB (exceptionally phase III)

Key translational directions:

i. unmodified cells
ii. genetically-modified cells
iii. immunotherapy
iv. gene therapy
v. devices and medical technologies

Cooperative research with top institutions:

- Czech Republic (CVUT, VŠCHT, IKEM, Institute of Experimental Medicine AS, Biotechnological Institute AS, FNUSA-ICRC, Masaryk Uni, UOCHB)
- EU/UK/USA (Cambridge Uni, Exeter Uni, MIT, N+S Carolina, D.C., NY, California)
- Israel (Ben Gurion University, Weizmann institut)
- Korea

J N I
M E D

PrimeCell focus and practice

Use of amniotic membrane in CR/SK

- **Ophthalmology**
- **Neurosurgery**

- Chronic wounds
- Skin burns

PrimeCell focus and practice

Perinatal tissues (AM/ACH) contain ≥ 200 biologically active molecules

| | Content (pg/mg) | | | |
| | Amnion | | Chorion | |
Cytokine	Average	Standard Deviation	Average	Standard Deviation
TIMP-4	5992.97	2414.42	5958.01	665.55
bFGF	4455.74	1382.91	4276.23	1354.65
TGF-α	3207.53	728.49	4215.82	343.1
PDGF-AA	2151.35	1382.41	4564.91	1465.36
TIMP-2	227.86	104.84	377.95	91.3
HGF	132.05	13.63	147.79	17.47
PIGF	118.72	27.77	114.63	28.28
PDGF-BB	82.93	60.67	151.47	55.82
EGF	77.08	7.26	5.13	4.05
VEGF	29.74	64.24	186.49	354.15
IL-8	28.36	21.01	84.88	22.13
SDF-1α	26.64	0	26.64	0
TGF-β1	16.64	0	25.77	27.08
TIMP-1	4.49	1.68	18.77	20.54
IL-6	2.96	0.92	9.93	2.13
IL-10	1.27	0.91	1.69	0.61
IL-4	0.86	0.1	0.99	0.51
GCSF	0.69	1.08	0.91	0.46

The amount of each cytokine in each sample was normalized to the dry mass of tissue.

Koob et al. J Biomed Mater Res Part B, 2015

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PrimeCell focus and practice
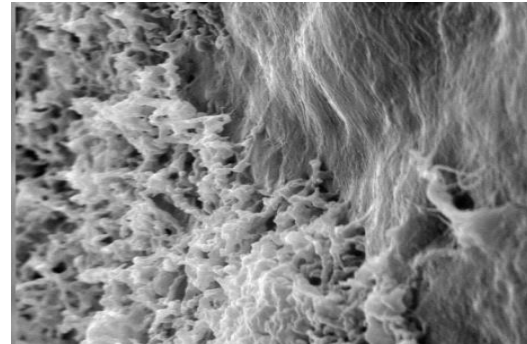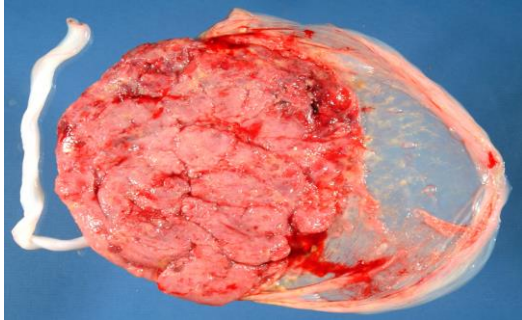
AMNIODERM®

– Manufacturing using a **patented technology AMNIPUR®** (patent number 307603)

– **GMP conditions**

– **SEC code** (Single European Code)

– **Safety** (complete serological testing:

- HIV 2
- HIV 1
- HBs Ag
- HBc total Ab
- HCV Ab
- HTLV 1,2 Ab
- *Treponema pallidium* Ab

- **5 Year shelf-life** (Expiration)

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Use of amniotic membrane in CR
Chronic wounds

Patients healing progression

Start of the AD application

- Prehealing period (granulation of the wound)
- Healing period (epithelization of the wound)
- Wounds that are not suited for AD therapy
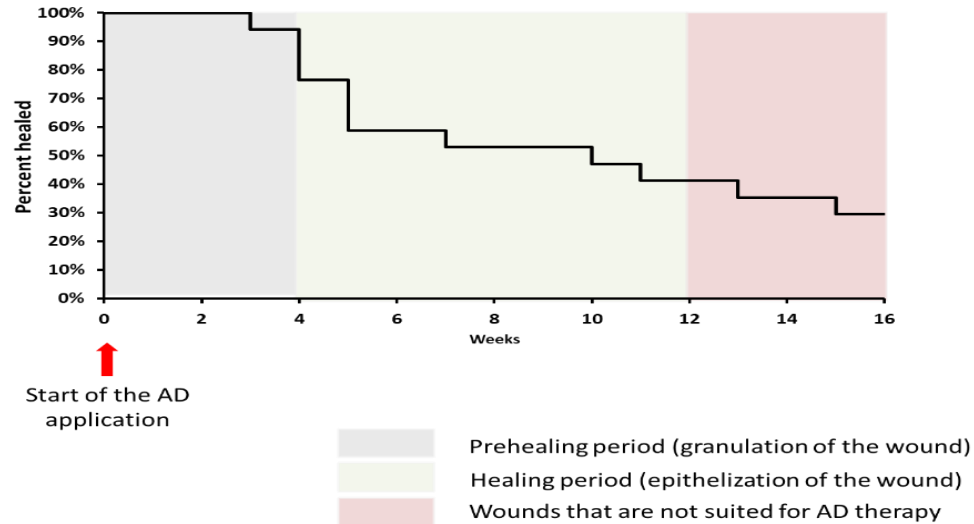
Percent of chronic wounds-size closure

