Histological Study of Novel Bone Grafts Based on β-Cyclodextrin/ Hydroxyapatite for Class II Furcation Defects in Dogs

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ABSTRACT

The aim of the present study is to histologically evaluate the effect of various grafting materials on the periodontal tissues, and examine the signs of their biodegradation, bony-graft integration and evidence of new bone formation. Materials and Methods: three different nanocomposites were synthesized based on β-Cyclodextrin (β-CD), glycidyl methacrylate (GMA), hydroxyethylmethacrylate (HEMA) and hydroxyapatite (HA). In six healthy mongrel dogs full thickness mucoperiosteal flaps were raised on the buccal aspects to create two-wall intrabony defects at the furcation areas. The mandibular premolars on both sides received the grafting materials. Histological analysis was carried out at 1, 2 and 3 months period. The results revealed the increased proliferation rate of osteoblast cells in the presence of the nano HA fillers inside the prepared nanocomposites. The hydrogel (poly GMA-co-HEMA) used did not enhance bone formation and was not completely resorbed and replaced by bone at the end of the 3 months experiment period. Conclusion: this study demonstrated that these novel nanocomposites, especially the grafted β-CD with the copolymer (GMA-co-HEMA) in presence of the HA nanoparticles possessed a bone forming ability and may serve as a delivery vehicle for osteogenic cells and osteogenic growth factor proteins in the bone development process.

Key words: Bone grafts-nanocomposites- furcation defects- histological

Introduction

Reconstruction of tooth supporting structures lost because of infection or trauma is one of the ultimate goals of periodontal treatment (Kasaj et al 2008).

Various graft materials have been used successfully to treat intrabony defects. Filling the regenerative spaces with a biocompatible material provides a scaffold for host resident cells and aid regeneration via osteoinductive or osteoconductive pathways (Fujinami et al 2007).

The autograft procedure has several disadvantages, not only because of the limited supply of the material but also because the surgery is time consuming and invades intra or extraoral sites (Brunsvold and Mellonig 2000). Although some allografts and xenografts have been reported to be osteoinductive /osteocductive, their use is still contr oversial controversial in some parts of the world. Consequently the use of synthetic materials has been proposed for grafting (Shirakata et al 2002).

In hard tissue applications, a variety of systems have been developed to mimic the specifically organized nanoscale structure of bone, which consists of collagenous fibers and mineralized apatite nanocrystals. Composites of apatite crystals and natural polymers have received a great deal of attention with the view that the composite system can provide compositional benefits and preserve structural and biological functions of the damaged tissues in a manner similar or better, to the natural system (Boskey 2000).

In the present study novel biodegradable pH-responsive polymeric hydrogels based on β-cyclodextrin (β-CD) and gelatin was used which was prepared by Haroun and El-Halwany 2010 for controlled protein drug delivery studies.

The use of hydrogels in biomedical and pharmaceutical applications has aided the solution of relatively complicated biocompatibility problems, because they exhibit some properties common to soft biological systems. Cyclodextrins (CD) are torus-shaped cyclic oligosaccharides made of six, seven, or eight glycosidic units linked by α (1→4) bonds and are called α-, β-, and γ-CD, respectively. This molecular structure has the capability to make inclusions (guest–host) compounds with aliphatic and especially aromatic molecules. Cyclodextrins are used in pharmaceutical applications for numerous purposes, including improving the bioavailability of drugs (Noomen et al 2008). Hydroxyapatite (HA), comprises the main inorganic component of hard tissues, which has a variety of applications as bone fillers and bone replacements due to its excellent
bioactivity and osteoconductivity. (HA) are useful alternatives to autogenous bone grafts due to their chemical and structural similarity to the mineral component of bone. Although these materials exhibit excellent bioactivity and osteoconductivity one of their problems is the release of crystals that can impair cell activity and hinder the regeneration processes. As natural bone comprises nanoscale features, it is believed that nanstructured HA can improve the properties of synthetic bone due to its higher surface area (Kasaj et al 2008). Advantages of such nanstructured material in comparison to bulk material are its close contact with the surrounding tissues, quick resorption characteristics and a high number of molecules on its inits surface. It was documented that undisturbed osseous-integration and complete resorption of nanohydroxyapatite paste occurs within 12 weeks (Chris Arts et al 2007). HA/collagen composites have attracted significant interest and hold great promise since they possess excellent bioactivity and osteoconductivity, as well as enhanced mechanical properties (Sotome et al 2004 and Chen et al 2006). Gelatin/HA composite powder was recently synthesized (Chang et al 2003 and Haroun and Migonney 2010).

