COST ACTION B23
”Oral-facial development and regeneration”

Working Groups, 3, and 4 and Management Committee
Meeting

“NEW FRONTIERS IN ORAL FACIAL REGENERATION:
From the lab’s bench to the patient’s bedside”

VENUE

“Gran Hotel Rey Don Jaime”
Avenida del Hotel, 22
Castelldefels,
Barcelona, Spain

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DATES
31 MAY-2 JUNE 2007
SCIENTIFIC PROGRAMME

“NEW FRONTIERS IN ORAL FACIAL REGENERATION:
From the lab’s bench to the patient’s bedside”

31 May 2007

10h’ Opening ceremony

10h30h Keynote Lecture: : Multidisciplinary approach to the facial clefts: the clinician’s touch.. Dr. T Coelho, Hospital Sao Joao-Facultade de Medicina, Universidade de Porto, PORTUGAL

11h30’: Coffe-break

12h: WORKING GROUP 3: GENETIC ANOMALIES

    Chairs: A Bloch-Zupan, JM Ustrell-Torrent. Oral Communications

12h10: New clinical features and new genetic events in Gorlin syndrome, P Gorry, Bordeaux, France.

12h25: Prevalence Of Tooth Agenesis And Related Pathologies In A Spanish Population, V Tallon, Barcelona, Spain

12h40: Causal Gene Evaluation Of Cleft Lip And/Or Palate Patients, I Grinfelde, Riga, Latvia

12h55: Craniofacial And Bone Phenotypes Associated To X-Linked Hypohidrotic Ectodermal Dysplasia (Xlhed), F Clauss, Strasbourg, France

13h10: Oral-dental Malformations. Prevalence, Phenotype-Genotype Relationship And Associated Pathologies, M Gouveia, Porto, Portugal

13h25: Prevalence of Tooth Agenesis and related pathologies in the Barcelona University Dental Clinic A Ureta, Barcelona, Spain

13h40: Cleft Lip And Palate Epidemiology Based On A Hospital Sample – Hospital São João Of Porto, J. Correia-Pinto, Porto, Portugal
14 h: Lunch

15h30: Chairs: K. Heikinheimo, F Unda. Oral Communications
15h40: The Clinical Relevance Of Demarcing The Frontonasal Field In The Human Face, Cranium And Dentition, I Kjaer, Copenhagen, Denmark
15h55: Genomic Characterization Of Keratocystic Odontogenic Tumour (Odontogenic Keratocyst) Associated With Nevoid Basal Cell Carcinoma Syndrome; J Jee, Turku, Finland
16h10: Is Unexpected Early Apical Resorption Of Primary Teeth A Clinical Sign Which Predispose For Resorption Of Permanent Teeth? ML Bille, Copenhague, Denmark,
16h25: Epiprofin/SP6, A Zinc-Finger Transcription Factor, Is Essential For Ectodermal Tissue Formation, L Jiménez, Vizcaya, Spain

16h30’: Coffee break

16H40: Tooth Phenotype On The Tbx1 Mouse Model For Digeorge Syndrome, J Catón, London UK
16H55: TGF-β1 and TGF-β3 in Cell Death During Palate Fusion, C Martínez, Madrid, Spain
17H10: Changes Observed Over Time In Nociceptive Fiber Development After Neonatal Capsaicin Treatment, T Krage, Duesseldorf, Germany

1 June 2007

10h WORKING GROUP 4: TISSUE REGENERATION/APPLICATIONS
:Keynote Lecture: "Role of microenvironment squamous epithelial stem cells". Prof. Dr Yann BARRANDON, École Polytechnique Fédérale de Lausanne, SWITZERLAND

11h: Coffee break
11h 30’ Chairs: I Binderman, F. Ventura-Pujol. Oral Communications
11h40: Dentonin, a MEPE fragment, initiates pulp healing response to injury; M Goldberg, Paris, France
11h55: Molecular Changes During Early Phases Of Bone Regeneration, H Bahar, Jerusalem, Israel
12h10: Human Gingival, Periodontal Ligament And Alveolar Bone Cell Cultures: A Comparative Study, MH Fernandes, Porto, Portugal
12h25: Alveolar bone loss is triggered by extracellular ATP and its receptor P2X4 in marginal gingival fibroblasts, I Binderman, Jerusalem, Israel
12h40: Ultrastructural Analysis Of Pericyte Distribution And Migration After Pulp-Capping In Rat Molars, I Lambriechts, Limburg, Belgium
12h55: Biological Response To Bone Substitutes: In Vitro Cell Culture Models, MH Fernandes, Porto, Portugal

13 h30: Lunch

15h: Chairs:.H Magloire, R Pérez-Tomás. Oral Communications
15h10: Are Ancient Mechanisms Of Replacement Tooth Formation Conserved In Bony Fish?, A Huysseune, Ghent, Belgium
15H25: Use of evolutionary analysis of AMEL to validate amelogenesis imperfecta and some comments on AMEL polymorphism in humans, JY Sire, Paris, France
15h40: Complete Fusion And Reshaping Of Vertebral Bodies In Atlantic Salmon (Salmo Salar): An Unusual Type Of Regeneration, P. Eckardt Witten, Hamburg, Germany
15h55: Expression Of Mineralization–Related Gla Proteins During Regeneration Of Zebrafish (Danio Rerio) Fins, AB Brito, Faro, Portugal
16H10’: Phenotypic Plasticity Of Cranial And Pharyngeal Structures In An African Cichlid Fish, F Galis, Leiden, The Netherlands

16h30’: Coffee break

SOCIAL PROGRAMME
2 June 2007


10h30’ Chair: Michel Goldberg, MP Ginebra: Oral Communications
10h40: Long-Term Biocompatibility Of Isfet Sensors Using Human Gingival Fibroblast Cells: Preliminary Results, MH Fernandes, Porto, Portugal
10H55: Osteogenic Induction Of Human Mesenchymal Stem Cells In Response To Titanium Implant Surface Roughness, I Wall, London, UK
11h10: Influence On Technological Parameters At Structure Of Calcium Phosphates, K Salma, Riga, Latvia

11h 30’ Coffee break

12h: Chairs: Ralph Radlanski, J. Franch. Oral Communications
12h10: ABA (Active Bone Area): a qualitative and quantitative integral analysis method of the osseous tissues around implants. P Carvalho, Barcelona, Spain
12h25: Computational Simulation Of Bone Remodelling And Growth: Influence Of Mechanical Factors, JM García-Aznar, Zaragoza, Spain
12h40: The Differentiation Potential Of The Periodontal Tissues, P Brett, London, UK
12h55: Calcified Tissues Reaction in Contact with an Osteoconductive Implant Surface. I. Valdivia, Barcelona, Spain
13h10: Experimental and FEA evaluation and comparison of three dental implants systems, made with different materials, L. Carvalho, Aveiro, Portugal.
13h25:Comparative mandibulometric data after unilateral transection of the lower alveolar nerve in post-weaning rabbit. I. Valdivia, Barcelona, Spain

13h40h: Lunch
DRAFT AGENDA

1. Welcome to the participants  
2. Adoption of the Agenda  
3. Approval of the Minutes of the 6th MC Meeting in Helsinki (4-7 May, 2006)  
4. Information from the COST OFFICE  
5. Information from the Chairman  
6. STSMs  
7. Dissemination of the results  
8. Preparation of the final report  
9. Proposal and discussion for a new year extension  
10. Organisation of 8th MC Meeting (Zurich)  
11. Web site of the COST ACTION B23  
12. Any other business
ABSTRACTS
New clinical features and new genetic events in Gorlin syndrome

Gorry P. 1, Lartigau M. 2, Richard B. 2, Musani V.3, Levanat S.3

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Gorlin syndrome or Nevoid Basal Cell Carcinoma Syndrome is an autosomal dominant disease with complete penetrance and variable expressivity. The diagnosis is based on the occurrence of basal cell nevus, porokeratosis, and jaw keratocysts. The severity is due to the tumoral risk, as basal cell carcinomas and brain tumors. The responsible gene is a tumor suppressor gene named PTCH encoding for the Sonic Hedgehog receptor. Mutation of PTCH gene conducts to dysregulation of the HH signaling pathway which is well known to be involved in many developmental events including craniofacial and tooth development. Early clinical diagnosis of the disease is difficult while the tumor risk is important. For the dentists, the first symptom is the apparition of multiples keratocyst.

Through a clinical research network, we screened for PTCH mutations in more than 100 unrelated Gorlin cases. Our results underlined the high frequency of de novo PTCH gene mutations.

The availability of the molecular diagnosis helps us to define new clinical features of the disease. For example, we were able to provide evidence that craniopharyngioma is linked to Gorlin syndrome, and that PTCH haploinsufficiency give rise to abnormal activation of the Wnt pathway in the tumor.