***
***

- Start
- 1 month
- 3 months

* $p \leq 0.05$
** $p \leq 0.01$
***$p \leq 0.001$

W1, 14 cm^2

W2, 12 cm^2

W6, 0 cm^2

Forostyak et al.,

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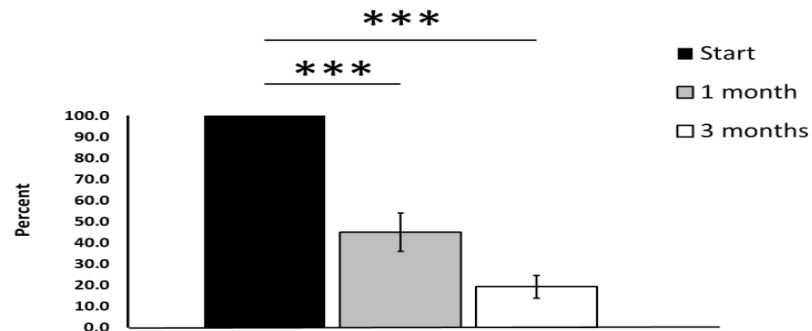
Use of amniotic membrane in CR Chronic wounds

Percent of healed/regenerated chronic wounds after AMNIODERM® application

Totally healed (N=37)

Non-healed wounds (N=11)

Forostyak et al., under revision

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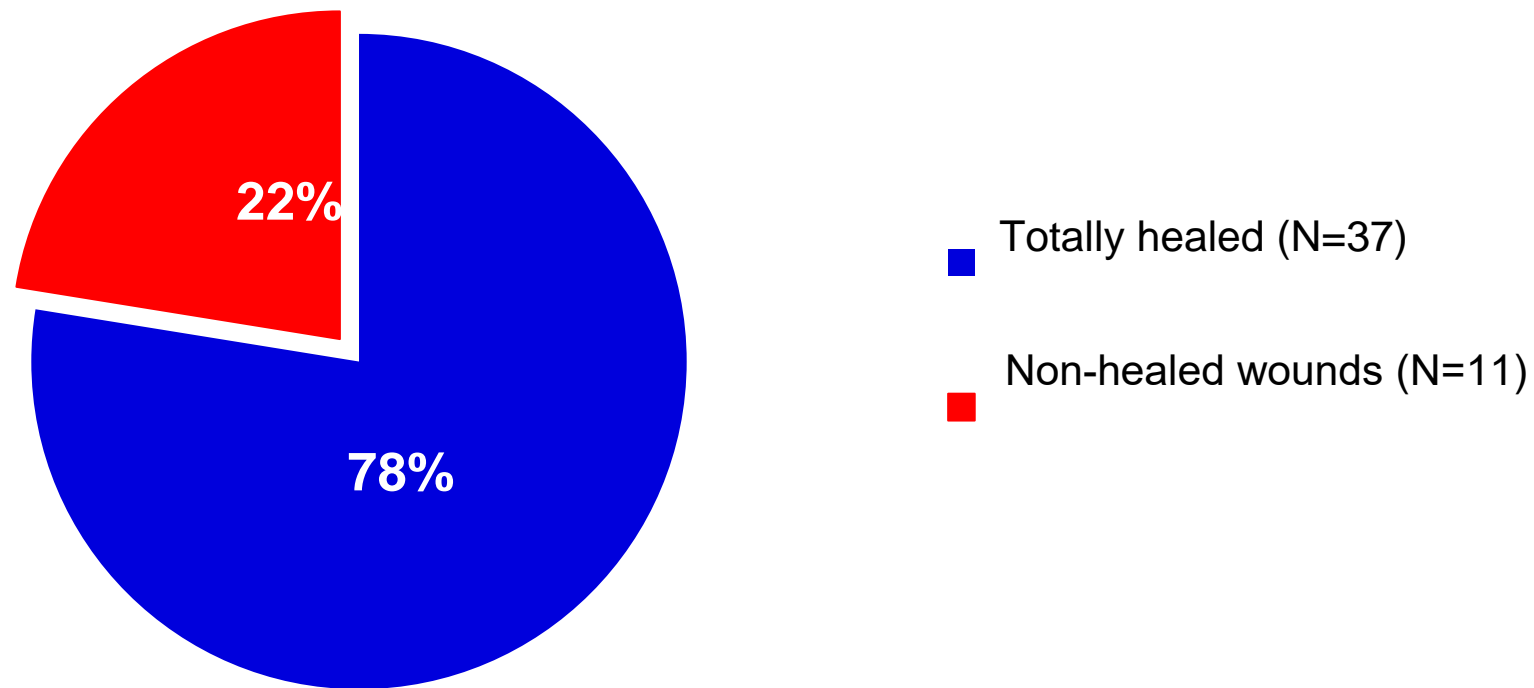
Use of amniotic membrane in CR
Chronic wounds

Fig. 1 Demonstrates a healing process of burns. A wound bed has been prepared with hydrosurgery (A), followed by immediate application of AmnioDerm® (B). On the 5th day of AmnioDerm® application has been observed robust epithelization of the wound (C) enabling a full wound closure and total healing at the 7th day of therapy (D).

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Use of amniotic membrane in CR
Chronic wounds

Amniotic membrane Prevents:
- Cerebrospinal fluid (CSF) leaks
- Formation of pseudomeningoceles
- Postoperative infections
- Dural scarring and intradural adhesions
- Disease transmission

Inhibits:
- inflammatory response

Male, 29	Defect location :	F-T l. sin.
	Etiology:	Decompression craniotomy + edema
	Dg.:	KCP + fr. scapula

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Use of amniotic membrane in CR Chronic wounds

paracentral corneal epithelial defect
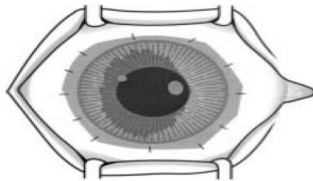
Whole corneal surface (after peritomy)
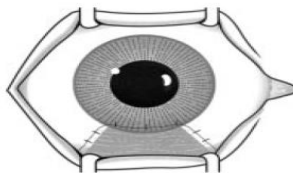
Conjunctival defect (after excision of pterygium

