ABSTRACTS: POSTER PRESENTATIONS

P1

Timing of cranial neural crest cell migration is critical for tooth type formation in mice

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Background: It was suggested the CNC cells that migrate into the first arch between the 10- and 12-somite stage have odontogenic potential to initiate tooth development, While the correlation between different timepoint of CNC cell migration and tooth type forming capability in mices is still unclear. The objective of the present study is to explore the correlation between different timepoint of cranial neural crest (CNC) cell migration and tooth type forming capability in mice. **Methods:** The first arches (E8.5-E10.0) or mandibular arches (E10.5, E11.5) were dissected out and were grafted for subrenal culture for 2 and 4 weeks, respectively. Explants were dissected, processed for Hematoxylin-eosin stainning and Heidenhain Azan staining to examine tooth formation. Tooth width and cusp number were measured by stereomicroscope with a digital camera. **Results:** The shape and size of formed teeth were analyzed. The first arch before E9.0 yielded only membranous bone. The first arch from E9.0 and E9.5 produced one multicuspid molar. The first arch from E9.75 (24-25 somites) or older embryos possessed the capability to form both molar and incisor. The size and cusp number of the molars increased from E9.0 to E11.5 grafts. Moreover, association of CNC cell number with tooth-forming capability was examined by dissecting the first arch of E9.75 and E9.5 into two halves. The grafts of half-arches of E9.5 and E9.75 failed to form tooth. When the number of CNC cells increased by transplanting the E9.5 first arch with its dorsally adjacent tissue, it was able to form one molar and one incisor. **Conclusion:** These results suggested that the timing of CNC migration is critical for dental patterning. This research was supported by the National Natural Science Foundation of China (Grant No.30700947) and Research Fund for the Doctoral Program of Higher Education of China (Grant No.20060486038).

P2

Neural crest like cells from induced pluripotent stem cells for tooth regeneration

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Induced pluripotent stem cells (iPS cells) are candidate to serve as a valuable source in regenerative medicine, because they can differentiate into various cell types of the body upon proper induction as well as embryonic stem cells. However, it has not been reported that iPS cells differentiate into odontogenic cells for tooth regeneration. In this study, we have designed to produce the neural crest like cells (NCLC), which could be an origin of odontogenic mesenchymal cells, using iPS cells. We maintained mouse iPS cells under the culture medium including leukemia inhibitory factor and the feeder cell layer. For NCLC differentiation, iPS cells formed spheroid body under suspension culture using HydroCell culture dish (CellSeed, Tokyo) for 4 days. Then, the spheroid bodies were seeded on fibronectin coated culture plates and were cultured in NCLC differentiation medium including B-27 for 6-9 days. The spheroid bodies spontaneously attached and expanded, and furthermore gave rise to stellate-morphology migratory cells. Immunofluorescence staining revealed that their cells prominently expressed neural crest marker, nestin (>90%, Fig.). Real time RT-PCR showed that mRNA of neural crest cell markers dramatically increased compared with that of undifferentiated iPS cells. These data suggest this culture condition could induce NCLC from iPS cells efficiently. Furthermore, to confirm whether NCLC can differentiate into odontogenic mesenchymal cells, we are planning to transplant them with dental epithelial cells.

P3

Patterning of the heterodont dentition in the house shrew (*Suncus murinus*)

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Introduction: The most commonly upheld criteria for tooth class (type) homology have focused on the position of teeth in the upper jaw. Upper incisors are the teeth anchored in the premaxilla; the canine is the first tooth behind the premaxillary-maxillary (incisive) suture, behind which is some number of premolar, then molar teeth. The permanent dentition of the house shrew (*Suncus murinus*) comprises all tooth classes without diastema region (I3/1 C1/1 P2/1 M3/3□30). During the dentition development of the house shrew, dental epithelium of upper deciduous first incisor originates from the frontonasal process, whereas that of second incisor originates from the maxillary process [1]. In this study, using the house shrew, we investigated (1) the expression pattern of the several genes (*Shh, Bmp4, Fgf8, Msx1, Barx1*) in the developing jaw, and (2) the positional relationship between the premaxillary-maxillary suture and the developing upper dentition. We discuss the patterning of the heterodont dentition in mammals both in terms of molecular control and the traditional criteria in comparative odontology. **Materials & methods:** *Suncus murinus* orthologs of *Bmp4, Fgf8, Msx1* and *Barx1* were isolated from the house shrew embryos. Expressions of these genes in the developing jaws were detected by *in situ* hybridization. H-E stained serial histological sections of the head were prepared for each embryonic age. Three-dimensional morphology of developing dentition and bone in the upper jaw was reconstructed from these serial sections. **Results:** In the upper jaw primordia prior to morphological initiation of tooth development, *Bmp4/Msx1* was expressed across the fusion site of the frontonasal and maxillary processes. *Bmp4/Msx1* was also expressed in the mesial part of the mandibular process. *Fgf8/Barx1* was detected in the distal parts of both upper and lower jaws. On the other hand, the ossification in the jaws started 3 days behind the initiation of tooth development. The ossification center of the premaxilla appeared in the mesenchyme adjacent to the enamel organ of the upper first incisor. The premaxillary bone grew distally beyond the fusion site of the frontonasal and maxillary processes, and finally reached just mesial to the canine germ and formed the premaxillary-maxillary suture there. **Discussion:** The model for tooth type determination is well established in the lower jaw of the mouse [2]. This model is applicable to both upper and lower jaws of the house shrew. The expression domain of *Bmp4/Msx1* corresponds to the incisor-forming region, whereas that of *Fgf8/Barx1* corresponds to the molar-forming region. Although the fusion site of the frontonasal and maxillary processes runs through between the first and second incisors, the premaxillary-maxillary suture is shifted distally to the canine-forming site. The above model is, therefore, not contradictory to the traditional criteria for tooth class homology in mammals.

References: 1. Yamanaka A, Yasui K, Sonomura T, Uemura M. (2007) Development of heterodont dentition in house shrew (*Suncus murinus*). *Eur J Oral Sci* 115: 433-440. 2. Tucker AS, Matthews KL, Sharpe PT. (1998) Transformation of tooth type induced by inhibition of BMP signaling. *Science* 282: 1136-1138.

P4

Development of the oral cavity: From gene to clinical phenotype in human

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Tooth development is under strict genetic control and is mediated by epithelio-mesenchymal interactions between first pharyngeal arch oral ectoderm and ectomesenchymal cells originating from cephalic neural crest. Signaling molecules of the main developmental pathways (Tgfβ, Shh, Wnt, Fgf, Notch…) and transcription factors are involved. Oro-dental anomalies are one aspect of the 5000 known rare diseases or syndromes and 700 of these diseases have an oral phenotype. Our goal is to combine complementary approaches in developmental biology especially via the study of animal models and bioinformatics to improve the understanding of etiopathogenic mechanisms involved in oral development. We used two approaches to target relevant genes: (1) selection of known genes responsible for rare diseases but for which the

expression and/or roles are not (or insufficiently) characterised; (2) identification of new candidate genes, through a systematic analysis of their craniofacial and dental expression patterns using the EURExpress mouse transcriptome-wide atlas (http://www.eurexpress.org/ee/). We then analysed by non radioactive manual or automated *in situ* hybridization the detailed expression patterns of these genes during mouse odontogenesis at E12.5, 14.5, 16.5, P0, P6. This method provides an accurate spatio-temporal description and cellular resolution of the expression of individual gene product.(1) *NSD1*, gene (nuclear receptor-binding SET domain protein 1 gene; locus 5q35), coding a retinoic acid co-regulator protein, when mutated is responsible for Sotos syndrome (OMIM #117550). This overgrowth condition combines excessive growth during childhood, macrocephaly, distinctive facial gestalt, various degrees of learning difficulty and variable minor features. An increased risk of tumors is reported. The oro-dental phenotype encompasses dental anomalies like hypodontia especially agenesis of premolars, supernumerary teeth, enamel hypoplasia, pulpal anomalies, premature tooth eruption, associated with high arched palate and prognathism. In the mouse, the localization of *nsd1* transcripts was detected throughout odontogenesis both in epithelial and in mesenchymal compartments. Transcripts were also detected in differenciating and differenciated cells (2) The screening of the EURExpress mouse transcriptome-wide atlas pointed towards the *Ap1m2* gene encoding the adaptor-related protein complex 1, mu 2 subunit. This protein, localized within the trans-golgi network clathrin coat and belonging to the adaptor complexes medium subunits family is capable of interacting with tyrosine-based sorting signals.The transcripts of *Ap1m2* were located within the enamel organ throughout odontogenesis and were also detected in differenciating and differenciated cells.To decipher further the role of these genes, interference with the function of these genes and associated proteins/pathways will be performed using *in vitro* siRNA silencing technics in tooth organ culture. We acknowledge the University of Strasbourg, API HUS and IFRO for their support. Huang N, vom Baur E, Garnier JM, Lerouge T, Vonesch JL, Lutz Y, Chambon P, Losson R.Two distinct nuclear receptor interaction domains in NSD1, a novel SET protein that exhibits characteristics of both corepressors and coactivators. EMBO J. 1998 Jun 15;17(12):3398-412. Laugel V et al., Bloch Zupan A, Développement de la cavité buccale : du gène à l'expression clinique chez l'Homme, IFRO, Cahiers de l'ADF, 2010 (in press).

P5

Molecular regulation of tooth replacement

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The tooth replacement capacity of different species varies greatly. Fishes, snakes and amphibians can replace their dentition several times during their lifespan while most mammals, including humans, have two sets of dentitions. However the mouse has no tooth replacement. The molecular mechanisms regulating tooth replacement have long been poorly understood because of the limitations of the traditional mouse model. Roles for Wnt signaling have been indicated in congenital human disorders affecting tooth replacement and in transgenic mice with extensive formation of supernumerary teeth. We have studied the role of Wnt signaling using both the mouse and the ferret (Mustela putorius furo) as model animals. I will present our results showing that the initiation of the primary teeth and tooth replacement are controlled by both common and unique signaling patterns.

P6

Developmental analysis of limb development after temporal blocking of Hedgehog signaling

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Background: The vertebrate limb skeleton develops through the endochondral bone formation, which involves in the substitution a cartilage primordium with the calcified bone matrix. The cartilage formation, called chondrogenesis, is crucial in determining the shape and size of definitive bones in vertebrate. During mouse limb chondrogenesis, mesenchymal progenitor cells are condensed differentiates into chondrocytes to form the cartilage primordium, and chondrocytes undergo sequential proliferation and differentiation along with secretion of extracellular matrix. Many studies have reported extensively the molecular mechanism on the endochondral bone formation rather than chondrogenesis. Es-

pecially, Indian hedgehog (Ihh) signaling is known to control both hypertrophy of chondrocytes and bone replacement which is particularly important in postnatal endochondral bone formation. **Methods:** In this study, maternal transfer of 5E1 (a hedgehog-blocking antibody) to E12 mouse embryo causes an attenuation of the Indian hedgehog activity, and causes malformation of the mouse limb. **Results:** We analysed genetic relationship during chondrogenesis in limb buds with mRNA microarray. As a result, we found some genes interacting with Ihh ; *Glo1, Dkk4, Mapk8, Ltbp3, Col1a2, Wasl, Dmkn, Flnc, Eltd1, Egfl7, Col22a1, Krt1, Col22a1, Krt1, Col19a1, Krt10, Hspa8, Fbxo2, Ocrl, map3k4, Garnl4, Fgf7, Krt4, Klf7* were up-regulated, and *Hhip, Hoxd13, Casp1, Nrg4, Alpl, Rassf8, Foxc2, Bmp5, Ddef1, Ptch1, Sdc4, Tbx5, Runx2, Ikkbkg, Ubc, Nog, Runx3, Fgfr1op2, Nfia, Gh* were down-regulated. Furthermore, we treated exogenous IHH and 5E1 protein into mesenchymal cells of limb bud in the micromass culture system. We suggested that Ihh increased condensation of mesenchymal cells and proliferation of prechondrocytes in early chondrogenesis. **Conclusion:** Ihh signaling positively regulates early chondrogenesis, as well as hypertrophy of chondrocytes in the mouse limb development.

P7

Methylation status of the Runx2 P2 promoter in a family with ectopic maxillary canines

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Aims and objectives: To determine the effect of methylation status of the Runx2 proximal promoter in buccal cell/lymphocyte DNA on ectopic eruption of the maxillary permanent canine (EC). **Introduction:** Genetic factors contribute to the aetiology of EC, the inheritance pattern being autosomal dominant with variable expression and incomplete penetrance. However there is also an epigenetic component. The Runx2 gene is intimately involved in the mechanism of tooth eruption and mutations of this gene result in delayed and ectopic eruption of teeth. There is a large CpG island spanning its proximal promoter, first exon and part of the first intron. Similarities between the inheritance patterns of EC and other methylation disorders raises the hypothesis that differential methylation of the Runx2 promoter may contribute to EC. **Materials and methods:** DNA from a 21 member, three-generation family, exhibiting 8 cases of EC was extracted from saliva (Oragene, DNAGenotek) using the phenol-chloroform method and converted with bisulphite (Methyleasy Xceed, Human Genetic Signatures) in order to determine 5mC content. Three primer pairs were designed, one being 2kb upstream of the transcription start site (TSS), one less than 1kb upstream of the TSS, within the promoter region and another in first intron. Polymerase chain reaction was used to amplify the relevant sites and the product directly sequenced using the Applied Biosystems 3130 Genetic Analyser. Analysis was carried out using the BIQ Analyzer program[1]. **Results:** There was no difference in the methylation status of the P2 promoter regardless of age or eruption status of the maxillary canines. **Conclusions:** The Runx2 P2 promoter in buccal cells and lymphocytes is unmethylated regardless of age or eruption status of the teeth. As promoter methylation is tissue as well as age specific, further research should analyse dental tissues. **Reference:** [1]Bock, C et .al. (2005). "BiQ Analyzer: visualization and quality control for DNA methylation data from bisulfite sequencing." Bioinformatics 21(21): 4067-8.

