WORKING GROUP 2. STEM CELLS / HARD TISSUE FORMATION

Chairpersons: K. Fried and ALJJ Bronkers

12 May 2005

18h : Poster session. Chair: C Martínez-Álvarez

- WG2: INHIBITION OF PTHRP EXPRESSION BY BMP-2 AND ITS IMPLICATION IN OSTEOBLAST DIFFERENTIATION
  R. G. Susperregui, F. Viñals and F. Ventura
- WG2: BONE CELL TRANSPLANTATION IN RECONSTRUCTIVE SURGERY IN OROFACIAL REGION
  Jenca A., Hanusínová V., Orenzak M., Kizek P., Jenkova J.

14 May 2005

9h30' Keynote Lecture: speaker: Dr Catherine MILLER:The human X-linked hypophosphatemia (XLH): structure and immunohistochemistry

10h30' **Chair: H Magloire: Oral Communications**

10H30' TWIST-1 EXPRESSION DURING PULP REPAIR
H. Løvschall, E.-M. Füchtbauer

10h45': ERUPTION OF TEETH IS LINKED TO THE FORMATION OF A SUCCESSIONAL DENTAL LAMINA IN THE ZEBRAFISH (DANIO RERIO): SUPPORT FOR THE STEM CELL CONCEPT
A Huysseune

11h00': EXPRESSION OF ADRENOMEDULLIN IN HUMAN DENTAL TISSUES
AJ Sloan, J McLachlan, AJ Smith, PR Cooper

11h15': CHONDROID BONE FACILITATES FAST GROWTH OF THE DENTARY IN MATURE MALE ATLANTIC SALMON AND IS ALREADY PRESENT IN EARLIER DEVELOPMENTAL STAGES.
P. Eckhard Witten, J. Andrew Gillis and Brian K. Hall

11h 30' Coffee break

12h00' Chair: AJJ Bronkers. Oral Communications

12h00' THE TWO LOW MOLECULAR WEIGHT AMELOGENIN PEPTIDES (A+4) AND (A-4) ACTIVATE THE EXPRESSION OF GENES INVOLVED IN TOOTH DEVELOPMENT, IN IMMORTALIZED MESENCHYMAL PROGENITORS CELLS

Sally Lacerda-Pinheiro, Arthur Veis, Odile Kellerman, Michel Goldberg and Anne Poliard.

12H15': EPITHELIAL-MESENCHYMAL INTERACTIONS REGULATE DENTAL AXON PATHFINDING
Päivi Kettunen, Inger Hals Kvinnsland, Keijo Luukko

12h30' HUMAN ODONTOBLASTS EXPRESS b1, aVb3 AND aVb5 INTEGRINS.
Staquet M-J, Couble M-L, Romeas A, Connolly M, Lucchini M, Durand S, Magloire H, Hynes R, Bleicher F, Farges J-C.

12h45' WHAT IS THE OPTIMAL END RESULT OF THE TRANSDENTINAL BIOACTIVE STIMULATION?
M. Kalyva, A. Alvanou, S. Papadimitriou, D. Tziafas

13h00': DENTIN MATRIX IN DIFFERENT TYPES OF ABNORMAL COLLAGEN I METABOLISM: A COMPARATIVE LIGHT MICROSCOPY (LM), IMMUNO-HISTOCHEMISTRY (IHC) AND TRANSMISSION ELECTRON MICROSCOPY (TEM) ANALYSIS
DeCoster PJ, Cornelissen M, DePaepe A, Martens LC, Vral A

13h15': DIMETHYLBENZ(A)ANTHRACENE DISTURBS THE FORMATION OF DENTAL MATRICES AND REDUCES THE SIZE OF CULTURED MOUSE LOWER MOLARS
Eija Peltonen, Carin Sahlberg, Anna-Maija Partanen, Satu Alaluusua, Pirjo-Liisa Lukinmaa.

13h30': GENE EXPRESSION ANALYSIS OF ENDOCHONDRAL BONE FORMATION.

Rachael Sugars, Ulrika Petersson, Alistair Chalk, Eszter Somogyi and Mikael Wendel.

DENTAL ABNORMALITIES IN PATIENTS WITH FAMILIAL HYPOPHOSPHATEMIC VITAMIN D RESISTANT RICKETS.

Catherine Chaussain-Miller[1,3], Sana Bagga[1], Dominique Septier[1], Christiane Sinding[2] , Maryse Wolikow[4], Michèle Garabédian[2] and Michel Goldberg[1] .