Indications:
- Mechanical & chemical eye injuries,
- Pterygium
- Corneal erosions and ulcers
- Eye keratopathy
- Keratitis of different origin

Effects
- Promoter of epithelialization
- Inhibitor of fibrosis

Results:
- Facilitates corneal regeneration
- Prevents vascularization of cornea
- Prevents scaring and visual defects

Harminder S Dua, BJO, 1999

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4. Tissue transplants: bone and bone products
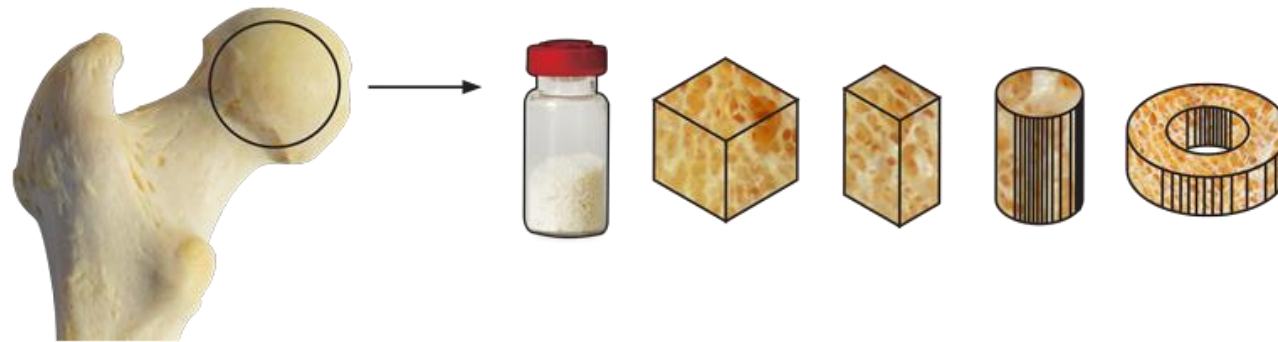
Tissue transplants: bone and bone products
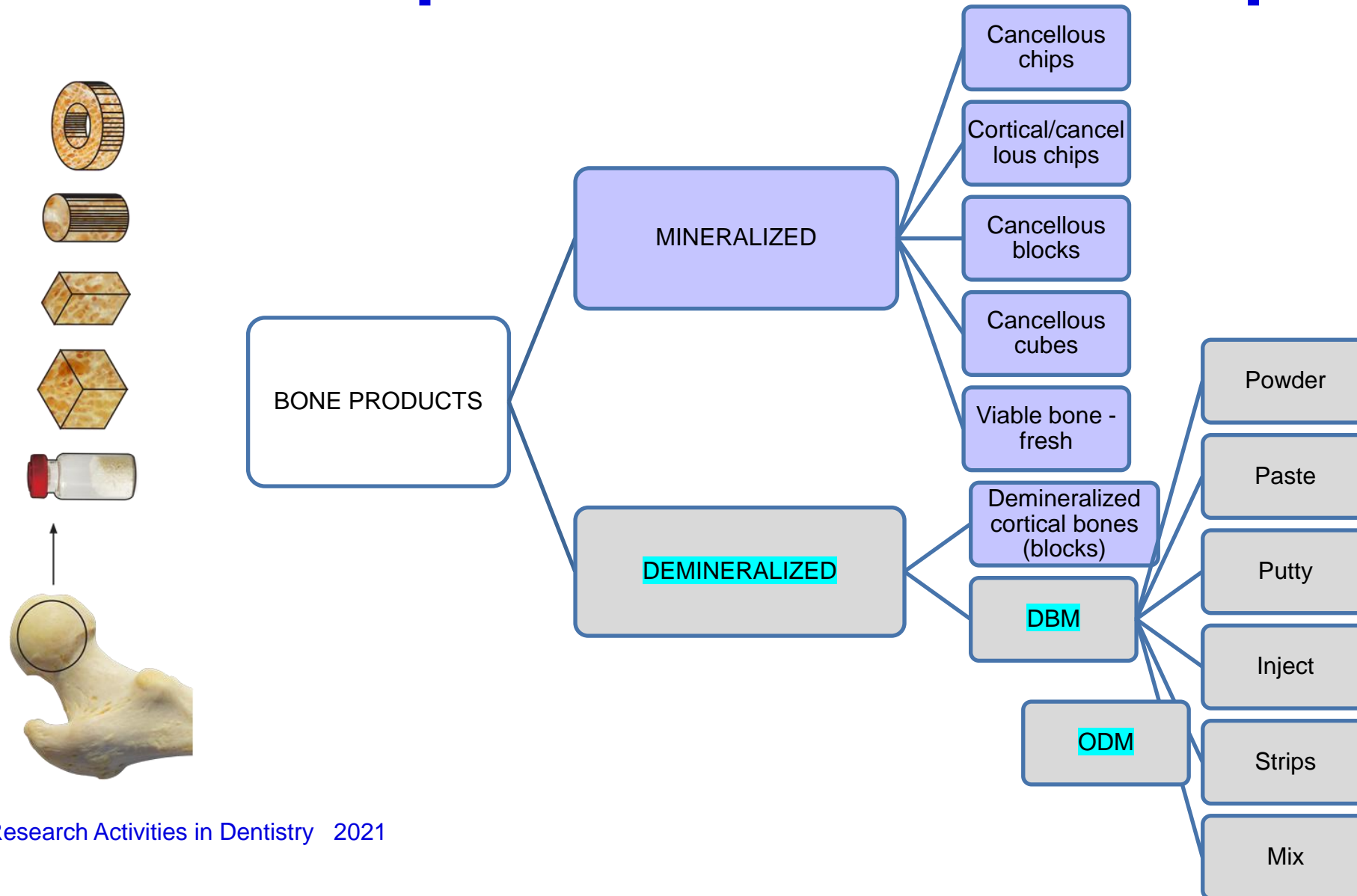
Tissue transplants: bone and bone products

1. Development strategy

- LTB

- Product for spinal surgery,

- Product for dental or oro-
  maxillofacial surgery

- Product for orthopedics /
  traumatology

2. Regulatory strategy & pathway

- Act 296/2008 Coll. (LTB)

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Tissue transplants: bone and bone products

- **Allogeneic materials:**
- Allogeneic bone grafts - not all patients agree with the use of bone grafts from the donor.
- Bone substitutes (tricalcium phosphate or hydroxyapatite) do not match their own bone tissue by osteoconduction and osteoinduction, and at the same time do not have osteoproduction.
- **Xenogenic**

Spondylosurgery: Actifuse, ProOssal, atd.