P8

Alteration of tooth development in two-phase organotypic cultures by transient Glycogen Synthase Kinase-3 (GSK-3) inhibition

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Background: Tooth development depends on intercellular cross-talk signalling between dental epithelium and mesenchyme. Canonical Wnt pathway has been found to present an outermost importance to coordinate dental morphogenesis and differentiation. It activates when secreted Wnt proteins bind to their transmembrane receptors in the target cell. This inactivates a multiheteromeric destruction complex that, in absence of Wnt signals, triggers the degradation of β-catenin, a gene transcription cofactor. When Wnt pathway is active, β-catenin accumulates in target cells and is eventually translocated to the nucleus, where it induces transcription of Lymphoid Enhancement Factor (LEF) controlled genes. One of the

main components of the β-catenin destruction complex is Glycogen Synthase Kinase-3 (GSK-3), which phosphorylates β-catenin, thereby sending it to degradation. Consequently, pharmacological inhibition of GSK-3 activates Wnt signalling, by preventing β-catenin elimination. The importance of Wnt pathway for dental development is underscored by the fact that transgenic mice that inactivate Wnt signalling fail to develop teeth past bud stage. On the contrary, Wnt overactivating mutants often present abundant supernumerary teeth. Genetically modified mouse models constitute an invaluable tool for the study of dental malformations, but since the majority of these reported modifications are also permanently expressed in the animal, it remains unclear at what specific stages of dental development is Wnt activation required, and for what specific functions, in the context of tooth development. **Methods:** In order to specifically determine the role of an activation of Wnt pathway at different stages of dental development, we performed organotypic mouse tooth cultures extracted at E14.5 and E17.5, at the onset of cap and bell stages, respectively. In a first phase, we cultured these tooth germs in vitro for 6 and 12 days, in the absence or presence of pharmacological inhibitors of GSK-3 activity, such as Lithium Chloride (LiCl; 2mM) and 6-bromoindirubin-3´-oxime (BIO; 5 μM and 10μM). In a second phase, we used these in vitro cultured teeth to transplant them to testicles of surrogate male mice, to eventually achieve full-term tooth development. This two-phase procedure culminated with the production of a calcified mature dental piece. **Results:** We assessed that GSK-3 inhibition effectively activated Wnt signalling in cultured teeth, by measuring increased β-catenin protein levels and nuclear translocation. Additionally, we analized different developmental parameters such as tooth morphogenesis (histology) cell proliferation levels (BrdU), apoptosis (active caspase 3), and differentiation of odontoblasts and ameloblasts (nestin and amelogenin). Treatment with GSK-3 inhibitors dramatically increased cell proliferation and delayed differentiation of dental cell types. Finally, these morphogenetical alterations induced the formation of abnormal teeth. **Conclusion:** Our experimental model allows to perform transient activation of Wnt signalling at different tooth developmental stages, and assess the outcome of these manipulations in the fully developed dental piece. Understanding how Wnt activation affects dental development may help develop new strategies for prevention of developmental malformations, in tooth and other related ectodermal organs. This work was funded by the Basque Country University (UPV/EHU), Jesús Gangoiti Foundation and Basque Government.

P9

Reduced expression of tight junction proteins ZO-1 and claudin-1 in ameloblasts and odontoblasts of Epiprofin/Sp6 deficient mice

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Background: Odontoblasts and ameloblasts are tall and columnar cells that characterize by a highly polarized distribution of cellular organelles. These cells differentiate terminally and deposit the organic matrix of dentin and enamel respectivelly. The contacts between odontoblasts are found at the junction area between the cell body and the odontoblast process in the form of a modified junctional complex. Cell adhesions between ameloblasts are present at the two junctional complexes between these cells, the proximal one that is adjacent to the stratum intermedium and the distal one that is located at their secretory pole. Junctional complexes and the cytoskeleton are important in maintaining cell polarity and cell-cell interactions. In addition, cell adhesión is a key regulador of cell differentiation. These interactions with neighboring cells and the extracellular matrix regulate gene expression, cell proliferation, polarity and apoptosis. Tight junctions are one type of such cell-cell junctions and several signaling complexes have been identified to associate with them. **Methods:** In this work, we have studied the odontoblast and ameloblast polarization and differentiation in Epiprofin/Sp6 null mice and the correlation with the decreased expression of tight junctins proteins, such as zonnula ocludens-1 (ZO-1) and claudin-1. **Results:** We found that odontoblast features were altered in Epiprofin/Sp6 null mice. Odontoblast differentiation was clearly delayed when compared to wild type mice. Both collagen I and DSPP dramatically decreased in E19.5 Epiprofin/Sp6 deficient first molars, whereas in

normal molars these proteins were normally secreted. In adult mutant mice, some odontoblasts either lost the polarization or polarization was changed with the nuclei located at the secretory side facing dentin. Several layers of odontoblasts were formed, and the dentin layer was thin and incomplete. The pattern of dentinal tubules was altered. The inner enamel epithelium remained undifferentiated in mutant embryo mice teeth and polarized and differentiated ameloblasts were absent during odontogenesis. Consequently, no enamel was detected in Epiprofin/Sp6 -/- teeth. In wild type mice, ZO-1 protein is expressed in preameloblasts and ameloblasts and localizes at the basal and apical sides of the cell membrane. In mutant mice, ZO-1 protein was barely detectable in the inner enamel epithelium, and its expression was decreased in odontoblasts and dental mesenchymal cells. Claudin-1 was expressed in the outer enamel epithelium and stratum intermedium in wild type molars, but its expression was clearly reduced in Epiprofin/Sp6 deficient molars. **Conclusion:** We report defects in polarization and differentation of odontoblasts and ameloblasts in *Epiprofin/Sp6* (-/-) mice, that correlate with a decreased expresion of tight junction proteins. We hypothesize that this defective polarization is linked to an impaired formation of the tight junction adhesion complex, and therefore to a decreased membrane recruitment of signaling proteins triggering cell differentiation. This work has been supported by the University of the Basque Country, the Basque Government and the Jesus Gangoiti Foundation. L Jimenez and M Aurrekoetxea contibuted equally to this work.

P10

Caspase knock-outs and dental apoptosis

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Background: Apoptosis (programmed cell death) has been shown to play an important role at a number of stages during tooth development. We are interested in the specific roles of caspases during odontogenesis. Caspases are a large protein family of cysteine aspartate proteases involved in inflammation or/and apoptosis. Initiator apoptotic caspases (caspase-8, -9, and -10) are activated via death adaptor molecules proximity and oligomerization induced proteolytic processing. Once activated, the initiator caspases cleave other members of the caspase family called effector caspases. Subsequently, effector caspases (caspase-3, -6, -7) cause degradation of several cellular polypeptides that are essential for cell survival (lamin, PARP) resulting to DNA fragmentation, cytoskeleton break-up, and cell death. **Methods:** Mouse knock-out phenotypes for individual caspases were studied with a special focus on embryonic day E15.5. At this stage the primary enamel knot of the first mouse molar is eliminated by apoptosis and a typical cluster of apoptotic (TUNEL positive) cells is apparent. Hematoxylin-eosin staining was used to evaluate any alterations in morphology of the tooth germ, TUNEL assay to follow changes in the apoptosis pattern and PCNA immunohistochemical labelling to detect proliferating cells. Caspase-3 immunohistochemistry (Cell Signalling) was used to follow the temporospatial pattern of activation of this central caspase. Caspase-8 knock-outs die at E11.5 before morphological onset of odontogenesis, therefore an explants culture approach of pharmaceutical inhibition is necessary. **Results:** In caspase-9 deficient mice, no TUNEL positive cells were detected in the primary enamel knot suggesting involvement of caspase-9 and the intrinsic pathway in dental apoptosis activation. The importance of this pathway was confirmed by the lack of TUNEL positive cells in mutant molars deficient in Apaf-1, another pro-apoptotic molecule in the intrinsic signalling. Proliferation in both mutants was not altered.Caspase-3 activation in odontogenesis correlates with apoptosis distribution based on TUNEL labelling. In caspase-3 deficient mice, temporal, strain-dependent alterations in tooth shape were found and TUNEL labelling confirmed a complete inhibition of apoptosis in the primary enamel knot of these mutants. Caspase-7 mutants are also under study to reveal further details related to the role of the caspase network in dental apoptotic machinery, while specific caspase-8 inhibition experiments are underway to investigate the role of the extrinsic apoptotic pathway. **Conclusion:** Caspases play important role in the mechanism of apoptosis during death of the enamel knot. Loss of specific caspases results

in loss of apoptosis and in some cases changes in tooth shape. However, compensatory mechanisms must be taken into account in the case of individual caspase inhibition and alternative cell death in the case of general caspase inhibition. Supported by the Grant Agency of the Czech Academy of Sciences (KJB500450802 - primary enamel knot apoptosis research, IAA600450904 - embryonic apoptosis research). International cooperation runs under the Royal Society grant (JP080875) and the IAPG lab under IRP IPAG No. AVOZ 50450515.

P11

1,25α(OH)2 vitaminD3 regulate Msx1 antisense RNA expression in mouse incisor

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Background: Clinical investigations report the existence of enamel and dentin alterations in vitamin D-related pathologies. Tooth morphogenesis results from the combinatorial action of different homeobox genes expressed in the facial neural-crest-derived mesenchyme. One of them, Msx1, is involved in cell proliferation and inhibits cell differentiation. A sense and a cis-antisens (AS) RNA are transcribed from Msx1 gene. They are present during tooth morphogenesis, but AS fonction is not clearly identified. It can modulate the distribution of the protein. Most of target organs of Msx1 are also known for their sensitivity to 1,25α(OH)2VitaminD3 (vitD). The aim of this study was to elucidate the influence of the vitamin D hormone on the sense and antisense Msx1 RNA expression pattern during dental cell differentiation. **Methods:** For this purpose we used the mouse incisor continuous growing tooth. This model provides a continuous proliferation and differentiation of dental cells with well established zones of pre-secretion, secretion and maturation. **Results:** For the first time the expression pattern of Msx1 sense and AS RNAs was investigated in mouse post-natal incisor, and the regulation of Msx1 AS expression in mice incisor by 1,25(OH)2D3 was shown. In vivo, Msx1 AS RNA expression is up-regulated in the microdissected epithelium from incisors of vitamin D-deficient mice, and rescued with 1,25(OH)2D3 repletion. In vitro, the treatment of ameloblast-like cell line (LS8) by 1,25(OH)2D3 decreased Msx1 AS RNA expression. **Conclusions:** Our results suggest that the presence of Msx1 sense and AS RNAs in bone cells might be considered in the complex mechanism of vitamin D and bone physiology.

P12

Expression and function of microRNAs in tooth development

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MicroRNAs are 19-25 nucleotide non-coding small signle-stranded RNAs that negatively regulate gene expression by binding target mRNAs. MicroRNAs have been implicated in numerous biological processes including development. The biological role of most individual mRNAs and the targets they modulate are largely unknown in tooth development. A comprehensive expression analysis of microRNAs in tooth development was therefore carried out as a starting point to understand the role of individual microRNAs. We have examined the temporo-spatial expression of 6 different microRNAs (miR-27560, miR-193, miR-378, miR-148a, miR-218 and miR-98) based on preliminary microarray data in murine tooth development. All 6 microRNAs show dynamic temporo-spatial expression in tooth development. MiR-27560 is expressed in both epithelium and mesenchyme throughout the early stages of tooth development. MiR-193 expression is observed in both epithelium and mesenchyme at initiation and bud stages whereas it could not be detected at the cap. MiR-378 and miR-148 show expression in presumptive tooth epithelium at initiation and basal epithelium at the bud stage whereas they are expressed in inner and outer enamel epithelium except the enamel knot at the cap stage. MiR-218 and miR-98 are strongly expressed in lingual cervical loops at the cap stage. Dicer, an RNase III endonuclease, is the essential emzyme that cleaves microRNA precursors into 19-25 nucleotides. In order to investigate the role of miRNA in tooth development, we further examined mice with conditional Dicer de-

letion (BF1-Cre/Dicer$^{flox/flox}$). The collar of tooth germ epithelium is lacking in both incisors and molars in BF1-Cre/Dicer$^{flox/flox}$. These suggest a specific role for microRNAs in maintaining the epithelial collar during tooth development.

P13

Ultrastructural localization of osteoadherin during early tooth development

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Background: The small leucine-rich proteoglycans (SLRP) have been extensively implicated in the biomineralization process of bone and dentin, albeit the complex mechanism of mineralization remains to be fully elucidated. Specifically, biglycan, decorin, and fibromodulin have been shown to be involved in dentinogenesis, and ultrastructural immunohistochemistry has shown that they are differently distributed across the mineralizing tooth, from the odontoblast cell layer to pre-dentin, pre-dentin/dentin interface and dentin. Furthermore, keratan sulphate, the glycosaminoglycan associated with the class II family of SLRPs, demonstrated an increasing gradient towards the mineralization front. Osteoadherin (OSAD), a keratan sulphate-substituted SLRP is also reported to be present in the tooth and has been postulated to be involved in dentinogenesis. However, the exact role and distribution of OSAD during tooth formation remains to be fully determined. **Aims:** This study aimed to clarify the ultrastructural localization of OSAD within the developing tooth; cell layer, pre-dentin, pre-dentin/dentin interface and dentine, and enamel, with a view to provide further evidence for the role of OSAD during dentinogenesis. **Methods:** Mouse mandibles were collected at different time points; embryonic day 15 (E15), newborn (NB), postnatal day 5 (d5) and adult tissue (A), and prepared for transmission electron microscopy (TEM). Ultrathin sections were probed with an antibody against OSAD and the signal detected using an immunogold-conjugated secondary antibody. Controls were performed by blocking the primary antibody with recombinant OSAD. Immunogold-labeled particles were counted and expressed as the number of gold particles/μm^2. These were scored per different compartment (cell layer, pre-dentin, pre-dentin/dentin interface and dentin, and enamel). **Results:** Quantification of OSAD density revealed considerable differences between the different developmental stages. At all developmental stages, few OSAD-labeled gold particles were detected in the cell layer and the enamel. With early dentinogenesis of the incisor at E15, OSAD was localized in the developing pre-dentin. However, by d5, OSAD was localized across all compartments, demonstrating an increase from the pre-dentin to pre-dentin/dentin interface and dentin. A similar pattern of OSAD localization was observed in molars from all developmental stages, with the highest number of immuno-gold-labeled OSAD particles observed in the pre-dentin and dentin layers. Of note, OSAD was found in close localization to collagen fibers in the pre-dentin and dentin layers of d5 mice, supporting the notion that OSAD, maybe involved in collagen fibrillogenesis, although this remains to be determined. **Conclusion:** These novel data for the first time demonstrate the ultrastructural localization pattern of OSAD throughout the tooth. The gradient expression follows that previously reported for other SLPRs in dentinogenesis and provides useful information to aid in the elucidation of the role of OSAD in this dynamic mineralization process.