However, the non-detection of PTCH mutations in half of the typical familial cases underlined also the non-exploration of other genetic events. So we set up new molecular diagnosis tool and look for the involvement of a new gene.

References:


Keywords : Gorlin syndrome, keratocyst, PTCH gene

Acknowledgements:
This work was supported by COSTB23, GIS “Maladies Rares” and Ministry of Health.
Prevalence of tooth agenesis and related pathologies in a Spanish population


Dental agenesis is defined as the radiological and clinical absence of a dental organ without its extraction or accidental loss; its etiology is related to environmental, phylogenetic or genetic factors. Recent studies have established the relationship between certain dental anomalies, such as family dental agenesis, with genetic mutations, which in turn are associated with systemic entities and multi-organ syndromes.

For this study of the first five years of the Primary Health Centre of Cassà de la Selva (Girona – Spain), which takes care of an approximated population of 14,000, 1660 clinical records were initially reviewed. 1518 were selected, 683 males, 835 females, ranging in age from 6 to 83 years.

The prevalence of congenital missing teeth and other forms of related oral and dental anomalies such as peg-shaped teeth, micro and macrodontia, mandibular prognatism and retrognatism, ectopic eruption, supernumerary teeth and taurodontic teeth were recorded. As well as the presence of the following systemic entities: allergies, endocrinal alterations, congenital cardiac valve anomalies and proliferative colonic pathologies (cancer and poliposis).

The prevalence of congenital missing teeth is of 9,48%, 7,25% if we exclude the third molars. The prevalence of endocrinal entities and congenital cardiac anomalies is also slightly higher in the patients that presented multiple congenitally missing teeth than in the general population.

The fact that individual members of the same family have congenital missing teeth and other dental anomalies, and also present específic systemic entities, reinforces the genetic theory about the etiology of dental agenesis.

The authors wish to aknowledge the collaboration of ACESBELL Project (Research Commission, Bellvitge Campus, University of Barcelona) and the COST B23 Action STSM
Causal gene evaluation of cleft lip and/or palate patients

Baiba Lace, Indra Dundure, Inga Prane, Inta Vasiljeva, Linda Piekuse, Ilze Akota, Biruta Barkane, Astrida Krumina

Scientific laboratory of Molecular Genetics, Department of Medical Biology and Genetics at Riga Stradins University, Latvia

Cleft lip and/or cleft palate (CLP) is one of the most common malformations among newborns. The estimated prevalence in Latvia is 1/700. Nonsyndromic CLP is a complex trait determined by multiple, interacting genetic and environmental factors. Many risk factors influencing development of cleft lip and/or palate have been analyzed recently. In the past decade strongly increased interest about etiological role of genetic factors in the development cleft lip and/or palate.

The objective of our study was to investigate role of MSX1 and BCL3 genes in the development of cleft lip and/or palate.

Material
DNA was extracted from 100 CLP patients and both their parents. Questionnaire and database was developed and completed for each family. It contained information about pregnancy, risk factors, genealogy etc. Facial measurements were performed for the both parents.

Methods
Promoter and coding regions of MSX1 gene were sequenced. Results compared with reference sequence Nr. AF426432 (NCBI).

Nine SNPs were analyzed for the BCL3 gene with MALDI-TOF technique. TDT test performed for the analyzed families.

Results and discussion
Two mutations were identified in MSX1 gene, which correlates with previous publications about MSX1 gene role in the development of CLP.

15 SNPs were identified within the gene coding region, two of the analyzed SNPs (11bpdel and A3696G) more frequently were met in patients with cleft lip and cleft lip/palate. They were considerably seldom in patients with isolated cleft palate.

From 9 analyzed SNPs in BCL3 gene, only six showed polymorphism in Latvian population. TDT test showed linkage disequilibrium for three of them.

Keywords: cleft lip/ palate, MSX1 gene, BCL3 gene

Acknowledgements
This study was realized within a framework of Baltic – Taiwan joint research Project.Cost Action B23
CRANIOFACIAL AND BONE PHENOTYPES ASSOCIATED TO X-LINKED HYPOHIDROTIC ECTODERMAL DYSPLASIA (XLHED)

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2: INSERM Unit UMR 595, Faculty of Medicine, Louis Pasteur University, Strasbourg, France

Key words: ectodermal dysplasia, bone phenotype, computed tomography (CT)

The X-linked hypohidrotic ectodermal dysplasia (XLHED) is ascribed to a mutation of ED1 locus coding for Ectodysplasin (EDA), a morphogenetic TNF-like factor. It is the most prevalent form of ectodermal dysplasia and is characterized by a large genotypic and phenotypic spectrum. Besides dental developmental defects, XLHED can lead to alveolar bone hypotrophy and craniofacial dysmorphies. These abnormalities may result from structural and metabolic bone modifications, which have been described in XLHED patients and the Tabby mouse experimental model.

Aim: To investigate XLHED-related bone modifications in humans, craniofacial standard radiographic and CT exams as well as quantitative bone densitometric analysis were performed on a group of eight XLHED patients and compared to a group of control patients.

Results: CT exams revealed bone structural modifications of the jaws, including an increase of cortex thickness associated to hyper and hypo-mineralization of medullary areas. Such bone alterations were not restricted to the jaw bone, but also observed in various parts of the craniofacial complex including the zygomatic complex. Multiplanar CT-slices and lateral skull radiographies demonstrated craniofacial dysmorphic features such as maxillary hypoplasia, maxillary sinus asymmetry, reduced cranial base length associated to smaller cranial vault. Quantitative bone densitometric analysis revealed an increase of cortical bone mineral density in XLHED compared to control patients.

Conclusions: The bone phenotype observed in XLHED was characterized by morphological and structural modifications that might result from bone developmental defects. These alterations should be taken into account for the management and survey of the dental implant osteointegration, which constitutes the sound basis of successful oral rehabilitation in XLHED patients.

Acknowledgements: The authors thank the supports of the COST Action B23.
ORAL-DENTAL MALFORMATIONS, PREVALENCE, PHENOTYPE-GENOTYPE RELATIONSHIP AND ASSOCIATED PATHOLOGIES

MC Manzanares Céspedes; MA Gouveia; S Barreto; V Tallón Walton; P Carvalho Lobato; JM Ustrell Torrent; A Almeida Dias


The oral-dental malformations can be early detected at the dentist's consulting-room or even in oral health school programmes. The detection of these pathologies, common to several family members, gives rise to the genealogical study and promotes the genetic analysis, both of the affected patients, and relatives apparently not affected by these oral-dental malformations. These studies can facilitate the precocious diagnosis, both of the genetic mutations responsible for the oral-dental malformations, and of the mutations which cause related systemic pathologies.

The hypothesis from which the present study sets is that some oral-dental malformations common to different members of the family obey to genetic mutations and can be seen in patients and/or in families with systemic pathologies, which are also caused by genetic mutations. It is important to ascertain the reciprocal influence of the phenotypic expression of the genes responsible for oral-dental malformations and of the systemic alterations is.

The aims of this study are:
- Analyse a clinic diagnosis and establish a fiable record of the oral-dental malformations;
- Study its prevalence in the Portuguese population and compare it to other countries;
- Define the existence of a hereditary pattern and perform a genetic study;
- Establish a phenotype-genotype relationship;
- Expose a possible association to other systemic pathologies.

The anamnesis files of the dental clinics from the littoral North of Portugal, which contributed to the study, is going to be observed. The historic record (for a period of five years) and the recent files, for a period of one year will be analysed. The aim is to obtain warrantable values of the oral-dental pathologies from genetic origin and of the pathologies associated to them such genetic causes as epigenetic. Among these, the allergies, the endocrine alteration, the congenital cardiac malformations and the digestive pathology must be distinguished.

Until now, five hundred clinical files of patients treated by our team, have been revised. Preliminary results and a familiar history of a particular case are presented.

Keywords: oral-dental malformations, agenesis, systemic pathologies

Acknowledgements: The authors wish to acknowledge the families who participated in this study. The collaboration of GCAID-CESPU, Unidad de Anatomia y Embriologia Humana de la Facultad de Medicina UB, COST B23 is gratefully acknowledged.
CLEFT LIP AND PALATE EPIDEMIOLOGY BASED ON A HOSPITAL evaluation
Rowney Furfuro¹, Jorge Carneiro¹, Josep Ustrell², João Correia-Pinto¹
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Introduction: The study of the cleft lip and palate is an inexhaustible source of information about craniofacial development. The new research team formed in Hospital of São João, has sought to register, classify and reorganize all the information obtained during the long years of activity treating those patients, according to a new protocol.

