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Efficacy of reconstruction of alveolar bone using an alloplastic hydroxyapatite tricalcium phosphate graft under biodegradable chambers

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Abstract

Our aim was to test the efficacy of a synthetic alloplastic graft under biodegradable chambers to reconstruct a horizontal bony deficiency as an alternative to autogenous, allogeneic, or xenogenic grafts. We used 11 New Zealand white rabbits. On each rabbit's mandible one test sample (grafted chamber) was placed on the (right or left) body, while its control sample (empty (E) chamber) was placed on the other side. Twelve weeks postoperatively the animals were sacrificed and the samples extracted for gross assessment, micro-computed tomographic imaging, and histological and histomorphometric analyses. There was significantly more new bone with a greater surface area in the test group than in the control group, and the alloplastic graft was osteoconductive when used as an onlay graft under a synthetic biodegradable chamber. Synthetic products can be efficient alternatives to autogenic, allogeneic, or xenogenic grafts.

Keywords: Onlay graft; Osteoconductive; Alveolar bone; Reconstruction

Introduction

The placement of dental implants is often compromised by deficiencies in alveolar bone in the horizontal or vertical dimension, or both, as a result of atrophy, periodontal disease, trauma, or infections.[1] Reconstruction of the alveolar ridge has been attempted using autogenous bone grafts, allogeneic bone grafts, xenografts, alloplasts, and the concept of guided bone regeneration.[2] Autogenous bone has been considered to be the gold standard because of its unique osteogenic, osteoconductive, osteoinductive, and continuous remodelling capabilities.[3] However, autogenous bone grafts have some disadvantages, such as morbidity at the donor site, limited quantity, and rapid resorption when compared with non-autogenous grafts.[4] These drawbacks were enough for some groups of patients to refuse autogenous grafts and prefer non-autogenic sources such as allogeneic, xenogeneic, and alloplastic grafts.[5] The fact that allogeneic and xenogeneic grafts are derived from humans and animals, respectively, means that they became a concern to other groups of patients.[2,5] We are therefore investigating a third option which is the alloplastic and synthetic grafts.

We used hydroxyapatite/tricalcium phosphate (HA/TCP; Bone Ceramic, Straumann®, Canada), which is composed of a combination of hydroxyapatite (Ca_5-$(PO_4)_3$-OH) 60% and beta tricalcium phosphate (bTCP; Ca_3-$(PO_4)_2$) 40%. The hydroxyapatite component supports the bulk of the graft with its hard particles, but the faster degradation of the TCP increases the subsequent replacement of its degradation products with blood vessels and mature lamellar bone.

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The osteoconductivity of the HA/TCP mixture has been confirmed by many studies[4–6]; Gosain et al.[7] illustrated it by successfully filling defects in calvarial bone in sheep. Using it as an onlay graft, Gosain et al.[8] compared facial augmentation in 10 sheep using autogenous calvarial bone and HA/TCP. Blocks 16.8 mm × 5 mm were constructed, and implanted at different sites on the faces and craniums. One year later, samples were harvested for analysis of volume, and the results showed that the HA/TCP blocks had more predictable volumes of graft than the autogenous calvarial blocks, which had significantly reduced volume ($p < 0.0001$). This indicates that the HA/TCP graft may be an excellent option for operators to consider according to the demands of the case and the patient's preference.

Another important consideration when planning alveolar reconstruction using particulate grafts is the type of membrane (mesh) that covers the graft. These membranes/meshes are used to stabilise and protect the particulate graft in place. There are several types of membrane available for clinical use that include resorbable, non-resorbable, and hard or soft products. The most common hard and non-resorbable form is titanium mesh, which has the following advantages[4,9]: it is biocompatible; it is easy to manipulate to form a volumetric chamber; it gives excellent support to the underlying graft; and it has a low resorption rate.

The chief disadvantage of titanium meshes is that they do not resorb,[9] so 3–5 months after placement they must be removed before dental implants are placed. The process of removal is usually traumatic to the bone graft and the soft tissue flap, so care should be taken while this is being done. This can be avoided if polylactic acid/polyglycolic acid (PLA/PGA) biodegradable membranes are used. PLA/PGA products are biocompatible,[10] can maintain proper geometric stability,[11] and degrade favourably.[12]

We investigated the combination of two alloplastic synthetic materials—the HA/TCP graft under PLA/PGA biodegradable chambers. To our knowledge this is the first experimental trial that has investigated this combination.