P14

c-Myb in prenatal odontogenesis: a pilot study in mouse and minipig models

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Background: The c-*myb* gene encodes a transcription factor involved in control of cell proliferation, differentiation, and survival. It is essential for control of hematopoiesis in vertebrates, however, its role in non-hematopoietic organs is less clear. There are multiple data suggesting participation of c-Myb in control of developmental processes. c-Myb mRNA was found in

mouse embryos and there is one report indicating the presence of c-Myb mRNA in mouse incisors at E14.5 (Ess et al. 1999, Oncogene 18: 1103-1111). This study aims to further investigate c-Myb expression pattern and potential role of this protein in odontogenesis based on c-Myb immunohistochemistry and temporo-spatial correlation with proliferation and apoptosis in two animal models: the mouse as a monophyodont species and the minipig as a diphyodont species. **Methods:** Cell morphology was analysed in serial histological sections after hematoxylin eosin staining. c-Myb expression was examined by immunohistochemistry (anti-c-Myb antibodies purchased from Abcam and Epitomics) and *in situ* hybridisation. Cell proliferation was addressed by immunohistochemistry of PCNA protein (DAKO antibody). Apoptotic cells were labeled by TUNEL assay (Chemicon). The study was supplemented by analysis of c-Myb in floxed mutant mice. **Results:** The c-Myb protein was found both, in epithelial and mesenchymal parts of the mouse and pig tooth germs. Analysis of cell proliferation showed that c-Myb expression is linked to proliferation, however, c-Myb positive cells were abundant to PCNA positive cells. Apoptotic cells were c-Myb negative. In the minipig, interestingly, an asymmetric c-Myb expression was found in the epithelial part of the tooth germ. Moreover, c-Myb-positive cells in the minipig degrading dental lamina were situated on the side facing the tooth germ.Absence of proper c-Myb function is lethal; c-Myb deficient mice die *in utero* at day 14.5 from failure of fetal hematopoiesis. Compared to the wild type, the tooth mutant phenotype at E14.5 did not display any morphological alterations in budding or in the presence of proliferating and apoptotic cells. **Conclusion:** Distribution pattern of c-Myb during tooth development positively correlates with proliferation, negatively with apoptosis and differentiation. c-Myb seems to have a specific role in diphyodont species related to the asymmetric growth of the tooth germ and dental lamina degradation. As c-Myb expression remains compatible with differentiation, the apparent lack of the first molar disturbance in mutant mice may reflect the fact that such events would arise after day 14.5. Supported by the Grant Agency of the Czech Republic (524/08/J032 and 304/08/P289), the Grant Agency of the Czech Academy of Sciences (KJB500450802) and Ministry of Education (MSM0021622415). International cooperation runs under the Royal Society grant (JP080875) and the IAPG lab under IRP IPAG No. AVOZ 50450515.

P15

Roles of Wnt/ß-Catenin signaling in the formation of dentin and periodontium

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Background: Wnt/ß-Catenin signaling is essential mediator of epithelial-mesenchymal interactions during tooth development. It has been well known that Wnt/ß-Catenin signaling is critical for the normal tooth morphogenesis in early tooth development. However, its roles in the formation of dentin and cementum are not completely understood. The objective of present study is to understand the roles of Wnt/ß-Catenin signaling in the crown and root formation. **Methods:** We generated and analyzed the mice with tissue-specific activation of *ß-Catenin*. The key mediator of Wnt signaling, *ß-Catenin*, was tempospatially activated by *OC-Cre* with the activity of *Cre* recombinase under the control of *OC* promoter. **Results:** Mutant mice exhibited prominent tooth phenotypes. In the crown of mutant molar, well-differentiated odontoblasts were observed but mineralized dentin was thinner than that of wild type. Instead of mineralized dentin, wide predentin was found underneath the dentin, which showed globular mineralized pattern. Root of mutant molar was more severely affected than its crown. Roots were shorter than those of wild type and the most part of roots were atypically surrounded by the cellular cememtum. In addition, periodontal space between cementum and alveolar bone was widened between the mutant molars. **Conclusions:** These results indicated that appropriate *ß-Catenin* mediated signaling is essential for the mineralization of dentin and periodontium formation. Therefore, it is strongly suggested that Wnt/ß-Catenin signaling may be important in the formation of dentin and periodontium as well as in the early tooth development. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (M10646010003-06N4601-00310 and 2009-

0085733)

P16

Smad4 mediated signaling is essential for dentin formation

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Background: Accumulating evidences indicate that TGF-β/BMP signaling play key roles in the early tooth morphogenesis. However, their roles in the differentiation of odontoblasts and dentin formation are not clearly understood. The objective of this research is to understand the roles of TGF-β/BMP signaling in vivo during dentin formation. **Methods:** We generated and analyzed the mice with odontoblast-specific inactivation of *Smad4*. The key mediator of TGF-β/BMP signaling, Smad4, was inactivated by three kinds of *Cre* mice lines (*Col1a1-Cre, Dmp1-Cre,* and *Dspp-Cre*) with the activity of *Cre* recombinase under the control of each matrix protein promoter. **Results:** All of three mutant lines exhibited similar tooth phenotype, particularly in coronal dentin of molars. Although no differences in the size and shape of molars, dentin formation was severely affected in mutants. The coronal dentin of mutant molars was thinner than those of wild type littermate whereas the radicular dentin was normal. In addition, number of differentiated odontoblasts was apparently reduced in mutant molar and some odontoblasts were included in the dentin matrix. In some case, ossifying masses and inflammatory cells were observed in the pulp cavity. In other case, pulpal degeneration and periapical invasion of inflammatory cells were also observed. **Conclusions:** These results indicated that *Smad4* mediated signaling is essential for dentin formation. Therefore, it is strongly suggested that TGF-β/BMP signaling may play as an essential regulator in the dentin formation. This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MEST) (2007-313-E00487 and M10646010003-06N4601-00310)

P17

Expression patterns of the *Fam83h* gene during murine tooth development

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Background: Recently a novel gene, *FAM83H*, was identified by a genetic linkage study in the hypocalcified form of the amelogenesis imperfecta family with an autosomal dominant hereditary pattern. Little is known about this novel gene, and so we investigated the expression pattern of *Fam83h* in murine tooth development using serial sectional *in situ* hybridisation. **Methods:** Using mandibles of ICR mouse at specific developmental stages, *in situ* hybridisation was performed by DIG-labeled RNA probe. **Results:** Faint expression was detected in limited cells at embryonic day 14 (E14) in the molar. At the bell stage, E16, *Fam83h* was localized in the outer and inner enamel epithelium, as well as dental papilla. *Fam83h* expression begins on E15 in the developing incisor. At E18, *Fam83h* was expressed in the inner enamel epithelium of the apical bud, ameloblasts and odontoblasts. The expression was stronger in the presecretory stages than the secretory stages. **Conclusion:** *Fam83h* was detected in the ameloblasts from the presecretory to the secretory stage, and also the odontoblasts layer and surrounding alveolar bone.

P18

Expression of prion gene and presence of prion protein during development of mouse molar tooth germ

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Expression of Prnp (encoding the Prion protein) in the mouse first molar tooth germ increased several-fold during the secretory phase of odontogenesis. A similar time-course of expression

was found for genes coding for enamel matrix proteins (Amelx, Ambn, Enam) and for Aplp1, Clstn1, and Clu. Western-blot analysis suggested that the amounts of Prion protein (PrP) and Amyloid beta (A4) precursor-like protein 1 (APLP1) in the tooth germ followed similar time-courses to those of expression of the corresponding mRNAs. Immunohistochemical studies of PrP in cells of mouse molar and incisor tooth germs at E18.5 suggested this protein to be located in the cervical loop, outer enamel epithelium, and in preameloblasts. Immunolabelling for PrP was also observed in the dental papilla, initially at the base of the tooth germ, later more centrally. Different immunolabelling of preameloblasts on the mesial and distal aspects of a lower molar cusp may be related to different enamel configurations on the two aspects. The results suggest that PrP, as well as other extracellular proteins showing similar expression profiles, may have functions during secretory odontogenesis and amelogenesis.

P19

Early odontogenesis of *Chamaeleo calyptratus*

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Background: Veiled chameleons (*Chamaeleo calyptratus*) are omnivores with a diet consisting of insects, leaves and plants. They have monophyodont dentition in contrast to most toothed reptiles with polyphyodont dentition. Furthermore, chameleons possess a heterodont dentition with monocuspid and multicuspid teeth acrodontly ankylosed to jaw bones. The aim of recent study is to investigate odontogenesis in the chameleon, with specific focus on the comparative morphology of the dental lamina, the mechanism of transition from polyphyodont to monophyodont dentition and cusp formation. **Methods:** Embryos of veiled chameleon were collected during the first six months of incubation (from 5th to 24th week) over 7 day intervals. The *in ovo* development of this species lasts from 6-8 months depending on incubation conditions. Serial histological sections were prepared and alternative slides were used for immunohistological analysis (PCNA, TUNEL) and *in situ* hybridization. MicroCT analysis was performed on one and half month old specimen to analyze shape of individual teeth along the jaw. *In situ* hybridization was performed using a python *Shh* probe. **Results:** After five weeks of incubation, the dental lamina was present as a shallow epithelial thickening protruding into the mesenchyme. A week later the epithelium elongated more deeply into the mesenchyme to form the dental lamina. Dental lamina growth was lingually angled. The formation of tooth germs was initiated in close proximity to the oral epithelium in the 7th week of incubation. From early stages, tooth germs developed as asymmetrical structures with a large cervical loop on the lingual side. In contrast to gecko, the stellate reticulum contained numerous cells. Transversal sections revealed small clusters of cells with few apoptotic cells situated in the inner enamel epithelium that resembled the enamel knot area in mouse. Dentin and enamel production was well on the way by the 11th week. Development of a small successional dental lamina was initiated in the 14th week and overgrew the tooth germ in the lingual direction. Proliferation activity was high in the cervical loops and successional dental lamina. *Shh* expression was present in the inner enamel epithelium of cup and bell stages. Furthermore, *Shh* transcript was localized asymmetrically in the oral epithelium on the lingual side of lamina. MicroCT analysis showed the presence of heterodont dentition in young chameleons with tricuspid teeth in the more proximal jaw area. These tricuspid teeth were composed of the massive central cusp and two lateral smaller cusps localized in mesio-distal direction. In the distal jaw area, teeth were unicuspid. One and half month old animals showed twelve erupted teeth. New teeth were initiated in the caudal areas as the jaws became elongated during the post-hatching period. **Conclusion:** Reptiles exhibit large variability in the type of dental attachment from acrodont to pleurodont, in the shape of teeth from unicuspid to multicuspid as well as in the number of replacement teeth generations from monophyodont to polyphyodont replacement. This high variability enables us to study evolutionary and developmental mechanisms of odontogenesis and compare differences in developmental processes in relation to mammalian species. **Acknowledgements:** MB and OZ are support-

ed by GAASCR (grant KJB601110919) and IRP IPAG No. AVOZ 5045015, AST is supported by an International Joint grant from the Royal Society (JP080875).

P20

Mechanism of dental lamina degradation

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Background: Minipig has diphyodont and heterodont dentition resembling that of human. Elongation of the dental lamina into the mesenchyme is necessary process for replacement teeth formation. Successional lamina overgrows the deciduous anlagen in the lingual direction when primary dentition reaches the late bell stage. At the same developmental stage as the second generation is initiated, the dental lamina is disconnected from oral epithelium and fragmentizes into several pieces. However, nothing is known about mechanism of dental lamina regression during development of diphyodont dentition. Here, we aim to evaluate possible processes during early stages of dental lamina regression by using immunohistochemical detection of candidate molecules. **Methods:** Minipig embryos and fetuses were obtained from Liběchov animal facility (Czech Republic, strain LiM). They were collected between the embryonic day (E) 19 and 67 and fixed in 4% neutral formaldehyde. After paraffin processing, 5 mm serial tissue sections were prepared, stained with Hematoxylin-Eosin and alternative slides were used for immunohistochemical analysis. The apoptotic DNA breaks were labeled by the TUNEL method (Chemicon). To study early apoptotic markers in the dental lamina we detected Fas (Santa Cruz Biotechnology), Fas-L (Santa Cruz Biotechnology) and FADD (Santa Cruz Biotechnology). As markers for the epithelial-mesenchymal transition (EMT) we chose E-cadherin (Abcam), MMP2 (Abcam) and Slug (Abcam). Primary antibody was incubated at room temperature in a humidified chamber for 1hour. Counterstaining with Hematoxylin was performed. **Results:** The first sign of the lamina degeneration and loss of its connection to the oral epithelium became obvious at E50. Between E56 and E67 the dental lamina fragmentized and lost the body mass. There were morphological differences between both sides of the dental lamina where the side facing the tooth anlagen became degraded as the first. We detected the presence of apoptotic cells during the regression of dental lamina. We expected to see condensation of apoptotic cells at area of connection the lamina to the oral epithelium and the differences in distribution of TUNEL positive cells on both sides of lamina. However, only few TUNEL positive cells were evident in the dental lamina at these developmental stages. To test the presence of pro-apoptotic markers we detected Fas, Fas-L and FADD at the late stages of lamina development when the signs of regression were already obvious. All markers were situated on the side of lamina facing the teeth. Next we tested the presence of epithelial-mesenchymal transformation markers during the degradation of lamina. MMP2 and Slug were increased in the side facing the tooth germ in E56 compared to E67 while E-cadherin was decreased in the whole mass of dental lamina. **Conclusion:** Based on TUNEL analysis results we propose that early disconnection of dental lamina with oral epithelium is not occurring by massive death of dental lamina cells and thus apoptosis does not seem to be the main mechanism involved in the dental lamina fragmentation. The localization and dynamics of EMT markers during the dental lamina degradation correspond to their pattern in the secondary palate seam disintegration and our results demonstrate the EMT as the process involved in the lamina breakup. **Acknowledgements:** This research was supported by the GACR (grant 304/08/P289) and the lab runs under IRP IPAG No. AVOZ 5045015.