Kim et al 2005 produced gelatin/HA nanocomposites as a porous scaffold using a biomimetic precipitation method.

Tanahashi et al 1994 found out that a HA layer with the desired thickness could be formed not only on ceramics and metals, but also on polymer films. One of the requirements of HA coating onto organic polymer substrates is good adhesion to the substrates. In order to enhance the interaction between the inorganic apatite and the organic polymers, some researchers have introduced hydrophilic polar groups such as phosphate, carboxyl and hydroxyl groups onto hydrophobic substrates (Kato et al 1996).

Because of the unique bioactivity properties bionanocomposites and the recommendation of using them as scaffolds in bone defects in vivo; (Haroun and Migonney 2010), this study was conducted to histologically evaluate their effect on the surrounding tissues, and examine the signs of their biodegradation, bony-graft integration and evidence of new bone formation.

Materials and Methods

I- Materials:

β-Cyclodextrin (β-CD) and Hydroxyapatite (99%, HA) nano-powder with particle size < 200 nm were obtained from Sigma-Aldrich. Glycidyl methacrylate (99.5%, GMA) and 2- hydroxyethylmethacrylate (96%, HEMA) monomers were obtained from BDH and Acros Organics, respectively. Ceric ammonium nitrate (CAN, 99%) as initiator was obtained from Analytical Univar Reagent, Ajax Chemicals. All chemicals and other reagents were used as received.

II- Methods:

Synthesis of novel β -Cyclodextrin based nanocomposites:

Equal volume of 2.5 ml of HEMA and GMA monomers were mixed in presence of 0.01 grams (g) CAN under a nitrogen gas atmosphere. The copolymer mixture was kept in water bath at 50°C for 2.5 hours (h) to achieve the copolymerization. A fixed weight (0.5 g) from nano filler HA and/or β-CD were well dispersed and mixed ultrasonically onto the copolymer mixture and kept at 50°C for further 30 minutes to complete the copolymerization in the presence of the filler powder. The two copolymer/filler mixture was left overnight at room temperature and then washed with hot ethanol with stirring for 1 hour (h) to remove homopolymer. The mixture was filtered, collected and dried at 60°C for 24 h. The prepared nanocomposites were denoted as follows:

Material I: β-CD-g-(GMA)/HA nanocomposites.
Material II: β-CD-g- poly (GMA- co-HEMA)/HA nanocomposites.
Material III: poly (GMA-co-HEMA) hydrogel copolymer.

Fig. 1: Scanning electron microscope (SEM) micrographs of the prepared bionanocomposites (I) and (II).
Characterization:

Scanning electron micrographs (SEM) were recorded using JXA-840A Electron Probe Micro analyzer JEOL-SEM. For SEM, the substrates were mounted on metal stubs and coated with gold-palladium with thickness of deposit about 75°Angstron at vacuum 7×10-2 millibar and 2.4 killovolt cathodic voltage before being examined using Polaron SEM Coating Instrument.

Experimental Design:

The study protocol was approved by the Ethical Committee of the National Research Center, Cairo, Egypt. Six healthy young adult male mongrel dogs with age range 19 -24 months and weighing from 13 to 16 kg, were selected for the study. Before the experiment, all dogs were examined by a veterinary and those showing with good systemic and periodontal health were included in the study. The mandibular right third premolar (RP3) received material I while the mandibular right fourth premolar (RP4) received material II. The mandibular left third premolar (LP3) received material III. Initially scaling and tooth brushing were performed to the selected teeth to control gingival inflammation for one week. During this period, plaque control was maintained by daily topical application of 0.12% chlorhexidine.