Dental implantology: Straumann Bone ceramic® – synthetic bone, **Geistlich Bio-Oss®,**

Fusion cages, for augmentation material

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Tissue transplants: bone and bone products

Autologous materials - the bone is the highest quality material. It is obtained most often from the shovel of the hip bone, which often leads to pain at the collection site (donor site pain). The result is the loss of pain of the operated segment, but the creation of a new source of pain in the harvesting area.

Basic functions of a bone graft:

a) Osteoinduction – the ability to promote the growth of new bone tissue.

b) Osteoconduction – the ability to promote the migration of osteogenic cells to the site of the bone defect or fracture site.

c) Osteogenesis – the ability to synthesise a new bone from donor graft cells. Clinically most important of these are the growth factors, especially bone morphogenic protein (BMP)
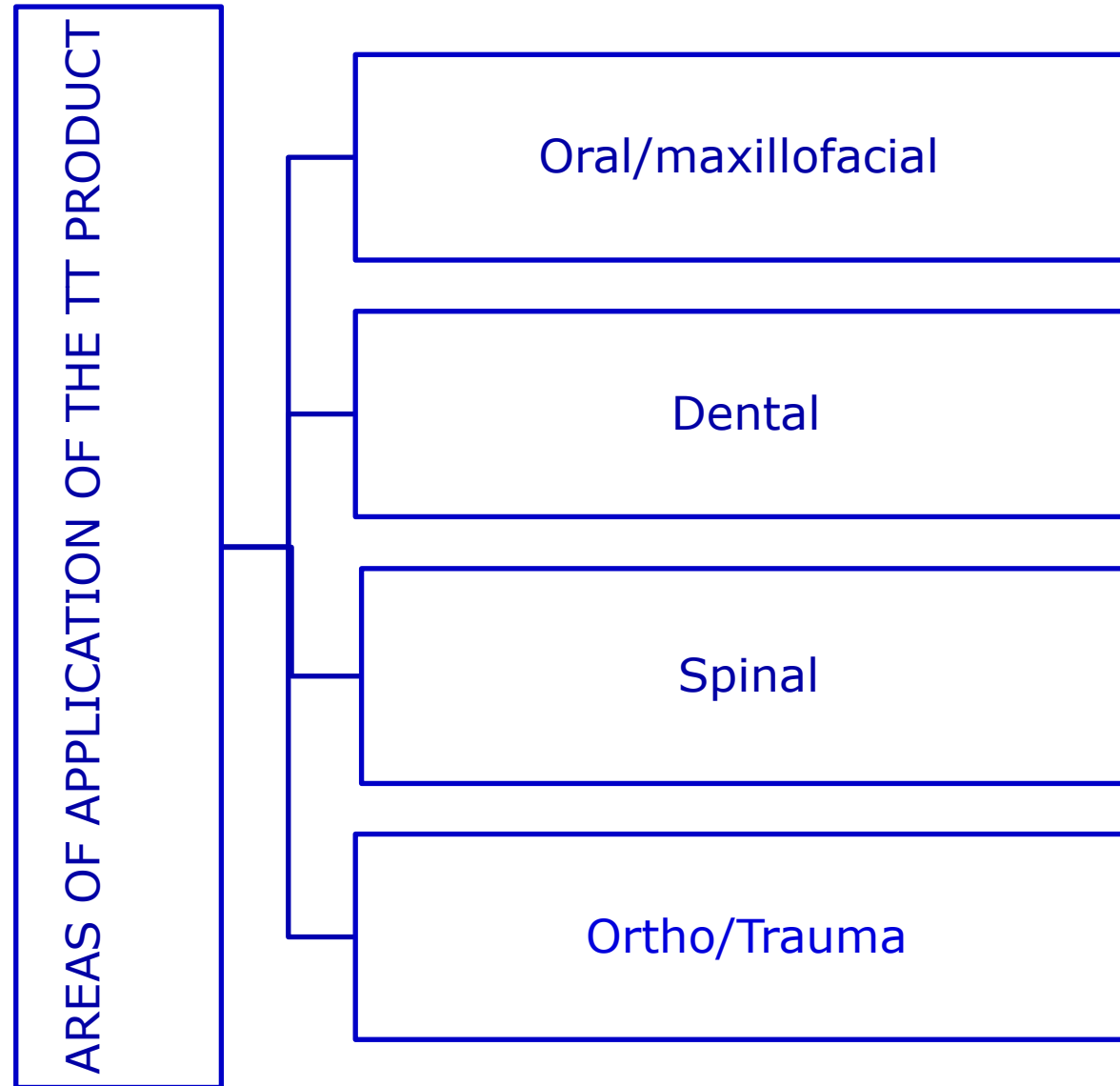
Bone morphogenetic protein (BMP-2)

- It ranks among beta growth factors, promotes bone and cartilage formation
- Significant in vivo regeneration effect when applied to the bone defect

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Tissue transplants: bone and bone products