P21

Dynamics movement of dental tissue during tooth development

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Background: Teeth develop from epithelium and neural-crest derived mesenchyme via a series of reciprocal epithelial-mesenchymal in-

teractions. Previous studies have shown the dynamic movement of cells in the dental follicle and their contribution to the periodontium. In this project we focus on the composition of the developing dental papilla, and movements in and out of this structure as it develops from the cap to the late bell stage. **Methods:** Using a slice culture technique to visualise the developing tooth germ we have followed the movement and fate of dental cells during tooth development. Our research combines cell labeling, using DiI and DiO, and the use of transgenic reporter mice, to follow cell movement and the origin of dental tissues. **Results:** Our results show that the tooth is a dynamic organ with dramatic movement of cells. We show the timing of migration of mesoderm cells into the tooth and the changing pattern of cells involved with epithelial-mesenchymal interactions. **Conclusions:** This work lays the ground work for our understanding of the tooth, and shows the contribution of a variety of cells to dental development. This work is funded by an Erasmus agreement between Charles University and King's College London and a grant from the European Science Foundation.

P22

Visualizing cellular dynamics of tooth development

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Rationale: Most of studies for tooth development have been mainly done with histological methods and cell culture. However, it has been difficult to observe the morphological changes and cellular dynamics properly during the organogenesis. We have therefore thought it´s been needed to design a new experimental system which can observe living cells keeping in vivo kinetics. In this study, we show the real time imaging system for a living tooth using the slice culture. **Material & methods:** We dissected the apical end of postnatal 2-4 days mouse lower incisors. The apical ends were embedded in a low melting point agarose. The embedded samples were then sliced to 150-200 mm thickness with vibratome and transferred to the culture chamber on confocal microscopy. The sliced sample was maintained at 37°C and exposed moisturized 5% CO_2 gas. Time-lapse 3D imaging was performed in the xyz-t mode using FV300 multiposition stage system. Data analysis and creation of the movies were performed using Metamorph software. **Results:** Time-lapse images of GFP mouse apical bud containing dental epithelial stem cells (Fig.), revealed that the stellate reticulum cells facing inner enamel epithelium were dividing. Regarding the divided cells, one daughter cell stayed on-site and the other one migrated into a layer of basal epithelial cells. These observations were in line with dental epithelial stem cell kinetics we proposed before (Harada, et al, JCB, 1999). Furthermore, we used Fucci mouse to determine the cell-cycle dynamics in apical bud. The cells with green fluorescence, which represents $S/G_2/M$ phases, were mainly located in the inner enamel epithelium and the peripheral stellate reticulum cells facing inner enamel epithelium. The location of the cells was consistent with that of dental epithelial stem cells, suggesting that this imaging system could allow chasing dental epithelial stem cells in real time. **Conclusion:** This imaging system for living tooth we established will be powerful tool to help us understand spatial and temporal phenomena during tooth development.

P23

Morphogenesis of the mouse third molar (M3)

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Background: In humans the third molar (M3) is the most commonly missing tooth type. All three molars in a quadrant (M1, M2, M3) develop from a single molar placode. Formation of the three molars has been followed in the mouse. M1 and M2 form during embryonic development, while M3 develops largely postnatally, once growth of the jaw has reached a size to accommodate all the teeth. Therefore, the mouse M3, unlike the M1 or M2, develops surrounded by fully mineralized bone. The aim of this study was to supplement our poor knowledge of M3 development and osseointegration in the mouse, with a special

focus on the stages of enamel knot formation. **Methods:** M3 development was evaluated by analysis of histology, proliferation (PCNA - proliferating cell nuclear antigen), apoptosis (TUNEL) and *in situ* hybridisation (*Shh, Fgf4*) from postnatal (P) 0 to P9. Osteoclasts were identified in the surrounding bone using tartrate resistant alkaline phosphatase activity (TRAP staining). The mouse mandibular molar dental primordia from P0-P20 were visualized in serial sections and reconstructed in 3D together with their surrounding bony structures. Regions of bone apposition and resorption were included. **Results:** The M3 is first visible as a bud extending from the M2 at P0. A cap stage forms by P3, at which stage the primary enamel knot undergoes apoptosis. The secondary enamel knots are visible at P5. Apoptosis in the surrounding bone showed a lingual-buccal asymmetry, correlating with the growth of the cervical loop as shown by PCNA staining. While in P0 the M3 is not completely surrounded by bone, it becomes gradually more encapsulated towards stage P20. The occlusal surface, however, remains free of bone. The bony crypt is characterized by bone resorption activity, while the crestal, the interdental and the periradicular bone is characterized by apposition. **Conclusions:** The morphology of the developing M3 and the surrounding bone were investigated by a variety of methods. The M3 primary enamel knot is visible at P3, while the secondary enamel knots form at P5. P3 and P5 for M3 therefore correspond to Embryonic day (E)15 and E17 for M1. Although M3 develops at a time of extensive jaw bone development, we have been able to show that it initiates budding in an area at the edge of the bone, and later becomes encapsulated. Supported by the Grant Agency of the Czech Republic (524/08/J032), the Deutsche Forschungsgemeinschaft (Ra 428/1-9), and a Royal Society Joint International Grant (JP080875). The IAPG lab runs under IRP IPAG No. AVOZ 50450515.

P24

The developmental stage-dependent increase in expression of soluble guanylate cyclase during mouse tooth development

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The nitric oxide-receptor enzyme soluble guanylate cyclase (sGC) contains one prosthetic heme group and two heterodimer isoforms ($\alpha_1\beta_1$, $\alpha_2\beta_1$) whose enzymatic activity have been characterized. There is no information available regarding the expression of sGC during tooth development. In order to clarify the expression of sGC during mouse tooth development, localizations of the α_1-, β_1- and α_2-subunit of sGC were examined in cells of the molar germs at prenatal (E20.5), postnatal (P3) and in adult (3 months) stages. In ameloblasts and odontoblasts of the molar germ at E20.5, a weak staining for α_1-, β_1- and α_2-subunit was detected. The localization of the β_1-subunit was identified in the nucleus of odontoblasts. In ameloblasts and odontoblasts of the molar germ at P3 stage, the staining intensities for the α_1- and β_1-subunit were increased. There were no changes in staining intensities for the α_2-subunit between E20.5 and P3 stages. In comparison to P3, higher staining intensities of the α_1-, β_1- and α_2-subunit of sGC were detected in adult molar odontoblasts. These data indicate that ameloblasts and odontoblasts may be regulated by the $\alpha_1\beta_1$-heterodimer of sGC during tooth development, while adult odontoblasts are regulated by $\alpha_1\beta_1$- as well as by $\alpha_2\beta_1$-heterodimer isoforms of sGC. We conclude that there is a developmental stage and cell type dependent expression of sGC during tooth development.

P25

Functional isolation of dental stem cells

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Background: One of the main challenges in making biological tooth replacement a reality is the isolation of potential stem cells for therapeutic application. Although many stem cell popula-

tions have been identified in anatomically distinct regions of the human tooth and its surrounding tissues, there is currently no reliable marker to isolate these cells. Markers used in literature reveal different populations with diverse stem cell properties which compromises broad clinical application of these cells due to heterogeneity. **Methods:** Rather than detecting cellular markers, our approach was to identify mesenchymal stem cells in rodent incisors using physical properties to isolate different cell populations. We aimed to identify the stem cells by performing growth curve, differentiation and migration experiments on cells isolated from cervical loop and body region of the rodent incisor and from the molar tooth. To determine whether a privileged population of very small embryonic-like (VSEL) stem cells also exists in the tooth, we have adopted a modified cell migration approach. Using invasion chambers with 8μm and 4μm pore sizes, cells which migrated through were assessed in terms of differentiation capacity. Growth curves were conducted to compare the proliferation rates of the different cell types. **Results:** Our results have demonstrated that the cells isolated from mesenchyme close to the cervical loop region are distinctly different from those isolated from the bone marrow or molar dental pulp. These differences are evident in terms of size, morphology and greater proliferation capacity of cervical loop cells. In addition, the migratory capacity is greatly altered indicating that these cells are a unique, privileged population that requires further characterisation to determine their clinical potential. **Conclusion:** In summary, identifying dental stem cells based on migration may enable isolation of a purer population with relative ease. In terms of future bioprocessing and translation to a reproducible therapy, the migration procedure is economical and quick to perform. The results obtained to date from rodent incisors suggest that it may be possible to obtain from adult dental tissue suitable stem cells for dental tissue engineering.

P26

Characterization of the putative epithelial stem cells involved in continuous tooth replacement in Atlantic salmon (*Salmo salar*)

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With the exception of mammals, vertebrates have the ability to replace their teeth throughout life. It has been suggested that adult epithelial stem cells are required for continuous tooth replacement (Huysseune & Thesleff 2004). The dentition of Atlantic salmon (*Salmo salar*), a teleost fish and member of the Salmonid family, has been well characterized (Huysseune et al. 2007). Similar to other salmonids (e.g. rainbow trout, *Oncorhynchus mykiss),* replacement teeth develop without the involvement of a successional dental lamina. Presumably epithelial stem cells reside in a cell layer between the inner and outer dental epithelium, termed the middle dental epithelium (Huysseune & Witten 2008). Their progeny is assumed to translocate to the outer dental epithelium to form a transient amplifying cell (TAC) population that eventually gives rise to a new tooth bud. To better understand the spatial relationship between the potential stem cell niche and the developing replacement tooth, we made 3-D reconstructions based on histological sections. To characterize cell dynamics within the different epithelial cell layers (including the putative stem cell compartment), we performed cell proliferation assays. Different stages of tooth replacement were labelled for proliferating cells in late G1-phase and S-phase of the cell cycle by means of PCNA (Proliferating Cell Nuclear Antigen) immunostaining. High numbers of labelled cells were observed in the local thickening (placode) of the outer dental epithelium of the functional predecessor, representing the initiation stage of the replacement tooth. At the morphogenesis stage, labelled cells were observed at the border of the middle dental epithelium and the outer dental epithelium of the predecessor tooth, in contrast to the inner dental epithelium and the bulk of the middle dental epithelium, which showed no labelling. Labelling in the replacement tooth was mostly restricted to the lingual side of the outer dental epithelium at the level of the cervical loop. The numbers of labelled cells in the different epithelial layers of the replacement tooth germ decreased during cytodifferentiation. These results are consistent with the behaviour of putative epithelial stem cells in the middle dental epithelium as hypothesized above. Ongoing experiments to further characterize putative stem cells include BrdU (Bromodeoxyuridine) proliferating assays to investigate label retention.

Huysseune, A. and Thesleff I. (2004). Continuous tooth replacement: the possible involvement of epithelial stem cells. BioEssays **26**(6): 665-671.

Huysseune A. , Hall B. K., Witten P. E. (2007). Establishment, maintenance and modifications of the lower jaw dentition of wild Atlantic salmon (*Salmo salar* L.) throughout its life cycle. Journal of Anatomy **211**: 471-484. Huysseune, A. and Witten P.E. (2008). An evolutionary view on tooth development and replacement in wild Atlantic salmon (*Salmo salar* L.). Evolution & Development **10**(1): 6-14.

P27

Cell culture of human dental epithelial cells and its approach for regenerative dentistry

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Teeth regeneration is an ideal treatment for patients with congenitally missing teeth. In the current state, teeth regeneration has been studied using animal model, however, human tooth regeneration has not yet been reported. Because it is known well that tooth development proceeds through epithelial-mesenchymal interactions, in the human tooth regeneration we also requires both of epithelial and mesenchymal stem cells derived from human tissues. In recent years, the human mesenchymal stem cell has been discovered from pulp, periodontal membrane, impacted third molar germs and exfoliated deciduous tooth, otherwise human dental epithelial stem cells has not been reported. Therefore, in this study, we have challenged an exploration of human dental epithelial stem cells and tried to culture the cells, and furthermore examined the possibility of human tooth regeneration using these dental epithelial stem cells and mesenchymal stem cells. Because some recent studies using a variety of animals suggested the possibility that dental lamina includes the dental epithelial stem cells, we examined if human dental lamina cells exist in enucleated third molar germs. In this study, we utilized impacted third molar germs extracted from patients (8-12 years old) undergoing orthodontic treatments, under sufficient informed consent in accordance with the Ethics Committee of School of dentistry, Iwate Medical University. The third molar germ was cut into two parts, an upper part including dental lamina and dental papilla. And we made paraffin embedded sections and carried out hematoxylen-eosin staining and Immnunostaining. The results showed that fragmented dental lamina epithelial cells existed in the tissues and were positive for cytokeratin14, E-cadherin and p63. Next, the upper part was digested by collagenase/dispase, and used for primary culture. The cells were successfully cultured under DMEM/hamF12 serum-free culture condition in the presence of EGF and FGF2, and at second passage dental lamina epithelial cells successfully purified from mesenchymal cells by the method utilizing difference of cell-dish adhesion property between these cells. Immunophenotypic analyses of cell surface antigens by FACS showed that more than 90% of the cells were positive for E-cadherin. Furthermore, these cells were positive for cytokeratin14, CD133, CD49f, and p63. The result was identical with that of human dental lamina epithelium, in vivo. In order to research the possibility of tooth regeneration using these cells, we transplanted the recombination of the dental lamina epithelial cells and dental papilla into abdominal cavity of SCID mice. 0ne month later, the epithelial cells developed dental epithelium of bud stage in the recombinant tissue. Taken together, the results suggests that human dental lamina cells are useful as a source of dental epithelial stem cells for human tooth regeneration.

P28

Complexity of chiropteran dentition: the GIS approach

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Background: Chiroptera represents the second largest mammalian order. Yet, most of its members bears the most primitive molar type, the tribosphenic one, which is the best type for insectivory (except myrmecophagy). Nevertheless, differences have occurred in the clade in various aspects of the molar shape during its evolutionary history: from differences in size and cusp height to finer changes, i.e. reorientation of shearing crests. The impact of these characters on the final dietary niche is not always easy to estimate. **Methods:** To attempt to do so, we have used

GIS techniques (already tested on Rodentia and Carnivora) to describe overall complexity of molar rows in bats and so to quantify the impact of the differences in molar crown shape and size. In addition, great morphological similarity among various molar rows of bat species can help us to test limitations of this new method.

