The Effect of Silica-substitution, Sintering and Particle Size on Bone Healing of Apatite Granules

Woods, S.A.[1]; Petticoffer, A.C.[1]; Arens, D[2];
[1]DePuy Synthes, West Chester, PA; [2]AO Research Institute, Switzerland.

Statement of Purpose: Using a well-established 1.5 cm segmental defect of the rabbit radius, the primary objective of this study was to evaluate the effect of size, silica substitution and sintering of apatite granules on bone healing.

Methods: A total of thirty-six (36) skeletally mature (>6 months) New Zealand white rabbits were enrolled in this study and subjected to a 1.5 cm critical sized diaphyseal bone defect created 2.5 cm proximal to the radio-carpal joint. Bone marrow aspirate (BMA) was obtained from the medullary cavity of the right femur and combined with the graft materials in a 1:1 ratio. A total of 0.3cc of graft material + BMA was then delivered into the defect site using a 1cc cut-off syringe. The six treatment groups evaluated in this study had a sample size of six (n=6), and all animals had an in-life duration of 8 weeks.

The six experimental groups were as follows:

- **Group 1**: 0.5-0.7mm apatite granules
- **Group 2:** 0.5-0.7mm 0.8% Si-apatite granules
- **Group 3:** 1.4-2.0mm apatite granules
- **Group 4:** 0.5-0.7mm sintered 0.8% Si-apatite granules
- **Group 5:** 0.5-0.7mm sintered apatite granules
- **Group 6:** 1.4-2.0mm Si-apatite granules

Post mortem contact radiographs were taken using a Faxitron X-ray cabinet (model # 43885A) at 36 KV for 3 minutes using a 0.5mm filter. The radiographs were scored consecutively by 3 independent investigators blinded to the treatment groups using an established scoring system outlined by Cook (1994).

Ex vivo micro-computed tomography (μCT) was performed on all samples after they were stripped of soft tissue and fixation in 70% ethanol. Bone density and bone volume were measured using a Scanco Micro-CT 40 (Scanco Medical, Switzerland) at a resolution of 15μm with settings of 70 kVp and 114 μA. The first slice was defined by a titanium reference pin which was placed 8mm proximal to the defect site during surgery. The ROI included the defect (15mm) and 5mm distal and proximal to the defect site. After reconstruction, the ulna was manually contoured and removed from the image data.

After micro-CT, the samples were dehydrated, infiltrated and embedded in methyl methacrylate for undecalcified histology. One 55-70 μm thick longitudinal section was made using an Exakt saw and grinder (Exakt Technologies, Oklahoma, OK) from the center of the defect mass. Each slide was stained with Sanderson's rapid bone stain and a Van Gieson counter stain for histopathological assessment.

High-resolution digital images were obtained for each histological slide using an Olympus SZX16 stereoscope (Olympus, Japan) with a DP25 digital camera. Quantitative histomorphometric measurements were obtained using Osteomeasure software V3.1.0.0 (OsteoMetrics, Decatur, GA) and included percent bone area and percent residual graft area.

For all data, significance was determined at the 95% confidence level, $p<0.05$.

Results: At necropsy, macroscopic examination of the tissues revealed complete and normal healing of the surgical wounds in all animals. There was no evidence of abnormal tissue reaction or inflammation in the tissues overlying the fusion site in any of the animals.

Mean semi-quantitative radiographic scoring for the defects showed no statistical difference between groups with respect to bone healing (Table 1).

No statistical differences between groups were observed in the mean bone volume (BV) or the mean bone density (BMD), as determined by micro-CT (Table 2). There were differences in the amount of residual biomaterial present with groups 4 and 5, both sintered, having significantly more residual biomaterial left within the defect site as compared to all other groups, $p<0.05$.

Histomorphometry of the defect also showed no statistical difference in new bone between treatment groups (Table 1).

Treatment	Radiographic Healing Score	Histomorphometry Bone Area (mm^2)
Group 1	4.5 ± 0.3	30.5 ± 8.5
Group 2	5.6 ± 0.5	26.7 ± 3.4
Group 3	5.2 ± 0.1	33.7 ± 6.6
Group 4	3.8 ± 0.2	31.8 ± 11.0
Group 5	3.8 ± 0.4	29.4 ± 14.1
Group 6	5.1 ± 0.2	36.2 ± 11.1

Table 1: Mean radiographic scores and histomorphometry.

Treatment	BV (mm^3)	BMD ($mgHA/cm^3$)	Biomaterial (mm^3)
Group 1	216.9 ± 34	873 ± 15	24.6 ± 4
Group 2	239.8 ± 36	871 ± 30	45.7 ± 9
Group 3	254.9 ± 48	854 ± 23	32.6 ± 11
Group 4	246.7 ± 36	868 ± 18	89.7 ± 30
Group 5	235.2 ± 59	831 ± 23	89.8 ± 21
Group 6	256.0 ± 73	848 ± 22	34 ± 20

Table 2: Bone volume (BV), bone density (BMD) and residual biomaterial volumes as determined by micro-CT.

Conclusions: The results of this study indicate that the affect of 0.8% silicate substitution, granule size and/or sintering had little effect on bone healing, with no statistical differences observed by radiograph, micro-CT, or histomorphometry. Sintering, however, did have an effect on the degradation rate of the apatite ceramic with significantly more sintered material remaining in the defect site at 8 weeks as appose to the un-sintered groups.

Reference: Cook SD, et. al. Clin Orthop Relat Res. 1994(301):302-312