Purpose: Establishment of a new diagnosis and treatment protocol.

Materials and method: We carried out an investigation through the records of the paediatric surgery ward, to study the incidence of cleft lip and palate in terms of: prevalence of the type of cleft, patient’s gender, the prevalence in the districts covered by our hospital and the incidence in the birth months. From this information it was possible to acquire a more accurate knowledge of the malformation incidence in the target population. The sample was made up of 203 records.

Results: This study acknowledged that the bilateral clefts had, in the group of studied patients, an incidence of 10.84%, and the unilateral clefts, an incidence of 47.29%. In the unilateral clefts, 42.7% of the cases were found on the right side; on the left side, 57.3% cases; 24.63% were preforamen cases, 33% transforamen and 40.4% postforamen. The incidence of the clefts occurs in 57.14% of the male patients and in 42.86% of females. In the female patients, the incidence of type I clefts is 20.69%, type II is 27.59% and type III is 49.42%. In male patients, the incidence of type I is 27.59%, type II is 37.07% and type III is 33.62%. The month which has registered the highest number of children born with a cleft is March. The area of Guimarães was the one with the highest incidence of cases.

Discussion: The prevalent incidence in the sample is of postforamen clefts. That result diverges from the result obtained from literature, reporting a higher incidence in both transforamen and preforamen. This divergence enhances the importance of the study that shows a possible local endemic characteristic in the appearance of these malformations. In some regions, such as the Andean countries, the appearance of the clefts is statistically more representative, acquiring typical characteristics of endemic pathologies.

Conclusion: The initial results obtained in the sample indicate that within the area surrounding the Hospital São João, the clefts are: more common in the male gender; the majority belonging to group III and with a higher occurrence in the children born in the month of March.

Keywords: Epidemiology; Cleft Lip and Palate; Occurrence

Acknowledgements: The authors wish to acknowledge the support of the COST Action B23.
The clinical relevance of demarcing the frontonasal field in the human face, cranium and dentition

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Keywords: Neural crest cells, holoprosencephaly, SMMCI

Holoprosencephaly and SMMCI (Single Median Maxillary Central Incisor) represent severely and less severely affected craniofacial structures located to the frontonasal field and include the frontonal lobe of the hemispheres. In order to demarcate the extent of the malformation and thereby the exact limitations of the frontonasal developmental field clinically, the purpose of this study was to analyze and compare human prenatal holoprosencephaly cases with human postnatal SMMCI cases.

Material
Seventeen human foetuses with Holoprosencephaly, representing all types from Cyclopia to Median Cleft Lip (gestational ages 10-22 weeks), and 24 children with SMMCI (aged 7-22 years) were included.

Method
Clinical, radiographic (lateral, frontal and axial projections) and histological investigations (survey staining with Toluidine Blue and Alcian Green) were performed.

Results
The frontonasal neural crest cells form the following parts of the face, oral cavity, dentition and cranial bones:

Face and oral cavity: The midaxial lower part of the forehead, the face between the eyes including the external nose, the philtrum of the upper lip, the frenulum labii superior (absent) and the incisal papilla (absent).

Dentition: In SMMCI the medial parts of the central incisors are absent in the primary and the permanent dentitions. The bilateral distal parts of the centrals are united resulting in one symmetric axial central incisor. In Holoprosencephaly the centrals as well as the laterals can be absent. In the maxillary dental arch, the frontonasal neural crest cells form the alveolar bone enclosing the incisors.

Cranial bones: The following structures are malformed: the interincisal maxillary suture (absent), the anterior nasal spine (short), nasal cavity (narrow), nasal septum (short and deviated), interfrontal suture (absent), anterior cranial base (short) and the anterior wall of the sella turcica (malformed). The other cranial structures are normal.

Conclusion
These pathological cases show that the frontonasal neural crest field in normal humans forms a fan-shaped part of the upper face and cranium from the sella turcica spreading out to the medial parts of the eyes. In the less severe phenotype (SMMCI) the midaxial part of this fan is always absent. Only in the most severe phenotype the whole fan is absent (Cyclopia). The question raised in this study is whether a special gene is responsible for the midaxial part of the fan.

Clinical perspectives
In the future it is important clinically to be aware of the origins of the different neural crest compartments of the face, cranium and dental arches. This will improve our understanding of craniofacial syndromes and the distinction between genetically inherited and non-inherited deviations in the dentition. Thus, agenesis of several neighboring teeth in a localized area might be explained by deviations in genes responsible for the actual neural crest developmental field.

Acknowledgements: COST Action B23.
Genomic characterization of keratocystic odontogenic tumour (odontogenic keratocyst) associated with nevoid basal cell carcinoma syndrome

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Nevoid basal cell carcinoma syndrome (NBCCS; also known as Gorlin syndrome) is a hereditary disease characterized by multiple keratocystic odontogenic tumours (KCOTs). Genetic aberrations described in both sporadic and syndrome-related KCOT include point mutations in tumor suppressor gene patched (PTCH). In sporadic KCOT, we recently described an amplicon in 12q13.2 spanning several cell growth-related genes, and overexpression of genes in 12q13 including KRT6B, ERBB3 and GLI1 (Heikinheimo et al., J Dent Res, in press). We have continued our series of genetic studies with syndrome-related KCOT and have so far analyzed KCOT samples from four NBCCS patients in oligonucleotide-based comparative genomic hybridization microarray. Amplicons in 6p21.33 (0.7 Mbp) and 16p telomere regions were found in three of the four cases studied. 6p21.33 spans an open reading frame (C6orf) which is susceptible to chromosomal changes. The amplified genes found in this region included lymphocyte antigen 6 complex (LY6G5B), known to be involved in Wnt signaling pathway which is reported to be aberrated in several tumours. In two KCOT cases, small amplicons (0.2Mb–1.2Mb) were found in 12q13. Consequently, syndrome-related KCOT and sporadic KCOT appear to differ in their genomic aberrations. However, amplifications in genes relating to cell proliferation as detected in 12q13 may be shared by sporadic and syndrome-related KCOTs.

Keywords: Genomic aberrations, Gorlin syndrome, keratocystic odontogenic tumour

Acknowledgements: The support of COST Action B23 is gratefully acknowledged
Is unexpected early apical resorption of primary teeth a clinical sign which predispose for resorption of permanent teeth?

Authors
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Keywords
Ectoderm, root resorption

Introduction
Early resorption of primary teeth in patients with multiple agenesis and root resorption of permanent teeth during orthodontic treatment occur in dentitions with ectodermally derived morphological characteristics of the permanent teeth (invagination, taurodontism, short roots, deviant shapes of crown and roots) as well as agenesis and ectopia of permanent teeth (Kjær, 1995; Kjær et al, 2007). The mechanism initiating and controlling root resorption is not known.

Aim
The aim of this study was to reveal whether there is an association between unexpected early apical resorption of the primary teeth in the presence of a permanent successor and ectodermally derived morphological characteristics and deviations of the permanent teeth within the same dentition.

Material and methods
Orthopantomograms of the early mixed dentition from 19 children (11 boys and eight girls aged six years and two months to 11 years and nine months) with unexpected early apical resorption of the primary teeth in the presence of permanent successors were identified from an archive of 588 patients referred to the Department of Orthodontics for diagnosis and treatment guidance.

After written request, follow-up radiographs (taken two to 15 years later) were obtained from 12 of the 19 children. These follow-up radiographs were evaluated with interobserver reliability for morphological characteristics and deviations of permanent teeth according to Kjær (1995). For seven children the evaluation of the permanent teeth was based on the radiograph of the early mixed dentition only.

Results and discussion
In 18 of the 19 children one or more ectodermally morphological characteristics and deviations of the permanent teeth were seen. In addition external apical root resorption was seen in three patients of whom two had received orthodontic treatment.

Unexpected early apical resorption of primary teeth in the presence of a permanent successor may predispose for resorption of permanent teeth also. It is not possible today to explain the link between increased root resorption tendency and the ectodermal morphological characteristics and deviations of the permanent teeth.