P29

Atavisms induced by *Fgf3* loss of function point to a central role for FGF signaling in evolution of tooth shape in mammals

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Background: Extant mammals exhibit an important range of dental morphologies adapted to different diets and masticatory movements. These morphologies derive from ancestral patterns of mammal ancestors. A central challenge is understanding how genetic mutations underlie these morphological changes and gave rise to the extant diversity. Previous studies have shown that genetic mutations can modify the number of teeth and lead to the development of lost premolars in mutant mice. However, many evolutionary modifications include the re-arrangement of cusps (pointed elements) present on occlusal surfaces of the molar teeth. Although they can potentially shed much light on evolutionary mechanisms, anomalies in tooth shape remain poorly studied. Understanding morphological changes will build an important bridge between the fossil record and developmental genetics. Because of its central role in tooth development, the Fibroblast Growth Factor (FGF) gene family is an attractive candidate for involvement in dental evolution. We therefore set out to evaluate the role of *Fgf3* in the morphogenesis of human and mouse dentition, and to compare the effects of changes in *Fgf3* expression with modifications that occurred during mammalian evolution. **Methods:** We studied the dental morphologies in mice and humans carrying *Fgf3* mutations and compared them with primitive rodent and primate fossils. Dental casts of one *Fgf3* null individual with homozygous c.616delG (p.V206SfsX117) mutation were obtained from Ankara University School of Medicine. We also studied mice carrying *Fgf3* null alleles. Studied fossils were from various collections and fossil sites. Tooth rows of mice were imaged using X-ray-synchrotron microtomography at the European Synchrotron Radiation Facility (Grenoble, France), beamlines ID19 and BM5; 3D-renderings were performed using VGStudiomax software. **Results:** Decreases in dosage of *Fgf3* in heterozygous and homozygous mutant mice led to phenotypes that resembled the progressive reappearance of ancestral morphologies. We also found that *FGF3* mutations in human patients led to the loss of the hypocone, which mimics the general ancestral organization of the molar occlusal surface in Primates. **Conclusion:** Together, our data from humans and mice showed that changes in FGF dosage can lead to the reappearance of morphological characters present in ancestral species. In light of these atavisms, we propose that increases in FGF signaling have played an important role in evolution of mammalian dentition by giving rise to new cusps and interconnecting cusps by new crests. Both the murine dental pattern in rodents and the acquisition of the hypocone in mammals are highly convergent characters that developed independently in a number of mammalian lineages, suggesting that changes in signaling by FGF3 or a similarly simple molecular modification may be involved in a number of speciation events. Comparative studies of both coding regions of FGF genes involved in dental development and noncoding regions that regulate these genes may lead to discovery of mutations linked with evolutionary relevant morphological modifications.
Acknowledgments: We thank Oztan Yasun from Numune Hospital in Ankara for casts, Jose Baruchel and ID 19 Staff at the European Synchrotron Radiation Facility (ESRF) for support, and Jean-Pierre Aguilar, Monique Vianey-Liaud and Pierre Mein for the loan of fossils.

P30

Exploring the roles of core-binding factor β (CBFB) in tooth development and ameloblast physiology

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Core-binding factor β (CBFB), a cofactor of RUNX gene family had attracted broad interest in recent years because of its critical roles in various developmental events. In the present study we investigated the possible roles of CBFB at the dental epithelium, using conditional null mutant mice (K14-Cre;RUNX1 flox, K14-Cre;CBFB flox). Rodent incisor featured with continuous elongation throughout life. This mechanism is support by labial cervical loop epithelium which contains proliferative cells and ameloblast precursor cells. CBFB deficiency in epithelium resulted in shorter incisor with poor enamel formation, non-polarized incisor ameloblast and less proliferating cells in labial cervical loop epithelium. In addition, FGF3 mRNA expression decreased at the mesenchyme which underlies the labial cervical loop. These results suggest CBFB contribute to ameloblast differentiation, cell proliferation of incisor cervical loop epithelium and FGF expression in underlying mesenchyme.

P31

Involvement of micro-RNAs in tooth morphogenesis and ameloblast differentiation

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Background: Teeth form as appendages of the ectoderm and their morphogenesis is regulated by tissue interactions mediated by networks of conserved signal pathways. The micro-RNA (miRNA) pathway has emerged as an important regulator of various aspects of embryonic development but its function in odontogenesis has not been elucidated. **Methods:** In order to examine the functions of miRNA during odontogenesis, we have used qPCR, miRNA microarray profiling and transgenic mouse lines. **Results:** The expressions of several RNAi pathway effectors and miRNAs were dynamic during tooth morphogenesis and epithelial cell differentiation. The conditional deletion of Dicer-1 in the dental epithelium in transgenic mice led to aberrations in the molar shape and cusp pattern as well as defects in the structure of enamel. Moreover, epithelial cell proliferation was increased in the continuously growing incisor and defects in ameloblast differentiation appeared in the pups and increased with the time. **Conclusion:** Our findings indicate that the miRNA pathway regulates several aspects of odontogenesis and plays important roles in epithelial morphogenesis and cell differentiation. The aberrations seen in molar cusp patterning in the Dicer1 mutant mice suggest roles for miRNAs in the evolution of the tooth crown.

P32

Distribution and structure of the initial dental enamel formed in incisors of young wild-type and Tabby mice

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Mouse incisor enamel can be divided into four layers: an inner prism-free layer; an inner enamel with prism decussation; outer enamel with parallel prisms; and a superficial prism-free layer. We wanted to study how this complex structural organization is established in the very first enamel formed in wild-type mice and also in Tabby mice where enamel coverage varies considerably. Unworn incisors from young female wild-type and Tabby mice were ground, etched, and analyzed using scanning electron microscopy. In both wild-type and Tabby mice, establishment of the enamel structural characteristics in the initially formed enamel proceeded as follows, going from the incisal tip in an apical direction: (i) a zone with prism-free enamel, (ii) a zone with occasional prisms most often inclined incisally, and (iii) a zone where prism decussation was gradually established in the inner enamel. The distribution of enamel in Tabby mice exhibited considerable variability. The sequence of initial enamel formation in mouse incisors mimics development from a primitive (prism-free) structure to an evolved structure. It is suggested that genes controlling enamel distribution are not associated with genes controlling enamel

structure. The control of ameloblast configuration, life span, organization in transverse rows, and movement is important for establishing the characteristic mature pattern of mouse incisor enamel.

P33

Fine structural and immunohistochemical observations of the collar enamel in Lepisosteus and Polypterus, actinopterygian fish

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Background: It is commonly accepted that ectodermal enamel covers the tooth crown in reptiles, amphibians and sarcopterygian fish, Latimeria and lungfish. A few actinopterygian fish, Lepisosteus and Polypterus, demonstrate collar enamel on the surface of the tooth shaft [1, 2, 3]. The structural features of collar enamel in Lepisosteus and Polypterus resemble those of the enamel in sarcopterygians and amphibians. The organic matrix of the collar enamel in Lepisosteus and Polypterus shows marked mammalian amelogenin immunoreactivity [2, 4]. Collar enamel in actinopterygians is thought to be a precursor of ectodermal enamel in higher vertebrates. However, the genes related to enamel protein that are present in amphibians, reptiles and mammals, are not found in fish. It is proposed that secretory calcium-binding phosphorprotein (SCPP) genes are involved in producing enamel-like tissues in teleost fish. A recent molecular genetic study reported that the strong expression of the odontogenic, ameloblast associate (ODAM) gene, which might be involved in the hypermineralization process at the late stage of enameloid maturation, was detected in the inner dental epithelial (IDE) cells of zebrafish [5]. In Lepisosteus and Polypterus, both enameloid and collar enamel are found in the same tooth. Therefore, they are suitable materials to study developmental relationships between enameloid and enamel. In this study, we focus on the morphological features of collar enamel, and on the chemical composition of collar enamel. **Methods:** Collar enamel and dental epithelial cells in Lepisosteus oculatus and Polypterus senegals were observed by light and transmission electron microscopy, and by light and electron microscopic immunohistochemistry using crude antiserum against porcine 25 kDa amelogenin, region-specific antibodies or antiserum against the C-terminus and central region of the porcine 25 kDa amelogenin, respectively [6]. **Results and conclusion:** The enamel layer, 600nm-5um thick, containing amorphous fine organic matrix was located between the dentin and IDE cells in the secretory stage. The layer also continues to the surface of enameloid near the dentin - enameloid junction, suggesting that the enamel covers both dentin and mature enameloid. It is assumed that the collar enamel in Lepisosteus and Polypterus is produced as a terminal product of the IDE cells at the late stage of enameloid maturation.

References:

[1]Prostak et al. (1989) In, Fearnhead R. W. (ed) Tooth Enamel V. 188-192, Florence.
[2]Ishiyama et al. (1999) Arch Histol Cytol, 62, 191-197.
[3]Sasagawa I. et al. (2008) Front Mater Sci China, 2, 134-142.
[4]Sasagawa I. et al. (2009) Front Mater Sci China, 3,174-182.
[5]Kawasaki K. (2009) Dev Genes Evol, 219. 147-157.
[6]Uchida et al. (1991) Histochemistry, 96, 129-138.

Acknowledgments: This study was supported in part by a Grant-in-Aid for Scientific Research, 16591844, from the Ministry of Education, Science, Sports and Culture, Japan, and by a Research Promotion Grant (NDUF-09-18, 08-19, 07-12) from Nippon Dental University.

P34

Identification of suppressor element in the amelogenin promoter.

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Background: Amelogenin expression is regulated at both the transcriptional and post-transcriptional levels. Previously, we demonstrated that the reuptake of amelogenin protein results in increased levels of amelogenin mRNA through

enhanced mRNA stabilization (1,3). Amelogenin expression is also regulated in a cell type-specific manner. Investigators have previously identified an enhancer element by using the 5' flanking sequence of the amelogenin promoter (4). However, the cell type-specific regulation of the amelogenin gene remains poorly understood. In some genes, the first intron regulates tissue-specific expression. We hypothesized that intron 1 is important for the cell type-specific regulation of amelogenin expression. **Results:** To understand the molecular mechanisms involved in the cell type-specific expression and developmental regulation of the amelogenin gene, we analyzed the effects of intron 1 on the amelogenin promoter activity in HAT-7 cells. We identified a suppressor element between -74 and -464 in amelogenin promoter. We also found enhancer activity in intron 1. Additionally, we found that the suppressor element in the promoter region suppresses intron 1 enhancer activity. The suppressor and the enhancers acted in an ameloblast-like cell line HAT-7, but not in HeLa cells. Mutation of the Oct-1 binding sites reversed the suppressor activity, suggesting that Oct-1 sites are essential for suppression. These results indicate that Oct-1 and intron 1 contribute to amelogenin expression. We performed immunostaining of Oct-1 and amelogenin in serial sections of PN d7 mouse incisors. The Oct-1 signal is observed in the inner enamel epithelium and is localized to the nuclei. The amelogenin signal is strongly observed in secretory ameloblasts concomitantly with the decrease in the Oct-1 signal (Figure 1). These findings suggest that Oct-1 as a cofactor might directly or indirectly collaborate in a cell type-specific manner with the intronic enhancer in the amelogenin gene (5).

Conclusion: We identified a suppressor element in the promoter region and enhancer elements in intron 1. The suppressor and enhancers acted in an ameloblast-like cell line, HAT-7, but not in HeLa cells, suggesting that these elements act in a cell type-specific manner.
References: 1. Xu L, Harada H, Tamaki T-Y, Matsumoto S, Tanaka J, Taniguchi A. (2006) J Biol Chem. 281: 2257-2262
2. Xu L, Harada H, Taniguchi A. (2006) J Biol Chem. 281: 32439-32444.
3. Xu L, Harada H, Taniguchi A. (2008) J Biochem. 144: 531-537.
4. Zhou YL, Snead ML. (2000) J Biol Chem. 275: 12273-12280.
5. Xu, L., Matsumoto, A., Sasaki, A., Harada, H., Taniguchi, A. (2010) J. Dent. Res., 89, 246-251.

P35

Loss of transglutaminase 2 in the mouse results in an accelerated enamel mineralization

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Tissue transglutaminase (TG2) is an ubiquitously expressed member of the large TG family exerting a number of physiological and/or pathological functions. The involvement of extracellular TG2 in cell adhesion, extracellular matrix assembly or bone ossification has been demonstrated previously. However, there are no earlier *in vivo / in vitro* studies to assess the role of TG2 in tooth development. Therefore we studied TG2 null mice to characterize its tooth phenotype. Mice were sacrificed at different developmental stages as follows: intaembryonic developmental stages (E 14, 15, 16), prior to tooth eruption (postnatal day 1), and adulthood (2 months postnatal). During early tooth development, when the tooth germs were going through bud, cap and bell morphogenetic stages, no obvious morphological alteration were observed due to the loss of TG2. The first significant differences in the hardness of the enamel, which showed accelerated enamel mineralisation, became apparent at postnatal stages. Furthermore, additional 3D analysis of incisors from the knockout mice revealed that they were slightly longer with a thicker enamel layer than their wild-type controls. Our findings reveal that TG2 may play a regulatory role in the enamel bio-mineralization processes.

P36

Pfeiffer syndrome and amelogenesis imperfecta

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Introduction: Pfeiffer syndrome is a rare autosomal dominantly inherited disorder that associates syndactyly on hands and feet, severe ocular proptosis, ankylosed elbows, abnormal viscera, and slow development. Hydrocephaly may be found occasionally, Based on the severity of the phenotype, Pfeiffer syndrome is divided into three clinical subtypes. Type 1 "classic" Pfeiffer syndrome involves, individuals with mild manifestations including brachycephaly, midface hypoplasia and finger and toe abnormalities; it is associated with normal intelligence and generally good outcome. Type 2 consists of cloverleaf skull, extreme proptosis, finger and toe abnormalities, elbow ankylosis or synostosis, developmental delay and neurological complications. Type 3 is similar to type 2 but without a cloverleaf skull. Clinical overlap between the three types may occur. Pfeiffer syndrome affects about 1 in 100,000 individuals. The disorder can be caused by mutations in the fibroblast growth factor receptor genes *FGFR-1* or *FGFR-2*. Craniofacial manifestations of Pfeiffer syndrome can include craniofacial asymmetry, maxillary hypoplasia, high arched palate and malocclusions. However, to our knowledge no report concerning Amelogenesis Imperfecta and Pfeiffer syndrome have been previously reported. **Objective:** The aim of this report is to characterize the dental phenotype of a consanguineous family with diagnostic hypothesis of Pfeiffer Syndrome and Hypocalcified Amelogenesis Imperfecta diagnosed by the Department of Medical Genetics and the Oral Care Center for Inherited Diseases at the University Hospital of Brasilia. **Subjects and methods:** Complete physical, oral and radiographic examinations were performed on all family members. **Results:** A family with two affected siblings was examined in this study. The parents reported to be first-degree cousins and to have three sons. The two older sons (10 and 9 years respectively) had similar systemic and oral features. Both presented ocular proptosis, developmental delay, craniosynostosis, craniofacial asymmetry, maxillary hipoplasia, high-arched palate, malocclusion and hearing impairment. Besides these typical features, they also presented visual impairment, high values of PTH, parestesia and seizures episodes. Permanent teeth appeared with a yellow discoloration, loss of interdental contact suggesting complete absence of enamel and dentin sensitivity. Gingivitis and severe dental decay was also observed. Radiographic analysis showed that some teeth exhibited periapical lesions and root formation delay. **Conclusion:** The examination of this family suggests a recessive form of Amelogenesis Imperfecta associated to Pfeiffer syndrome. Further molecular analysis is necessary to confirm Pfeiffer syndrome diagnosis and better understand the association with amelogenesis imperfecta.

