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ORIGINAL ARTICLE

Comparison of the osteoconductive properties of three particulate bone fillers in a rabbit model: Allograft, calcium carbonate (Biocoral®) and S53P4 bioactive glass

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Abstract

Aim. The aim of this study was to compare the osteoconductivity and suitability of three biomaterials used as particulate fillers; S53P4 bioactive glass, allogeneic fresh frozen bone and coral-derived calcium carbonate. ***Materials and methods.*** Materials were implanted into drill-holes in the femoral condyles of adult rabbits. Follow-ups were performed at 3, 6, 12 and 24 weeks. Host–response, osteoconductivity, bonding and filler-effect were evaluated by SEM, EDXA and histology and histomorphometry to evaluate. ***Results.*** All three materials were found to be biocompatible and osteoconductive. Defects filled with allograft seemed to have more bone at 24 weeks, although no statistically significant difference in new bone growth was found. In earlier time points, coral, however, was observed to degrade more quickly, leaving more empty space in the defects, thus making it a less suitable filler for cavitary defects. ***Conclusion.*** At all time points there was less filler material (i.e. biomaterial and new bone) in coral-filled defects than in BAG or allograft filled defects ($p < 0.05$).

Key Words: *bone, bioactive glass, biocoral, allograft*

Introduction

At present, research on biomaterials emphasizes the creation and characterization of new materials and enhancement of existing materials. Many materials are already in clinical use, yet there are relatively few comparative studies that evaluate the suitability of different bone substitutes for various conditions. A theoretical ideal bone substitute would have the same biomechanical properties as bone, would be degraded at the same rate as new bone is formed and would be at least osteoconductive and possibly osteoinductive. This investigation focused on three biomaterials, bioactive glass S53P4 (BAG), calcium carbonate derived from Porites-coral (Biocoral®, Inoteb, Noyal-Pontivy, France) and allogeneic (fresh-frozen) bone, all of which are used to fill defects in bone left for example by tumors, arthroplasty revision, trauma or infection as well as obliteration of bony cavities and spinal fusion [1–9]. The objective of this study was to compare *in vivo* the osteoconductive and filler properties in drilled trabecular bone-defects of three well-known biomaterials, namely allograft, calcium carbonate and BAG, in rabbits. Empty defects served as controls. Emphasis was on the suitability of the aforementioned materials for filling of cavitary defects. The hypothesis was that is has superior filler properties.

Methods

Calcium carbonate was from treated natural coral exoskeleton (Biocoral®, Inoteb). The granule size ranged from 630–1000 μm. BAG used was S53P4 and, thus, contained weight percentages of constituents as follows: SiO_2 53%, Na_2O 23%, CaO 20%, PO_4 4%. Allografts were obtained from iliac crests of previously sacrificed rabbits and freeze-dried. Allografts and BAG were prepared to the same granule-size as the calcium carbonate.

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Operative procedures

New-Zealand white female rabbits were used as the test animals. The operative procedure was performed under general anesthesia, which was accomplished with 5 ml diazepam i.p. (Diapam 5 mg/ml, Orion, Espoo, Finland), 0.1 ml/kg of buprenorphine s.c. (Temgesic 0.3 mg/ml) and 10 mg/kg ketaminehydrochloride i.m. (Ketalar, Parke-Davis).

Routine orthopedic aseptic measures were followed. The operative field of both knees was shaved and cleaned with 70% ethanol–water solution. An adhesive cut-through barrier was applied (Barrier®, Johnson & Johnson, New Brunswick, New Jersey, USA). Small medial and lateral incisions extending to the surface of the bone were made on both condyles. Defects of 4.5 mm in diameter and 8 mm in depth were drilled bilaterally into both femoral condyles with a small trepane-drill under continuous saline-irrigation. The biomaterials were then poured into the defects to the rim and covered with the elevated periosteum. The defects were then filled with the studied materials and covered with periosteal flaps. Soft tissues were then approximated with absorbable sutures and skin with non-absorbable continuous sutures. Animals were sacrificed at 3, 6, 12 and 24 weeks with an overdose of pentobarbital (Mebunat®, Orion Pharma, Espoo, Finland). The study protocol was approved by the institutional animal study committee on December 12th 2003 (Nr LSLH-2003-10931/Ym-23).

A total of 69 specimens from 42 knees of 21 adult female rabbits were studied. Fifteen specimens obtained at 12 or 24 had to be discarded due to poor technical quality of the blocks or slides.