References

Acknowledgements
COST Action B23
Epiprofin/SP6, a zinc-finger transcription factor, is essential for ectodermal tissue formation

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We previously identified epiprofin (Epfn) from a tooth germ cDNA microarray as a zinc-finger transcription factor that is expressed in certain developing ectodermal tissues such as the dental epithelium, apical ectodermal ridge of the limbs, and matrix epithelium of hair follicles, and it also promotes cell growth in culture. In our present study, we show that Epfn is essential for the development of these ectodermal organs by creating Epfn-deficient mice. These mice survived but were abnormally small, and they developed severe ectodermal organ defects, including dental tissues. Null mice were hairless and had supernumerary teeth with enamel hypoplasia, irregular dentin structure, and a hyperthick epidermis. We found that Epfn was essential for developing the proper number of teeth by controlling branching of tooth buds and for promoting differentiation of dental epithelium and mesenchyme. In addition, we observed that adult mutant mice had malformed molars and incisors. These teeth were missing the enamel, and the molars had poorly developed cusps and malocclusion. Moreover, there were severe defects in the dental root structures and alveolar bones. Odontoblasts were not fully developed, and they secreted dentin; the secretion was delayed when compared to that of normal teeth. Several layers of altered odontoblasts were found, and dentin tubules were not correctly formed. These results suggest that Epfn is essential for proliferation, cell fate determination and cell differentiation in teeth. The mechanism of action of this transcription factor is involved in producing the correct number of incisors and molars, the shape of molars, enamel formation and dentin matrix secretion.

Acknowledgements: This work has been supported by the University of the Basque Country (GIU05/27) and Intramural Research Program of the NIH (NIDCR) grants. Susana de Vega and Lucia Jimenez were supported by fellowships of the Basque Government and the University of the Basque Country, respectively. We are also grateful to COST B23 for its support.
Tooth phenotype on the Tbx1 mouse model for DiGeorge syndrome

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DiGeorge syndrome is associated with the deletion of a portion of the 22q11 chromosome in humans. This syndrome is characterized by abnormalities in tissues developing from the pharyngeal apparatus. Patients with this syndrome display, amongst others, craniofacial anomalies including ear malformation, short mandible and cleft palate. Some of these patients also display enamel hypoplasia along with enamel hypocalcification. The portion of the deleted 22q11 chromosome has numerous candidate genes, but recent studies suggest Tbx1 as the most likely candidate. There is a mouse model with a null mutation on the Tbx1 gene that mimics the DiGeorge syndrome phenotype. In this study we look at the relationship between Tbx1 and tooth development at the stage of enamel formation. Tbx1 expression is restricted to the epithelial component of tooth primordia and appears to mark the epithelial cells destined to give rise to the enamel matrix producing ameloblasts. Here we show the importance of Tbx1 in the regulation of genes involved in tooth development and differentiation as well as its effects in the expression on tooth specific genes and cell proliferation in the epithelial layer of the continuously growing mouse incisors. Tbx1 mutant incisors grown to maturity in kidney capsules display a tooth phenotype. Taken together these results suggest a relationship between Tbx1 and tooth development and mineralization.

Key Words. Tbx-1, DiGeorge Syndrome, Tooth, Enamel
TGF-β1 and TGF-β3 in Cell Death During Palate Fusion

During palate fusion, the midline epithelial seam (MES) cell death is a crucial event for its disappearance. At this time point, both TGF-β1 and TGF-β3 are expressed in this epithelium and have been considered cell death inductors. However, it is unknown whether there is an interaction between them to accomplish this function.

**Objectives:** The aim of this work has been to determine the relationship between TGF-β1 and TGF-β3 with the cell death observed in the MES prior to palate fusion.

**Methods:** By using Tgf-b3 wild type and null mutant mice, we have modified the presence of TGF-b1 or TGF-b3 in palate cultures and then analysed through TUNEL, morphometry and *in situ* hybridization the presence of cell death in the MES and the expression of Tgf-β1 and Tgf-β3. We have also performed double labelling of embryonic day 14.5 mouse palates with antibodies against TGF-β1 and TGF-β3 and TUNEL.

**Results:** Our results demonstrate that both the increase and the decrease of TGF-b1 induce the overexpression of TGF-b3, whilst the MES cell death increases. High doses of TGF-b1, however, do not increase cell death. In the absence of TGF-b3, neither the increase nor the neutralisation of TGF-b1 modifies the presence of dead cells, which is scarce. In physiological conditions, the MES cells that die do not contain TGF-b3. We thus think that TGF-b3 is the cell death inductor in the MES and that TGF-b1 alone is not: the induction of cell death observed after adding moderate doses of TGF-b1 occurs because TGF-b1 increases the expression of TGF-b3. In physiological conditions, TGF-b1 might control the expression of TGF-b3 and the MES cell death, since in the absence of TGF-b1 the expression of TGF-b3 increases. **Conclusion:** There seems to be a delicate balance between TGF-b1 and TGF-b3 in the production of cell death in the MES, and, even when both are necessary, TGF-b3 is the main inductor.

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Changes observed over time in nociceptive fiber development after neonatal capsaicin treatment

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The neonatal application of capsaicin has been known to produce a selective desensitisation of nociceptive fibers in a physiological system. Additionally, it has been observed that a lack of functional nociceptive fibers in the pulp has a detrimental effect on dentine development after 120 days. The purpose of this study was to determine if age has an influence on the reduction or elimination of nociceptive innervation in the neonatally capsaicin-treated rat over time. This was carried out with the subcutaneous application of capsaicin at a dose of 50 mg/Kg body weight in a sterile vehicle in 48 Wistar rats on the 3rd day of life. This group served as the experimental group in which 12 rats lived to 30 days, 12 to 60 days, 12 to 90 days and 12 to 155 days. The control group was made up of 48 rats which were treated with the sterile vehicle of identical volume which did not contain capsaicin. Furthermore, in the control group 12 rats lived to 30 days, 12 to 60 days, 12 to 90 days and 12 to 155 days. All rats were deeply anaesthetized before being sacrificed with cardiac puncture and intravital perfusion and fixation. Jaws were then immediately dissected and further fixed for no more than 24 hours in 4% buffered paraformaldehyde/0.2% picric acid. Jaws were washed in PBS, saturated with 30% sucrose and frozen. Immunocytochemistry was performed on frozen cryosections with primary antibodies against calcitonin gene related peptide (CGRP) and substance P (SP) in order to localize nociceptive fiber proteins within the dental pulp of each specimen. Micrographs were produced from jaws at magnifications of 10X and 25X. Scion image was applied to micrographs of the distal pulp horn of the first molar in the left lower jaw of each animal. Here the pixel numbers per inch² of positive-immunohistologish staining was measured. SPSS was used to calculate student T-test in order to analyse differences between the experimental and control groups within each age category, while the Bonferroni Post-Hoc test was used to measure differences between age groups in the experimental group and in the control group. Our results demonstrate that a significant difference is found in all age categories between the capsaicin-treated group and the control group for both CGRP and SP. Furthermore, the immunocytochemistry for CGRP showed a significant difference between the ages of 60 and 90 days in the capsaicin-treated group, while no differences for CGRP were found between age categories in the control group. The immunocytochemistry results for SP showed a significant difference between the capsaicin-treated group and the control group in all age categories examined. Furthermore, within the capsaicin-treated group significant differences were observed between the 30 and 155 day group, the 60 and 155 day group, as well as the 90 and 155 day group. Within the control group a significant difference for SP was only observed between the 30 and 155 day group. From our results we may conclude that neonatal systemic application of capsaicin significantly reduces the nociceptive fibers within the dental pulp, which is influenced by age in the rat.

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Dentonin, a MEPE fragment, initiates pulp healing response to injury

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Phosphorylated extracellular matrix proteins, including matrix extracellular phosphoprotein (MEPE) are involved in the formation and mineralization of dental tissues. In this study, we evaluated the potential of Dentonin, a synthetic peptide derived from MEPE to promote the formation of reparative dentin. Agarose beads either soaked with Dentonin or unloaded, were implanted in the pulps of rat molars, and examined 8, 15 and 30 days after treatment. As most bioactive molecules so far studied (Goldberg & Smith Crit Rev Oral Biol Med, 2004), implantation into the pulp induces a cascade of events involving the commitment of odontoblast/osteoblast progenitors, the proliferation of these cells, and their differentiation. This series of events lead to the synthesis of an extracellular matrix that will further mineralize and form a reparative dentinal bridge. At day 8, Dentonin promoted the proliferation of pulp cells, as visualized by PCNA-labeling. Many of these labeled cells were located near and around the Dentonin-soaked beads. They were also RP59-positive, a labeling suggesting that they were putative osteoblast-like progenitors. PCNA- and RP59- labeling were decreased at day 15, while osteopontin, weakly labeled at day 8, was increased at 15 days. In contrast, dentin sialoprotein remains undetectable after Dentonin implantation for all the periods of time studied. At 8 days, precocious reparative dentin formation occurred in pulps containing Dentonin-soaked bead, with formation slowing after 15 days. These results suggest that Dentonin affects mostly the initial cascade of events leading to pulp healing.
Molecular changes during early phases of bone regeneration