Surgical procedures:

All surgical procedures were performed under systemic anesthesia with an intravenous injection of sodium thiopental solution following sedation. The systemic anesthesia was complemented with infiltration anesthesia to ensure local homeostasis. Following the intrasulcular incisions around the selected teeth, full thickness mucoperiosteal flap was raised on the buccal aspect to create two-wall intrabony defects” box-type” (4 mm inferior, 3 mm horizontal from the buccal surface of the roots in a bucco-lingual direction to the lingual bone wall, using a line tangential to the buccal root surface as reference and 5 mm height from the alveolar crestal bone to the base of the defect) at the furcation area using a water-cooled diamond fissure bur. Each root surface was scaled and planed completely to remove remaining cementum and periodontal ligament. An experimental notch was placed at the most apical part of the exposed root using a round diamond bur with abundant irrigation. These notches were placed on the buccal aspects of the roots and extended interproximally and into the furcation areas as deep as the involvement of the furcation defect permitted, as a guideline for histological analysis. Following placement of the notches, the surgical sites were thoroughly irrigated with sterile saline. The study and control materials were inserted in the created defects. The flaps were repositioned and sutured with non-absorbable sutures. From the evening prior to surgery and for 4 days after surgery intramuscular injections with vibramycin (0.1g/15 kg weight) were administrated. The animals were fed only water-softened dog food to reduce the possibility of local trauma to the site of operation. After 7 days the sutures were removed. Plaque control was maintained by daily topical application of 0.12% chlorhexidine solution until the time of sacrifice, which was performed by a thiopental overdose at 1, 2, and 3 months after surgery. Using a computer program, the intrabony periodontal defects were randomly assigned to treatment with the grafting materials.

Tissue Processing:

After euthanasia, which was performed by a thiopental sodium overdose 1, 2 and 3 months after the regenerative surgeries, the mandibles were dissected, and the tissues containing the experimental specimens were obtained. The tissues were fixed in a 10% formalin solution and decalcified in multiple baths of 10% trichloroacetic acid. After decalcification and dehydration, the tissues were immersed in paraffin, and semi-serial 7-mm-wide histological sections were made in a mesio-distal direction and some of the sections were stained with hematoxylin and eosin (H&E) and other sections were stained with Masson Trichrome stained for histological examination for signs of biodegradation as well as bony graft integration of the new material and evidence of new bone formation.

Histological Analysis:

Nine non-serial histological sections of each block, corresponding to the first three, central and last three sections, which contained notches in the roots were examined with a light microscope under conventional and polarized light to evaluate the areas of new bone formation in the periodontal defects.

Histological analysis was performed by an examiner with no prior knowledge of the experimental design using the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd, Cambridge, England) with optical magnification of 200 x on the H&E and the Masson Trichrome slides.
Statistical Analysis:

Data were presented as mean and standard deviation values. Friedman's test was used for comparison between scores in the three groups. Wilcoxon signed-rank test was used for pair-wise comparison between the groups if Friedman's test renders a statistically significant result. The significance level was set at $p \leq 0.05$. Statistical analysis was performed with PASW Statistics 18.0® (Predictive Analytics Software) for Windows.  

® SPSS: An IBM Company, Chicago, IL, USA.

Results:

I. Clinical findings:

Clinical healing proceeded uneventfully. The animals tolerated the surgical procedures well and postoperative signs were consistent with these following a localized periodontal flap surgery. At the time of sacrifice, all dogs showed a healthy periodontal condition without gingival recession or inflammation. There were no significant difference between the different experimental surgical sites based on clinical observation.

II. Histological findings:

A. Material I:

1. One Month after Surgery:

The grafted material partially converted to new bone. Varying amount of trabecular shaped bone was seen throughout the entire grafted area. These bone trabeculae illustrated distinctive features as enclosed widened osteocytic lacunae enfolding multiple irregular sized and shaped fibrovascular connective tissue spaces. These spaces are outlined by a thin rim of newly formed osteoid tissue which was stained blue by Masson Trichrome as shown in figures 8 and 9.

