Pre-clinical *(in vitro & in vivo)* studies

   


   The aim of this experimental animal study was to assess guided bone regeneration (GBR) and implant stability (ISQ) around two dental implants with different macrogeometries.

   MATERIAL AND METHODS: Forty-eight dental implants were placed within six Beagle dogs. GBR was performed to fill buccal defects using maxresorb® bone grafting material and Jason® membrane to cover the graft. The implants were divided into two groups (n = 24 per group): G1 group implants presented semi-conical macrogeometry, a low apical self-tapping portion, and an external hexagonal connection (whereby the cervical portion was bigger than the implant body). G2 group implants presented parallel walls macrogeometry, a strong apical self-tapping portion, and an external hexagonal connection (with the cervical portion parallel to the implant body). Buccal (mouth-related) defects of 2 mm (c2 condition) and 5 mm (c3 condition) were created. For the control condition with no defect (c1), implants were installed at crestal bone level. Eight implants in each group were installed under each condition. The implant stability quotient (ISQ) was measured immediately after implant placement, and on the day of sacrifice (3 months after the implant placement). Histological and histomorphometric procedures and analysis were performed to assess all samples, measuring crestal bone loss (CBL) and bone-to-implant contact (BIC).

   RESULTS: The data obtained were compared with statistical significance set at p < 0.05. The ISQ results showed a similar evolution between the groups at the two evaluation times, although higher values were found in the G1 group under all conditions. Within the limitations of this animal study, it may be concluded that implant macrogeometry is an important factor influencing guided bone regeneration in buccal defects. Group G1 showed better buccal bone regeneration (CBL) and BIC [%] at 3 months follow up, also parallel collar design can stimulate bone regeneration more than divergent collar design implants.

   CONCLUSION: The apical portion of the implant, with a stronger self-tapping feature, may provide better initial stability, even in the presence of a bone defect in the buccal area.
2. Comparison of titanium dioxide scaffold with commercial bone graft materials through micro-finite element modelling in flow perfusion.


TiO2 scaffolds have previously shown to have promising osteoconductive properties in previous in vivo experiments. Appropriate mechanical stimuli can further promote this osteoconductive behaviour. However, the complex mechanical environment and the mechanical stimuli enhancing bone regeneration for porous bioceramics have not yet been fully elucidated. This paper aims to compare and evaluate mechanical environment of TiO2 scaffold with three commercial CaP biomaterials, i.e. Bio-Oss, cerabone® and maxresorb® under simulated perfusion culture conditions.

Material and Methods: The solid phase and fluid phase were modelled as linear elastic material and Newtonian fluid, respectively. The mechanical stimulus was analysed within these porous scaffolds quantitatively.

Results: The results showed that the TiO2 had nearly heterogeneous stress distributions, however lower effective Young’s modulus than cerabone® and maxresorb®. The permeability and wall shear stress (WSS) for the TiO2 scaffold was significantly higher than other commercial bone substitute materials. maxresorb® and Bio-Oss showed lowest permeability and local areas of very high WSS. The detailed description of the mechanical performance of these scaffolds could help researchers to predict cell behaviour and to select the most appropriate scaffold for different in vitro and in vivo performances.

Graphical abstract Schematic representation of the establishment procedure. Take the establishment process of cerabone® as an example. Left shows a slice of micro-CT image from cerabone®, and 1.5 mm × 1.5 mm region of interest was shown in the red box. A 1.5-mm3 cube was cut out by Boolean operation in Mimics (Materialise, Belgium), and the cubic model was remeshed in 3-Matic 6.0 (Materialise, Belgium). The cubic model is shown in blue, and the empty space in red.

3. Bone regeneration using composite non-demineralized xenogenic dentin with beta-tricalcium phosphate in experimental alveolar cleft repair in a rabbit model.