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Abstract

Regeneration is the ability of cells to restore lost or damaged tissues and organs in the adult life by pathways that mimic developmental processes. Many of the molecular mechanisms that control cellular differentiation and growth during embryogenesis recur during bone regeneration; however, these processes take place in a post-natal environment that is unique and distinct from those which exist during embryogenesis. We have developed an in vivo bone regeneration model at ectopic sites in the rat. Whole fresh marrow is placed inside cylinders of demineralized rat femurs (DBM) and implanted at subcutaneous thoracic region of DA rat. An "ossicle" develops spontaneously in 3 to 4 weeks, composed of cortical bone, trabecular bone and newly formed bone marrow. The aim of this study was to explore gene expression profile in early stages of bone regeneration following marrow transplantation. Affymetrix gene array analysis of the bone regenerating in DBM at day 7 and 12 days post transplantation revealed up regulation of genes that are known to participate in endochondral bone formation process. Importantly, parathyroid receptor 1 (PTH1-Rc) was up regulated early during bone regeneration. Also, key genes in muscle differentiation pathway were found to be down regulated, simultaneously. It seems that commitment of marrow cells to generate bone represses their commitment to form muscle. It should be noted that recently PTH and its related peptides are efficient in treatment of osteopenia. The present studies support the notion that they can support bone formation in regenerative procedures of bone.
Human gingival, periodontal ligament and alveolar bone cell cultures: a comparative study

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Introduction. Human in vitro models representative of the periodontal tissues are useful tools to perform research on periodontal cells/biomaterials, biologicals interactions. The present work compares the long-term behaviour of human gingival (HG), periodontal ligament (HPL) and alveolar bone (HAB) cells cultured in the presence of ascorbic acid (AA) β-glycerophosphate (βGP) and dexamethasone (Dex), supplements frequently used in the culture of connective tissues.

Material and Methods. HG, HPL and HAB cells (first subculture) were cultured (10^4 cells/cm^2) for 35 days in α-Minimal Essential Medium supplemented with 10% fetal bovine serum in the presence of (i) AA, 50 µg/mL, (ii) AA + βGP, 10 mM and (iii) AA + βGP + Dex, 10 nM. Cell behaviour was assessed in terms of attachment and spreading, proliferation, acid phosphatase (ACP) and alkaline phosphatase (ALP) activities and extracellular matrix mineralization.

Results. HG cell cultures presented a high proliferation rate with the production of an abundant non-mineralized matrix. HPL cell cultures showed the ability to express moderate osteoblastic features upon appropriate stimulation, i.e. in the presence of dexamethasone. HAB cell cultures presented the typical behaviour of an osteoblastic cell system with the ready expression of an osteogenic phenotype. βGP and Dex allowed the modulation of the proliferation/differentiation behaviour of periodontal cells within the proposed physiological and regenerative capabilities of these cell types.

Conclusion. Long-term HG, HPL and HAB cells maintained their distinct phenotypic characteristics in vitro and were sensitive to the presence of bioactive compounds, suggesting a potential utility of this model to perform research in periodontal regeneration procedures.

Key words: gingiva; periodontal ligament; alveolar bone; cell cultures
Alveolar bone loss is triggered by extracellular ATP and its receptor p2x4 in marginal gingival fibroblasts.

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Alveolar bone resorption and loss of dentition are the major consequences of periodontitis and periodontal surgery, in humans. Based on clinical observations in humans and animal experiments we have recently proposed that the signal for alveolar bone resorption starts by disruption of dento-gingival collagen bundles of fibers, by surgery or by metallo-proteinases(MMP’s), specifically in the marginal gingiva, producing an abrupt fall in cell physiological strain. **Our aim** was to identify the sensor and signaling molecules of strain deprivation in marginal gingival fibroblasts. **Methods:** Mucoperiosteum surgery was performed next to molars in 3 month old wistar rats disrupting the dento-gingival fibers. The tissue was removed after 20 minutes and processed for RNA or the mandibles were x-rayed and processed for histology 3 weeks later. Similar surgery in the apical region not disrupting the marginal gingival fibers was served as control. **Results:** Using molecular techniques like differential gene display (Affymetrix), real time RT-PCR and immunostaining, we have shown that the ligand-gated ionic channel P2X4, which is activated by extracellular ATP was up-regulated in rat marginal gingiva. We demonstrated an increase in real time RT-PCR and intense immunostaining with specific antibodies to P2X4 of fibroblasts aligned on collagen fibers in the marginal gingiva. ATP flowed out can act between sensor cells in marginal gingiva and bone cells through Ca²⁺ signaling or by direct activation of osteoclastic bone resorption. It seems that a wave of intercellular calcium propagating to adjacent cells are finally translated in neighboring cells into biological signals activating osteoclasts spatially and temporarily. Local application at time of surgery of Apyrase which degrades ATP or Coomasie Brilliant Blue, antagonists of purinoreceptors reduced significantly alveolar bone loss while suramin had no effect, three weeks after surgery. **Conclusion:** We have shown here for the first time that fibroblasts in the marginal gingiva are sensing the mechanical strain deprivation by up-regulating the P2X4 receptors which are activated by extracellular ATP. The signal is then propagated by ATP and intercellular calcium toward the alveolar bone surface; it was shown before that ATP activates also other purinoreceptors on the osteoblasts and osteoclasts resulting in bone resorption. We propose therefore new modes of local therapeutic intervention to prevent alveolar bone loss.

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Ultrastructural analysis of pericyte distribution and migration after pulp-capping in rat molars

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Keywords: pericyte, pulp capping, electron microscopy

Introduction: Many studies address the dentin-pulp complex to possess a strong regenerative capacity following tooth damage. One of the basic needs to fully understand this regenerative process is to target the residual stem / progenitor cell population in adult teeth. It has already been suggested that perivascular cells might be this stem cell source of dentinogenic precursor cells. In this study we analyse the distribution and migration of pericytes after pulp-capping in rat molars.

Methods: In this study 25 male Wistar rats at 2-3 months of age were used. Pulp exposure was made through the mesio-buccal pulp horn of the first upper molar and the cavities were capped with calcium hydroxide and were filled with IRM cement. Animals were killed 1, 3, 7, 14 or 28 days after pulp treatment by means of intra-vital perfusion with 3% glutaraldehyde. After dissection of the jaws, specimens were prepared for electron microscopic examination using routine decalcifying, embedding and staining methods.

Results: On day 1 and 3, pericytes are located at their normal sites. Starting on day 7, we observed that pericytes start to migrate away from the blood vessels and loose contact with the endothelial cells but they still maintain their basal membrane. Finally on day 28 hardly any pericytes can be localised around the blood vessels. Furthermore we see the presence of a granular cell type during the whole process of dentin bridge formation. Similar granules are observed in the dentinal tubules of dentin fragments that remain in the pulp after the cavity preparation.

Conclusion: As a general conclusion we can state that pericytes do migrate as a result of damage in the dental pulp and this migration suggests an active role in the healing process after pulp capping. The granular cell type present both during the healing process and the tooth slice organ culture might be a cell type involved in the production of the ground substance preceding the tertiary dentin formation.

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Biological response to bone substitutes: in vitro cell culture models

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Introduction. The process of bone graft incorporation involves an early inflammatory/revascularization stage, the repair stage and the late remodelling stage. Ingrowth of vascular tissue favours the migration of mesenchymal stem cells which contributes to the establishment of the repair stage, with the adhesion of osteoprogenitor cells to the material surface and their proliferation and differentiation into mature osteoblasts which secrete a collagenous matrix that is subsequently mineralized. During the remodelling stage, this primitive bone is gradually replaced by organized lamellar bone. This work presents results regarding relevant human cell culture models to evaluate cell response to bone implant materials, namely endothelial, osteoblastic and osteoclastic cell cultures.

Material and Methods. Cultures of human endothelial, osteoblastic and osteoclastic cells were established from umbilical vein, bone marrow and peripheral blood, respectively, in appropriated experimental conditions and characterized for phenotypic parameters.

Results. Endothelial cell cultures presented a characteristic pattern of cell proliferation, with the formation of viable tubular-like structures and positive immunostaining of the adhesion molecule PECAM-1. Bone marrow osteoblastic cell cultures showed a high cell growth rate, synthesis of high levels of alkaline phosphatase and formation of a mineralized extracellular matrix. Osteoclastic cell cultures showed the formation of multinucleated cells and characteristic actin rings.

Conclusion. Studies involving the utilization of well-characterized culture models of human endothelial, osteoblastic and osteoclastic cells might contribute to a more complete and integrate knowledge of the cell response leading to graft osseointegration and maintenance.

Key words: cell cultures, endothelial cells, osteoblastic cells, osteoclastic cells

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Are ancient mechanisms of replacement tooth formation conserved in bony fish?