Growth Factor	Source	Receptor Class	Function
Transforming growth factor beta (TGF-β)	Platelets, bone extracellular matrix, cartilage matrix	Serine threonine sulfate	Pleiotropic growth factor stimulates undifferentiated mesenchymal cell proliferation
Bone morphogenetic protein (BMP)	Osteoprogenitor cells, osteoblasts, bone extra-cellular matrix	Serine threonine sulfate	Promotes differentiation of mesenchymal cells into chon-drocytes and osteoblasts, promotes differentiation of osteoprogenitors into osteo-blasts, influences skeletal pattern formation
Fibroblast growth factors (FGF)	Macrophages, mesenchymal cells, chondrocytes, osteoblasts	Tyrosine kinase	Mitogenic for mesenchymal cells, chondrocytes, and osteoblasts
Insulin-like growth factors (IGF)	Bone matrix, osteoblasts, chondrocytes	Tyrosine kinase	Promotes proliferation and differentiation of osteopro-genitor cells
Platelet-derived growth factor (PDGF)	Platelets, osteoblasts	Tyrosine kinase	Mitogen for mesenchymal cells and osteoblasts; macrophage chemotaxis

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Tissue transplants: bone and bone products

AREAS OF APPLICATION OF THE TT PRODUCT

- Oral/maxillofacial
- Dental
- Spinal
- Ortho/Trauma

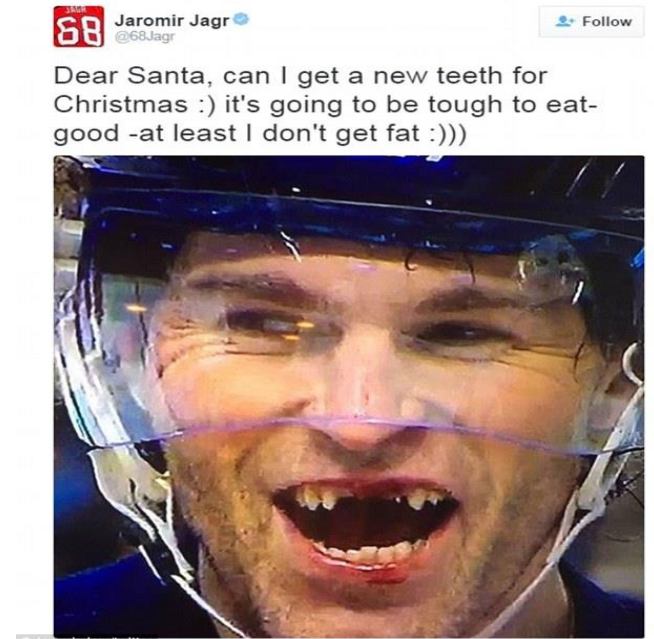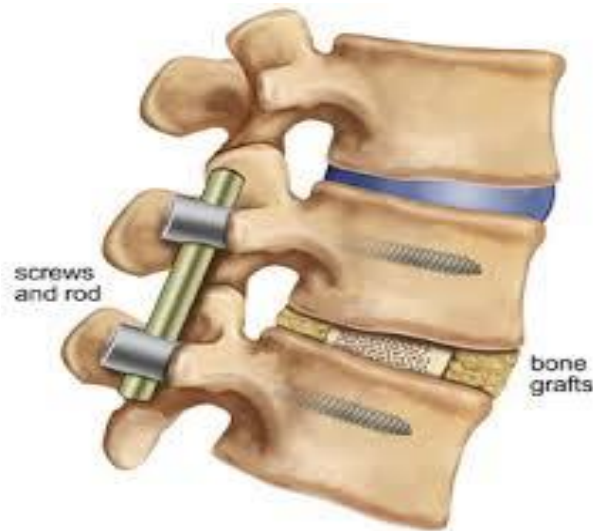MUNI
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Tissue transplantation: bone and bone products

Examples of use:

1. Spinal fusion (connection of two or more vertebrae by a bone graft)

2. Conditions after tooth loss (tooth extraction, periodontitis, trauma, other)

3. Cysts, tumors, etc.

Tissue transplantation: bone and bone products

Degenerative changes in the spine (lumbar or thoracic), vertebral fractures, tumors

- The most common: intervertebral disc herniation and narrowing of the spinal canal (stenosis) - both diseases result from these degenerative changes
  - **Disc prolapse** - release of the inner - soft part of the disc into the spinal canal through a crack in the circumferential - firmer part of the disc. In the spinal canal, this loose part irritates or oppresses the nerve. This leads to pain and in some cases to neural dysfunction
  - **Narrowing of the spinal canal** is caused by arching intervertebral discs, enlarged and degeneratively altered intervertebral joints

Healthy disc   Disc degeneration

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Tissue transplantation: bone and bone products

Spondylosurgery – surgical field dealing with surgical therapy of spinal diseases - injuries, congenital defects and deformities, tumors, post-traumatic deformities, degenerative changes

Degenerative changes in the spine (lumbar or thoracic), vertebral fractures, tumors

- Degenerative changes (congenital or acquired by a hitherto unknown process) can lead to the so-called instability of a certain section of the spine.

- Neighboring vertebrae move more than normal due to changes in the intervertebral disc and the vertebrae themselves. A situation can occur when one vertebra moves forward relative to the other, creating a kind of "step" or "shift" of the spondylolist.

Pars Interarticularis   Spondylolysis   Spondylolisthesis

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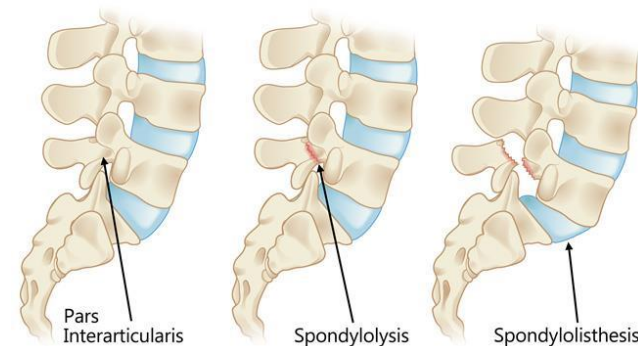
Tissue transplantation: bone and bone products

- Surgical treatment - where non-surgical treatment fails, the problems persist or worsen despite treatment.

- The essence of every operation is always to remove the oppression of nerves (or spinal cord) while maintaining the stability of the spine. During surgery, various fixators are often used - implants to ensure the stability of the spine, screws, rods or plates.

