In Vivo Absorbability of 45S5 Bioglass® Bioactive Glass – A Histologic Analysis

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Introduction

Synthetic graft materials for use in osseous defect repair have been developed to reduce dependency on autograft or allograft tissues and the complications associated with their use. These synthetics run the spectrum ranging from calcium hydroxyapatite, a non-absorbable permanent graft material, to those that are absorbed within a matter of weeks, such as with calcium sulfate. Bioactive glasses remain *in situ* for extended periods to stimulate bone formation, but ultimately are absorbed to leave only new bone tissue in their place. As part of a comprehensive study, the *in vivo* absorption profile of bioactive glass was evaluated out to one year.

Materials and Methods

Bilateral defects were created in the distal femur of skeletally-mature female goats. The medial femoral condyle was exposed and a 10mm diameter transverse defect was created from the medial cortex to the lateral cortical wall.

The site was irrigated and the defect was grafted with 45S5 Bioglass (NovaBone, USBiomaterials Corp., 710-90µm) mixed with sterile saline. The prepared bioactive glass was placed into the site, minimizing compression or compaction of the particulate material. Eighteen animals received NovaBone in one femur, the contralateral limb receiving either another material or left empty as a control. Six animals were sacrificed at each period of 6, 26, and 52 weeks.

Evaluation Methods

Histology

At sacrifice, the distal femur was resected and radiographs were taken. The medial condyle was submitted for undecalcified histology. The samples were dehydrated in increasing concentrations of ethyl alcohol, embedded in methyl methacrylate, sectioned in the coronal plane and ground to a 20 µm thickness. Tissue sections were stained with Van Gieson bone stain. Histologic evaluations were conducted using a standardized defect region of 8mm x 10mm. Histomorphometry was conducted with a semi-automated digitizing system to measure the amount of bone and residual graft material in the defect site. The number of graft particles and their individual areas were also measured. Calculated values included: bone-to-defect area ratio; graft-to-defect ratio; and bone-to-graft ratio.

Results

Histomorphometry

Percent Bone / Graft Material

Table I summarizes the results of the histomorphometric analyses in terms of the percentage of the available graft space filled either by bone or graft material over the standardized 8 mm x 10 mm defect area. The graft area decreased throughout the course of the study, while the bone content was greater at the later periods when compared to six weeks. There was no statistically significant difference between the six and twelve month values for either parameter.

Table I. Histomorphometric Analysis – Percentage of Bone and Graft Material in Defect Site

Period (weeks)	% Bone (mean ± s.d.)	% Graft (mean ± s.d.)
6	[a] 9.75 ± 8.00	[b] 17.10 ± 11.34
26	[a] 38.21 ± 4.50	[b] 1.58 ± 1.47
52	[a] 24.64 ± 10.98	[b] 0.32 ± 0.004

a: 6 week < 26, 52 weeks ($p < 0.05$)
b: 6 week > 26, 52 weeks ($p < 0.05$)

Results

Histomorphometry

Residual Graft Content

The total graft material area and the average number of residual NovaBone graft particles area reported in Table II. In addition, the averages for the minimum, maximum, and mean particle size also are reported for each period. The total graft area and the mean particle size are also shown graphically in Figure 1. Both the number of particles and the average particle size decreased over the course of the study, resulting in the overall graft area decrease. That this is due to the absorption of the particles is shown by the reduction in average particle size and the continued decrease in size of the largest particles.

Table II. Histomorphometric Analysis – Residual Graft Material Measurements

	6 weeks (Mean ± SD)	6 months (Mean ± SD)	12 months (Mean ± SD)
Total Graft Area (mm^2)	13.82 ± 9.17	1.28 ± 1.19	0.26 ± 0.23
Particle Count	468.5 ± 274.2[a]	90.3 ± 91.3[b]	54.4 ± 45.9[b]
Average Particle Size ($mm^2 \times 10^{-2}$)	28.6 ± 10.0[b]	14.5 ± 2.7[b]	4.5 ± 1.1[b]
Maximum Particle Area ($mm^2 \times 10^{-2}$)	178.0 ± 58.0[b]	106.1 ± 39.3[b]	23.7 ± 12.7[b]
Minimum Particle Area ($mm^2 \times 10^{-2}$)	0.4 ± 0.3[c]	1.0 ± 0.6[c]	0.6 ± 0.1

a. 6 weeks > 6 months, 12 months
b. 6 weeks > 6 months > 12 months
c. 6 months > 6 weeks

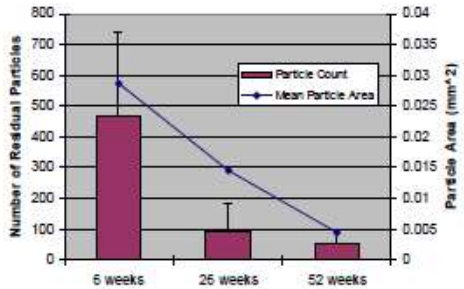

Figure 1. Number of Residual NovaBone Particles and their Average Cross Sectional Area

Results

Histology

Figures 2 - 4 are representative images of the residual bioactive glass particles at 6 weeks, 6 months, and 12 months. At six weeks, the bioactive glass particles are larger and present in greater numbers than in later samples. The particles are generally connected by bone bridges, although some at the defect center are still not involved in bone formation. At the later periods, the residual particles are completely embedded in new bone and are smaller, due to absorption and fragmentation.

Figure 2. Histologic Sections at Six Weeks - Sample 12R. A) 2x composite image; B) 100x

Figure 3. Histologic Sections at Six Months - Sample 43L. A) 2x composite image; B) 100x

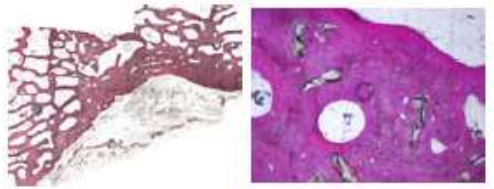

Figure 4. Histologic Sections at Twelve Months - Sample 35L. A) 2x composite image; B) 100x

Discussion

45S5 Bioglass was implanted in transfemoral osseous defects and the progression of graft absorption was evaluated. At six weeks, approximately 10% of the defect area was filled with new bone, which increased and remodeled to an average of 25% at one year. At the same time, the amount of bioactive glass continually decreased, with a ten-fold decrease in graft area percent between 6 and 26 weeks. Based on a pre-implantation mean particle size of 0.096mm^2 (350µm), the average particle area decreased by 70% at 6 weeks and 85% by 26 weeks. While a small amount of graft material remains at one year, it occupies less than 0.3% of the graft space.

Previous tests of this material at shorter periods have demonstrated initial packing densities of 35-40%[1]. This would indicate that for this study, upwards of 50% of the initial graft volume may be absorbed by six weeks and replaced by bone. This initial material absorption and early formation of new bone, coupled with the sustained presence of small particles of bioactive glass, combine to promote a prolonged period of bone stimulation.

Conclusions

Bioactive glass has been shown to stimulate new bone formation in osseous defects. While the majority of graft material is absorbed within the first six months, a small amount (<1%) may be present after one year. The residual particles present out to six months and beyond are embedded in new bone tissue and may continue to provide an osteostimulative response well after implantation.

References

1. Wheeler, J. Orthop., Res., 18:140-148, 2000

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