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To gain insight into the evolution of tooth replacement mechanisms, we have studied the histogenesis of first-generation and replacement teeth on the dentary of wild Atlantic salmon (Salmo salar L.), a primitive teleost fish, and compared these results to observations on the continuous replacement of teeth in a shark (Squalus acanthias, spiny dogfish).

First-generation teeth in Atlantic salmon develop within the oral epithelium. The anlage of the replacement tooth is first seen as a placode-like thickening of the outer dental epithelium of the predecessor, at its lingual and caudal side. Ongoing development of the replacement tooth germ is characterized by the elaboration of multiple layers of epithelial cells apposed to the inner dental epithelium on the lingual side of the tooth germ, termed here the “middle dental epithelium”. Prior to the formation of the new successor, a single-layered outer dental epithelium segregates from the middle dental epithelium, which next thickens into the placode for the new tooth germ. The dental organ of predecessor and successor remain broadly interconnected.

The absence of a discrete successional dental lamina in salmon stands in sharp contrast to what is observed in other teleosts, even those that share with salmon the extraosseous formation of replacement teeth. We propose that the mode of tooth replacement in Atlantic salmon displays several ancient characters similar to those observed in sharks, and that differences between Atlantic salmon and sharks can be explained by a heterochronic shift. The possibility that the middle dental epithelium functionally substitutes for a successional lamina, and could be a source of stem cells, whose descendants subsequently contribute to the placode of the new replacement tooth, needs to be explored.

This work was carried out within the frame of COST Action B23 “Oral-Facial Development and Regeneration”.

Key-words: tooth replacement – stem cells – regeneration
A study of amelogenin polymorphism in humans and validation of x-linked amelogenesis imperfecta inferred from evolutionary analysis

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Amelogenin (AMEL), the main enamel protein, plays important roles in organization and structure of this hypermineralized tissue. Recently, an evolutionary analysis of AMEL in mammals (Delgado et al., 2005) allowed to identify a variable, central region and two highly-conserved regions at the N- and C-termini. The various changes observed in the central region suggested a possible polymorphism in human AMELX. In contrast, unchanged residues indicated functional constraints, principally in relation to protein interactions with its environment (cell membrane and mineral crystals). The crucial role of conserved amino acids located in these two regions is well illustrated by 15 mutations leading to various types of X-linked amelogenesis imperfecta (AIH1) (hypoplasic or hypomatured enamel).

First, we have tested the hypothesis of AMEL polymorphism in the central region of the encoding gene in humans, with the aim to avoid a polymorphism to be erroneously identified as responsible for AIH1. We analysed 100 alleles in an European population randomly sampled. No variants were detected and only two synonym substitutions were found in databanks. In addition, AMEL analysis in primates revealed only a few substitutions in this region. Such an unexpected strong constraint in this region, although it is subjected to variations in other mammalian lineages, can only be explained by the location of AMEL on sex chromosomes, in a region close to the non allelic homologous recombination between X and Y.

Second, we compared 50 mammalian and more than 30 reptilian AMEL sequences in an evolutionary perspective. We identified 35 amino acids which were unchanged during circa 310 millions years. These residues are certainly subjected to important functional constraints and are, as a consequence, all candidates for AIH1 if substituted. Among the AIH1 reported to date, five cases are single amino acid substitutions. They are validated by our evolutionary analysis.

These two studies illustrate how interesting are the evolutionary analyses, not only for improving the understanding of the evolutionary pattern of the varous regions of a protein, but also to put the light on residues, which have certainly an important role, but that remains still unknown.

Reference:

Key words: amelogenin, amelogenesis imperfecta, polymorphism.
Complete fusion and reshaping of vertebral bodies in atlantic salmon (*salmo salar*): an unusual type of regeneration.

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As has been shown for continuous tooth formation and for the regeneration of fin rays, lifelong growing bony fish are excellent models to study pathways of skeletal development and regeneration. In this study we have examined the capacity of Atlantic salmon to contain the fusion of vertebral bodies, a pathology common to fish that also occurs in humans. To clarify the pathogenesis of vertebral fusion, we have studied the development and progress of the disease in farmed Atlantic salmon by tracing vertebral fusion in individual fish at three different life stages and by analysing vertebral fusion in adult animals 12 months after seawater transfer.

Vertebral fusion was observed to develop at any time in the life of a salmon. It is thus not just an early developmental disorder. Vertebral fusion involves replacement of intervertebral notochord tissue by cartilage, shape alterations of vertebral body endplates, mineralisation of the intervertebral cartilage, and finally replacement of intervertebral cartilage by bone. Fused vertebral columns can develop into a centre of severe malformation through the continuous amalgamation of neighbouring vertebrae. Alternatively, animals have the capacity to contain the problem through reshaping and remodelling of two fused vertebral bodies into a single, regularly structured and jointed element. Despite the fact that the fish only reshape the vertebral bodies, but not the haemal and neural arches, successfully reshaped vertebrae apparently do not inflict further spine malformations.

We here demonstrate for the first time that the onset of vertebral fusion must not inevitably lead to fish with deformed vertebral columns. The complete fusion and reshaping of vertebral bodies may be viewed as an unusual type of regeneration since a regularly shaped vertebral body is created from two malformed units.

**Key words:** Vertebral Body Fusion, Vertebral Body Remodelling, Modularity, Intervertebral Tissue.

**Acknowledgements:** This work was carried out within the frame of COST Action B23: Oral-Facial Development and Regeneration.
Expression of mineralization–related gla proteins during regeneration of zebrafish (danio rerio) fins

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The ability to regenerate epidermal injuries is a general feature of most organisms yet only a few can regenerate largely severed appendages that comprise several different tissues. Nowadays, zebrafish is one of the most used metazoan models for regeneration studies, in particular to investigate molecular events occurring during fin regeneration. Fin regeneration starts with the formation of a blastema, a set of heterogeneous mesenchymal-like cells located between stump tissues and wounded epidermis. This event, denominated epimorphic regeneration, comprises strict growth control and cell reprogramming leading to faithful restoration of the lost parts.

Matrix Gla Protein (Mgp) and Bone Gla Protein (Bgp, osteocalcin) are small extracellular matrix proteins, members of the vitamin K-dependent (VKD) proteins family, which are known to be related to bone formation and mineralization, and more recently, to vascular calcification. Expression of bgp is specific to bone tissue and dentine while expression of mgp is associated with cartilage, soft tissues, and vascular muscle cells.

The typical teleost caudal fin, such as in zebrafish, is composed of multiple fin rays with a bony part named lepidotrichium, so it is of great relevance to determine mgp and bgp expression patterns during regeneration events.

Our main objective was to determine the gene expression levels for mgp and bgp in the first 96 hours of the regeneration process. Real-time PCR was used to determine relative expression for each of these genes while the histological markers alizarin red and alcian blue allowed detailed detection of both calcium deposition and cartilage formation in the regenerating fin. The results obtained suggest a correlation between expression of Gla-proteins and fin regeneration providing possible molecular markers for different phases of this process.

Keywords: Bone, Regeneration, Zebrafish, Gla proteins

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Phenotypic plasticity of cranial and pharyngeal structures in an African cichlid fish

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The role of phenotypic plasticity in evolution is highly controversial, even though models have shown rather convincingly that plasticity will sometimes facilitate and sometimes constrain evolutionary changes. In our study we are investigating the possible role of phenotypic plasticity in the process of adaptation and evolutionary change in the cichlid Pseudocrenilabrus multiclor.

We examined the plasticity in response to alternative oxygen environments for fishes from three habitats in Uganda that differed widely in stability and dissolved oxygen (DO) availability. One population occurs in a stable hypoxic environment, a swamp, the second in a stable well-oxygenated environment, a lake and the third population in an environment that fluctuates seasonally from almost as hypoxic as the swamp to almost as well-oxygenated as the lake, a river. Broods were split and each half was grown under hypoxic or well-oxygenated conditions. We measured morphological parameters of three categories: (a) the gill apparatus, (b) the surrounding structural elements, i.e. the pharyngeal jaws and muscle, the eye and the brain and (c) the outer shape of the fish.

The amount of phenotypic plasticity varied for the different morphological parameters and the different populations. We discuss the results in the light of the costs and benefits of plasticity. Furthermore, we discuss the absence and presence of indications for genetic assimilation.
Long-term biocompatibility of isfet sensors using human gingival fibroblast cells: preliminary results

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Introduction. The development of microsystem-based oral health diagnostic devices is a relevant issue as the immediate evaluation of biochemical oral parameters will clearly improve the practitioners knowledge about the natural history and the early stages of the more frequent oral pathologies and facilitate its treatment and its prevention. Standardized toxicity tests carried out with the relevant cell phenotype will ensure the complete safety of such devices in the oral cavity. In this work, human gingival fibroblast cell cultures were used as a preliminary approach of the biocompatibility testing of a prototype ISFET sensor.