[1]EA 2496 Groupe Matrices extracellulaires et biominéralisation, Faculté de Chirurgie Dentaire, Université René Descartes, France
[2]Inserm U561, Hôpital St Vincent de Paul, Paris 14ème, France
[3] Hôpital Hôtel-Dieu- Jean Délibéros, AP-HP, Paris 9ème, France
[4] Hôpital Charles Foix, AP-HP, 94200 Ivry/Seine, France

Hypophosphatemic rickets, the most common form of rickets in the industrialized countries, is in most cases transmitted as an X linked dominant trait and results from the mutation of the PHEX gene located in Xp22.2-22.1. The PHEX gene encodes an endopeptidase, predominantly expressed in bone and teeth but not in kidney[1]. Its precise mode of action is uncertain, though recent publications [2] had proposed that FGF-23 might be a substrate of this endopeptidase and inhibit renal Pi reabsorption leading to hyperphosphaturia and hypophosphatemia, as well as impaired 1□-hydroxylation of 25-hydroxyvitamin D. These abnormalities lead to discordance between the production and the rate of mineralization of bone matrix, hence the accumulation of unmineralized matrix and poorly mineralized bone. The main clinical features are bony deformities, especially bowing of the legs, impaired growth and short stature. Severe dental troubles have been reported mainly related to impaired dentin mineralization, enlarged pulp chambers, prominent pulp horns, and spontaneous pulp necrosis with periapical infections[3]. Beginning in the 1970s, 1-hydroxylated forms of vitamin D have progressively replaced vitamin D itself for the treatment of hypophosphatemic rickets. This later strategy clearly improved the prevention and correction of bone deformities in children with hypophosphatemic rickets, as well as their skeletal growth. But its influence on teeth abnormalities has not been specifically addressed. The clinical aspect of our work was devoted to the evaluation of the dental impact of 1-hydroxylated vitamin D3 treatment in patients with familial hypophosphatemic vitamin D resistant rickets (VDRR). 48 hypophosphatemic patients were included in the study. 16 adults had received no treatment or phosphate supplements + vitamin D/25- (OH) D3 before puberty. The 32 younger patients had received phosphate supplements +1alfa-(OH)D3 from infancy. All patients were clinically examined and panoramic and periapical radiographs were made. Evaluations of DMFT (decayed, missing, filled teeth) and dft (decayed, filled teeth) indexes, and the Pulp/crown Ratios, allowed comparison with healthy age-matched subjects. Poor dental health and characteristic dental anomalies were found in the 16 older patients. In contrast, the 32 younger patients had a normal dental status comparable to healthy age-matched populations, although they still showed prominent pulp horns in deciduous teeth and increased pulp area/tooth area ratios. As primary conclusion, this clinical investigation showed the beneficial impact of 1alfa-(OH)D3 treatment on the dental status of VDRR patients and emphasized the necessity of an early onset of treatment [4]. Remaining defects may result from early exposure of odontoblasts and surrounding osteoblasts to hypophosphatemia, before the onset of treatment, and/or from intrinsic cell disturbances linked to the genetic alteration(s).
The second step of our work was to better characterize the dentin structure associated with correct and defective 1alfa-(0H) vitamin D treatment. Teeth from hypophosphatemic children

with good or poor compliance to therapy were compared to control age-matched patients. A part of extracted teeth was prepared for SEM examination[5]. Direct SEM and backscattering examination revealed that the outer dentin layers were identical in the controls and in pathological teeth. These areas included the coronal mantle dentin, the radicular Hopewell Smith and Tome's granular layers. The circumpulpal dentin was severely disturbed in poorly compliant hypophosphatemic patients in both deciduous and permanent teeth. Large interglobular defects appeared between calcospherites. For compliant patients, the outer circumpulpal dentin was altered, but to a lesser degree than in non-compliant patients, whereas the inner layer was normal.

Other hypophosphatemic teeth were demineralised with acetic acid (0.5 M, pH 5) and subsequently embedded in paraffin. Serial 10□m thick sections were prepared for indirect immunodetection. In order to visualize dentin matrix molecules implicated in mineralization[6] immunolabeling was carried out with antibodies raised against 5 SIBLINGs (DSP, DMP-1, BSP, OPN and MEPE), an anti-glycosaminoglycan antibody (2B6), and an anti-osteocalcin. For compliant patients, whatever the molecules visualized, the staining was similar to the unaffected teeth. For SIBLINGs, a strong labelling was obtained in dentin at the mineralization front, in odontoblast cell bodies and in the inner third of odontoblast processes. However, even for the most compliant patients, hardly detectable but still remaining calcospherites were observed in the outer third of circumpulpal dentin. In contrast, for non compliant patients, interglobular spaces were strongly labelled. For 2B6, odontoblast processes were labelled along tubules in the circumpulpal dentin in compliant patients and in controls. In both cases predentin was labelled. For poor compliant patients, the staining was localized at the border of calcospherites. Finally, osteocalcin was found in predentin, in odontoblast cell bodies and processes in control as well as in the teeth of treated hypophosphatemic patients. Interglobular spaces were strongly labelled in non compliant hypophosphatemic patients pointing out the implication of this molecule in mineralization.