The knees were evaluated macroscopically, with light and scanning electron microscopy, Energy dispersive x-ray analysis (EDXA) and computer-assisted histomorphometry. Specimens were fixated in 70% ethanol and embedded in plastic (Technovit®, Kulzer GmBH, Wehrheim, Germany). Twenty micrometer longitudinal histological sections were prepared using a cutting-grinding technique used for undecalcified hard-tissue specimens (Exakt-Apparatebau, Norderstedt, Germany). Samples received van Gieson staining and were evaluated using light microscopy. Blocks were polished and carbon sputtered for SEM-evaluation. Scanning electron micrographs were made from samples obtained at 3 weeks. A Princeton Gamma-Tech Prism 2000 electron microscope was used (PGT Inc., Princeton, NJ). A computerized analysis system (Microscale TC, Digithurst Ltd., Royston, UK) was used to evaluate the amount of different tissue components in the cross-section of the defect. New bone, remaining biomaterial and other tissue (i.e. mostly connective tissue of varying maturity, some bone marrow and adipous tissue) were identified and measured as a percentage of defect area and subtracted from 100, giving the value for empty space. Special care was taken in identifying the allograft from new bone.

Between-group differences in bone formation and amounts of biomaterial, other tissue and filler (bone + remaining biomaterial) were analyzed using one-way ANOVA followed by Tukey's HSD post-hoc tests and Kruskall-Wallis. A p-value of ≤ 0.05 was considered a criterion for statistical significance.

Results

All the wounds healed without complications and macroscopically no difference in cartilage repair was seen. All cartilage defects were covered with grey opaque tissue. No hydrops or synovial irritation was observed.

Histological evaluation revealed new trabeculous bone formation in all defects already at 3 weeks. All three biomaterials showed biocompatibility and osteoconductivity suggested by apposition of new bone on the granules surface. No signs of infection or other adverse tissue reaction were visible at any time point.

Empty defects (control group) healed with a significant amounts of loose connective tissue at first, which was gradually substituted with bone. Some amount of empty space remained still at 24 weeks (Figure 1).

The allograft granules were rapidly coated with new bone already at 3 weeks with gradual replacement of allogeneic bone by host bone (Figure 2). At 24 weeks the allograft granules were very difficult to differentiate from surrounding bone as remodeling had occurred. Phase-contrast microscopy was used to confirm identification of the allograft from host bone. On SEM, the allograft granules showed resorption lacunae on all surfaces. Host bone trabeculae were in tight contact with the allograft. EDXA showed identical stoichiometric distribution on both sides of the interface and an identical Ca/P ratio, confirming that no demineralization of the allograft had occurred. The interface could not be discerned with EDXA, suggesting a tight bond.

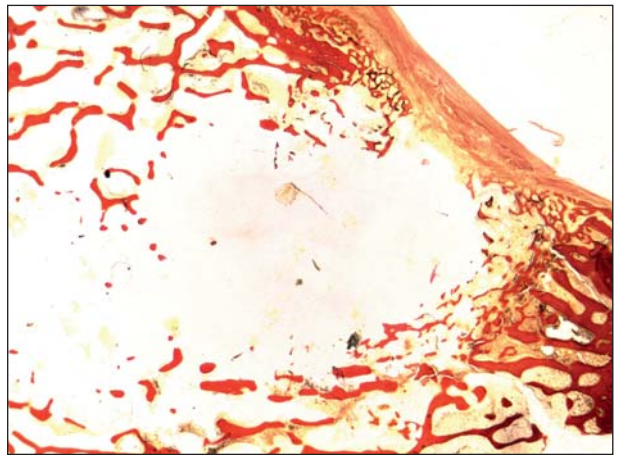

Figure 1. Empty defect at 24 weeks, van Giesson stain.

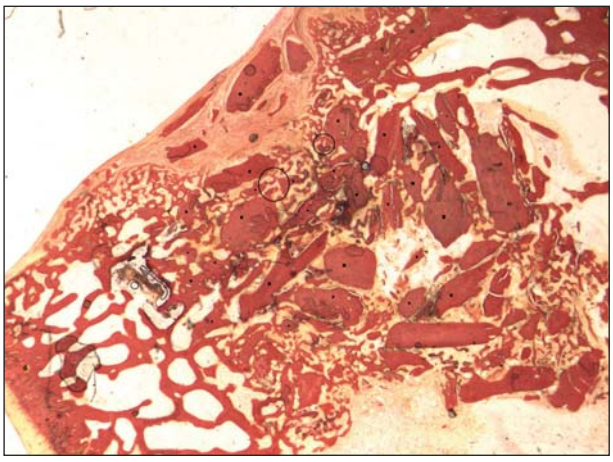

Figure 2. Allograft at 3 weeks, van Giesson stain. Black dots = allograft, new bone encircled.