Material and methods. Human gingival fibroblast cells (1st subculture) were maintained for 14 days in -Minimal Essential Medium, 10% fetal bovine serum, 50 µg.ml⁻¹ ascorbic acid, 50 µg.ml⁻¹ gentamicin and 2.5 µg.ml⁻¹ fungizone, at 37ºC in a humidified atmosphere of 5% CO₂ in air, and seeded (10⁴ cells.cm⁻²) in control conditions (standard plastic culture plates) and on the surface of the ISFET. Cultures were observed at days 3, 7 and 14 by scanning electron microscopy (SEM). ISFET sensors were fabricated in the IMB-CNM using standard NMOS technology, with Si₃N₄ gate material. The packaging material protecting all the electrical parts of the chip is a photocurable polymer formed by acrylate and epoxy oligomers (Ebecryl 600).

Results. Gingival cells cultured in control conditions showed a fibroblastic appearance and a high cell growth rate, with the formation of a continuous layer of parallel-oriented cells at day 14. Evident signs of cytotoxicity were observed both on the ISFET gate surface and the surrounding encapsulating material: the few adherent cells presented an altered morphology (decreased cytoplasmic volume) and hardly survived on long incubation times.

Conclusion. Results showed that the prepared ISFET sensor and surrounding material, and/or released products, appear to be toxic to human gingival fibroblast cells on a direct-contact biocompatibility assay.

Key words: ISFET; cytotoxicity assays; human gingival cells

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COST Action B23.
Osteogenic induction of human mesenchymal stem cells in response to titanium implant surface roughness

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**Introduction:** Surface properties of titanium (Ti) implants are commonly recognised to influence cellular responses and accelerate bone regeneration. Implant roughness is a key parameter contributing to accelerated osteogenesis reported both *in vitro* and *in vivo.*

**Objectives:** to assess the effects of Ti surface roughness on the phenotypic responses of human mesenchymal stem cells (hMSCs) *in vitro.* In addition to the “standard” rough surface, a chemically modified hydrophilic rough surface was assessed for its ability to induce bone regeneration.

**Methods:** hMSCs from three individuals were cultured on three different titanium surfaces: polished (SMO), rough (SLA) and rough hydrophilic (SLActive). RNA was extracted after 3 and 24h for analysis of gene expression using Affymetrix™ U133 Plus 2.0 chips. Several phenotypic parameters were assessed including cellular attachment, morphology, and mineralised matrix deposition over a 4 week period.

**Results:** Whilst initial attachment of hMSCs to Ti surfaces was similar in terms of relative cell number, SEM revealed morphological differences in response to the three surfaces examined. Gene expression analysis revealed that several groups of genes were differentially regulated between the three material groups including osteogenic markers (p<0.05). Rough surfaces induced a greater degree of mineralised matrix deposition over a 4 week period as assessed by Alizarin red staining, with the SLActive proving more effective than SLA (p<0.05).

**Conclusions:** This study demonstrates that the physical properties of Ti surfaces greatly affect the behaviour of hMSCs. The osteogenic response of these cell cultures to Ti is favourably affected by surface roughness and by further modifying the rough surface (by incorporating hydrophilic properties), even greater beneficial effects on osteogenesis may be observed.

**Keywords:** stem cells, osteogenesis

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Influence on technological parameters at structure of calcium phosphates

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Abstract

At the present work powders of calcium phosphate have been synthesized with wet chemical precipitation method using CaCO$_3$ and H$_3$PO$_4$ as starting materials. Influence of technological parameters on structure of final product of calcium phosphate was investigated.

Design for three different conditions of technological synthesis – the temperature of suspension in process of synthesis, ending pH value of suspension and aging time of suspension has been made.

The identification and characterization of obtained calcium phosphate powders structure has been made with X-ray powder diffraction.

All investigated diffraction patterns of synthesized calcium phosphates exhibited crystalline phases with significant peaks of diffraction. Identification results of crystalline structure of calcium phosphates powders are determined pure hydroxylapatite (HAp) phase with hexagonal crystalline structure. The phase diagram of first synthesized calcium phosphate strongly verified from others powders of calcium phosphates. There was not observed characteristic crystalline phase of HAp. It could be explained with differential technological parameters of synthesis – addition rate of acid solution and stabilization time of suspension ending pH value, which influenced stability of pH and homogeneity of suspension.

Keywords: Calcium phosphates, synthesis, technological parameters
Aba (active bone area): a qualitative and quantitative integral analysis method of the osseous tissues around implants.

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A new method for assessing the interaction between titanium dental implants and bone tissues is proposed. In order evaluate their biocompatibility, 8 screw implants with new surface treatment were placed in tibia and femur of 4 rabbits. After a 30 days healing period, the implants within their bone segment were extracted. For the analysis of the implant-bone interaction, a methodology was designed involving the use of Backscattering Scanning Electron Microscopy (BS-SEM). The bone surface was divided in triangular areas situated between each two threads of the screw implant surface. These zones were used like isolated study units and were called “Active Bone Areas” (ABA). For each ABA the following criteria were evaluated: percentage of Bone-to-metal contact, number and size of vascular spaces, percentage of area refilled by osseous tissues and within it, the respective amount of Chondroid Tissue, Woven Bone and Lamellar Bone. A total of 109 ABA were analyzed. The results obtained indicate an adequate microarchitectural organization of periimplantar bone; moreover, this method allows the osteoconduction and osteoinduction phenomena to be clearly evaluated. In conclusion, methodology proposed allows a qualitative and quantitative integral analysis of the tissular response to the implant. By this way, aspects related with time and cost in this kind of research are favoured and the 3R in animal experimentation are applied.

Key words: Osseointegration, Scanning Electron Microscopy-Backscattering, Anatomy.

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Computational simulation of bone remodelling and growth: influence of mechanical factors

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Aragon Institute of Engineering Research (I3A)
University of Zaragoza (Spain)

ABSTRACT
Bone tissue is a hierarchical structural composite material, with the ability to modify its internal properties, size and shape in function of the environment that is supporting. Although this adaptative process, characteristic of bone, is mainly controlled by biochemical factors, the mechanical effects can also regulate it. Therefore, mechanical forces can modulate morphological and structural fitness of the skeletal tissues (bone, cartilage, ligament and tendon) (van der Meulen and Huiskes, 2002). In fact, mechanical forces may act within tissues to regulate biological processes at different spatial scales: at molecular level (Bao and Suresh, 2003); at cellular level (Suresh et al, 2005); cell-extracellular matrix interaction (Engler et al, 2006); at tissue level (Gómez-Benito et al., 2005); at organ level (Chalmers and Ray, 1962). The interaction between mechanical and biological factors is usually described through the concept of Mechanobiology that tries to predict the evolution of the microstructure and biological constitution of a tissue or an organ as consequence of the mechanical environment.

One way to understand mechanobiological effects is through computational modelling, which is complementary to traditional approaches of theory and experiment. In fact, computational simulation in mechanobiology presents many advantages: possibility of comparing many different conditions, factors and interactions (mechanical, biological, pharmacological, etc), simulations closer to reality in some cases due to the possibility of considering and controlling factors that cannot be controlled or measure in experimental tests, economical impact (low cost) and reduction of animal experiments.

In this work we present several computational examples of application in bone mechanobiology through Finite Element Analysis: bone remodelling after prosthesis implantation (García-Aznar et al., 2005); bone fracture healing (Gómez-Benito et al., 2005); bone ingrowth (Moreo et al., in press); and bone morphogenesis.

KEYWORDS:
Computational Mechanobiology, Bone Remodelling, Bone Growth, Bone Fracture Healing

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The differentiation potential of the periodontal tissues

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The role of adult stem cells (multi-potent cells that are resident in tissues throughout the body) in wound healing and tissue regeneration is becoming an area of great interest. These multi-potent cells are good target for use in repair and regeneration of lost or damaged tissues. Evidence suggests that these cells have the ability to differentiate to form different tissues from their origins. Currently bone is repaired using cadaveric allografts; autografts from the ileac crest; and bone marrow derived cells; however, these types of cells can be fraught with health risks, so an alternative source is desirable.

Objectives: The aim of this study was to assess the multi-potency of the cell types comprising the periodontal apparatus (Osteoblasts (OB); periodontal ligament (PDL); and gingival fibroblasts (HGF)).