We conclude that the formation of the peripheral dentin layers is not under the control of this genetic disease, hence unrelated to the phosphate status of the patient. The target is clearly the circumpulpal dentin and even if the 1alfa-(0H) treatment has a beneficial impact on the dental structure of hypophosphatemic patients, structural alterations are still observed, revealing that abnormal dentin is a good indicator for this genetic disorder.

Key words: Familial Hypophosphatemia, 1-hydroxylated vitamin D treatment, PHEX gene.

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TWIST-1 EXPRESSION DURING PULP REPAIR

H. Løvschall[1], E.-M. Füchtbauer[2],

1. Department of Dental Pathology, Operative Dentistry and Endodontics, Royal Dental College, Faculty of Health Sciences. 2. Department of Molecular Biology, Faculty of Sciences, University of Aarhus, Denmark

loev@odont.au.dk

Basic helix-loop-helix (bHLH) transcription factors have been shown to play an important role in controlling cell type determination and differentiation. In a previous study our observations suggested that Notch signaling may activate the downstream target *Hes1*, a bHLH transcription factor, in subodontoblasts near the lesion and in odontoblasts during reparative dentinogenesis (H. Løvschall, M.Tummers, I. Thesleff, E.-M. Füchtbauer, K. Poulsen. Eur J Oral Sci, aug 2005). In this study, our aim was to examine gene expression of another bHLH protein, Twist, during repair of pulp perforation. Twist proteins are involved as transcription factors in development of neural-crest derived tissues and the branchial arches. Twist proteins antiosteogenic function is mediated by a novel domain, the *Twist* box. In this study we explored *Twist* expression after pulp exposure of adult first upper rat molars. The wound was capped with calcium hydroxide. *In situ* hybridization of *Twist-1* in control molars revealed it was not present in pulp stroma and it had only a weak expression in the odontoblast layers. In comparison, the periodontal tissues had *Twist-1* expressed on the bone walls, and especially in the PDL and bone marrow. *Twist-1* expression was absent in the superficial defense zone with groups of inflammatory cells, dentin chips, and necrotic tissue, whereas, it increased close to the injury in the reparative zone of the upper pulp stroma. In conclusion, our observations confirm that *Twist-1* expression is activated after pulp injury. The observations suggest Twist signaling is involved in the control of stem cell progenitors during reparative dentinogenesis.

Key words: pulp injury, stem cells, hybridization, wound, odontoblasts

Acknowledgments the support of the COST Action B23 is kindly acknowledged.

ERUPTION OF TEETH IS LINKED TO THE FORMATION OF A SUCCESSIONAL DENTAL LAMINA IN THE ZEBRAFISH (DANIO RERIO): SUPPORT FOR THE STEM CELL CONCEPT

A. Huysseune

Biology Department, Ghent University, 9000 Gent, Belgium.

Recently, we have hypothesized that epithelial stem cells give rise to replacement teeth in continuously replacing dentitions, such as in the zebrafish (*Danio rerio*) (Huysseune & Thesleff, 2004, BioEssays 26: 665-671). In order to test whether activation of putative stem cells – to give rise to a replacement tooth bud – is linked to the developmental stage of the tooth predecessor, I examined the timing of development of replacement teeth with respect to their predecessors in the pharyngeal dentition of the zebrafish.

Observations based on serial semithin sections of ten specimens, ranging in age from young juveniles (approx. four weeks old) to adults, indicate that (i) replacement tooth germs develop from an epithelial structure, resembling a successional dental lamina; (ii) a successional dental lamina, or a tooth germ developing from it, is present at every single tooth locus; (iii) erupted, functional teeth that are still in the process of ankylosis are invariably associated with a successional dental lamina only; (iv) well attached but young functional teeth are always associated with young replacement teeth; (v) older functional teeth are not necessarily associated with advanced replacement teeth; they can also have a successor that has not progressed beyond the initiation stages. These observations suggest a developmental link between eruption of a tooth and development of a successional dental lamina, thus supporting assumptions of the epithelial stem cell hypothesis. At the same time they, however, indicate that factors other than the state of functionality of the erupted tooth influence the further differentiation of the germ.

