In-vivo evaluation of nano-coated calcium phosphate scaffolds for bone regeneration

S Oh[1], N Oh[2], H Yoon[1], Y Yang[1], M Appleford[1], J Bumgardner[3], W Haggard[3], JL Ong[1]
[1]The University of Tennessee Health Science Center, Memphis, TN 38163, USA.
[2]Inha University, Incheon, S Korea,
[3]University of Memphis, TN 38152, USA.

Introduction

The limitation of current grafting materials has driven the search for synthetic alternatives for the regeneration of trabecular bone [1, 2]. Due to a low availability of autografts and issues of biocompatibility surrounding allografts and xenografts, there is a substantial need for an alternative scaffold material to promote bone repair [3, 4]. As such, three dimensional (3-D) bioactive scaffolds are found to have increasing applications in clinical orthopedics and tissue engineering. One bioactive material that is found primarily in the mineral component of bone is hydroxyapatite (HA). The HA materials can be formed into porous scaffolds, with one application being to repair segmental defect. In order for the scaffolds to be effective in bone regeneration, it is also critical that there is enhanced control over the material crystalline structure as well as many other factors such as interconnectivity and porosity. As such, to elucidate the factors needed to optimize bone regeneration, the purpose of this study was to evaluate bone regenerate in dogs' mandible using HA scaffolds having nano- and micro-crystals on the surface.

Materials and Methods

Highly porous micro-crystalline HA scaffolds were prepared by polyurethane coating method. The nano-crystalline HA scaffold surface was fabricated by spin coating HA sol on micro-crystalline HA scaffolds. Porosity and pore size were determined using a micro-CT and scanning electron microscopy (SEM), respectively. Four mandibular defects (5 x 4 mm) were created in 12 dogs and scaffolds (5 x 4 mm) were then placed in these defects. The controls were defects without any scaffolds. The implanted scaffolds were harvested from each dog after 3, 6 and 12 weeks. After sacrifice, the samples were fixed with 10% neutral formalin and dehydrated with 100% EtOH, followed by embedding. Samples were then sectioned to 50 μm thick, polished, followed with Vilanueva staining and were examined under a light microscope.

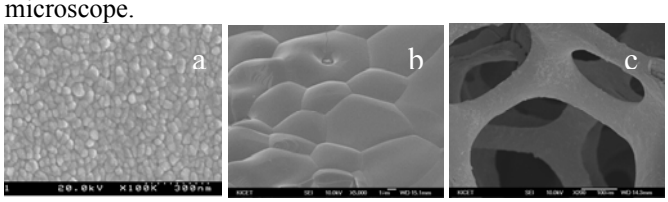

Fig 1. SEM micrographs of nano-(a), micro-HA surface (b), and scaffolds structure (c).

Results and Discussion

Figure 1 shows the SEM micrographs of the nano- and micro-crystalline HA scaffold surfaces. The average porosity value for the scaffolds was 90% as measured by Micro-CT with an average pore size of 250 μm measured by SEM. Scaffold pore was observed to be fully interconnective. From the light microscope, ingrowth of vascularized connective tissue into the scaffolds were observed As shown in Table 1, HA scaffolds with nano-crystalline surface exhibited statistically higher bone formation as compared to HA scaffolds with micro-crystalline surface. However, no new bone was observed for defects in the control group at 3 and 6 weeks, with some new bone regeneration in defects for the control group at 12 weeks. Figure 2 shows the lack of bone regeneration for the controls, whereas micro- and nano-crystalline scaffold surface scaffold exhibited bone filling up the pores after 12 weeks post-operation.

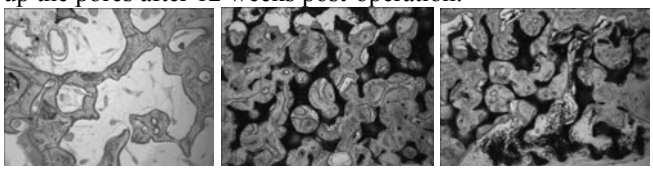

Fig 2. Histology showing defect site without scaffold (a), new bone growth on HA Scaffold with micro-crystalline surface (b), and new bone growth HA Scaffold with nano-crystalline surface (c) after 12 weeks post-operation.

	Total Bone Area (%)	Total Scaffold Area (%)	Total Porosity (%)
Control	35.6±2.9	-	64.4±2.9
HA Scaffold with micro-crystalline surface	59.0±2.4	23.3±2.9	17.7±2.5
HA Scaffold with nano-crystalline surface	69.7±2.0	22.6±1.8	7.8±3.7

Table 1. Evaluation of bone regeneration area after 12 weeks post-operation.

Conclusions

At 12 weeks, more bone regeneration occurred in mandibular defects when the defect site was implanted with HA scaffold having nano-crystalline surface as compared to HA scaffold having micro-crystalline surface. However, healing occurs in defects in the control group. In conclusion, with the growing demands of bioactive materials for orthopedic as well as maxillofacial surgery, the utilization of macroporous nano-coated HA scaffolds with an average pore size of 250 μm may be an appropriate scaffolding material for repairing bone defects.

References

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