Methods: Primary cell cultures of OBs, PDLs and HGF were established from redundant tissue collected from four unrelated Caucasian patients seen at the EDI for wisdom tooth extraction. The cells were cultured in standard media; osteogenic media; adipogenic media and chondrogenic media. Osteogenesis was assessed by alizarin red and von Kossa staining; adipogenesis by oil red-o staining and chondrogenesis by histological staining. RNA was collected and used for Q-PCR analysis of osteogenic; chondrogenic and adipogenic gene markers.

Results: Significant alizarin red staining and chondrogenic staining was exhibited by all cell cultures, and oil red-o staining was seen in OB and PDL cultures. The Rq-PCR results supported the phenotypic observations.

Conclusion: OBs, PDLs and HGFs all exhibited osteogenic and chondrogenic potential, but only OBs and PDLs exhibited adipogenic potential. Suggesting that all three cell types are either multi-potent or contain a population of multi-potent cells and may be source of cells for tissue engineering strategies.

Keywords: periodontal; adult stem cells; regeneration.

Acknowledgements: COST Action B23 for their support of the conference; research supported by grants from the ITI Foundation, Switzerland and the CRDC, UK.
Calcified tissues reaction in contact with an osteoconductive implant surface.


**Introduction**: the aim of this communication is to present the biological properties of a new surface of dental implant denominated Biomimetic Advance Surface (BAS) studied in an animal model. This surface has demonstrated favourable outcomes in studies in vitro.

**Material and Methods.** Thirty-six implants were inserted in three pigs, immediately post-extraction of the premolar teeth from mandibular and maxillary bones, 18 with BAS treatment (Avinent®) and 18 without surface treatment. The animals were sacrificed after 90 days post-insertion of biomaterial. The histomorphologic analyses of the implant-bone interaction were performed by Backscattering-Scanning Electron Microscopy (BS-SEM) following the procedure described by Manzanares et al (1998). The image of the bone surface was divided into triangular areas situated between each two threads of the screw implant surface. These zones were used as isolated study units called “Active Bone Areas” (ABA).

**Results.** The implant with BAS treatment has shown excellent properties of osteoconduction. The ABA analyses indicate an adequate maturity process of the osseous tissues after 90 days, leading to the implant osseointegration. This is not observed among the implants without treatment. Another interesting collateral result was observed when the biomaterial contacted the tooth organ: the implants submitted to the BAS treatment were completely integrated into the dental tissues, keeping them vital. These phenomena are not observed in untreated implants.

**Conclusions.** The implants with BAS treatment, show better characteristics of osseointegration when compared with untreated implants. In addition, the findings obtained about the contact between implants and dental organs indicate interesting properties for calcified tissues conduction of BAS implants.

The authors wish to acknowledge AVINENT S.L. for the financement of the present study, and COST Action B23 for its kind support of our research and presentation.
Experimental and FEA evaluation and comparison of three dental implants systems, made with different materials

L. Carvalho, J. Merino, P. Carvalho, I. Abe, J. Simões

Introduction

The main goal of this study was to evaluate the influence of different dental implant materials stiffness, in the load transfer mechanism to the surrounding bone media. The evaluation was performed experimentally, using a new kind of biomechanical sensor, based on fiber Bragg grating sensors. The experimental results were validated numerically.

Materials and Methods

For this study were manufacture three macromodels of dental implants, based in the standard model of the Brånemark system, with different materials. The first one, was made of steel, the second had the core in steel an the halo was made of plastic ABS and the third one was made completely of plastic. All of the macromodels were inserted in a fresh bovine cancellous bone, with a maximum torque of 1.4 N.m. The experimental measurements were done with fiber Bragg grating sensors, glued in the surface of bone. The signal was measured using a dedicated optoelectronic system that dynamically demodulates optical intensity for an impulsive signal. The acquisition system acquires data at a sample rate of 1 MHz and can measure dynamic signals at a maximum frequency of 100 KHz.

In order to validate the experimental data, it was created and tested a numerical model, reproducing the experimental setup. For the numerical model all the materials were considered isotropic and linear. The comparison between numerical and experimental data has been done considering a static load of 50 N.

Results

The experimental results, obtained for a dynamic load, are listed in table 1.

<table>
<thead>
<tr>
<th>Height (mm)</th>
<th>Combined implant</th>
<th>Plastic implant</th>
<th>Metallic implant</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>-46</td>
<td>-41</td>
<td>-45</td>
</tr>
<tr>
<td>15</td>
<td>-139</td>
<td>-121</td>
<td>-99</td>
</tr>
<tr>
<td>25</td>
<td>-303</td>
<td>-312</td>
<td>-249</td>
</tr>
<tr>
<td>35</td>
<td>-272</td>
<td>-251</td>
<td>-201</td>
</tr>
<tr>
<td>45</td>
<td>-3</td>
<td>-1</td>
<td>-1</td>
</tr>
</tbody>
</table>

Table 1: Peak strain values for the three dental implant system, for a dynamic load.

According with these results, the metallic implant appears to have a more uniform strain distribution. But all the tree implants had the highest value at 25 mm height.

According with these results, the plastic implant exhibit its highest strain value, near the collar region and for the other two implants is located at 35 mm height. From a mechanical point of view, it is not recommended to have highest strain values near the collar, because it can lead to implant failure. Regarding this, it seems that the plastic implant it is not a good solution.

In order to validate the experimental data, it was compared with results obtained numerically (table 2), for the combined implant.

<table>
<thead>
<tr>
<th>5 mm</th>
<th>15 mm</th>
<th>25 mm</th>
<th>35 mm</th>
<th>45 mm</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental</td>
<td>-2</td>
<td>-11</td>
<td>-30</td>
<td>-26</td>
</tr>
<tr>
<td>Numerical</td>
<td>-5</td>
<td>-9</td>
<td>-18</td>
<td>-21</td>
</tr>
</tbody>
</table>

Table 2: Comparison of experimental and numerical strain values for the combined implant.
Table 2: Comparison of strain values, obtained experimentally and numerically, for the combined implant, for a static load of 50 N.

| Difference (%) | 250 | -22 | -63 | -24 | -700 |

The greatest difference is located at the extremes, near the collar and the apical region. In the others locations, there is a good agreement, nevertheless the numerical model is a simplified approach of the real system.

Conclusions

From the numerical results obtained, the combined implant is the one with a more uniform strain distribution. But according with the experimental results, it was not totally establish that the use of a dental implant, combining two different materials, is better in terms of the performance of the load transfer mechanism, between implant and the surrounding bone media.

It was establish that the experimental technique, implemented with fiber Bragg grating sensors, appears to be fishable and be able to measure in places of difficult access.
Comparative mandibulometric data after unilateral transection of lower alveolar nerve in post-weaning rabbits. Preliminary report.


Introduction: Several factors have been involved in the bone growth. Muscular forces (Moore 1973, Kiliaridis 1985), hormonal action (Shibata et al 2003, Ramirez et al 2004), nutrition (Pucciarelli 1980), and other factors play fundamental roles in the bone development. In the mandibular bone, the presence of the dental organ is added to the factors implicated in the bone growth. The innervation has an important role in the development and maintenance of dental arch (Chiego 1995, Parner et al 2002, Fujiyama et al 2004). That structure forms a great part of the mandibular bone in rabbits. The principal aim of this preliminary inform is to show the difference in the macrostructural growth between surgical denervated hemi-mandibular bone (DM) and its contralateral non-denervated mandibular bone (NDM), 3 months after weaning, by mandibulometric measures in rabbits.

Material and method. Fourteen rabbits, 7 female and 7 male, were included in the study. The alveolar nerves were transacted unilaterally by surgical intervention one week post-weaning. Careful surgical design was made in order to avoid the musculoskeletal damage. After 3 month of growth, the animals were sacrificed and the hemi-mandibular bones were dissected for mandibulometric study. In the first time, anterior- posterior measures were obtained. Nine mandibulometric points were established for the measurements. In the second time, transversal sections of the mandibular bone were obtained for transversal measures. All the measures were carried out three times by the same examiner and instrument. The results were analyzed by statistics software.

Results. Twelve animals completed the study. The average growth of the DM were smaller than the NDM in 7 of the 9 anteroposterior measurements. For these measures, no relevant statistical differences were found. In the transversal measures information, the average growth of the DM is smaller than NDM but without statistical difference. An interesting find in the molar region was observed. The size measures of molar region indicate statistical difference in the distribution of measures by Kolmogorov-Smirnov Test. Greater measures of molar region in DM were revealed by this information.

Conclusion. In general, non significant differences were observed between DM and NDM after three months of macrostructural growth. Only the molar region samples showed important differences. In fact, this region is the most important section innervated by the lower alveolar nerve. In order to obtain more information about the sensitive innervation role in the rabbit mandibular development we are presently continuig the studies about the microstructural morphology and cell biology of the tissues involved.

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