Key words: replacement teeth - epithelial stem cells – zebrafish – *Danio rerio*

EXPRESSION OF ADRENOMEDULLIN IN HUMAN DENTAL TISSUES

AJ Sloan, J McLachlan, AJ Smith, PR Cooper

Tissue Injury & Repair, School Of Dentistry. The University Of Birmingham, Uk

Adrenomedullin (ADM) is a multifunctional peptide produced by many tissues and is abundantly expressed during tooth development and by oral epithelium. ADM has been suggested to regulate bone formation *in vivo* and *in vitro* and may also be important of the control of inflammation. Recent microarray analysis of healthy and carious human pulp tissue has indicated a 3.47 fold up-regulation of ADM with respect to carious disease. ADM may have a role in dental tissue repair, however information regarding the expression and localisation of ADM in mature dental tissues is still relatively limited. This study aimed to further characterise the expression of ADM in both human dental tissues. To confirm recent microarray data, real-time quantitative PCR analysis for ADM was performed on 5 healthy and carious pulpal samples and immune cells and fold change obtained by comparison with the sample exhibiting the lowest level of expression. Immunohistochemical expression of ADM was examined in 7 healthy and carious human teeth. 5mm wax embedded sections were stained with a rabbit polyclonal antibody to human ADM prior to detection using the StrAviGen method. Controls included omission of the primary antibody and substitution of the primary antibody with non-immune rabbit IgG. Real-time quantitative PCR indicated increased expression of ADM in dental tissues following carious disease and significant differences between carious and healthy gene expression levels was observed. Gene expression of ADM was observed in immune system cells, with the greatest expression seen in neutrophils and neutrophils stimulated with bacterial LPS. The presence of ADM in odontoblasts and pulp fibroblasts was demonstrated immunohistochemically, with strong staining seen in the odontoblast layer. Similar patterns of staining were observed in carious tissue, with positive staining observed in the inflammatory cell infiltrate. These studies demonstrate the expression of ADM in odontoblasts and pulpal tissue of healthy and carious teeth and that expression is increased following carious disease. The relative expression of ADM in these tissues may be important in the modulation of the tissue response to injury during caries.

CHONDROID BONE FACILITATES FAST GROWTH OF THE DENTARY IN MATURE MALE ATLANTIC SALMON AND IS ALREADY PRESENT IN EARLIER DEVELOPMENTAL STAGES.

P. Eckhard Witten[1,3], J. Andrew Gillis[2] and Brian K. Hall[3]

[1]Zoological Institute, Univ. of Hamburg (Ger), [2]Earth Sciences, Univ. of Bristol (UK), [3]Department of Biology, Dalhousie Univ. Halifax (Ca)

While returning to their home rivers for spawning, starving adult female and male Atlantic salmon resorb several bone structures. At the same time, rapid skeletal growth at the tip of the lower jaw of male salmon facilitates the development of a prominent kype (hook); an element that is regarded as a secondary sexual character.

Analysis of the kype reveals fast growing skeletal structures that are composed of chondroid bone and that display resemblance to deer antlers and to periosteal bone tumours. Different from deer antlers, the kype of male Atlantic salmon that survive spawning is not shed. Unlike in bone tumours, the fast growth of the kype skeleton is reversed through resorption of apical parts and through remodelling of basal parts into regular bone tissue. The conversion of basal parts of the kype skeleton into regular (compact) dentary bone - with no traces of chondroid bone - contributes to the elongation of the dentary. It also raises the question whether this special mechanism of dentary bone growth is sex-, age-, and/or maturity-related or whether it is part of the normal dentary development in salmon.

A study of salmon larvae and of three groups of juvenile salmon (immature males, immature females, and premature males) reveals the presence of chondroid bone at the tip of the dentary in all groups. Thus, apical dentary growth in Atlantic salmon apparently does not occur by typical intramembranous ossification, but by a modified periosteal bone growth involving chondroid bone, and by a transformation of this tissue into regular bone. While parts of the chondroid bone derive from the periosteum/perichondrium and thus qualify as secondary cartilage, the examination of salmon larvae also suggests a possible developmental relationship between chondroid bone and Meckel's cartilage. Further studies should reveal if the unusual mode of fast dentary bone growth is a feature restricted to salmon or if this mode of dentary growth is also present in other vertebrates.

THE TWO LOW MOLECULAR WEIGHT AMELOGENIN PEPTIDES (A+4) AND (A-4) ACTIVATE THE EXPRESSION OF GENES INVOLVED IN TOOTH DEVELOPMENT, IN IMMORTALIZED MESENCHYMAL PROGENITORS CELLS

Sally Lacerda-Pinheiro(1), Arthur Veis(2), Odile Kellerman (1), Michel Goldberg(3) and Anne Poliard(1).