Materials and methods

Eleven adult female New Zealand white rabbits 3–4 months old and weighing 3–4 kg were used. To reduce variability all the operations were done by one surgeon, and the animal health team dealt with all the handling, preparation, feeding, drug injections, and general anaesthetics. Water and food were withheld from each animal 12 h preoperatively and each animal was given a preoperative dose of a first-generation cephalosporin (cefazolin 12.5 mg/kg) intravenously (Novopharm Ltd., Toronto, Canada).

The PLA/PGA membrane was prepared according to the manufacturer's instruction (Inion GTR^{TM}, Finland). The manufacturer's plasticiser, N-methyl-pyrolidone (NMP), a widely used solvent for water insoluble drugs, was used to soften a 0.2 mm dense PLA/PGA membrane (Inion GTR^{TM}, Finland). Subsequently, the softened form was molded to the desired shape by press-forming the material over a metallic template (Fig. 1). The metallic chamber had a total volume of 48 mm^3 and a dimension of 4 mm × 4 mm × 3 mm. The molded Inion membrane had an approximate dimension of 4.5 mm × 4.5 mm × 3.2 mm, and resulted in a chamber volume of 64.8 mm^3 (Fig. 1).

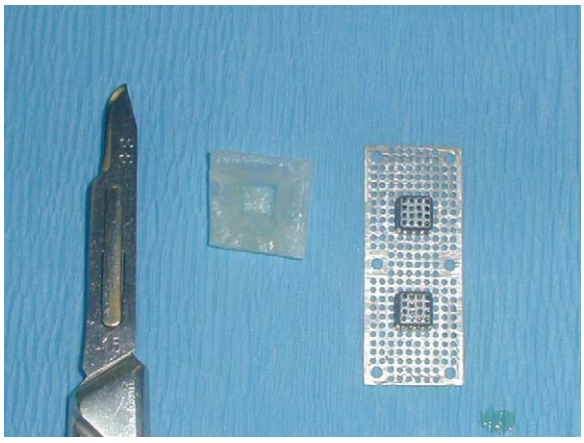

Fig. 1. The PLA/PGA chamber in the middle, showed the size of the replica chamber on the right. The size of the chamber is compared with that of a No. 15 blade.

Just before the operation, the skin over the submandibular area was shaved and disinfected using povidine iodine solution. The animal was then placed on one side laterally and the surgical site was prepared and draped. A 3 cm submandibular skin incision was made along the inferior border of the mandible and carried through skin, subcutaneous tissue, muscle, and periosteum, to expose the lateral aspect of the mandibular body. The HA/TCP graft was taken from its sterile pack, poured into a small dish, wetted with 0.9% normal saline to ease its manipulation, and packed gently into one PLA/PGA biodegradable chamber without excessive force. The chamber was secured over a physiologically bleeding cortical surface on the body of the mandible using 3–4 titanium screws (4–6 mm long/1.7 mm in diameter). The periosteum was protected and kept intact, then carefully laid over the chamber. The incision was closed in layers. The rabbit was then turned on to the other side for placement of the control sample (the E chamber) using the same technique. As all the chambers were placed over a freshly blood oozing cortical surface, the E chamber was expected to be filled out with an organised blood clot that would subsequently stimulate the formation of bone. The bone formed into an organised blood clot, possibly in an extraction socket, and is an example of secondary bony wound healing. The samples were placed randomly in the right and left bodies of the mandible and identified.

Postoperatively all the animals resumed their regular diet on time, and none had any local or systemic complications. Twelve weeks after the grafting procedures all 11 animals were killed and samples taken. All samples were sent to the McGill University Bone Laboratory for micro-computed

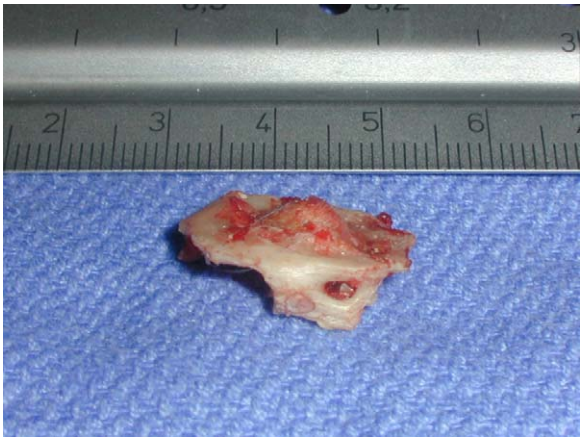

Fig. 2. Lateral view showing the vertical height of a test sample.

