WORKING GROUP 4. TISSUE REGENERATION / APPLICATIONS
Chairpersons: M Goldberg, AJ Smith

12 May 2005

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

- WG4: CALCIFIED TISSUES INVOLVED IN THE DENTAL ANKYLOSIS PROCESS
  P. Carvalho-Lobato, C. Pilipili, V Tallon Walton, J. Franch-Serracanta, M. Lafuente-Baigorri, I. Serra-Renom, M. Manzanares-Cespedes.
- WG4: BIOLOGICAL BEHAVIOR OF A POROUS BONE CEMENT IMPLANTED IN RAT *TIBIA*
  Puska M, Aho AJ, Tirri T, Yli-Urpo A, Vallittu PK.
- WG4: PHOTOPOLYMERISABLE FIBER-REINFORCED COMPOSITE AS BONE REPAIR MATERIAL
  Tuusa SM-R, Lassila LVJ, Puska MA, Matinlinna JP, Peltola M, Tirri T, Meretoja V and Vallittu PK
- WG4: SURFACE POROUS FIBRE-REINFORCED COMPOSITE AS BONE REPLACEMENT IMPLANT: AN ANIMAL STUDY ON INTERPHASE HISTOLOGY
  Mattila RH, Aho AJ, Hautamäki M, Rekola J, Gunn J, Strandberg N, Lassila LVJ and Vallittu PK
- WG4: SURFACE MODIFICATION AND IN VITRO STUDIES OF FIBRE-REINFORCED COMPOSITE SCAFFOLDS FOR BONE TISSUE ENGINEERING
  Kokkari A, Heikinheimo K, Lassila LVJ, Mattila RH, Meretoja VV, Vallittu PK
- WG4: .MULTIPLE DENTOFACIAL DEFORMITIES
  Jenca A., Orenzak M., Danko J., Kizek P., Jenková V., Hanusínová V

14 May 2005

15h30': **Keynote Lecture. Biomaterials and Oral-Facial Regeneration: New Aspects. Speaker: Dra Mª Pau GINEBRA, (UPC, Spain)**

16h30': Coffee break

17h **Oral Communications Chair: Fernando Unda**

17h00': Morphometric Analysis of the Palate using Dense Surface Models
Peter Hammond, Pavel Trefny and Stepanka Balkova

17H15': ROLE OF HUMAN PULP FIBROBLASTS IN ANGIOGENESIS
Lam Hung Tran, Sylvie Mathieu, Imad About

17h30': PROLIFERATION OF EPITHELIAL RESTS OF MALASSEZ FOLLOWING AUTOTRANSPLANTATION
T. Struys, C. Politis, L. Vrielinck, S. Schepers and I. Lambrichts

17h45': HYDROPHOBICITY AS A DESIGN CRITERION FOR POLYMER SCAFFOLDS IN BONE TISSUE ENGINEERING.
Jansen EJ, Sladek RE, Bahar H, Yaffe A, Gijbels MJ, Kuijer R, Bulstra SK, Guldemond NA, Binderman I, Koole LH.

18h00' INTEGRATION OF HA GRANULES ON/IN ALVEOLAR BONE IN EXPERIMENT AND CLINIC
A.Skagers, L.Feldman, V.Groma, M.Pilmane, G.Salms, K.Stamers, L.Berzina, R.Cimdins

NEW TRENDS IN BIOMATERIALS FOR BONE REGENERATION: CALCIUM PHOSPHATE BONE SUBSTITUTES

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Nowadays, one of the main concerns of the Biomaterials community is to be able not only to replace damaged tissues, but also to regenerate them. For this purpose, it is necessary to find materials that are able to elicit a specific response from the host, being at the same time bioresorbable: during the healing process, the material should disappear, being replaced by the newly formed healthy tissue. In the specific area of bone regeneration, Calcium Phosphate based materials are the best fitted candidates. They are not only biocompatible, bioactive, and can be considered as bioinspired materials, since nonstoichiometric hydroxyapatite is the mineral phase of bone, but they can also be bioresorbable. In this work, four different approaches to the application of Calcium Phosphates in the Bone regeneration field are presented.