(1)Laboratoire de Differentiation Cellulaire et Prions – UPR1983 CNRS, Villejuif, FR (2) Cell &Molecular Biology, Northwestern, Chicago, (3)Faculté de Chirurgie Dentaire – Université René Descartes, Paris, FR

ABSTRACT
Amelogenins are the major proteins of the developing tooth enamel matrix. Although, first considered to be ameloblast-specific, amelogenin expression has recently been demonstrated in odontoblasts. Over 10 amelogenin isoforms have been characterized, derived by alternative splicing at the mRNA level. These isoforms, their extensive posttranslational possible modifications and their degradation products produce the variability of amelogenin proteins found in the tooth. Two low molecular weight amelogenin [A+4](8.1kDa) and [A-4](6.9kDa) , produced by alternative splicing of the exon 4 of the amelogenin (AMEL) gene, have recently been postulated to be tissue-specific epithelial mesenchymal signaling molecules (1). We have shown that, implanted in the dental pulp or the oral mucosa, these peptides promoted the expression of different proteins involved in osteogenesis (2, 3). The aim of the present study is to get an understanding of the molecular mechanisms underlying the action of the AMEL peptides. To this end, we have first monitored the effect of the A+4 and A-4 peptides on the expression of a series of genes involved in the chondrogenic, osteogenic and/or odontoblastic program in a set of different mesenchymal progenitor cell lines derived from embryonic multipotential cells, bone marrow or dental pulp(4,5). All cell lines were treated for 6, 24 and 48 hours with 2µg of A+4 or A-4). Analyses by semi-quantitative RT-PCR showed a specific activation of the transcripts encoding Sox9 and Runx 2 as already observed in embryonic fibroblasts (1) but also of early tooth development transcription factors, (Lhx6, Lhx7, Pax9, Msx1) and bone and dentin matrix proteins (osteocalcin, and DSP). The increase in gene expression was in all cases dependent on the peptide (A+4 orA-4), the time of treatment and the cell type. These studies pave the way for more quantitative studies at the mRNA and protein levels (real time RT-PCR and western blots). Gene chip microarray analysis performed with this cell system should furthermore permit an identification of the signaling mechanisms by which the two AMEL peptides act on the differentiation of mesenchymal progenitors towards odontoblast or osteoblast cells.
Keywords: amelogenin, tooth developpement

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EPITHELIAL-MESENCHYMAL INTERACTIONS REGULATE DENTAL AXON PATHFINDING

Päivi Kettunen, Inger Hals Kvinnsland, Keijo Luukko

Division of Anatomy and Cell Biology, Department of Biomedicine, University of Bergen, 5009 Bergen, Norway

The development of the sensory trigeminal innervation of the tooth is tightly coupled with tooth morphogenesis. By analyzing knockout mouse embryos, we found that the expression of Semaphorin3a (Sema3a), a secreted repulsive axon guidance molecule, regulates timing and navigation of the trigeminal axons. Tissue recombination experiments showed that the early oral and dental epithelium, and epithelial Wnt4 induce Sema3a in the molar mesenchyme before arrival of first nerve fibres. Later, on during pioneer dental axon navigation epithelial signaling as well as Wnt4 and Tgfß1 control Sema3a expression in the dental mesenchyme. Thus, epithelial-mesenchymal interactions in developing tooth provide the instructions for the trigeminal axon navigation and patterning, and may coordinate establishment of tooth nerve supply with tooth formation.

Acknowledgements: This study was supported by the Norwegian Cancer Society, the L. Meltzer's foundation, the Research Council of Norway, Helse-Vest and Sparebanken Vest. The support of the COST B23 action is gratefully acknowledged.

Key words: axon guidance, tissue interactions, odontogenesis

HUMAN ODONTOBLASTS EXPRESS b1, aVb3 AND aVb5 INTEGRINS.

Staquet[1] M-J, Couble[1] M-L, Romeas[1] A, Connolly[2] M, Lucchini[1] M, Durand[1] S, Magloire[1] H, Hynes[2] R, Bleicher[1] F, Farges[1] J-C.

[1]Faculty of Odontology, Lyon1 University, France, [2]Howard Hughes Medical Institute, Cambridge, MA, USA.

Cellular homeostasis is maintained in the organism through the correct responses to extra-, intra-, and intercellular signals. Integrin ab heterodimers are membrane receptors that play an important role in the adhesive interactions between neighbouring cells and their surrounding extracellular matrix (ECM), and are known to initiate intracellular signalling cascades in response to a variety of factors. This study sought to characterise the way human odontoblasts could bind to their cellular and matrix environment by analysing the expression and distribution of the av subfamily integrins in sound human dental pulp *in vivo* and odontoblasts differentiated *in vitro*. Messenger RNA expression determined by RT-PCR revealed the presence of av, b1, b3, b5, and b8 integrin genes, but not b6, in the whole pulp cells. Alpha v, b1, b3, avb3 and avb5 were detected by flow cytometry analysis. Immunostainings on sections of human teeth demonstrated that these integrins were clearly localised in the membrane of mature odontoblasts, including the intradentinal cell processes, and in blood vessels. The b8 subunit was only detected in nerves.