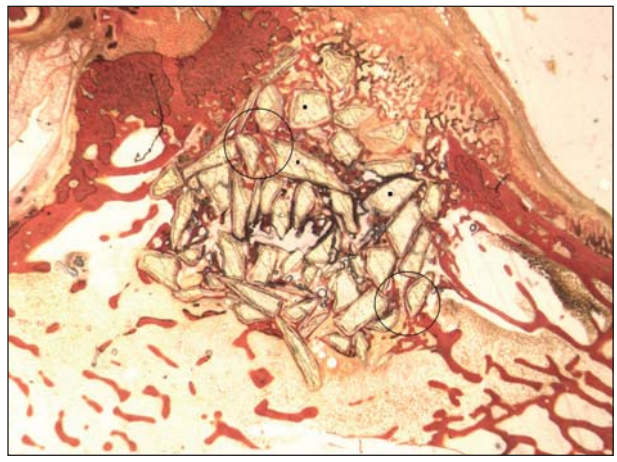

Figure 4. BAG at 3 weeks, van Giesson stain. Black dots = glass granules, bone ongrowth encircled.

Coral granules showed good bone ongrowth and a significant reduction in size of the granules, suggesting resorption at 3 weeks (Figure 3). At 6 weeks the calcium carbonate had been resorbed to a great extent with only a minimal amount of biomaterial left at 12 weeks in one specimen. At 24 weeks no coral could be found. On SEM coral granules showed considerable resorption both on the surfaces and inside the granules (Figure 4). Already at 3 weeks the particles had broken down into pieces ranging from 100–300 microns. Coralline calcium carbonate was not replaced at the same rate as it was resorbed. Host bone was in tight contact with the granules. EDXA showed a steep change in the amount of calcium and phosphorus at the interface, suggesting a lack of a reactive layer, although tight bonding seemed to occur. A measurable amount (5.4 weight%) of strontium, which could not be found in surrounding bone or the other studied materials, was found in the calcium carbonate granules.

Glass granules were almost completely coated with bone at 3 weeks (Figure 5). Also a significant amount of connective tissue was apparent at all time points. Unlike in the other groups, the defects filled with glass showed virtually no empty space at any time. No significant resorption of the glass granules was apparent at 24 weeks, although the reactive layer appeared to be thicker than at 3 weeks. On scanning electron microscopy glass granules showed osteocytes on the surface. The granules showed the typical core composed mainly of calcium, sodium and silicon and the silicon-rich reactive layer. Host bone was in tight contact.

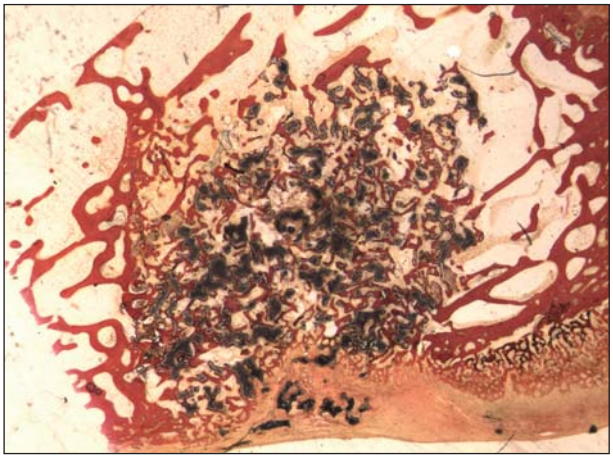

Figure 3. Coral at 3 weeks, van Giesson stain. Coral granules dark grey.

No statistically significant difference between groups in new bone formation or connective tissue formation was found at 3, 12 or 24 weeks, although at 24 weeks the allograft group seemed to have the most new bone. At 6 weeks, however, there was less new bone in the allograft group (8.1%, SD = 3.2) than in empty defects (25.5%, SD = 6.1) or coral-filled defects (24.7%, SD = 9.4). This can be explained by the shift from relatively rapid ongrowth to the slower creeping substitution as, at 3 weeks, the allograft granules appeared to be completely coated with a new bone layer. At 24 weeks the BAG group showed more other tissue (mainly loose connective tissue) than other groups. At 3 and 6 weeks the empty controls had more empty space than the filled defects ($p > 0.05$). At 12 and 24 weeks, defects filled with allograft or BAG showed significantly less empty space than controls or coral-filled defects (p 0.035 and 0.028). At all time points there was less filler material (i.e. biomaterial and new bone) in coral-filled defects than in BAG- or allograft-filled defects ($p < 0.05$).