The purpose of this study was to evaluate bone regeneration pattern and quantify bone formation after grafting pre-established experimental alveolar clefts defects model in rabbits using composite xenogenic dentin and β-TCP in comparison to β-TCP alone.
METHODS: Unilateral alveolar cleft defects were created in 16 New Zealand rabbits according to previously described methodology. Alveolar clefts were allowed 8 weeks healing period. 8 defects were filled with β-TCP, whereas 8 defects filled with composite xenogenic dentin with β-TCP. Bone regeneration of the healed defects was compared at the 8 weeks after intervention. Quantification of bone formation was analyzed using micro-computed tomography (µCT) and histomorphometric analysis.

RESULTS: µCT and histomorphometric analysis revealed that defects filled with composite dentin/β-TCP showed statistically higher bone volume fraction, bone mineral density and percentage residual graft volume when compared to β-TCP alone. An improved surgical handling of the composite dentin/β-TCP graft was also noted.

CONCLUSIONS: Composite xenogenic dentin/β-TCP putty expresses enhanced bone regeneration compared to β-TCP alone in the reconstruction of rabbit alveolar clefts defects.

4. In vitro evaluation of an injectable biphasic calcium phosphate (BCP) carrier system combined with recombinant human bone morphogenetic protein (rhBMP)-9.


The aim of the present study was to evaluate the possibility of combining rhBMP9 with an injectable biphasic calcium phosphate (I-BCP, maxresorb inject®), since I-BCP is an easy to handle biomaterial with ideal properties for bone augmentation procedures.

The adsorption potential of rhBMP9 as well as the cell behavior of bone stromal ST2 cells were investigated on cell viability, adhesion, proliferation and osteogenic differentiation for I-BCP combined with/without rhBMP9 in vitro. I-BCP demonstrated excellent adsorption/retention potential of rhBMP9 with a slow and steady release over a 10-day period by ELISA. Cell attachment at 8 hours and cell proliferation at one, 3 and 5 days was decreased on I-BCP with/without rhBMP9 when compared to control tissue-culture plastic. While I-BCP had little influence on osteoblast differentiation, its combination with rhBMP9 significantly increased ALP activity at 7 days and mRNA levels of osteoblast differentiation markers including ALP and osteocalcin at 14 days. I-BCP served as an excellent carrier for rhBMP9 clearly demonstrating its osteoinductive potential. We therefore confirm the great potential of rhBMP9 to serve as a future regenerative growth factor for bone applications.


Investigation of the dimensional changes and molecular mobility by Dynamic Mechanical Analysis (DMA) of xenograft (cerabone®), synthetic (maxresorb®), and allograft (maxgraft®, Puros®) blocks in a wet and dry state. While no significant differences could be seen in dry state, cerabone® and maxresorb® blocks showed a slight height decrease in wet state, whereas both maxgraft® and Puros® had an almost identical height increase. In addition, cerabone® and maxresorb® blocks remained highly rigid and their damping behaviour was not influenced by the water. On the other hand, both maxgraft® and Puros® had a strong increase in their molecular mobility with different damping behaviour profiles during the wet state. A high-speed microscopical imaging system was used to analyze the hydrophilicity in several naturally derived (cerabone®, Bio-Oss®, NuOss®, SIC® nature graft) and synthetic DBGS granules (maxresorb®, BoneCeramic®, NanoBone®, Ceros®). The highest level of hydrophilicity was detected in cerabone® and maxresorb®, while Bio-Oss® and BoneCeramic® had the lowest level of hydrophilicity among both naturally derived and synthetic DBGS groups. Deviations among the DBGS were also addressed via physicochemical differences recorded by Micro Computed Tomography, Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy, X-ray powder Diffractometry, and Thermogravimetric Analysis. Such DBGS variations could influence the volume stability at the grafting site, handling as well as the speed of vascularization and bone regeneration. Therefore, this study initiates a new insight into the DBGS differences and their importance for successful clinical results.

6. Recombinant human bone morphogenetic protein (rhBMP) 9 induces osteoblast differentiation when combined with demineralized freeze-dried bone allografts (DFDBAs) or biphasic calcium phosphate (BCP).