- As a replacement for the intervertebral disc, special sprouts filled with bone marrow are used, which allow the adjacent vertebrae to coalesce.

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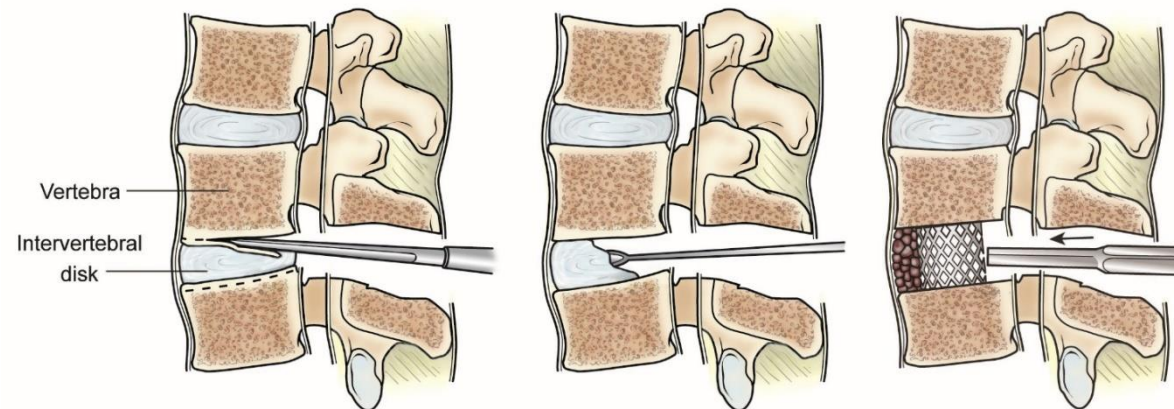
Tissue transplantation: bone and bone products

- **The following operating techniques are used:**

- PLIF (Posterior Lumbar Interbody Fusion) - through the posterior approach, the surgeon reaches the spine, then relaxes the nerves by removing the vertebral arch, strengthened ligaments and parts of enlarged joints, removes the intervertebral disc and replaces it with the above-mentioned clavicle
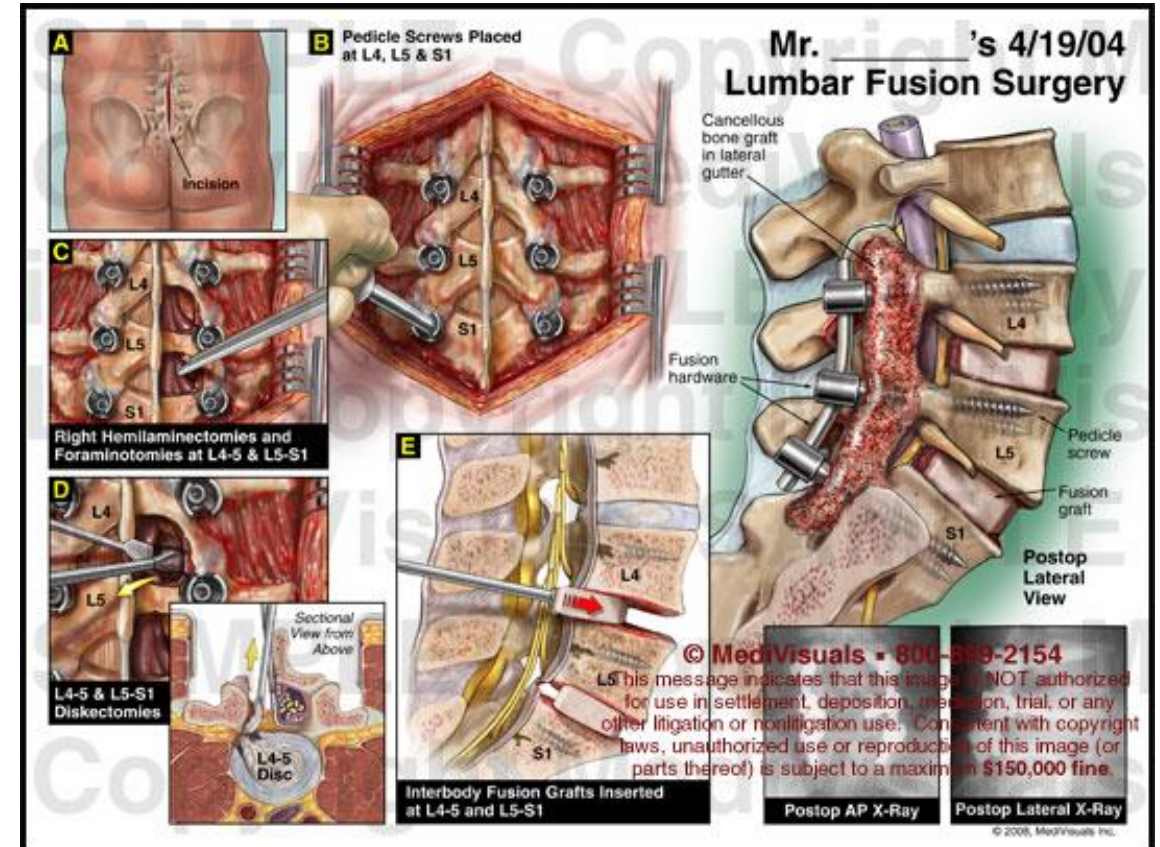
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Tissue transplantation: bone and bone products

- TLIF (Transforaminal Lumbar Interbody Fusion) - a similar technique as PLIF. It differs in that it is not necessary to open the spinal canal by removing the vertebral arch.

- The intervertebral disc is removed and the restoration is inserted with a more lateral approach.

- Instrumented and uninstrumented posterolateral dissa - the intervertebral disc is not removed and the fusion of the adjacent vertebrae is achieved by means of bone pulp deposited on the transverse protrusions of the vertebra.