2. Two Months after Surgery:

Wide patches of newly formed bone were the hallmark feature in these specimens as seen in the H&E as well as the Masson Trichrome stain histological sections. The woven bone present stood clearly demarcated from the mature Haverisan system aside. The newly formed bone exhibited widened osteocytic lacunae, irregular collagen fibers, irregular sized and shaped marrow cavities and early lamellation in close proximity to some marrow cavities. New attachment can be seen between the tooth and the newly formed bone. Areas of remodeled old bone can be noticed beside the newly formed bone. This old bone surfaces showed festooning which reflected preceding remodeling activity by osteoclasts. No inflammatory reaction can be seen during this stage as revealed in figures 13, 14 and 15.

3. Three Months after Surgery:

The grafted area was completely occupied with bone which showed the woven nature otherwise areas of lamellar bone can be distinguished. Resting and reversal lines can be spotted. Condensation of mesenchyme and fibrous connective tissue were seen overlying the bone surfaces. The mature lamellar areas exhibited in some way regular marrow cavities with osteoblastic rimming. Multinuclear giant cells with osteoclastic features were also seen. The coexistence of both types of cells affirms that the remodeling activity took place. Overall mild inflammatory reaction can be scored during this stage as seen in figure 18.

B. Material II:

1. One Month after Surgery:

Mild to moderate inflammatory reaction was seen in the graft vicinity. Areas of the grafted material were resorbed and replaced by bone trabeculae exhibiting widened osteocytic lacunae outlined by a thinner rim of osteoid than that observed in material I. Modest osteoclastic activity was noticed on the bone surfaces facing the graft material as seen in figures 10 and 11. The fibrovascular spaces monitored during this period were larger and even more irregular than that seen in material I.
2. Two Months after Surgery:

The graft material is completely resorbed and replaced with large areas of woven bone. The newly formed bone enclosed large osteocytic lacunae and irregular marrow cavities. In contrast to material I larger areas of fibrous connective tissue can be seen in the graft area (FCT). The FCT showed a boost of angiogenic activity and mild inflammatory cell infiltration. Cuboid shaped osteoblasts outlining both the marrow cavities and the bone surfaces is one of the characteristic features in these sections. Areas of fully mineralized bone stood clearly in contrast to the newly formed woven bone as seen with Masson Trichrome. Early lamellation can be seen in close proximity to marrow cavities reflecting bone maturation and lamellation as demonstrated in figure 16.

3. Three Months after Surgery:

No material II residues whatsoever were detected in the graft area as all was replaced with normal bone. Sporadic condensations of inflammatory cells were seen. More outsized areas of lamellar bone than that detected in material I were covered by large amount of condensed fibrous connective tissue and collagen bundles. Furthermore, marrow cavities exhibited more regularity in size and shape. Osteoblasts and osteoclasts coexisted to illustrate the remodeling activity of bone as shown in figure 19. As an overall observation worth to note that the bone trabeculae found in material II exhibited more bulk and mature features than that seen in material I.

C. Material III:

1. One Month after Surgery:

The grafted material remained in the graft area. The unresorbed material was predominantly surrounded by granulation tissue which demonstrated significant inflammatory cell infiltration. Bone festooning (indicator of resorption) and multinucleated giant cells having the histological features of osteoclasts were seen residing in their lacunae on the brink of material III. Residual bone spicules bordered the bone defect and extended into the graft area as seen in figure 7.

2. Two Months after Surgery:

Outsized amount of material III can still be seen in the grafted area. No sign of bone was seen in the area whatsoever. Instead, exaggerated granulation tissue reaction can be clearly seen next to material III. Dense infiltration of inflammatory cells predominates in the surgical field. Finally, intercellular edema was one of the distinctive features during this period as reported in figure 12.