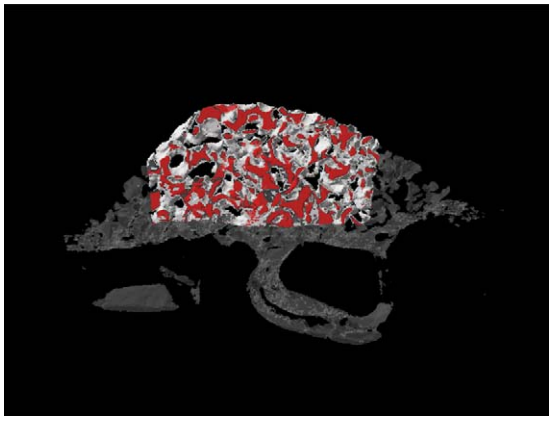

Fig. 3. Micro-computed tomographic three-dimensional image build by the Creator software. Once the bony deposits have been identified (red), measurement of the total volume and height of the bone in the sample is feasible.

tomographic imaging, histological analysis, and histomorphometric measurements.

Student's paired *t* test was used to help with statistical calculations, and probabilities of less than 0.05 were accepted as significant.

Results

Gross description

When the masseter muscle and periosteum had been dissected off the mandible, the titanium screws were identified, and these helped to locate the chambers. The samples were harvested using bone clippers and were trimmed to produce 2 cm × 2 cm specimens (Fig. 2). Next, the remaining muscle layer and fibrous tissue were dissected from the sample, which uncovered partial degradation of all the PLA/PGA chambers. The screws were removed easily using a screwdriver and the specimens were stable.

Micro-computed tomographic (CT) analysis

All the micro-CT digital images were downloaded into three-dimensional Creator software (SkyScan, Kontich, Belgium) which created a three-dimensional model from a sequence of two-dimensional images from each sample (Fig. 3). The areas of bone were calculated from the three-dimensional image together with the total volume of bone. The total percentage of bone volume was defined as the percentage of bone that occupied the total volume of the chamber. The same technique was used to measure the generated bone height, that indicated the height of the bone from the cortical base to the roof of the chambers. The percentage of bone found in the E chambers ranged from 0 to 9.5% (mean (SD) 3.1 (2.6)%). The height of bony growth in the chamber (the mean of the original chamber's height was 3.2 mm), ranged from 0 to 2.0 mm (mean (SD) 1.1 (0.6) mm).

The results of the 11 test samples showed that the percentage of bone found in the chambers ranged from 11.5 to 37.5 (mean (SD) 25.8 (7.3%). The height of bone in the test chambers ranged from 1.6 to 3.2 mm (mean (SD) 2.6 (0.4 mm).

When we compared the HA/TCP bone ceramic and the E groups, the test group had significantly more bony volume that the E group ($p < 0.0001$) and the bone had grown significantly higher ($p < 0.0001$).

Histological examination

The E samples had de novo vital bone growth in the form of multiple linear deposits. In the test samples, bony growths took the shape of multiple circular deposits that were larger and denser closer to the base of the samples than the roof.

Histomorphometric examination

Image-J software (version 1.37) was used for the histomorphometric analysis. The histological two-dimensional slide image from the centre of the chamber was captured and the software was calibrated so that 245.65 pixels = 1 mm. The bony deposits were shaded, and the particle analyser feature in the software was used to calculate the bone surface area. The percentage of bone surface area indicated the percentage of bone that occupied the total chamber's surface area in two dimensions.

The values from the E chambers varied, ranging from completely collapsed chambers to non-collapsed chambers (Fig. 4). The percentage of bone surface area ranged from 0 to 15.1% (mean (SD) 6.2 (5.2)%). Most of the bone was in the lower third of the chamber.

The results from the examination of the 11 test samples showed that the percentage of bone surface area ranged from 7.1 to 33.9% (mean (SD) 20.7 (7.9)%). The bone was distributed throughout the chamber (Fig. 5). The BC group

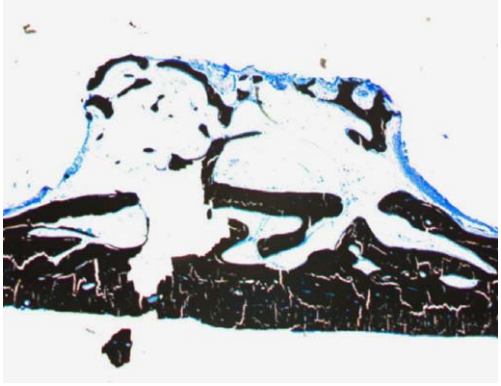

Fig. 4. Histological slide of a control (E) specimen (Von Kossa and toluidine blue stain, original magnification 2.5×). The figure shows a nicely collapsed chamber; the limits of bone are shown as multiple linear deposits.

had significantly more bony surface area than the E group ($p < 0.0001$).