In vitro differentiated odontoblasts expressed av, b1, b3, and b5 mRNAs, but not b6 and b8. Cell membranes were stained with anti-av, -b1, -avb3, and -avb5 antibodies. Flow cytometry analysis revealed that cultured non differentiated pulp cells and odontoblasts displayed similar phenotypic profiles but the levels of expression, assessed by the mean fluorescence intensity, revealed that differentiated odontoblasts exhibited a greater amount of av subunit (x 1.2), b1 (x 2.1) and avb5 (x 1.5) integrin molecules. Histological analysis of teeth from av knockout mice (embryonic day 19, 1 h after birth) and b3/b5 knockout mice (embryonic day 19, postnatal day 2 and 5) did not show obvious modifications in the odontoblast layer.

These results suggest that avb3, avb5, and possibly avb1 integrins could play similar roles in interodontoblast adhesion and odontoblast binding to the surrounding pulp, predentin/dentin matrices, thus maintaining the organisation of the odontoblast layer necessary to the integrity of the peripheral dental pulp.

WHAT IS THE OPTIMAL END RESULT OF THE TRANSDENTINAL BIOACTIVE STIMULATION?

M. Kalyva, A. Alvanou, S. Papadimitriou, D. Tziafas

Department of Endodontology, School of Dentistry, Department of Histology& Embryology, School of Medicine, Aristotle University of Thessaloniki, Greece

Biologically active molecules released during the natural reparative mechanisms in the dentin-pulp complex have been shown to signal cellular events leading to tertiary dentin formation. Numerous studies during the last two decades were designed to evaluate whether the exogenous application of bio-active molecules in direct contact with the traumatized dental pulp or in deep non-exposed cavities might regulate targeted regeneration of the dentin-pulp complex. However, the ultimate goal of such a therapeutic strategy remains to be confirmed. In particular it has been reported that the aim of a regenerative treatment strategy in case of dentinal injuries seems to be a regional and time-limited effect on surviving odontoblasts in order to up-regulate their biosynthetic activity. The natural defensive mechanisms taking place in dentinal injuries where odontoblasts may survive, e.g. non-cavitated stages of enamel caries, slowly progressing dentinal caries, mild abrasion and erosion, could provide us with a master plan for the achievement of regenerative approaches in the non-exposed dentin-pulp complex.

In this presentation, experimental observations from previous works will be critically reviewed and discussed together with data from our recent experimental attempts. Our data are based on light microscopic and SEM observations, 3-8 weeks after experimental application of TGF-beta1, or bFGF, or IGF-II, or OP1 in acid-etched bucall class V cavities of dog teeth (remaining dentin thickness ranged between 0,30 to 0,60 mm), where non-acid etched and untreated acid-etched cavities were used as controls.

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DENTIN MATRIX IN DIFFERENT TYPES OF ABNORMAL COLLAGEN I METABOLISM: A COMPARATIVE LIGHT MICROSCOPY (LM), IMMUNO-HISTOCHEMISTRY (IHC) AND TRANSMISSION ELECTRON MICROSCOPY (TEM) ANALYSIS

DeCoster PJ [(1)], Cornelissen M [(2)], DePaepe A [(3)], Martens LC [(1)], Vral A [(2)]

(1) Dept. Paediatric Dentistry & Special Care, Paecamed Research, Ghent University, Ghent, Belgium; (2) Dept. Anatomy, Embryology, Histology and Medical Physics, Ghent University, Ghent, Belgium; (3) Centre of Medical Genetics, Ghent University Hospital, Ghent, Belgium

Collagen I makes up the bulk of dentin, providing a scaffold for intra- and interfibrillar depostion of hydroxyapatite crystals during dentinogenesis. Both aberrations in collagen fibril formation and mineralisation of the predentin matrix may result in structural defects of dentin. These defects have been linked with mutations in *DSPP* gene on chromosome 4 (mineralisation defect) or have been associated with several syndromes, e.g. caused by deficiency of collagen I. The purpose of this study was to examine and compare the histology and ultrastructure of dentin matrix of teeth from patients with different types of collagen I deficiency.
Decalcified sections of the dentin matrix of five teeth from three patients with Type III OI and DI, two from a patient with Type I EDS with unusual *COL1A1* mutation, one from a patient with Type VIIC EDS (*ADAMTS2* mutation), and three control teeth were compared by LM, IHC and TEM.
The dentin matrix of the different pathological samples was commonly characterized by structural aberrations with a distinct variety of forms, which did not necessarily correlate with the dental clinical manifestations. The greatest histological similarities were found between dentin samples from Type III OI and Type VIIC EDS. The structural anomalies reflect the different abnormalities in the organisation of the collagen I meshwork, serving as a scaffold for dentin mineralisation.
The present results suggest that different types of collagen I deficiency may cause similar abnormalities of dentin structure. Ultrastructural evidence of disrupted dentinogenesis may also be found in clinically unaffected teeth in these patients. Confirmation on a larger scale is needed, however, to establish a clear-cut genotype-phenotype correlation.