Calcium Phosphate Cements

In 1983 Brown and Chow showed the possibility to form hydroxyapatite in a monolithic form, at room or body temperature, by means of a cementitious reaction. This was an important breakthrough in the field of bioceramics research, since it supplied a material which was mouldable, and therefore could adapt to the bone cavity, presenting a good fixation and an optimum tissue-biomaterial contact, necessary for stimulating the bone ingrowth. Since then, calcium phosphate cements have attracted much attention and different formulations have been developed. More recently, injectable calcium phosphate cement formulations have established good prospects for injectable bone substitution materials, which are used in percutaneous techniques developed in recent years, less aggressive than the classical surgical methods. An additional advantage of calcium phosphate cements, is that the product of the reaction, usually a hydroxyapatite, is obtained at low temperature. Consequently, it is much more similar to the biological hydroxyapatite than the hydroxyapatite prepared by the conventional high temperature sintering methods: it is nonstoichiometric, and it has a low crystallinity and a high specific surface (Ginebra et al, 2004). These characteristics increase the reactivity and resorbability of these materials.

Calcium Phosphate Glasses

Calcium phosphate glasses have also a chemical composition which can be adjusted to resemble that of the mineral phase of bone. These materials offer a distinct advantage in relation to crystalline calcium phosphates, since they allow a gradual adjustment of their chemical composition and consequently of their solubility, in wide ranges (Navarro et al, 2003). Indeed, they have been proposed as bone cavity filling materials, as tissue engineering scaffolds and as a reinforcing phase of fully degradable composite materials.

Biodegradable Composites

Biodegradable composites have been prepared by using calcium phosphate glasses as the reinforcing phase of polylactic acid. The composites are prepared by solving and casting Poly(95L/5DL)lactic acid with the glass in chloroform. The volume fraction of glass can be varied to adjust the mechanical properties of such composites. Macroporous scaffolds can also be prepared with porosities above 90 vol%, by different processing techniques (Navarro et al, 2004).

Biomimetic Calcium Phosphate Coatings

Initially, the bone-bonding ability was restricted to some materials, such as calcium phosphates and Si based glasses. However, in the last years some surface treatments have been developed, which confer this ability to other materials, such as metals and polymers. It is necessary to generate the chemical conditions at the interface that induce the precipitation a calcium phosphate layer in vivo, which is known to be an essential requirement for the bone bonding formation. In this approach, the surface modification techniques allow for the modulation of the biological response, whereas other properties, such as the mechanical ones, are determined by the substrate used (Aparicio et al, 2002).

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MORPHOMETRIC ANALYSIS OF THE PALATE USING DENSE SURFACE MODELS

Peter Hammond[1], Pavel Trefny[2] and Stepanka Balkova[2]

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Subjects: 117 healthy females (5.0 to 25.5 years old) with an average age of 12.8 years and standard deviation of 4.5 years; 113 healthy males (6.4 to 25.3 years old) with an average age of 12.8 years and standard deviation of 4.2 years.
Maxillary dental stone casts were digitized using a Roland LPX-250 3D laser scanner (Roland DG Corp.) with a lateral resolution of 200 microns. The scanned surfaces were processed using the Pixform reverse engineering software (Roland DG Corp.).
Four landmarks were added manually to each surface: incisive papilla (IP); intersection of the lingual groove and gingival margin on the first left and right permanent molars (ML, MR); orthogonal projection relative to the IP-ML-MR plane of the mid-point of the line ML-MR onto the palate surface (MP). Dense surface models (DSMs) of various subsets of the landmarked surfaces were generated using software developed in-house (Hammond et al, 2004).
For the purposes of visualisation of shape differences, two DSMs were computed for all 230 palate surfaces using two base meshes, one with posterior dental surfaces and one without. For both models, the average male and female surfaces were computed.
Twenty random splits of the 230 surfaces into 90%-10% training-test set pairs, stratified with respect to sex, were generated. The DSM mode representations of the cast surfaces computed from each training set were presented as input to three pattern-recognition algorithms (closest mean, linear discriminant analysis (LDA) and support vector machines (SVM)) to build discrimination models for classifying the associated test set unseen as male or female. The average equal error rate (EER), the point on an ROC curve where true positive and false positive rates sum to unity, was computed for each algorithm from the discrimination performance of the 20 training-test set pairs. The same calculations were repeated for the subset of individuals aged over 14 years.
Dynamic morphs between the male and female mean surfaces for the two visualisation DSMs show anterior shape and height differences of the palate surfaces in particular. The inclusion/exclusion of teeth surfaces did not affect classification performance. For discrimination testing using all 230 palate surfaces, the average EER was 24.0% for closest mean and 24.5% for both LDA and SVM. By comparison, for individuals over the age of 14 years, the average EER was lower at 20.5% for each algorithm.
Sexual dimorphism in some palatal dimensions is well established. Previous studies of palate shape in 3D have used linear measurements or curves on the surface to compare male-female palate shape. Our study using the full palate surface both visualises surface shape differences and demonstrates that gender classification of palate surface shape can be achieved with a success rate of 80%.