<table>
<thead>
<tr>
<th>Table 1:</th>
<th>Mean and standard deviation (SD) results of the comparisons between the different materials.</th>
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<tr>
<td>Variable</td>
<td>Group Period</td>
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<tr>
<td>Non-resorbed material</td>
<td>1month</td>
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<td></td>
<td>2 months</td>
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<td></td>
<td>3 months</td>
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<td>3 months</td>
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<tr>
<td>Osteoclasts</td>
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<td></td>
<td>3 months</td>
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<td>Inflammation</td>
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*: Significant at p ≤ 0.05

3. Three Months after Surgery:

Thinned bone trabeculae are seen radiating from material III. At a standstill, the grafted material can be seen in the grafted area. Mild to moderate inflammatory reaction was demonstrated. These slim trabeculae enclosed irregular sized and shaped marrow spaces; as well they showed widened osteocytic lacunae. A
considerably thick collagen bundle is seen walling off material III from the surrounding bone tissues as reported in figure 17.

**Non-resorbed material:**

After 1, 2 and 3 months, material III showed the statistically significant highest mean score. There was no statistically significant difference between material (I) and material (II); both showed the statistically significant lowest mean scores.

**New bone formation:**

After 1 month, material (I) showed the statistically significant highest mean score. There was no statistically significant difference between material II and III; both showed the statistically significant lowest mean scores. After 2 months, there was no statistically significant difference between the groups. After 3 months, material (I) showed the statistically significant highest mean score. Material III showed the statistically significant lowest mean score.

**Osteoclasts:**

There was no statistically significant difference between the groups through all periods.

**Inflammation:**

After 1, 2 and 3 months, material III showed the statistically significant highest mean score. There was no statistically significant difference between material (I) and material (II); both showed the statistically significant lowest mean scores.

![Fig. 2: Non-resorbed material scores in the three groups.](image)

![Fig. 3: New bone formation scores in the three groups.](image)
Fig. 4: Osteoclasts scores in the three groups.

Fig. 5: Inflammation scores in the three groups.

Fig. 6: New attachment scores in the three groups.

Discussion:

Over the last few decades, a wide range of procedures have been proposed for the treatment of periodontal disease to promote bone formation in intra-osseous defects, among them the use of bone replacement graft materials. These materials should support attachment and proliferation of PDL cells, which are involved in the repair of damaged periodontal tissues (Kasaj et al 2008). An ideal scaffold should have porous structure
comparable to that of human bone. Polymer/ceramic composite materials are gaining increased attention as synthetic alternatives to bone grafting materials (Wang 2003 and Khan et al 2004). Promising results have been achieved indicating that these novel biocomposites have unique bioactive properties, recommending their application in tissue engineering (Haroun and Migonney 2010).

In the present study materials I and II were biocompatible and osteoconductive when placed into intrabony defects, as they were resorbed and converted to bone as demonstrated histologically. Material I being a simple composite demonstrated the highest mean score of new bone formation after one month than material II, which is a copolymer with greater structural complexity than material I. New bone formation with material II was significantly greater than that in the material I, exhibiting more bulk and mature features than material I, suggesting that material II enhanced differentiation of the periodontal ligament cells (PDL) to osteoblasts, thus augmenting the periodontal regenerative potential in the late phase of the healing process. In this study, the microstructure of porous β-CD-g- poly (GMA-co-HEMA) matrix led to vascularization of the matrix itself and this might have been the most important geometrical basic fibroblast growth factor (FGF-2) induced osteogenesis. Consequently, over 3 months with FGF-2/material II, many osteoblasts were observed to attach via the pores of the materials including β-CD cavities. Higher porosity was shown to promote resorption and replacement with bone tissue as previously discussed by Saito et al 2013.

Results of this study revealed that adhesion of osteoblastic cells was greater with FGF-2/material II matrix than with material I matrix. This suggested that poly (GMA-co-HEMA) matrix offers greater absorbance of FGF-2 when grafted with β-CD in presence of HA nanoparticles than other matrices. Similarly, Kim et al 2005 reported that synthetic bone graft material based on poly (lactic-co-glycolic)/HA scaffold implanted in rats had a comparable bone regeneration potential to the bovine-derived bone graft material as demonstrated histologically eight weeks post implantation.

Vascularization of the grafted site ensures adequate supply of nutrients, prompt removal of metabolic by products and delivery of cells and growth factors that support the formation of osseous tissue as presented by Saito et al 2013. In our study many fibrovascular spaces were observed histologically in material II and were larger and more irregular than that seen in material I. Moreover a boost of angiogenic activity was demonstrated in the graft area of material II two months post-surgery. This might have supplied cells and signaling factors that supported the formation of osseous tissue towards the furcation defect.