Keywords: Type I collagen, dentin matrix, Osteogenesis Imperfecta (OI), Ehlers- Danlos Syndrome (EDS), Dentinogenesis Imperfecta (DI)

ACKNOWLEDGEMENTS: THE STUDY WAS SUSTAINED BY GRANT NR. B/05088 FROM THE SPECIAL RESEARCH FUND OF GHENT UNIVERSITY, SECTION IV.1.

DIMETHYLBENZ(A)ANTHRACENE DISTURBS THE FORMATION OF DENTAL MATRICES AND REDUCES THE SIZE OF CULTURED MOUSE LOWER MOLARS

Eija Peltonen, Carin Sahlberg, Anna-Maija Partanen, Satu Alaluusua, Pirjo-Liisa Lukinmaa.
Institute of Dentistry, University of Helsinki, and Helsinki University Central Hospital

Clinical studies suggest that maternal smoking during pregnancy can reduce the crown size of the child's teeth. One of the main components of tobacco smoke is the group of polycyclic aromatic hydrocarbons (PAHs), in part toxic chemicals, which are formed by incomplete combustion and carbonization processes of organic matter. Apart from some natural sources like smoke from forest fires and volcano eruptions these widely spread environmental contaminants are also present in industry- and traffic-derived exhaust fumes, industrial by-products and food. The aim of our study was to investigate the effect of PAHs on tooth formation and the function of tooth-forming cells *in vitro*.
E18 mouse (NMRI) mandibular molars were cultured in a Trowell type organ culture with dimethylbenz(a)anthracene (DMBA), a toxic PAH compound. DMBA was used at the concentrations of 0.1, 0.5, 1.0, 2.0, and 4.0 μM. After 12 days of culture the explants were digitally photographed and fixed with 4% paraformaldehyde, demineralized with EDTA, embedded in paraffin, serially sectioned and stained with hematoxylin and eosin. The mesio-distal width of each tooth was measured from the photographs by Analysis software and for statistical analysis Pearson's Chi-Square test was used.
At the start of culture, on E18, morphogenesis of the first molar crown had been completed but deposition of predentin had not started yet. The second molar was undergoing transition from the cap stage to the bell stage of morphogenesis.
After 12 days of culture, dentinogenesis was in progress throughout the crown of the first molars. In those regions where dentin mineralization was ongoing, ameloblasts had also become secretory. Extent of predentin mineralization and amelogenesis, having started at the tip of the mesial cusp, varied from one culture experiment to another. The combined thickness of predentin and dentin equalled that of the enamel. DMBA at the concentrations of 0.1 and 0.5 μM had no detectable effect on hard tissue formation or tooth morphology. At higher concentrations up to 4.0 μM, hard tissue formation was reduced, proportionally more of enamel than dentin. In some teeth, no enamel was visible. The severity of effects increased with DMBA concentration. The cusps became thin and curled and tooth size was reduced.
In the second molars cultured for 12 days, a layer of coronal predentin was visible and ameloblasts were elongated. Occasionally, enamel facing mineralized dentin was already seen. Beginning from DMBA concentration of 1.0 μM, polarization and differentiation of the dental cells and dentinogenesis were retarded. As in the first molars, cusps were sharp and thin. Some second molars exposed to higher DMBA concentrations were retained at the bell stage compared to the secretory stage reached by unexposed teeth.
DMBA exposure significantly reduced the mesio-distal width of germs of the first molar teeth dose-dependently.
In conclusion: i) DMBA interferes with the formation of dental hard tissues in mouse mandibular molars *in vitro*. Ameloblasts seem to be more sensitive than odontoblasts; ii) DMBA affects morphology of cultured mouse mandibular molars. DMBA dose-dependently reduces the width of tooth germs and alters cusp morphology.

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GENE EXPRESSION ANALYSIS OF ENDOCHONDRAL BONE FORMATION.

Rachael Sugars[1], Ulrika Petersson[2], Alistair Chalk[3], Eszter Somogyi[1] and Mikael Wendel[1].
[1.]Center for Oral Biology, Karolinska Institute, Sweden, [2.] Oral Biochemistry, Göteborg University, Sweden, [3.]Center for Genomics and Bioinformatics, Karolinska Institute, Sweden.