Discussion

All three biomaterials were observed to be biocompatible and osteoconductive, as previous studies have already shown [16,17]. Comparative studies have found bioactive glass to perform in a similar fashion to allogenous and even autologous bone [10–12]. A previous study comparing coralline calcium carbonate and BAG showed similar results to ours: the calcium carbonate granules were resorbed already at

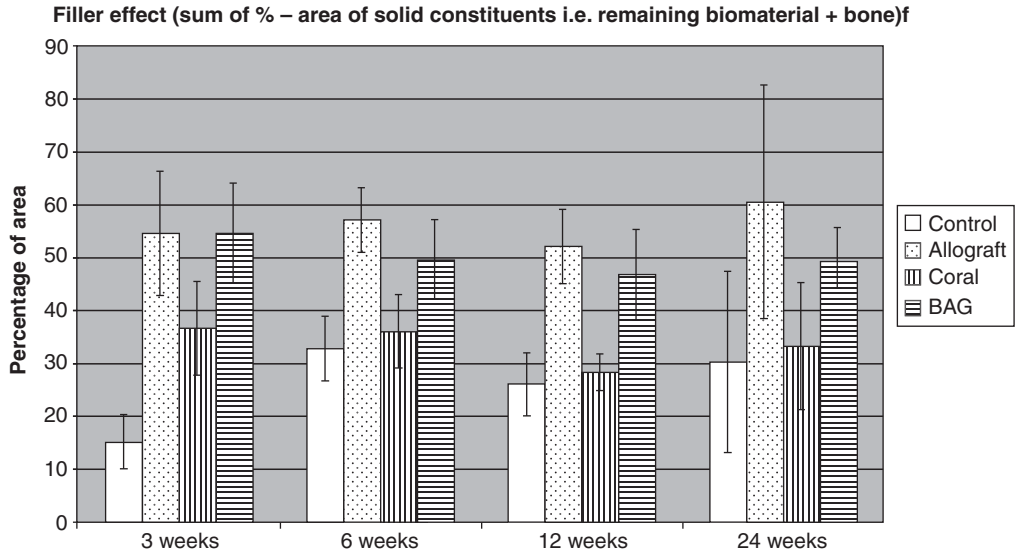

Figure 5. Filler effect, significant difference at all time points between coral and BAG/Allograft.

6 weeks, resulting in loss of new bone stock [13]. Gao et al. [14] compared tricalcium phosphate and Biocoral® enhanced with bone morphogeneic protein in an experimental segmental defect in sheep. They achieved satisfactory mechanical integrity with coral, but also noted rapid resorbtion of the biomaterial. Differences in new bone growth between groups did not reach statistical significance in our study, possibly due to the relatively small sample sizes and on the other hand possibly due to the size of the defect. It might be that a larger critical size defect would create a larger difference as it might better demonstrate the osteoconductive properties. However, also many clinical series in cavity filling do not study actual critical size defects. If no great difference in bone formation could be discerned, the rate at which the biomaterials degraded, however, did differ. At all time points the coral-filled defects had less solid filler material. Although particulate bone substitutes aren't intended for immediate load-bearing purposes, the cavitary defects often treated with these materials are ultimately meant to regain some structural integrity. While an ideal biomaterial should be resorbed *in vivo* it should do so at the same rate as new bone is formed [15]. Granular BAG and allogeneic bone are very slowly degraded, but without leaving empty space in their wake. Biocoral® was almost completely resorbed as early as at 6 weeks, leaving a similar filler effect as was seen in the empty control defects. It can, thus, be debated as to whether it is a useful filler if the effect is neglectable. The amount of strontium found in the calcium carbonate is probably a result of normal coralline metabolism and the relevance of this finding is unclear. The results of this study suggest that the properties of different filler materials are not equal and care should be taken in the choice, especially in defects that are under mechanical stress, for example near joints and where fusion is desired.

This study can be argued to confirm the biocompatibility and osteoconductivity of S53P4 bioactive glass, allogeneic bone and coral-derived calcium carbonate. Our findings, however, suggest that bioactive glass S53P4 can be considered a better bone filler than coral-derived calcium carbonate, which is resorbed too quickly, thus making it less ideal material for filling cavitary defects.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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