The aim of the present study was to investigate the effects of rhBMP9 in comparison to the clinically 2 on in vitro cell behavior when combined with two bone graft materials including demineralized freeze-dried bone allografts (DFDBAs) and biphasic calcium phosphate (BCP).

MATERIALS AND METHODS: The absorption and release kinetics of rhBMPs from DFDBA and BCP were investigated by ELISA. Moreover, murine bone stromal ST2 cell behavior was investigated on DFDBA or BCP seeded on (1) graft only, (2) rhBMP2 (10 ng/ml), (3) rhBMP2 (100 ng/ml), (4) rhBMP9 (10 ng/ml), and (5) rhBMP9 (100 ng/ml). The effects of rhBMPs on DFDBA and BCP were assessed for cell adhesion,
proliferation, and osteoblast differentiation by alkaline phosphatase (ALP) activity, alizarin red staining, and real-time PCR for genes encoding Runx2, ALP, and bone sialoprotein (BSP).

RESULTS: While both BMPs were gradually released from DFDBA and BCP over time, significantly higher adsorption was observed on BCP when compared to DFDBA. Cell attachment and proliferation was higher on BCP with little influence of either rhBMP2/9. Despite rhBMPs having relatively no effect on cell attachment/proliferation, a pronounced and marked effect was observed on osteoblast differentiation for both rhBMP2/9. Interestingly, it was observed that rhBMP9 induced significantly higher ALP activity, alizarin red staining, and messenger RNA (mRNA) levels of ALP and BSP when compared to rhBMP2. Our results also revealed higher differentiation for rhBMP2/9 with BCP when compared to DFDBA most likely as a result of higher growth factor adsorption.

CONCLUSION: While both rhBMP2/9 combined with DFDBA or BCP induced osteoblast differentiation, rhBMP9 induced greater osteoblast differentiation when compared to rhBMP2.

CLINICAL RELEVANCE: rhBMP9 may be a recombinant growth factor with higher potential to induce new bone formation when compared to rhBMP2. Further in vivo studies are necessary to characterize its regenerative potential in various animal models.

7. Bone substitute material composition and morphology differentially modulate calcium and phosphate release through osteoclast-like cells.


The aim of this study was to determine the material composition and cell-mediated remodelling of different calcium phosphate-based bone substitutes.

Osteoclasts were cultivated on bone substitutes (cerabone®, maxresorb®, and NanoBone) for up to 5 days. Bafilomycin A1 addition served as the control. To determine cellular activity, the supernatant content of calcium and phosphate was measured by inductively coupled plasma optical emission spectrometry. Cells were visualized on the materials by scanning electron microscopy. Material composition and surface characteristics were assessed by energy-dispersive X-ray spectroscopy. Osteoclast-induced calcium and phosphate release was material-specific. maxresorb® exhibited the highest ion release to the medium (P=0.034; calcium 40.25mg/l day 5, phosphate 102.08mg/l day 5) and NanoBone the lowest (P=0.021; calcium 8.43mg/l day 5, phosphate 15.15mg/l day 5); cerabone® was intermediate (P=0.034; calcium 16.34mg/l day 5, phosphate 30.6mg/l day 5). All investigated materials showed unique resorption behaviours. The presented methodology provides a new perspective on the investigation of bone substitute biodegradation, maintaining the material-specific micro- and macrostructure.
Donor age-related biological properties of human dental pulp stem cells change in nanostructured scaffolds.