Nanophase HA had better compatibility and dissolvability than dense HA. Recent research has shown that synthetic nanostructured HA enhance osteoblasts function and has higher biocompatibility for microvascular endothelium, representing a promising class of bone substitutes. Furthermore, it has been shown that proteins (collagen) interact differently with nanophase materials than with conventional ceramics having similar chemical properties (Thorwarth et al 2005). When bioactive nanocomposites come in contact with tissue fluids the hydroxycarbonate apatite layer formed on the surface of the particles incorporate organic proteins such as collagen which attract osteoblasts and in turn release organic constituents followed by mineralization as explained by Felipe et al 2009. The present study demonstrated the increased proliferation rate of osteoblast cells in the presence of nano HA fillers inside the prepared nanocomposites I and II. In a study by Kasaj et al 2008, the increased proliferation rate of PDL cells in the presence of nano-HA paste was mechanically linked to the epidermal growth factor receptor (EGF).

Although poly (GMA-co-HEMA) hydrogels are bioactive and are widely used in the drug- delivery systems, its similarity to native bone mineral is generally poor and their biological properties are far from those of bone. In our study the hydrogel (material III) used did not enhance bone formation and was not completely resorbed and replaced by bone at the end of the experiment 3 months later as demonstrated histologically by the persisting inflammatory cells and the considerably thick collagen bundles walling off the grafted material from the surrounding bone. This hydrogel does not contain HA nanoparticles and hence has a low affinity for bone formation. The high molecular weight and large particle size may have attributed to its incomplete resorption and the persistence of inflammation.

On the other hand, new bone formation, after grafting with β-CD over 3 months was very similar to native bone and thus may provide a more conductive environment for bone regeneration than synthetic HA alone. Chris Arts et al 2007 demonstrated that undisturbed osseous integration and complete resorption of nano-HA paste occurs within 12 weeks. In our study materials I and II were completely resorbed and replaced by woven bone only 8 weeks after surgery which might highlight the effect of grafting with β-CD. HA/collagen composites hold great promise since they possess excellent bioactivity and osteoconductivity, as well as enhanced mechanical properties as documented by Chen et al 2006. Also, β-CD plays an important role for bone regeneration (Kim et al 2005).
Conclusion:

This study demonstrated that these novel nanocomposites, especially the grafted β-CD with the copolymer (GMA-co-HEMA) in presence of HA nanoparticles possessed a bone forming ability and may serve as a delivery vehicle for osteogenic cells and osteogenic growth factor proteins in the bone development process.

Recommendations:

Additional studies are recommended to determine the therapeutic potential of these graft materials in the future.

Fig. 7: Histopathological photomicrograph of the unresorbed material III 1 m after surgery surrounded by granulation tissue GT with significant inflammatory cell infiltration I. Residual bone spicules RBS bordered the bone defect and extended into the graft area.

Fig. 8: Histopathological photomicrograph showing that material I 1 m after surgery partially converted to new bone. Varying amount of trabecular shaped bone TB was seen throughout the entire grafted area with enclosed widened osteocytic lacunae L and multiple irregular sized fibrovascular connective tissue CT spaces.

Fig. 9: Histopathological photomicrograph showing that material I partially converted to new bone 1 month after surgery. Varying amount of trabecular shaped bone TB was seen throughout the entire grafted area outlined by a thin rim of newly formed osteoid tissue Os (stained blue by Masson Trichrome).
Fig. 10: Histopathological photomicrograph showing mild to moderate inflammatory reaction in the graft vicinity 1 month after surgery. Areas of the graft material II were resorbed and replaced by bone trabeculae TB exhibiting widened osteocytic lacunae L. The fibrovascular spaces (FVS) were large and irregular.

Fig. 11: Mild to moderate inflammatory reaction was seen in the graft vicinity 1 m after surgery. Areas of the graft material II were resorbed and replaced by bone trabeculae TB exhibiting widened osteocytic lacunae L outlined by a thinner rim of osteoid Os than that observed in material I. Modest osteoclastic activity was noticed on the bone surfaces facing the graft material Oc. The fibrovascular spaces FVS monitored during this period were larger and even more irregular than that seen in material I.