P37

A frameshift mutation in DSPP associated with dentinogenesis Imperfecta type II

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Background: Dentinogenesis imperfecta (DI) types I, II and III, is a congenital rare disease which affects the organic dentine matrix. Its etiology has been associated with defects in the dentin sialophosphoprotein (DSPP) gene (chromosome 4) (Kim et al, 2007, Barron et al, 2008). The aim of the present work was to study the phenotype and the genotype of three generations of a family affected by DI type II, showing a dominant transmission pattern. **Methods:** The genomic DNA was isolated from peripheral lymphocytes or buccal swabs. The PCR product had to be inserted into pJET plasmid (Fermentas, Burlington, Canada) and cloned into Stbl2 cells (Invitrogen, Carlsbad, USA) to detect the mutation. The results were compared to the previously published human DSPP genomic sequence. **Results:** Amber colour and total obliteration of the pulps and root canals in both dentitions were observed. Attrition was slight. Shortened and abnormally thin roots were observed in some permanent teeth. Periapical infections and dental fractures had caused several dental extractions. A heterozygous frameshift mutation (c.2349delT) causing a deletion in the region coding for DPP was identified. **Conclusion:** We concluded that the identified mutation was the etiological cause of DI type II, suggesting that its location could be reflected in

the phenotypic features as a severity gradient from dentine anomalies.

P38

Fragile fracture of dental pulp chamber: a new method to obtain dental pulp stem cells

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Objective: The traditional technique to obtain Dental Pulp Stem Cells DPSC, with rotary tools for opening pulp chamber has been compared with a new fragile tooth fracture technique in order to improve dental pulp collection. **Methods:** We have obtained a brittle fracture by an unstable propagation of a concentrated stress by the application of a calibrated increasing load on the teeth surface. Moreover we tested different notch techniques adapted to the different types of tooth morphology. The improvement in the cell collection has been tested by: 1) Identification of human dental stem cells using appropriate cell markers (STRO-1, CD34, CD44, CD106, CD146 and c-kit) assayed by cytofluorimetric analyses. 2) Assessment of the consequences of the pulp Collection timing, by the measurement of differences between DPSCs obtained immediately after tooth extraction and DPSCs from teeth cryopreserved following several protocols reported in literature. 3) Assessment of the effects on the vitality and the DPSC differentiation level due to different pulp extraction methods. **Results:** We obtained a significant increase in cell vitality with our fragile fracture method when compared with the previously described technique. **Conclusion:** The lessening of the thermal stress on the DPSC because of the reduction of temperature when opening the pulp chamber for stem cells extraction is probably the main cause of the improvement the dental pulp collection as well as the lowering of the differentiation induction. This study was supported by a Spain-Italy Integrated Research Action Grant HI2007-0089.

P39

The vascularization of embryonic mouse molar papilla evaluated by CD34, CD31 antigen expression and morphological arrangement

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Background: Mouse teeth develop as a result of sequential and reciprocal interactions between oral epithelium and cranial neural crest-derived mesenchyme. The vasculature is very important for embryonic organ development, for it is in charge of maintaining metabolic homeostasis by supplying oxygen and nutrients as well as excreting waste products. Although the fine structures and three-dimensional organization of the capillaries in the enamel organ, the dental epithelial structure, have been previously investigated, the process of vascularization of the tooth mesenchyme remains poorly understood. **Methods:** In the hope of better understanding the mechanism of formation of tooth mesenchyme capillaries, mouse mandibular molars during embryonic (E) 13.5 to E16.5 were harvested and processed for CD34, CD31 immunohitochemistry and conventional transmission electron microscopy (TEM). **Results:** At E13.5, molar tooth germ comprises condensed mesenchyme and a typical epithelial bud. At this stage, CD34- and CD31- positive cells are distributed in the prospective dental follicle around the tooth epithelium and condensed mesenchyme; By E14.5, the bud epithelium progressively takes the form of the cap configuration and develops into the internal and the external enamel epithelium, while the mesenchyme develops into the dental papilla and follicle. At this stage, in addition to the localization of CD34- and CD31- positive cells in the follicle, a few positive cells appeared within the follicle at the bottom of the tooth germ sprouting toward the dental papilla underlying the cap epithelium; From E15.5 to E16.5, molar tooth germ develops into early bell stage. And during this period, the CD34- and CD31- positive cells have entered the dental papilla, some of which are even progressively close to the prospective odontoblasts. Negative staining was found in the dental epithelium during the stages investigated. For TEM, abundant ribosomes, mitochondria, rough endoplasmic reticulum (RER), Golgi complexes, pinocytic vesicles, gly-

cogen granules are present in the cytoplasm of the endothelial cells. The basal lamina of most capillaries was discontinuous or absent. The presence of cells containing vacuoles suggests vasculogenesis is taking place; Loss of basal lamina, presence of filopodia and lateral sprouting suggests angiogenesis is also occurring. **Conclusion:** Neoformation of capillaries of molar mesenchymal papilla seems to occur simultaneously by mechanisms of vasculogenesis and angiogenesis. This research was supported by the National Natural Science Foundation of China (Grant No. 30572043).

P40

Non-invasive imaging of human dental pulp stem cells

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Stem cells, with the capacity to differentiate into different types of cells and finally tissue, raise the hope of cell-based regenerative medicine for many diseases such as ischemic heart disease, neural disease, cancer, and many other disorders. Especially, adult mesenchymal stem cells, which have been described to be present in almost every organ including teeth, are gaining more interest because of their ease of isolation and elaborate differentiation potential and plasticity. As determining the fate of stem cells or their lineage committed progeny transplanted in vivo will be of utmost importance, stem cell labeling and non-invasive imaging will play a crucial role in determining the possible therapeutic potential of these stem cells. Magnetic resonance imaging (MRI) is one of the most powerful diagnostic imaging techniques currently available for in vivo diagnosis. Unfortunately, the excellent spatial resolution and detailed structural information obtained with this technique has to be paid with a decrease in sensitivity. Furthermore, diffusion of contrast agent out of labeled cells can provide non-specific signals. In contrast, optical imaging making use of bioluminescence offers a methodology which is highly sensitive and specific but lacks any structural 3D information. Therefore, a combination of both MRI and bioluminescence imaging will give detailed information on the in vivo location/migration of stem cells thereby providing both a high resolution and high sensitivity. As little is known about the applicability of both MRI and optical bioluminescence imaging on human dental pulp stem cells, this study will investigate the efficacy by which these postnatal stem cells can be labeled and imaged making use of both techniques. For MRI, cells will be labeled with a commercially available MRI contrast agent named Endorem® which in fact are iron oxide based nanoparticles. Cells will be analysed using transmission electron microscopy (TEM), high resolution MRI and inductively coupled plasma spectrometry in order to quantify the amount of endocytosed iron. For bioluminescence imaging, cells will be transduced with a lenti-viral vector containing both GFP and Luciferase. Cells will be analysed using an IVIS optical imaging system. For both techniques, the optimal labeling strategies will be discussed. The effect of both labeling techniques on cell viability will be assessed by an MTT assay.

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Dental pulp progenitor / stem cells - dentin interactions in vivo

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Introduction: Dentinogenesis, represents a complex sequence of biological processes, including the proliferation, migration and differentiation of pulpal progenitor cells into a new generation of dentin-forming cells. To date, numerous experimental approaches have demonstrated that isolated Dental Pulp Stem Cells (DPSCs) can be directed to differentiate into cells of the odontoblastic lineage forming dentin-like structures and associated pulp tissue in transplants. **Objectives:** The purpose of the present study was to approach interactions between dentin matrix and dental pulp progenitor / stem cells seeded on organic or synthetic scaffolds and implanted as hybrid root implants in the jaw bone of mini pigs. **Methods:** All animal and stem cell experiments were approved by the National Institute of Animal Care and the Ethic Committee of Aristotle University of Thessaloniki. Immature permanent incisor teeth and unerupted premolars at the

early root forming stage of three, 6-8 month-old miniature pigs were extracted and a number of teeth were evaluated histologically. The expression pattern of the extracellular matrix protein tenascin and the cell surface proteoglycan syndecan was evaluated using immunohistochemistry. Mesenchymal Stem / Progenitor Cells were isolated from Dental Pulp. Cells were cryopreserved at -196^{0}C. The expression of cell surface antigen markers for mesenchymal stem cells STRO-1, CD90, CD105, and CD146 was examined using flow cytometric analysis before and after the cryopreservation procedure. A year later, autologous cryopreserved DPSCs were implanted into the jaw bone of the adult mini pigs using organic (collagen) and synthetic (PLGA) scaffolds in a new hybrid root implant model. In detail, pieces of root canals containing scaffolds seeded with the autologous DPSCs were implanted into the fresh extraction socket of the mini pigs. The resulting constructs were harvested after 2, 6 and 12 weeks and evaluated by x-ray, histological and mmunohistochemical analyses. **Results:** 9 - 15 x 10^6 cells were obtained from each tooth. Porcine pulp Stem Cells were positive for CD90 (strong expression), CD105 and CD146 (slight expression) and negative for STRO-1. Similar results were obtained before and after the cryopreservation procedure. Immunohistochemical analysis showed the specific distribution pattern of these cells both in pulp and apical papilla tissues. Upon histological examination of the root implants, new extracellular matrix had been deposited in a polar predentin-like pattern on the canal dentinal walls by cuboidal non-polarized cells. **Conclusions:** The interactions of dental pulp progenitor cells with the dentin matrix may provide evidence to investigate stem cell-mediated dentin regeneration. Since the unique odontoblast phenotypic expression is defined by specific epithelio-mesenchymal interactions that are difficult to replicate during experimental conditions, dentin- or pulp- like tissue formation resulting from stem cell-mediated tissue engineering approaches should be further characterized for its specific nature.

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Influence of TGF-ß1 and culturing media on ALP expression in human pulp fibroblasts with different root development *in vitro*

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Background: TGF-ß1 is one of the most important growth factors in human pulp fibroblast differentiation. The influence of TGF-ß1 is modulated by its concentration. Furthermore the regenerative potential of human pulp fibroblasts depends on their origin. The aim of the present *in vitro* study was to compare the expression of ALP (alkaline phosphatase) in cultures of human pulp fibroblasts with different root development under the influence of various TGF-ß1 concentrations and culturing media. **Methods:** Human pulp fibroblasts from third molars were distributed into 2 groups according to their root development and cultured in D-MEM with 25 mM Hepes, 10% FCS and 50 µg/ml Gentamycin. After the 3rd passage, cells were seeded to 25.000 in 24 well plates. A third of each culture was treated with D-MEM + 10% FCS, D-MEM + 0.1% FCS and D-MEM + ITS (Insulin-Selenit-Transferrin), respectivly. Additionally the cultures were treated with 0.5; 1; 5 and 10 ng/ml TGF-ß1. ALP expression was estimated by the 4-NPP method over a period of 32 days. **Results:** Irrespective of the root development, a specific mode of ALP expression was observed according to the different media applied. However from day 11 to day 32 the ALP expression in pulp fibroblast cultures from teeth with incomplete root development was significantly higher than in the other group. The onset in ALP expression was significantly different in both groups as well. In contrast TGF-ß1 had no significant influence on the ALP expression in each group, but the peak of ALP expression was recorded at a concentration of 0.5 ng/ml. Higher and lower concentrations of TGF-ß1 diminished the ALP expression. **Conclusions:** The donor specific origin of the cells is more important for their regenerative potential and characteristics in culture than the addition of growth factors like TGF-ß1. However, a certain concentration of TGF-ß1 (0.5 ng/ml in this study) improved the mineralizing properties of human pulp fibroblasts *in vitro* as measured via ALP expression.

P43

Implantation of odontoblast progenitors in the rat molar pulp leads to the formation of reparative osteodentin

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The presence of mesenchymal-like stem cells has been demonstrated in the human dental pulp. Stem cell based therapies may be promising treatments for tooth injury since ectopic implantation of these cells have been shown to induce the formation of a dentin-like mineralized tissue. Yet their capacity to readily contribute to the formation of dentin, in a tooth lesion has never been directly explored. The aim of our study was to evaluate the feasibility of a cellular therapy for pulp injury. This was achieved by implanting pulpal progenitor cells into an injured pulp of a rat first molar and analyzing the histology of the implanted tooth 7, 15 and 28 days after treatment,. We used clonal dental pulp progenitors derived from the first molar of ED 18 mouse embryo (A4 cell line). These cells can be recruited alternatively towards the odonto/ osteoblast, chondrogenic or adipogenic differentiation programs depending on the type of induction and thus, behave as multipotent progenitors *in vitro*. Rats were randomized in two experimental groups: 1) controls, in whom only the tooth cavity was performed 2) treated rats, in whom 10^5 odontoblast progenitor cells were implanted in the cavity. Our data show that implantation of these progenitor cells lead to lesion repair through the formation of an osteodentin bridge. Inflammation can be evidenced during the first week. At day 15, the formation of a dentinal barrier starts to be noticed. At day 28, the bridge completely fills the implantation site. The treated teeth keep their vitality since infection, necrosis or pulp retraction is not observed. To determine whether the grafted cells were directly responsible for the neodentin formation or whether they provided signals recruiting local progenitors, GFP-transfected A4 cells were implanted and followed after 3 and 7 days. GFP positive cells could not be revealed neither by fluorescence nor by immunohistochemistry using a GFP specific antibody. These results could be explained by a destruction of the implanted cells due to an immune reaction or the inflammatory process present in the first week post-implantation. Dental pulp stem cells, as other mesenchymal stem cells, have been described as immunotolerant. Rat/mouse and human/mouse xenotransplantations are documented in bone and muscle but nothing is known on the behaviour of pulpal progenitors in the tooth microenvironment. Experiments in immunodepressive and/or anti-inflammatory conditions are in progress to understand the cellular mechanisms underlying the formation of a reparative dentin after stem cell implantation. Altogether, our study paves the way for the future development of stem cell based therapies of tooth injuries.