The aim of the present work is to study how biological properties, such as proliferation and commitment ability, of human adult dental pulp stem cells (DPSCs) relate to the age of the donor. Human dental pulps were extracted from molars of healthy adult subjects aged 16 to >66 years. DPSCs were isolated and cultured in the presence of osteogenic, neurogenic, or vasculogenic differentiation medium. Proliferation ability was evaluated by determining doubling time, and commitment ability was evaluated by gene expression and morphological analyses for tissue-specific markers. The results confirm a well-defined proliferative ability for each donor age group at an early in vitro passage (p2). DPSCs from younger donors (up to 35 years) maintain this ability in long-term cultures (p8). Stem cells of all age donor groups maintain their commitment ability during in vitro culture. In vivo tests on the critical size defect repair process confirmed that DPSCs of all donor ages are a potent tool for bone tissue regeneration when mixed with 3D nanostructured scaffolds.

CONCLUSIONS: Biphasic calcium phosphate functioned well as a scaffolding material allowing mineralized tissue formation. Furthermore, the addiction of absorbable collagen membranes enhanced bone gain compared with non-membrane-treated sites.

*Study refers to Ossceram (Bredent), which was a former private label of maxresorb®.


INTRODUCTION: One of the most important goals of periodontitis therapy is the elimination of deep periodontal pockets. In regenerative periodontal therapy, different types of bone grafts, membranes, growth factors, etc. are used to improve regeneration of lost periodontal tissue. The aim of this study was to evaluate the effect of surgical therapy supported by the use of bone replacement material in the treatment of deep intrabony pockets, compared to surgical treatment (flap surgery) without the use of bone replacement in advanced periodontitis.

METHODS AND MATERIALS: The study included 50 patients of both sexes with advanced periodontitis, divided into two groups. After initial periodontal therapy was performed, plaque index (PI), papillary bleeding index (PBI) were verified, and depth of periodontal pockets was measured in both groups. One group (group 1) of the patients underwent surgical therapy, open flap surgery, while the other group (group 2) underwent the same surgical treatment method (open flap surgery), during which bone defects were filled with bone replacement material.

RESULTS: The results showed that both group 1 and group 2 experienced improvements after periodontal surgical therapy. In group 1, there are no statistically significant changes in all three plaque index measurements (PI), while there has been a significant reduction in PI in group 2 following the surgery. For the PBI index, it was determined that there were statistically significant changes in values in group 1, both after surgical procedures and six months later, as well as in group 2. Statistical analysis of the results of the probing depth of pockets has shown that there are significant changes in the measurement of the depth of periodontal pocket one month after the surgery, as well as six months later, meaning that there has been a significant reduction in the depth of the periodontal pocket one month following the surgery as well as six months later, for both groups. However, we did not determine a statistically significant difference in the probing depth of pockets between these two groups.

CONCLUSION: Six months after a surgical therapy, clinical parameters showed a reduction of the probing depth of the periodontal pocket in both examined groups. The use of bone replacement did not yield significantly better results in reducing the depth of probing compared to the standard flap surgery. We believe that future research should focus on testing the effectiveness of new regenerative methods and materials (bone replacements with various properties, membranes, and surgical methods) that will result in better treatment results with predictable outcomes.
10. Investigation of peri-implant tissue conditions and peri-implant tissue stability in implants placed with simultaneous augmentation procedure: a 3-year retrospective follow-up analysis of a newly developed bone level implant system. 


The aim of the present retrospective analysis was to assess peri-implant tissue conditions and document peri-implant tissue stability in C-Tech implants when placed simultaneously with a GBR augmentation procedure.

METHODS: A total of 47 implants, which were placed simultaneously with a GBR procedure with a synthetic bone substitute material in 20 patients, were investigated clinically and radiologically at least 3 years after loading. Implant survival, the width and thickness of peri-implant keratinized gingiva, probing depth, bleeding on probing (BOP), the Pink Esthetic Score (PES), peri-implant bone loss, and the presence of peri-implant osteolysis were determined.

RESULTS: The follow-up investigation revealed a survival rate of 100% and only low median rates for probing depths (2.7 mm) and BOP (30%). The mean PES was 10.1 from the maximum value of 14. No osseous peri-implant defects were obvious, and the mean bone loss was 0.55 mm.