Fig. 12: Histopathological photomicrograph showing outsized amount of material III in the graft area 2 m after surgery. Exaggerated granulation tissue GT reaction can be clearly seen next to the graft material. Dense accumulation of fibrous tissue DFT was observed walling off the material from the surrounding tissues. Heavy infiltration of inflammatory cells I predominate in the surgical field. Finally, intercellular edema E was one of the distinctive features during this period.

Fig. 13: Histopathological photomicrograph 2 m after surgery showing wide patches of newly formed bone NB in the H&E sections after grafting of material I. The woven bone present stood clearly demarcated from the mature Haverisan system aside H.
Fig. 14: Histopathological photomicrograph 2 m after surgery showing wide patches of newly formed bone NB in the Masson Trichrome stained sections after grafting of material I. The newly formed bone exhibited widened osteocytic lacunae, irregular collagen fibers, irregular sized and shaped marrow cavities M and early lamellation LM in close proximity to some marrow cavities. New attachment NA can be seen between the tooth T and the newly formed bone. Areas of remodeled old bone ROB can be noticed beside the newly formed bone.

Fig. 15: Histopathological photomicrograph 2 m after surgery showing the newly formed bone exhibiting widened osteocytic lacunae L, irregular collagen fibers, irregular sized and shaped marrow cavities and early lamellation L in close proximity to some marrow cavities after grafting with material I. New attachment NA can be seen between the tooth T and the newly formed bone after grafting. Areas of remodeled old bone ROB can be noticed beside the newly formed bone. This old bone surfaces showed festooning F which reflected preceding remodeling activity by osteoclasts. No inflammatory reaction can be seen during this stage in the Masson Trichrome sections.

Fig. 16: a,b and c Histopathological photomicrograph showing that the graft material II 2 m after surgery was completely resorbed and replaced with large areas of woven bone WB. The newly formed bone enclosed large osteocytic lacunae L and irregular marrow cavities M. Large areas of fibrous CT can be seen in the graft area FCT. The FCT showed a boost of angiogenic activity A and mild inflammatory cell infiltration I. Cuboid shaped osteoblasts Ob outlining both the marrow cavities and the bone surfaces is one of the characteristic features in these sections; where a&b) H&E stain, and c) Masson Trichrome stain.
Fig. 16: (b) H &E stain.

Fig. 17: (c) Masson Trichrome stain.

Fig. 16: Histopathological photomicrograph showing thinned bone trabeculae TTB radiating from material III 3 m after surgery. Material III can be seen in the graft area. Mild to moderate inflammatory reaction I was demonstrated. These slim trabeculae enclosed irregular sized and shaped marrow spaces M; as well as widened osteocytic lacunae L. Thick collagen bundles CB are seen walling off the material from the surrounding bone tissues.

Fig. 18: Histopathological photomicrograph showing material I 3 m after surgery which was completely occupied with bone which still show the woven nature WB otherwise areas of lamellar bone LM can be distinguished. Resting and reversal lines can be spotted RL. Condensation of mesenchyme and fibrous connective tissue FCT were seen overlying the bone surfaces. The mature lamellar areas exhibited regular marrow cavities M with osteoblastic rimming Ob. Multinuclear giant cells with osteoclast Oc features were also detected.
Fig. 19: a,b,c: Histopathological photomicrograph showing no graft material II residues 3 m after surgery as all was replaced with normal bone NB as seen in H&E sections. Sporadic condensations of inflammatory cells I were seen. Oustsized areas of lamellar bone LM were covered by large amount of condensed fibrous connective tissue FCT and collagen bundles. Marrow cavities M were regular in size and shape. Osteoblasts Ob and osteoclasts Oc coexisted to illustrate the remodeling activity of bone where a) H&E stain and b&c) Masson Trichrome stain.

Fig. 19b: Masson Trichrome sections.

Fig. 19c: Masson Trichrome sections.

References