P44

Expression pattern of Apin and Amelotin during formation and regeneration of the junctional epithelium

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Background: The junctional epithelium (JE) is the component of the dentogingival junction that adheres to the tooth surface, and seals off periodontal tissues from the oral environment. This unique, incompletely differentiated epithelium is formed by the fusion of the reduced enamel organ (REO) with the oral epithelium. Attachment of the gingiva to the enamel surface is provided by a structural complex called the epithelial attachment. This complex consists of an inner basal lamina (BL) formed and maintained by the superficial JE cells to which they are attached by hemidesmosomes. This BL is considered atypical because it contains laminin-5 but not other typical components, such as γ1 chain-containing laminins, and type IV and VII collagens. However, the exact mechanisms by which the JE maintains its adhesive relationship with the tooth surface through the BL and establishes its unique incompletely differentiated cellular status are still not known. Efforts to identify the secretome of the epithelial cells responsible for creating tooth enamel, have led to the identification of genes encoding for two novel proteins called Apin (APIN) and amelotin (AMTN). Unexpectedly, they are also strongly expressed in the JE. The objective of this study was therefore to investigate the presence and distribution of APIN and AMTN at various stages of tooth eruption and in regenerating JE following gingivectomy. **Methods:** Immunohistochemistry was carried out on erupting rat molars, and in samples of regenerating JE following gingival wound healing. Cell proliferation activity involved in these two processes was also examined by immunolabeling for the Ki67 marker. **Results:** During tooth eruption, both APIN and AMTN were immunodetected at the REO-tooth

interface. APIN was also distinctively expressed by cell clusters present between the REO and oral epithelium. In established JE, APIN and AMTN were likewise found at the cell-tooth interface, where the inner BL is found, but APIN was additionally observed among JE cells. During early JE regeneration, only APIN was detected in association with cells at the leading wound edge. At the later phase, both APIN and AMTN were present at the interface with the enamel surface, but only APIN was observed among cells of the reforming JE. Cells associated with JE formation and regeneration exhibited higher cell division activity than adjacent epithelial cells. **Conclusion:** The dual localization of APIN is consistent with the idea that it may be implicated in both cellular activities and in the molecular mechanisms that allow the JE to adhere to tooth surface. It may influence the apical extension of the gingival wound edge to the reestablishment of a functional JE and may play a role in modulating the cell differentiation status of the JE. Instead, the late appearance of AMTN when the reforming JE reestablishes contact with the tooth, and its conspicuous localization in the area of the inner BL suggest that the role of AMTN in the JE is restricted to events taking place at the epithelium-tooth interface. As components of the inner BL of the JE, both APIN and AMTN may contribute, directly or indirectly by interacting with other components of the BL, to the attachment mechanism of the JE. Supported by the Department of Foreign Affairs and International Trade, Government of Canada, and by the Canadian Institutes of Health Research.

P45

Cellular events in tooth root morphogenesis

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Objectives: Most of studies in tooth development have been restricted in and focused on the early development of crown formation. However, developmental mechanisms and cellular events were not examined properly in tooth root formation, known as a following event after the completion of the crown formation. In this study, morphogenesis and cellular events such as cell proliferation, apoptosis and cytoskeletal formation have been examined using mice lower molar development. **Methods:** Localization patterns of pan-cytokeratins, as an epithelial marker and Ki-67, cell proliferation marker showed the distinguished boundary of a root and a bifurcation forming regions of developing molar tooth. In addition, treatments of pharmacological inhibitors including cytochalasin D and nocodazole, inhibitors of actin filaments and microtubules respectively, while in vitro tooth root culture. **Results:** In tooth root development at PN3, PN5 and PN8, showed the specific localization patterns of cellular events markers with the specific morphological changes. In vitro tooth root culture could confirm that tooth root formation would be modulated by the fundamental mechanisms of cellular events regulated by signalling molecules. **Conclusion:** These dynamic morphological alteration patterns of tooth root formation suggest that region specific cellular events, regulated by signalling molecules, would determine the multiple rooted tooth formation.

P46

Genome-wide screening of key molecules for tooth root development

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Background: The tooth root is one of the most critical parts that support the force of tribosphenic functions, however, even molecular mechanisms of the root development remains unknown. Although various studies on tooth regeneration have been carried out, the tooth root structure remains to be difficult to regenerate. We tried to identify specific molecules involved in root morphogenesis to present basic informations as a first step to approach to regenerate tooth root structure. **Methods:** Laser capture micro-dissection was employed to isolate cementoblasts and PDL cells from undecalcified frozen sections of murine mandible, and primary culture of neu-

ral crest cells(NCC) and bone marrow stroma cell (BMSC) were performed to collect odontogenic mesenchyme. The expression level of mRNA was compared between these tissues by genechip. We compared each sets of transcripts and isolated cementum(+)/PDL(-)/NCC(+)/BMSC(+) genes as candidate molecules involved in root development. Then, we examined the expression of these candidates by real-time PCR and *in situ* hybridization. **Results:** A comparative analysis of mRNA expression by genechip showed that about 67 genes were differentially expressed between cementoblasts and PDL cells. By the further analysis, the cementum(+)/PDL(-)/NCC(+)/BMSC(+) genes were remained as 19 candidates. In the molar roots, Chd3 was specifically expressed in the Hertwig's epithelial root sheath (HERS) which is recently thought to differentiate into cementoblasts. In the incisor roots, Chd3 expression was identified until adult stage, suggesting that Chd3 play unknown function in developing roots. Next, we examined the expression of Chd3 at embryonic stage, the signal was very weak as background level. These expression pattern suggests that Chd3 is particular important for root formation process. We hypothesized that this molecule may also play a important role in transcriptional regulation during the process of root formation. It is reported that chd3 form the nucleosome remodeling deacetylation (NuRD) corepressor complex that mediates posttranslational modifications of histones and nonhistone proteins resulting in transcriptional repression in drosophila. We think that in mouse NuRD complex would repress the proliferation and differentiation of HERS during root formation. Further study will focus on the biological function of Chd3 in HERS cells. In preliminary experiment, we identified that Chd3 express in the HERS derived cells. We are studying gene function by using knockdown system with siRNA to examine molecular functions in vitro using HERS derived cells. **Conclusion:** We isolated candidates involved in tooth root morphogenesis by genechip. Although further functional analysis is required, chd3 might contribute to the apical extension of tooth root and might be a specific factor for the initiation of cementogenesis.

P47

Establishment of immortalized Hertwig's epithelial root sheath cells and character of cell line

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Tooth root formation starts after the completion of crown morphogenesis. The transition from crown to root is seen as the formation of Hertwig's epithelial root sheath (HERS), consisting of two epithelial layers. HERS development plays a key role of inducing odontogenesis in the root formation. We developed the original organ culture system to observe postnatal tooth development, and have studied the relationship between HERS development and the regulations by growth factors. From the studies, we reported that epidermal growth factor (EGF) signaling regulated transition from crown to root in mouse molars (Fujiwara, et al.,2009), and insulin-like growth factor (IGF)-I accelerated HERS elongation during early root development (Fujiwara, et al., 2005). However, it is difficult to analyze the characteristics of HERS cells by the culture method. Here, to discover the problem, we planed the establishment of HERS cell line and examined the characteristics of HERS cells. HERS cells were isolated from mandibular first molar of a PN 6d mouse and immortalized naturally under serum-free culture condition, and a cell line, HERS01a is cloned by a cloning cylinder. HERS01a proliferated actively in the presence of EGF, IGF-I, hepatocyte growth factor (HGF). Next, we examined the gene and protein expression of HERS01a, and compared with that of HERS in vivo. Real-time PCR showed that HERS01a expressed growth factor receptors of EGF, IGF-I, and HGF. Interestingly, HERS01a expressed mesenchymal markers, vimentin and N-cadherin, as well as cytokeratin 14, E-cadherin and p63 of epithelial stem cell markers. Immunohistochemical staining also showed that HERS01a expressed both of cytokeratin 14 and vimentin. We speculate that HERS01a cells have unique feature associated with epithelial-mesenchymal transition. Accordingly, these data suggested that HERS01a reflected the characteristic of intact HERS cells *in vivo*, and is useful for analyzing the biological characteristic of HERS and for elucidating the mechanisms of root development and the molecular mechanisms of EMT. **References:**

1. Fujiwara, N., Tabata, M.J., Endoh, M., Ishizeki, K., and Nawa, T. (2005). Cell Tissue Research *320*, 69-75.
2. Fujiwara, N., Akimoto, T., Otsu, K., Kagiya, T., Ishizeki, K., and Harada, H. (2009). J Exp Zoolog B Mol Dev Evol *312B*, 4886-4494.

P48

Biological effects of cyclic diarylheptanoids on tooth root formation

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The mastication is one of the essential factors for maintenance of better quality of life. Healthy tooth roots and periodontal tissues are important for support the applied force and to keep the function of tooth. As tooth roots are fundamental structures for tooth development, it is important to find compounds that specially target functional tooth root formation to be used as ideal candidates for subsidiary agents for clinical applications. Therefore, we study about the biological effects of natural products on tooth root formation *in vitro* and *in vivo*. Bone diseases such as osteoporosis and periodontitis result from an imbalance in bone remodeling caused by excessive bone resorption relative to bone formation. There are two potential categories of pharmacological treatments for bone diseases: Anti-resorptive agents that inhibit osteoclast differentiation and bone resorption, and anabolic agents that stimulate osteoblast differentiation and bone formation. In the screening for anabolic agents from natural compounds, we found acerogenin, cyclic diarylheptanoids isolated from stem barks of *Acer nikoense*, have the biological activities to induce the differentiation of mouse MC3T3-E1 osteoblast cells into mature osteoblasts by evaluating alkaline phosphatase (ALP) activity and mineralization activities by the Alizalin Red S staining. In this study, we applied these compounds to developing tooth buds of post-natal 7 days pups and performed the kidney capsule grafting[1)]. We found that these compounds stimulated tooth root formation by micro CT analysis. Then, we performed HE staining and immuno-histochemistry of anti-keratin antibody for the histological analysis of the samples, suggesting that acerogenin maintained HERS cell population. Next, we examined growth stimulation activities of acerogenin on HERS derived cell line by MTT assay and observed unique activities. In addition, acerogenin stimulated ALP activities and mineralization activities of periodontal ligament primary cells derived from Sostdc1-/- mutants comparing to wild type, suggesting functional relationship between acerogenin and BMP signaling pathway.
Acknowlegement: This study was supported by a Grant for Supporting Project for Strategic Research of Nihon Univ. Sch. Dentstry at Matsudo by the Ministry of Education, Culture, Sports, Science and Technology, 2008-2012 (Team: Dental morphogenesis; to MO) and partially funded by a grant for Biodefense Programs of the Ministry of Education, Science and Technology of the Republic of Korea.
References: 1) J. Oral Tissue Engin 2007; 4(3):137-142

P49

Essential roles of *Osx/Sp7* in the root dentin formation

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Background: Dentin, a major component of tooth, is formed by odontoblasts. Although dentin is physiologically different from bone, its matrix proteins show biochemical similarities with those of bone. Recently we found that an essential transcription factor for osteoblast differentiation, *Osterix/Sp7(Osx)* is also temporospatially expressed in the differentiating odontoblasts during tooth development. The aim of present study is to understand the roles of *Osx* in the odontoblast differentiation and dentin formation. **Methods:** We generated and analyzed the mice with odontoblast-specific inactivation of *Osx*. *Osx* was inactivated in the odontoblasts by the transgenic *Cre* mice lines (*Col1a1-Cre* and *OC-Cre*) with the activity of *Cre* recombinase under the control of each matrix protein promoter. **Results:** Tooth phenotypes of two independent *Osx* conditional knockout mice were very similar in each other. Incisors as well as mandibles of both mutant lines were short in their length. In both mutant lines, any remarkable pheno-

type was not found in the crown. However, roots of mutant mice were shorter than those of wild type littermates. In histological analysis, inter-radicular dentin of mutant molar was severely affected in contrast to the coronal dentin. **Conclusions:** These results indicated that transcriptional regulation of *Osx* is necessary for the radicular dentin formation. Therefore, it is strongly suggested that *Osx* may play as an essential regulator in the differentiation of odontoblasts and dentin formation, particularly in root formation. This work was supported by the National Research Foundation of Korea(NRF) grant funded by the Korea government(MEST) (R01-2007-000-20005-0)

P50

Formation of the maxillary alveolar bone in humans

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There is a mutual relation between teeth and bone, not only in postnatal life, but also during stages of initial formation of teeth and alveolar bone. It is unknown how the interdental ridges and the bony crypts form, in which the dental primordia develop. The crypts may form due to bone resorption underneath the expanding dental primordia, and the interdental bony ridges may be remnants of adjacent bone resorption. On the other hand, the ridges may be active outgrows of the maxillary bone. It is known that bone as a tissue is being formed in interaction with its surrounding structures; however, the morphology of the peridental bony structures during the stages of dental morphogenesis, is not known. Therefore, the development of the human maxillary bone together with the developing tooth primordia was examined for the prenatal stages of 19 to 270 mm CRL, with special reference to the regions of bone resorption and apposition. 3D reconstructions from serial sections showing regions of bone remodelling revealed that the formation of the dental crypts, the interdental and the interradicular bone is a result of a mixture of resorptive and appositional processes. These results serve as a basis for further research focused on the mechanical and molecular control mechanisms leading to formation of form. Supported by grant Ra 428/1-9 Deutsche Forschungsgemeinschaft

P51

Formation of the mandibular alveolar bone in mice

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There is a mutual relation between teeth and bone, not only in postnatal life, but also during stages of initial formation of teeth and alveolar bone. It is unknown how the interdental ridges and the bony crypts form, in which the dental primordia develop. The crypts may form due to bone resorption underneath the expanding dental primordia, and the interdental bony ridges may be remnants of adjacent bone resorption. On the other hand, the ridges may be active outgrows of the mandibular bone. It is known that bone as a tissue is being formed in interaction with its surrounding structures; however, the morphology of the peridental bony structures during the stages of dental morphogenesis, is not known. Therefore, the development of the murine mandibular bone together with the developing molar tooth primordia was examined for the prenatal stages of E13 to postnatal stages P20, with special reference to the regions of bone resorption and apposition. 3D reconstructions from serial sections showing regions of bone remodelling revealed that the formation of the dental crypts, the interdental and the interradicular bone is a result of a mixture of resorptive and appositional processes. These results serve as a basis for further research focused on the mechanical and molecular control mechanisms leading to formation of form. Supported by grant Ra 428/1-9 Deutsche Forschungsgemeinschaft
Deutsche Gesellschaft für Zahn-, Mund- und Kieferheilkunde (DGZMK)
Czech Academy of Sciences GACR 524/08/J032