CONCLUSIONS: In conclusion, implants placed in combination with a GBR procedure can achieve long-term stable functionally and esthetically satisfying results for replacing missing teeth in cases of atrophy of the alveolar crest.

11. Monophasic ß-TCP vs. biphasic HA/ß-TCP in two-stage sinus floor augmentation procedures - a prospective randomized clinical trial. 


The study compares a monophasic (100% ß-TCP) and a biphasic (60% HA and 40% ß-TCP) bone substitute material (BSM) regarding biocompatibility, osteoconductivity and implant stability using histological, radiological and resonance frequency analysis.

MATERIAL AND METHODS: Sixty-seven sinus floor elevations were performed in 60 patients. One patient group (monophasic bone substitute [MBS], 30 patients, 32 sinuses) was augmented by the use of the monophasic material (Bioresorb®, Sybron Implant Solutions, Bremen, Germany), while the second group (biphasic bone substitute [BBS], 30 patients, 35 sinuses) received a biphasic material (maxresorb®, botiss biomaterials, Berlin, Germany). Cone beam CT images were taken immediately after augmentation and prior to implant placement after 6 months. Trephines were harvested, while
the implant bed was prepared. Resonance frequency analysis was performed immediately after implant placement and 6 months later. Descriptive analysis was performed on all augmented sinus (n = 67). For statistical comparison of the groups, one sinus of each bilaterally treated patient was randomly excluded, resulting in 30 sinuses grafted with MBS and 30 sinuses grafted with BBS (n = 60). RESULTS: Histomorphometrical analysis of all sinuses displayed comparable results for both groups regarding new bone matrix (MBS 36.16 ± 19.37%, BBS 38.42 ± 12.61%), residual BSM (MBS 30.26 ± 11.7%, BBS 32.66 ± 12.57%) and non-mineralized tissue (MBS 34.29 ± 18.32%, BBS 28.92 ± 15.04%) (P > 0.05, respectively). Radiological volume of BBS was significantly more stable (volume loss of 22.2% for MBS, 6.66% for BBS; P < 0.001), and homogeneity of the graft after 6 months was higher for BBS than that for MBS (P < 0.05). Resonance frequency analysis endorsed a higher implant stability quotient for BBS after 6 months than that for MBS (MBS 78.31 ± 5.81, BBS 80.42 ± 6.31; P < 0.05, Mann-Whitney U-test, respectively). CONCLUSION: Both monophasic and biphasic materials show good biocompatibility and osteoconductivity with satisfactory support on implant stability. BBS remains more stable in terms of volume maintenance and radiological graft homogeneity after a healing period of 6 months.


The objective of this research was to evaluate implant stability following sinus lift with two grafting materials, and to compare it with the results obtained for the implants placed in a pristine posterior maxilla.

MATERIALS AND METHODS: The study included 44 healthy patients with an existing indication for sinus lift procedure (test group). 46 implants were placed following sinus lift with a pure-phase beta-tricalcium phosphate, while 39 implants were placed following augmentation with 60% hydroxyapatite with 40% beta-tricalcium phosphate material. The control group consisted of 48 healthy patients who were treated with 85 implants but without bone augmentation in posterior maxilla. Astra Tech OsseoSpeed implants were placed in all subjects. Resonance frequency analysis was used in both groups for determining implant stability 4 months after insertion. A mean implant stability quotient (ISQ) was calculated on the basis of 3 measurements.

RESULTS: No statistical difference was observed in ISQ values of implants placed with and without augmentation procedure (p=0.789). Statistically significant difference was not found when ISQ values of implants placed following particular grafting material were compared with ISQ values of corresponding implants in a pristine bone (p=0.697 and p=0.402).

CONCLUSIONS: This study demonstrated that the implant stability is comparable among implants placed in the posterior maxilla regardless of sinus lift and grafting procedure. Implants placed in the
grafted posterior maxilla can be predictably loaded as the implants placed in a non-grafted, pristine maxilla.

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