ROLE OF HUMAN PULP FIBROBLASTS IN ANGIOGENESIS

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Angiogenesis occurs during embryonic development, chronic inflammation, tumor growth and wound healing. It is a complex process with extracellular matrix remodeling, secretion of proteolytic enzymes, endothelial cell migration and proliferation, capillary differentiation and anastomosis. This process is regulated by interplay of numerous cytokines and growth factors.
The dental pulp is a highly vascularised tissue and pulp cavities preparations result in a subsequent injury of pulp tissue including the blood vessels. It is well established that injured endothelial cells release signaling molecules to initiate inflammatory reactions and the healing process. Moreover, pulp fibroblasts secrete growth factors which may be involved in angiogenesis. Our hypothesis was that pulp fibroblasts may be involved in endothelial cell organization by stimulating angiogenesis in the pulp particularly at the injury site.
For this study, we used an in vitro model of angiogenesis. Human pulp fibroblasts and L 929 cells were fluorescence-labeled by transduction with the Enhanced Green Fluorescent Protein (EGFP). Similarly, human umbilical vein endothelial cells (HUVEC) were labeled with the Discosoma Red Fluorescent Protein-2 (DsRed2). The human pulp fibroblasts or L 929 fibroblasts were co cultured with HUVEC cells at a ratio of 80%/20% on a Matrigel extracellular matrix. Angiogenesis was studied under a fluorescent microscope by examining the formation of tubular structures corresponding to capillaries *in vivo*.
HUVEC cells co cultured with pulp fibroblasts organize into tubular structures. This organization was time-dependent and tubular structures were already observed after 24 hours. HUVEC cells co cultured with L 929 cells remained separated from each other and no organization was found even after 7 days. In order to find out if the direct contact between HUVEC and pulp cells was necessary to induce angiogenesis, pulp fibroblasts were cultured in EGM- medium with or without artificial injuries. The medium was then used for the culture of HUVEC cells on Matrigel Extracellular matrix. The results show that the effects on angiogenesis are due to soluble factors in the culture medium. The effects on angiogenesis increase with media obtained from pulp cells artificial injuries. The effects of candidate molecules on angiogenesis were examined: FGF-2 and VEGF. Immunohistochemistry showed here that both are expressed in human third molar pulps while other works showed by ELISA that they may be secreted in the dental pulp. In the presence of both growth factors, HUVEC cells formed tubular structures within 3 hours. The tubular structures became larger and regular at 24 hours. In the absence of both factors, angiogenesis was not observed at 3 hours and cells remained isolated. A partial but discontinuous organization was observed after 24 hours. FGF-2 was more potent than VEGF in stimulating the HUVEC tubular organization.
These results suggest that, the pulp fibroblasts are involved in the special organization of endothelial cells in tubular structures particularly at the pulp injury site.

Keywords: pulp fibroblasts, angiogenesis, injury, growth factors.