Fourteen of the 22 chambers (64%) maintained their geometric shape (10 were test, and 4 were control, samples), while 8 of the 22 chambers had collapsed (7 control samples showed signs of severe collapse, and partial collapse was seen in 1 test sample).

Discussion

Implant surgeons often encounter patients for whom alveolar bone augmentation is necessary to place dental implants in the proper sites. Alveolar bone can be reconstructed with autogenous bone, or allogeneic, xenogeneic, or alloplastic grafts.[1]

Each grafting technique has advantages and disadvantages. For example, autogenous bone grafts, although considered to be the best grafting material, require a second operative donor site. Allogeneic and xenografts need no second surgical site, but the fact that they originate from other humans or animals makes these materials unacceptable to a lot of patients for religious, traditional, or personal reasons. Synthetic or alloplastic grafting products, such as HA/TCP and PLA/PGA, therefore provide another option for the patients and surgeons.

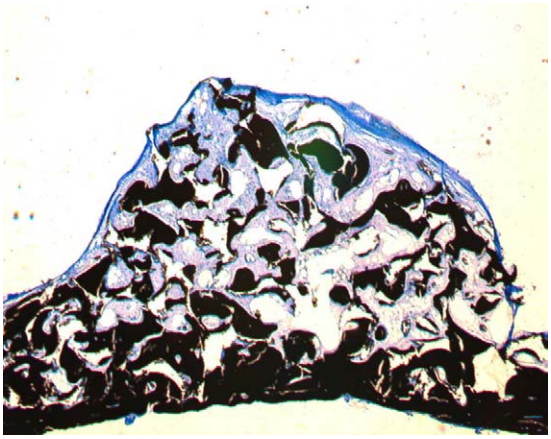

Fig. 5. Histological slide of a bone ceramic graft showing the distribution of bone (black islands stained with Von Kossa & Toluidine Blue) arranged in multiple semicircular deposits. There are denser bony aggregates closer to the base than the roof of the chamber.

The advantages of HA/TCP compared with autogenous grafts, are their synthetic origin, biocompatibility, osteoconductivity,[6] unlimited quantity, and avoidance of a second surgical site. The osteoconductivity of HA/TCP has been examined in many studies,[6–8] and as an onlay, Gosain et al.[7] found more predictable maintenance of volume within the HA/TCP samples than among the samples of autogenous bone. The bone replacement in HA/TCP grafts ranged between 16.4 and 23.9%, which is comparable to the results found in our experiment.

A particulate graft, unlike a block graft, usually requires a stabilising membrane, carrier, or chamber, to protect and localise the underlying graft. These membranes can be biodegradable, resorbable, or non-resorbable. The term "biodegradation" is used to explain the course of resorption or degradation of these membranes in the human body.

Two main categories of resorbable membranes are available for clinical use: synthetic and natural. The thickness can range from 0.05 to 1.5 mm, which leads to variable biodegradation or resorption times ranging from 3 to 36 weeks. The natural products are usually composed of animal type-I collagen,[9] which can be extracted from animals' Achilles tendons. These natural products are biodegraded by enzymatic biochemical processes. The main disadvantage of a collagen membrane is the lack of strength to support a particulate graft.

The other category of resorbable membranes comprises the synthetically fabricated ones. PLA/PGA products are copolymers made of PLA and PGA monomers. The copolymer PLA/PGA is soluble in most of the common solvents including chlorinated solvents, tetrahydrofuran, acetone, or ethyl acetate. PLA/PGA is degraded by hydrolysis in the body to produce the original monomers, lactic acid (LA) and glycolic acid (GA). The final by-products of the degradation of PLA/PGA are water and carbon dioxide.[12,13] The main advantage of a PLA/PGA membrane over a collagen membrane is better stability. However, membranes' physical properties are lost by 2–4 months after implantation, which is usually sufficient time for a graft to incorporate. Complete degradation is usually apparent by 12 months after implantation of the membrane.[6]

We noticed that after 3 months of healing, all the membranes had been partly degraded, though they maintained the bulk of the HA/TCP graft. This means that using PLA/PGA biodegradable membrane is easier than using titanium mesh, which has to be removed at the time that the implant is inserted. This can be a meticulous and demanding procedure, which is time-consuming and may injure the graft and the soft tissue envelope.

We found that the main drawbacks of the PLA/PGA membranes are: sensitive handling; preparation of the

chamber was difficult and relatively time-consuming (10 min/chamber) (Fig. 1); and the chambers were unstable when not packed with grafts.

To the best of our knowledge this is the first published experiment of the use of PLA/PGA membranes in the form of volumetric chambers to carry an underlying HA/TCP onlay graft. The advantages of these two materials have been tested together, and proved to be a suitable technique for the reconstruction of alveolar bone.

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