Introduction: Endochondral bone formation is a well-defined event, orchestrated by mesenchymal cell condensation to form a cartilage structure, which acts as template for mineralization. During this process, a vast number of molecules are involved, including those required for osteoblast recruitment, osteoclast activity and bone remodeling. To date, little information is known regarding the molecules involved in remodeling and the use of long bone cultures in conjunction with gene expression analysis provides an excellent opportunity to investigate those concerned. **Purpose:** To study the changes in gene expression with the progress of endochondral bone formation of metatarsal long bones. **Approaches:** Central mouse metatarsals from embryonic stages 15 and 19, pre and post-mineralization, were excised and total RNA isolated. Biotin-labeled cRNA from the long bones, at the different stages of mineralization, was subjected to gene chip analysis, using the Affymetrix GeneChip Mouse Expression Set 430 -GB (34,000 genes). The gene array data was analyzed using data mining tools and comparisons were made using the Gene Ontology database. RT-PCR and Northern blot analyzes were performed on a selected number of genes to verify the data obtained. **Results:** Microarray data showed a distinct up-regulation, of at least 5-fold, for several genes associated with bone remodeling and bone formation, including TRAP, MMP-13, bone sialoprotein, osteopontin, dentin matrix protein-1, MMP-9, and cathepsin K. Interestingly, osteonectin was found to be 4-fold down-regulated post-mineralization (E19). RT-PCR and Northern blot analysis of these molecules confirmed the microarray data. **Conclusions:** Biomineralization is a complex and dynamic process, and this study, using gene chip analyzes, has identified several genes of potential importance during bone remodeling, contributing to the ever-emerging picture of the molecules involved in this complicated event. **Keywords:** Bone, Microarray, Gene expression. **Acknowledgements:** Supported by the Swedish Research Council and the COST Action B23.

INHIBITION OF PTHRP EXPRESSION BY BMP-2 AND ITS IMPLICATION IN OSTEOBLAST DIFFERENTIATION (POSTER)

A. R. G. Susperregui, F. Viñals and F. Ventura
Laboratory of Bioquimica, Department of Ciencies Fisiologiques II, University of Barcelona, IDIBELL, 08907 L'Hospitalet de Llobregat, Spain. E-mail: antoniogarcia@ub.edu

Bone morphogenetic proteins (BMPs) constitute a family of multifunctional growth and differentiation factors related to transforming growth factor-beta. They are potent inhibitors of myoblast differentiation and inducers of osteoblast differentiation, both in vivo and in vitro. In this study we have identified the parathyroid hormone-related peptide (PTHrP) as a target gene regulated by BMP-2. PTHrP was originally described as a tumor derived agent responsible of hypercalcemia in patients with malignancy. Here we show a reduction in the expression of PTHrP mRNA after two hours of BMP-2 addition to the cell culture. Assays with the PTHrP mouse promoter using luciferase as reporter gene, and the degradation of PTHrP mRNA observed in presence of BMP-2 and actinomycin D reveals that this effect of BMP-2 occurs at the transcriptional level. As PTHrP is an important regulator of several processes in bone such as chondrocyte differentiation, osteoclast activation and bone resorption we suggest a posible role of this peptide in the transdifferentiation of myoblast cells into the osteoblastic phenotype promoted by BMP-2. We have analyzed different osteoblast markers in C2C12 cells stimulated with BMP-2 when adding exogenous PTHrP (1-34) to the culture. The induction of the alkaline phosphatase activity was reduced by the addition of PTHrP (1-34) as well as the expression of osteocalcin mRNA. This data suggest that BMP-2 decreases PTHrP expression as a permisive mechanism of osteoblast differentiation.

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BONE CELL TRANSPLANTATION IN RECONSTRUCTIVE SURGERY IN OROFACIAL REGION (POSTER)

Jenca A., Hanusínová V., Orencak M., Kizek P., Jencova J.
Department of Stomatology and Maxillofacial Surgery Medical Faculty
and University Hospital UPJS, Kosice, Slovakia.

Defects of facial bones have a variety of causes (trauma, cysts, benign osteodestructive tumors, malignant tumors, atrophic loss of bones, congenital deformities). The aim of this study is to evaluate possibilites of reconstruction of these defects.

There are three types of bone grafts used in reconstruction surgery of hard tissue defects in orofacial region. Autogenous grafts which are composed of tissues of the same individual. Allogeneic grafts which are taken from another individual of the same specie. Xenogeneic bone grafts, taken from one species and grafted to another are not very frequently used in human medicine. In the period within 1998 – 2003, 150 patients were treated at our clinic. In 82 cases autografts were used. In 68 cases the allografts were used. Xenogenic bone grafts were not used. In 64 % of cases supporting of osteointegration with autogenic osteoblasts was used.

Time after operation was in range 6-48 months. The healing success was in 73 % of cases, but in combination with autogenic osteoblasts were more than 91% and the period of healing activated and time decrease to 32 %.

Using a bone grafts in hard tissue reconstruction surgery in orofacial region is very popular in these days. The correct choice of type of bone graft depends on many factors (evaluation of the defect, type of surgical intervention, possibility of supportive therapy using a autogenic osteoblasts). One of the biggest disadvantages of autografting is the mutilation of the patients. But allografting is linked with technical problems and the healing phase is longer and healing success is not as big as in autografting.