PROLIFERATION OF EPITHELIAL RESTS OF MALASSEZ FOLLOWING AUTOTRANSPLANTATION

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The periodontal ligament (PDL) is the dense fibrous connective tissue that occupies the periodontal space between the root of the tooth and the alveolar bone. Its main function lies in the attachment of the tooth to the alveolus. A special feature of the PDL is the epithelial rests of Malassez (ERM). The precise function of the ERM is not known yet, but it is believed they are involved in preventing root resorption and maintaining the width of the periodontal ligament, thereby preventing ankylosis. Since it is reported that proliferation of ERM occurs during experimental tooth movement (1), we were interested to see whether autotransplantation has the same effect.
Autotransplanted teeth were extracted with informed consent of the patient and collected in formol (6%). Later we decided to study the periodontal ligament with the light (LM) and transmission electron microscopy (TEM). Tissue was collected by removing PDL from the root and fixed in 2% glutaraldehyde in 0.05M cacodylate buffer (pH 7.3). Semithin and ultrathin sections were prepared using routine embedding and sectioning techniques.
We described the morphology of the PDL, and in particular the ERM. From light microscopic examination, we could conclude that the ERM were slightly larger than in normal PDL, with a mean value of 20 cells per island. Compartimentalization of collagen bundles in the PDL was also noted. From LM and TEM analysis we concluded that the autotransplantation were successful because fully developed PDL components (cells, connective tissue fibres, interstitial tissue, blood and lymphatic vessels, and nerves). The enlargement of the ERM seen with the light microscope was confirmed by the TEM images. Another interesting feature was the close apposition of fine neural structures to the ERM after transplantation.
It is known that ERM cells produce inflammatory mediators like prostaglandin E2, which are capable of activating osteoclasts and in this way stimulating bone breakdown and bone remodelling (3). In case of autotransplantation, the alveolar bone around the implantation-site has to be remodelled to provide a good fit for the implanted tooth. Bone breakdown is involved in this remodelling-process. This bone breakdown can be stimulated by increased activity of the ERM. This could explain why the ERM in the PDL of transplanted teeth are proliferating. Not only the alveolar bone has to be remodelled, but also the PDL itself. The compartimentalization of the collagen bundles is a possible consequence of this remodelling-process. The neuro-epithelial relationships might be important in the remodelling process.

Keywords: periodontal ligament, epithelial rests of Malassez, autotransplantation

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HYDROPHOBICITY AS A DESIGN CRITERION FOR POLYMER SCAFFOLDS IN BONE TISSUE ENGINEERING.

Jansen EJ, Sladek RE, Bahar H, Yaffe A, Gijbels MJ, Kuijer R, Bulstra SK, Guldemond NA, Binderman I, Koole LH.

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Porous polymeric scaffolds play a key role in most tissue-engineering strategies. A series of non-degrading porous scaffolds was prepared, based on bulk-copolymerisation of 1-vinyl-2-pyrrolidinone (NVP) and n-butyl methacrylate (BMA), followed by a particulate-leaching step to generate porosity. Biocompatibility of these scaffolds was evaluated in vitro and in vivo. Furthermore, the scaffold materials were studied using the so-called demineralised bone matrix (DBM) as an evaluation system in vivo. The DBM, which is essentially a part of a rat femoral bone after processing with mineral acid, provides a suitable environment for ectopic bone formation, provided that the cavity of the DBM is filled with bone marrow prior to subcutaneous implantation in the thoracic region of rats. Various scaffold materials, differing with respect to composition and, hence, hydrophilicity, were introduced into the centre of DBMs. The ends were closed with rat bone marrow, and ectopic bone formation was monitored after 4, 6, and 8 weeks, both through X-ray microradiography and histology. The 50:50 scaffold particles were found to readily accommodate formation of bone tissue within their pores, whereas this was much less the case for the more hydrophilic 70:30 counterpart scaffolds. New healthy bone tissue was encountered inside the pores of the 50:50 scaffold material, not only at the periphery of the constructs but also in the center. Active osteoblast cells were found at the bone-biomaterial interfaces. These data indicate that the hydrophobicity of the biomaterial is, most likely, an important design criterion for polymeric scaffolds which should promote the healing of bone defects. Furthermore, it is argued that stable, non-degrading porous biomaterials, like those used in this study, provide an important tool to expand our comprehension of the role of biomaterials in scaffold-based tissue engineering approaches.