- ALIF (Anterior Lumbar Interbody Fusion) - the surgeon reaches the spine through anterior access through the abdominal cavity around the abdominal organs and blood vessels, removes the intervertebral disc and replaces it with a clavicle filled with bone-pulp.

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Tissue transplantation: bone and bone products
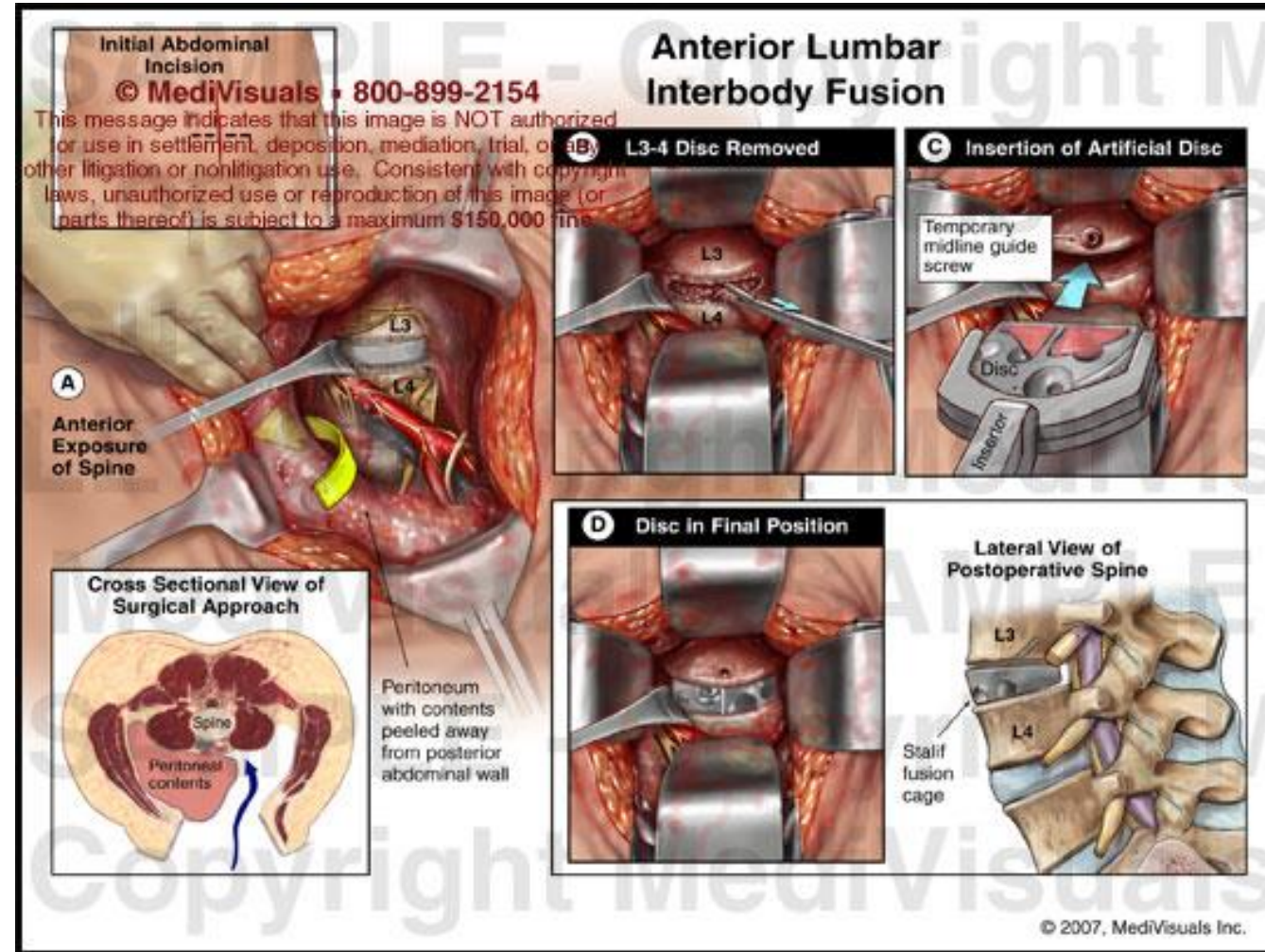
Tissue transplantation: bone and bone products

Implantology:

Missing teeth

Conditions after tooth loss for various causes:

Periodontitis

Tooth extraction

Trauma

Other

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Tissue transplantation: bone and bone products

AUGMENTATION - filling of the own bone in case of a lack of the alveolar bone due to atrophy.

1. **Bone autograft augmentation** - postoperative pain, impaired bone strength and stability,

    risk of infection

2. **Augmentation with allogeneic material** (synthetic, xenogeneic, allogeneic graft)

SINUS LIFT

formation of bone in the maxillary cavity, when

after lateral access, the lining of the cavity is

lifted and the resulting space is subsequently

filled with augmentation material

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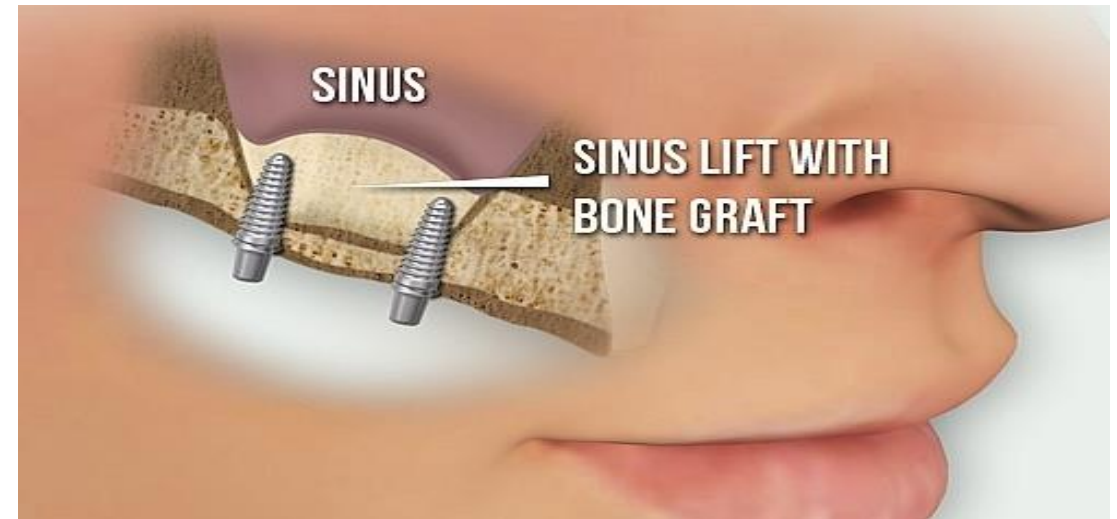
Tissue transplantation: bone and bone products