P52

Formation of the maxillary alveolar bone in mice

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There is a mutual relation between teeth and bone, not only in postnatal life, but also during stages of initial formation of teeth and alveolar bone. It is unknown how the interdental ridges and the bony crypts form, in which the dental primordia develop. The crypts may form due to bone resorption underneath the expanding dental primordia, and the interdental bony ridges may be remnants of adjacent bone resorption. On the other hand, the ridges may be active outgrows of the maxillary bone. It is known that bone as a tissue is being formed in interaction with its surrounding structures; however, the morphology of the peridental bony structures during the stages of dental morphogenesis, is not known. Therefore, the development of the murine maxillary bone together with the developing molar tooth primordia was examined for the prenatal stages of E13 to postnatal stages P20, with special reference to the regions of bone resorption and apposition. 3D reconstructions from serial sections showing regions of bone remodelling revealed that the formation of the dental crypts, the interdental and the interradicular bone is a result of a mixture of resorptive and appositional processes. These results serve as a basis for further research focused on the mechanical and molecular control mechanisms leading to formation of form. Supported by grant Ra 428/1-9 Deutsche Forschungsgemeinschaft
Deutsche Gesellschaft für Zahn,
Mund- und Kieferheilkunde (DGZMK)
Czech Academy of Sciences GACR 524/08/J032

P53

Formation of the mandibular alveolar bone in humans

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There is a mutual relation between teeth and bone, not only in postnatal life, but also during stages of initial formation of teeth and alveolar bone. It is unknown how the interdental ridges and the bony crypts form, in which the dental primordia develop. The crypts may form due to bone resorption underneath the expanding dental primordia, and the interdental bony ridges may be remnants of adjacent bone resorption. On the other hand, the ridges may be active outgrows of the maxillary bone. It is known that bone as a tissue is being formed in interaction with its surrounding structures; however, the morphology of the peridental bony structures during the stages of dental morphogenesis, is not known. Therefore, the development of the human maxillary bone together with the developing tooth primordia was examined for the prenatal stages of 19 to 270 mm CRL, with special reference to the regions of bone resorption and apposition. 3D reconstructions from serial sections showing regions of bone remodelling revealed that the formation of the dental crypts, the interdental and the interradicular bone is a result of a mixture of resorptive and appositional processes. These results serve as a basis for further research focused on the mechanical and molecular control mechanisms leading to formation of form. Supported by grant Ra 428/1-9 Deutsche Forschungsgemeinschaft

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A new experimental model for monitoring bone neogenesis and osseointegration of implants in rats (OSSI model)

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Background: The last decade promoted a tremendous development in bone and tooth reconstruction techniques including the increasing application of dental implants. During osseointegration of the implant, the quality of the newly formed and the timeframe of its formation is crucial. Therefore, it is important to develop new, well reproducible, low-cost experimental models to study bone regeneration and osseointegration of implant. The small size of rat maxillary and mandibular alveolar bones does not permit reproducible bone regeneration studies. But our structural analyses revealed that the rat tail bones are very similar to maxillary and mandibular bones but much more roboust. **Methods:** Based on these observations, we decided to model dental implantation in the rat

tail and developed a new model to study osseointegration. This novel method involves (1) implantation of titanium screw into the tail vertebrae, (2) followup of the integration process, and (3) quantitative biophysical measurements which mirror consolidation of implant, i.e. strength of fixation and changes in newly formed bone architecture using micro Computer Tomograph (mCT). The maximum force needed to extract the titanium implant is measured using a Tenzi TE 18.1 (TENZI Ltd. Hungary) apparatus and expressed in Newton (N). **Results:** We observed that the one millimeter diameter titanium mini-implants integrated into the rat tail bone. The strength of osseointegration increased gradually by time as measured by the force needed to extract the implant starting at week 3. It reached a plato value after 36 weeks of implantation. mCT investigations confirmed these functional observations showing a gradual increase of bone density during the investigated period. When Zometa, an amino-bisphosphonate - a test material known to inhibit osteoclast, and stimulate osteoblast activity in healthy rats - was applied for 6 weeks following implantation, it significantly increased the force needed to remove the implant. Also, when the amino-bisphosphonate was applied, there was a three-fold increase in new bone volume around the implant by week 6, as observed by mCT. **Conclusions:** We have developed a new, highly reproducible experimental model for studying bone regeneration and osseointegration of titanium implants in rats. This promising method is suitable to test various implant materials and surfaces, and also the effect of bioactive materials, scaffolds and even stem cells to investigate their action on the osseointegration process. Supported by the Hungarian National Scientific Research Fund (CK 80928).

P55

Osteoblastic differentiation of transgenic mice overexpressing Apert syndrome-type mutant FGFR2 and its soluble form

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Background: Apert syndrome is an autosomal dominant disease characterized by craniosynostosis and syndactyly. This syndrome is caused by one of two mutations (S252W or P253R) of fibroblast growth factor receptor 2, that leads to gain-of-function of FGF signal. Previous study reported that FGFR2IIIc-S252W induced the accelerated osteoblastic differentiation, and its soluble form (FGFR2IIIc-SOL), which lacks transmembrane and tyrosine kinase domains, inhibited the differentiation[1)]. Since it was the study using osteosarcoma cell line, particular interest is in the function of FGFR2IIIc-S252W and FGFR2IIIc-SOL on normal osteoblastic differentiation. To ask this question, we generated two lines of transgenic mice, S252W-Tg and SOL-Tg, which overexpress FGFR2IIIc-S252W and FGFR2IIIc-SOL, respectively. **Methods:** Primary calvarial osteoblasts were isolated from postnatal 2-day old mice of wild-type (WT), S252W-Tg, SOL-Tg, and mice crossed with S252W-Tg and SOL-Tg (S252WxSOL-Tg). After culturing cells in the differentiation inducing medium (50 mg/ml ascorbic acid, 10 nM dexamethasone and 10 mM b-glycerophosphate) for 3 weeks, osteoblastic differentiation was examined by the alkaline phosphatase (ALP) activity, alizarin red staining, and expression of osteoblast-related genes. Next, phosphorylation of signaling molecules of FGF, such as MEK, ERK, PLC-γ, and P38 by Western blot analysis. Finally, osteoblasts were implanted subcutaneously into immunodeficient mice to examine the ectopic bone formation. Eight week after implantation, histological analysis was carried out. **Results:**

1) Osteoblasts from S252W-Tg showed higher level of ALP activity, alizarin red staining, and expression of Runx2 and osteocalcin, than those from WT and SOL-Tg mice. Interestingly, all these levels were lower in osteoblasts isolated from S252Wx-SOL-Tg than those from S252W-Tg mice.
2) Osteoblasts from S252W-Tg showed higher phosphorylation of MEK, ERK, PLC-γ, and P38, whereas they were all suppressed in osteoblasts from S252W×SOL-Tg.
3) Osteoblasts from S252W-Tg formed much larger amount of bone-like tissue than those from WT mice, and osteoblats from S252W x SOL-Tg formed less amount of bone-like tissue than those from S252W-Tg.

Conclusion: These findings indicate that FGFR2IIIc-S252W promotes osteoblastic differentiation through MEK, ERK, PLC-γ, and P38 pathways, whereas SOL suppresses this process. Findings also suggest that SOL is an useful therapeutic target of Apert syndrome.
Reference: [1] Tanimoto Y, Yokozeki M, Hiura K, Matsumoto K, Nakanishi H, Matsumoto T, Marie PJ, Moriyama K (2004) A soluble form of fibroblast growth factor receptor 2 (FGFR2) with S252W mutation acts as an efficient inhibitor for the enhanced osteoblastic differentiation caused by FGFR2 activation in Apert syndrome. *J Biol Chem* 279; 45926-45934

P56

Characterizing the craniofacial and dental phenotype of Costello Syndrome patients

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Background: Costello Syndrome (CS) is a rare disease consisting of a wide range of craniofacial, cardiac, musculoskeletal, dermatological and functional abnormalities. Costello Syndrome is caused by a heterozygous de novo germline mutation in HRAS that results in a constitutively active Ras protein. Ras proteins function downstream of receptor tyrosine kinase (RTK) signaling, and RTK signaling is known to play an important role in tooth development. To date, no studies have systematically screened a cohort of CS patients for craniofacial and dental defects. Therefore, we set out to examine patients at the 2009 Costello Syndrome International Conference to comprehensively characterize the dental and craniofacial phenotype of CS. **Methods:** We performed craniofacial and dental exams on 29 patients at the 2009 Costello Syndrome International Conference, including intra and extraoral photographs, clinical examinations, xray reviews and alginate impressions. We next studied the craniofacial and dental defects in the CS mouse model. **Results:** The CS patients presented with a number of craniofacial findings, including relative macrocephaly, bitemporal narrowing, hypertelloric and telencanthic appearance, downslanting palpebral fissures, epicanthal folds, ptosis, short nose with depressed nasal bridge and anteverted nares, and low set, posteriorly rotated ears. During our craniofacial and dental examinations, we noted full cheeks with a large-appearing mouth and tongue and thick-appearing lips. Nearly 80% of patients presented with a convex facial profile, whereas 20% had a concave profile. There was a slight predilection towards Class II (35%) and III (35%) molar relationship compared to Class I (30%), and we detected a fairly high incidence of posterior crossbite (36%) and open bite (39%). In addition, delayed tooth development and eruption were noted. Many patients also presented with a high arched palate (81%) and thickening of the posterior alveolar ridge (32%). Patients also demonstrated habits including bruxism (45%), tongue thrusting (28%) and open mouth posture (39%). In light of the importance of RTK signaling in dental development, we were surprised to find that the morphology of teeth was normal. An interesting finding during our clinical examinations was enamel hypoplasia, which occurred in 84% of patients. Micro computed tomography (microCT) of exfoliated primary teeth from CS patients showed a significant decrease in enamel thickness compared to controls. The enamel defect was also seen in the CS mouse model, and further inspection revealed disorganization of the ameloblasts in the incisor. In order to determine the etiology of the ameloblast dysfunction and subsequent enamel defect, we are currently studying cell proliferation and polarity using incisors from mutant mice. In addition, we plan to transiently transfect an ameloblast-like cell line with plasmids encoding wild-type and mutant HRAS to determine how this affects signaling and behavior of the cells. **Conclusion:** We systematically examined a cohort of 29 CS patients and characterized their craniofacial and dental phenotypes. One of our most striking findings was a pronounced enamel hypoplasia, and we were surprised to find normal dental morphology. Ongoing experiments are focused on understanding the etiology of the enamel defect and the role of Ras signaling in tooth development.

P57

miR-200b integrates Tgf-b signaling pathway in mouse palate development

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Background: During palatogenesis, dynamic cellular and molecular events occur, including the elevation and fusion of the palate. These complex events are mediated by cell proliferation, cell death and epithelial-mesenchymal transformation (EMT). Failure of these processes can cause cleft palate, the most common birth defect in human. Epithelial-mesenchymal transformation (EMT) is a fundamental mechanism in many embryological processes. In palate formation, the epithelium of both sides palatal shelf adheres to form Midline Epithelial Seam (MES) and then disappear through EMT. Particularly, EMT cause seam to break away into small islands of epithelial cells that are in the process of transforming to mesenchyme. Many transcription factors, such as Snail, Slug, Zeb1, Zeb2 and Twist directly bind to the E-cadherin gene promoter to inhibit its transcription. **Methods:** miRNAs are non-coding, endogenous single-stranded RNAs of about 22 nucleotides that negatively regulate gene expression, mainly through post-transcription repression. **Results:** Particularly, we found predicted target genes of miR-200b on the miRGator website. Smad2 (factor of TGF-β pathway), Snail, Zeb1, Zeb2 were selected as a predicted target genes. We therefore hypothesized a role for regulation of EMT related genes and miR-200b. First of all, to see the expression of E-cadherin•Zeb1•Zeb2, we performed immunostaining and in situ hybridization. E-cadherin was observed in epithelium of palatal shelves. Where small islands were formed, there are the expressions of E-cadherin, miR-200b in small islands in the seam and epithelium of palatal region. Contrary to E-cadherin, ZEB1, 2, Snail was distributed in the mesenchyme cells according to in situ hybridization. In order to see effect of miR-200b during palatogenesis, miR-200b was transfected into palate shelves using miR-200b expressing lentivirus. Those genes were inhibited by miR-200b during palatogenesis but not E-cadherin expression. **Conclusion:** These results indicate that miR-200b modulates Smad2, Snail, Zeb1, Zeb2 as regulator of key factor of the TGF-β pathway during palatal EMT process.

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Phenotypic characterization of seventeen families with nonsyndromic tooth agenesis.

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Introduction: Tooth agenesis is one of the most common developmental anomalies in man. Nonsyndromic familial tooth agenesis is a heterogeneous condition that can affect various combinations of teeth and have been associated to other dental anomalies. Studies on inherited tooth agenesis as well as mouse null mutants have identified several of the genetic factors and helped to understand the molecular mechanisms of tooth development. However, so far success has only been made in identifying the genes involved in syndromic or dominant forms of tooth agenesis, while the genes and defects responsible for the majority of cases of tooth agenesis, especially the common and less severe forms, are largely unknown. **Objective:** The aim of this study was to characterize the phenotype of seventeen families with nonsyndromic tooth agenesis followed up since March 2002 in the Oral Care Center for Inherited Diseases, University Hospital of Brasília, Brazil**. Subjects and methods:** Complete physical, oral and radiographic examinations were performed on all family members after signature of the written informed consent. Taurodontism was verified according to Seow and Lai (1989). **Results:** In the seventeen nonrelated families, twelve showing autosomal-dominant inheritance mode and in five was not possible to define the inheritance mode. Two families presented congenital heart disease and one presented colorectal cancer. Seven families presented individuals with oligodontia and ten families presented hypodontia. All families showing at least one dental anomaly associated with tooth agenesis: enamel development defects in 14 families, peg-shaped teeth in ten, canine ectopic eruption in nine, taurodontism of mandibular permanent first in five and supernumerary in five. **Conclusions:** A detailed description of the dental anomalies associated to tooth agenesis in these families is essential to better correlate phenotype with genotype. The present families studied diverse phenotypes when considered the number of systemic alterations associated, type of tooth affected and tooth anomalies associated.