INTEGRATION OF HA GRANULES ON/IN ALVEOLAR BONE IN EXPERIMENT AND CLINIC

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The aim of this study was to evaluate the response of alveolar bone and surrounding tissue to subperiostal on- and inlay implantation of granules of porous synthetic hydroxyapatite (HA) produced in Laboratory of Biomaterials Riga Technical University in experimental and clinical conditions. In experimental part, samples for histomorphological evaluation using staining by HE, van Gieson, immunohistochemical reactions with anti-actin and anti-collagen type VI monoclonal antibodies and EXACT Grunding method were taken out two weeks, 1, 3 and 4 months after implantation. In clinical part consisting of 46 dental implant patients, HA granules were put around implants in fresh extraction sites, to fill extraction sites when implants were put near, to close the perforations of vestibular cortical plate and increase narrow alveolar ridge. Clinical evaluation was done at second stage surgery when also samples of alveolar crest soft tissue were taken out for histomorphological examination. In 8 cases, implantation of HA granules was done as separate stage to enlarge alveolar bone insufficient for same stage dental implant insertion which was done 6 months later.

There were no suppurative complications neither in experimental or clinical group. Pathomorphological picture after 2 weeks of soft tissue around HA granules included vasculitis, haemorrhagies, active macrophages and inflammatory cells. After one month acute inflammatory response was minimal and gigantocellular activity in peri-HA granules was observed. After 3 or more months, inflammatory response mostly was changed to reparative regeneration of bone and cartilaginous tissue also endochondral ossification, only some mononuclear inflammatory cells were found. In undecalcined samples after 3 months there were no inflammatory cells around HA granules which were encircled by irregular collagen and neural fibres, focus of bone formation and also normal muscle tissue. Only slight vacuolisation in superficial epithelial layer was present. Immunohistochemistry in patient material of overlapping gingival tissue showed actin expression in the vasculature of gingival papilla and in stratum spinosum of epithelial layer. Expression of collagen type VI was in fiber protrusions of papillar layer into the gingival epithelium.

Morphological evaluation of alveolar bone and soft tissue response to implantation of synthetic HA granules confirmed good biocompatibility and positive reactogenicity in experiment and clinical cases.

BIOLOGICAL BEHAVIOR OF A POROUS BONE CEMENT IMPLANTED IN RAT TIBIA (POSTER)

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Acrylic bone cements are generally used in fixation of total hip replacements. The plain unmodified acrylic bone cements prevent bone ingrowth into the cement layer, so new bone formation is limited to the interface between the bone cement and the bone. In this study, the biological behaviour of porous bone cement was investigated in vivo.

In vivo, the porous structure was in situ created using pore-generating filler (20 wt%), i.e. an experimental biodegradable polyamide of naturally occurring trans-4-hydroxy-L-proline, which was incorporated in non-degradable acrylic bone cement. The test specimens also contained 8 wt% of chopped E-glass fibres (length =1 mm). The pore-generating filler and chopped E-glass fibre modified acrylic bone cement (N=4) and control cement (i.e. the plain unmodified acrylic bone cement, N=4) were placed in tibia of rats. Eight weeks after the implantation the rats were sacrificed and the histological evaluation of both groups was conducted.

According to the histological evaluation of the modified bone cement, signs of bone ingrowth to the cement were noticed. The bone ingrowth filled the porous structure of the pore-generating filler and E-glass fibre modified bone cement. It was also remarked that the bone ingrowth seems to be guided by the E-glass fibres to some extend. Therefore, the possible osteoconductivity of E-glass fibres should be investigated in more detail in the future.

Within the limitations of the animal experiment by rat, the pore-generating filler modified acrylic bone cement demonstrated performance to bone ingrowth into the material and formed a direct contact between the cement and the bone tissue.