Mineral part of the bovine bone - successfully used for 25 years. The implantologist is responsible for the treatment

   - The conservatism of the surgeon, who is taught to use current materials

   - Long-used materials from renowned brands

   - Psychological barriers - tissue from a deceased donor / risk of transmission of infection

   - Informed consent in connection with a practically cosmetic procedure

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Tissue transplantation: bone and bone products

Duration of current standard treatment :

Spondylosurgery - fusion

The total length of stay in the hospital is 5-7 days, after which the patient is released for home treatment or transferred, for example, to the care at a rehabilitation or neurology department in the place of residence or in the catchment area hospital. The basis is active rehabilitation, individual exercises and appropriate movement, regime measures and often a change in lifestyle, including weight reduction

Augmentation in implantology

it is usually performed by dental clinics and dental surgery clinics - dental implants can be inserted at the same time and allowed to heal for nine months.

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Tissue transplantation: bone and bone products

Costs of current treatment :

Spondylosurgery

VZP price list - conventional bone grafts

Implantology

- Implantation with augmentation is not covered by VZP

- Private payers

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Tissue transplantation: bone and bone products

Verification of safety and efficacy according to ZoLTB:

Safety evidence

- setting up a donor screening system - serological examination,

- 3NAT examination (beyond the requirements of Czech legislation),

- medical documentation of the donor

- proof of personal history, examination of the donor's body, hemodilution

- The assessment of medical fitness is performed by a doctor

- Use of safe and approved materials in processing

- **Demonstration of product efficacy**

  - the product cannot be used in humans before the approval of SÚKL

  - literary sources

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DBM – PUTTY

– The source for production is the powder from the cortical layer of long bones

– Mix of bone powder and glycerol (hyaluronic acid, etc.)

– Ratio 65% DBM, 35% Glycerol (according to NHS)

– Ratio of 31% of DBM by weight, 93% by volume of DBM + hyaluronic acid (according to MTF)

– Promoting the growth of autologous cells that cause osteogenesis

– Hydrated DBM loses its osteogenic effect after approximately 5 weeks

– The average expiration of products with a carrier is 2-3 years, according to the manufacturer

1) https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/3750/170413-demineralised-bone-matrix-01.pdf
2) http://mtf.trellist-dev.com/docs/default-source/product/dbx-brochure.pdf
3) https://www.aesculapimplantsystems.com/assets/base/doc/DOC1248-ProSpace_DBM_Moldable_Putty_Paste_and_Cancellous_Chips_Data_Sheet.pdf
4) https://bonegrafttoday.com/product/dbm-paste-1cc/
5) http://mtf.trellist-dev.com/docs/default-source/product/dbx-brochure.pdf

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DBM – INJECT

— The source for production is powder from the cortical layer of long bones

— Overfilled glass syringe „DBP Putty with deliver system" Ratio of 31% by weight of DBM, 93% by volume of DBM + hyaluronic acid (according to MTF)

— Promoting the growth of autologous cells that cause osteogenesis

— Hydrated DBM loses its osteogenic effect after approximately 5 weeks

— The average expiration of products with a carrier is 2-3 years, according to the manufacturer

1) https://nhsbtdbe.blob.core.windows.net/umbraco-assets-corp/3750/170413-demineralised-bone-matrix-01.pdf
2) http://mtf.trellist-dev.com/docs/default-source/product/dbx-brochure.pdf
3) https://bonegrafttoday.com/product/dbm-paste-1cc/

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DBM – MIX

— The source for production is the pulp from the cortical and

parenchymatous parts of long bones

— Mix of bone powder and hyaluronic acid (according to MTF)

— Elimination of the need for a bone graft in combination with DBM

— The proportion of bones is 35%

— Promoting the growth of autologous cells that provide osteogenesis

http://mtf.trellist-dev.com/docs/default-source/product/dbx-brochure.pdf

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Summary

- The EU is a common space for translational and clinical research

- Partner projects are more successful towards clinical testing

- The current success rate for phase I clinical trials is 64%; The success rate of phase II clinical trials is 43%

- Nearly 14% of all drugs and 18% of biological products in clinical trials received EMA / FDA approval

- The approval rates of new medicines ranges from 33.4% (vaccines against infectious diseases) to 3.4% (cancer treatment under investigation)

- The success rate in translation of biologically active products was higher than that in small molecules trials

- Translation of know-how for clinical testing in CEE is significantly cheaper, high quality GCP and market access

- Regulatory support and basic research are the keys to the successful implementation of know-how into clinical practice

- Tissue transplantation, biological products and biomaterials differ in regulatory pathways

- Tissue-based materials and products have a wide range of applications, advantages and indications in regenerative medicine

- Proper regulatory classification of the product is a key for product transfer and routine use in patients

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Colloquium

Assoc. Prof. RNDr. Petra Bořilová Linhartová, Ph.D., MBA

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Opponents

— **RNDr. Mária Hovořáková, Ph.D.**

 Institute of Histology and Embryology, First Faculty of Medicine, Charles University

— **Assoc. Prof. MUDr. Jakub Suchánek, Ph.D.**

 Department of Dentistry, Faculty of Medicine in Hradec Kralove, Charles University

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