Keywords: Prosthesis, Acrylic Bone Cement, Porous Structure, Fibre Reinforced Composite

CALCIFIED TISSUES INVOLVED IN THE DENTAL ANKYLOSIS PROCESS (POSTER)

P. Carvalho-Lobato(1), C. Pilipili(3), V Tallon-Walton(1), J. Franch-Serracanta(2), M.P. Lafuente-Baigorri(2), I. Serra-Renom(1), M. C. Manzanares-Céspedes(1)
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The interactions between the alveolar osseous tissues and the dental tissues are determinant factors for the viability of both the ankylosed deciduous teeth and the eventual definitive teeth situated below. However, the studies aimed to apprehend the chronology of the tissular events in the ankylosis process around the teeth have been scarce until recently.We present the first results of a series of studies dealing with the tissue interactions established between the alveolar tissues and the teeth in an experiment in which a forced teeth reimplantation was surgically obtained. Twenty adult female dogs were operated, under general anesthesia. The periodontal ligament of both the second maxillary molars were removed after a surgical teeth extraction. Afterwards, the natural teeth were replaced and secured by way of a surgical suture of the gingiva. The ensuing dental ankylosis was evaluated clinically. The sacrifice of the animals was performed by anaesthesia overdose 2 to 18 weeks after the intervention. The alveolar bone samples were submitted to a scheduled procedure of embedding in plastic polymers without prior decalcification, in order to perform ultrastructural studies: scanning microscopy with secondary and backscattered electrons (BS-SEM).All our samples showed that the calcified tissues involved in the ankylosis process show a very similar pattern that has been previously described for the processes of sutural fusion, fracture healing, teeth eruption and osteointegration of various biomaterials. It involves a first phase of osteoclastic activity, followed by the formation of thin trabeculae of chondroid tissue. After that, the bone apposition occurs, starting with woven bone, then followed by lamellar bone. These tissues seem to be the responsibles of the adherence of the teeth to the dental socket. The ubiquitous presence of chondroid tissue in the samples strongly suggests that the mechanisms involved in the dental ankylosis are related to the endomembranous ossification process, rather than to the endochondral ossification, as is the case in other processes mentionned.

Keywords: Bone, Chondroid Tissue, Ankylosis, Teeth

The authors express their acknowledgement for the COST B23 Action support.

PHOTOPOLYMERISABLE FIBER-REINFORCED COMPOSITE AS BONE REPAIR MATERIAL (POSTER)

Tuusa SM-R[1], Lassila LVJ[1], Puska MA[1], Matinlinna JP[1], Peltola MJ[2], Tirri T[1], Meretoja V[1] and Vallittu PK[1].

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In this project, photopolymerisable fiber-reinforced composites have been tested as possible bone repairing material. In the experiments continuous random oriented E-glass fibre-reinforced composite (FRC) (veil) has been impregnated with BisGMA/TEGDMA/PMMA resin system or with dendrimer (DD1)/BDDMA resin system. DD1 is methacrylate functionalized polyester resin of hyperbranched type. In the series of studies, it was found that it was possible to photopolymerise the BisGMA based FRC by free radical polymerisation in contact with bone and blood in vitro. No formation of inhibited surface layer by existence of oxygen or proteins was found of the FRC. The same FRC material was implanted to artificially made frontal bone defects of NZW rabbits for follow-up times of 3, 6 and 8 weeks. The polymer surface of the FRC was coated with bioactive glass granules for promoting new bone formation. The animal experiment showed that the implant promoted new bone formation, but there were signs of lower biocompatibility where the polymer of BisGMA resin system was particularly examined. Some signs of inflammation reaction persisted on implant surface. For this reason, DD1/BDDMA resin mixture have been developed to replace the BisGMA-based resin system of the FRC. In a preliminary cell culture study, primary rat osteoblasts were able to attach and spread to the small flats on polymerised DD1/BDDMA between the reinforcing glass fibers, thus predicting better biocompatibility of this polymer than BisGMA-based polymer. The biocompatibility of the new resin system is going to be evaluated with animal experiments.

Keywords: FRC, resin, biocompatibility

MULTIPLE DENTOFACIAL DEFORMITIES(POSTER)

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The aim of this paper is to present our experience in the simultaneous surgical treatment for skeletal and soft tissue deformity. Patients with the cleft palate, mid-face hypoplasia and mandibular excess require complex surgical planning of maxillary advancement, transversal expansion and osteo-reconstruction of the cleft and prostetic rehabilitation.

We present case report of patient with multiple dentofacial deformity of the hard and soft tissue. We used allograft materials for osteo-reconstructive procces of cleft to induce osteoplastic correction. Our surgical treatment consists in Le Fort I and sagittal split osteotomies for the repositioning of the skeletal frame and surgical therapy of mandibular excess.

Complete therapy of this patient required 5 operations, using of osteotransplantates in combination with autologous osteoplastic implants. Clinical case illustrates the various solutions that can be achieved with correction of the maxillo–mandibular asymmetry combined surgical technique aiming at reconstruction of the soft and hard deformities.

The maxilo mandibular osteotomies are not sufficient to restore good facial symmetry. It is necessary to supplement therapy with autogenic osteotransplantative materials which helps to realized perfect facial remodelling.

Orthodontic and prosthetic therapy is also necessary to allow the patient maximal esthetical and functional benefit.

SURFACE MODIFICATION AND IN VITRO STUDIES OF FIBRE-REINFORCED COMPOSITE SCAFFOLDS FOR BONE TISSUE ENGINEERING (POSTER)

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Three crucial elements needed for bone regeneration include growth factors, cells and extracellular matrix. Bone tissue engineering relates to all these elements by developing controlled growth factor delivery systems, cell-based approaches and new biomimetic scaffolds. The aim of the present study was to examine collagen coated fibre-reinforced composite (FRC) scaffolds *in vitro*. Novel surface porous FRC scaffolds for bone replacement were prepared using random oriented chopped E-glass fibres (length 2mm) within polymethylmethacrylate (PMMA) matrix. Such FRC scaffolds has been shown to be biocompatible in animal studies. However, bioactivity of the scaffolds might be increased for example by using incorporated growth factors or bioactive glass. Of the first one, our earlier studies have revealed that native, hydrophobic PMMA surface can serve as neither an absorbtion nor a controlled release substrate for proteins. Therefore, before incorporation of growth factors, scaffold surfaces should be modified to more protein-friendly.

Thermosetting collagen liquid was used to coat porous surface of the composites. To reduce the dissolving rate of the coatings ultraviolet ligth was used to crosslink collagen molecules. Total amount of collagen and dissolving rates of crosslinked and non-crosslinked collagen coatings were examined *in vitro*. Material characteristics were also investigated under cell culture with primary rat osteoblasts. The dissolving rate of collagen was decreased to about half after the crosslinking procedure. Osteoblasts proliferated and differentiated normally with both coated and uncoated composites and no significant differences were observed. Thus, more research is going to be focused on the possible biological effects of surface modification of FRC.

Keywords: cell culture, fibre-reinforced composite, surface modification

SURFACE POROUS FIBRE-REINFORCED COMPOSITE AS BONE REPLACEMENT IMPLANT: AN ANIMAL STUDY ON INTERPHASE HISTOLOGY (POSTER)

Mattila RH[1], Aho AJ[1,2], Hautamäki M[1], Rekola J[1], Gunn J[1], Strandberg N[1], Lassila LVJ[1] and Vallittu PK[1]

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The aim of the present study was to analyze the histology and bone ingrowth into an artificial fibre-reinforced composite (FRC) bone material intended to be used as an implant. Biomechanical properties of FRC implant can be tailor-made to be equal to bone. A hollow-cylindrical glass fibre-reinforced polymethylmethacrylate (PMMA) composite implant was developed to be used as prosthesis for the reconstruction of large and middle size bone defects in load-bearing conditions. The interconnective surface porosity (pore size maximally ~ 500 μm) of the implant was obtained by the solvent treatment method to achieve the mechanical interlocking between bone and the implant. A surgical segment defect (length: 10 mm) in rabbit tibia was replaced by the surface porous FRC implant or by a control implant made of PMMA only. Fixation of the implant for healing period was made with titanium plate. Bone-implant interphase after 4, 8 and 20 weeks was evaluated by radiographs, conventional histology and histomorphometry quantification. The results revealed higher bone ingrowth values for the surface porous FRC implant than for the control implants. New bone formation was also detected in the medullary canal (hollow of the implant) of the FRC implant.

Keywords: animal study, bone, fibre-reinforced composite