The Integrative and Mechanical Potential of Bilayered Hydroxyapatite Scaffolds: An *In Vivo* Study

[+1]Walker, JA; [2]Guda, T; [2]Oh, S; [2]Appleford, MR; [2]Singleton, B; [2]Ong, JL; [1]Wenke, JC
[+1]U.S. Army Institute of Surgical Research, San Antonio, TX, [2]The University of Texas at San Antonio, San Antonio, TX

INTRODUCTION: The growing demand to use synthetic bone substitutes in lieu of conventional grafts for bony defect repairs has lead to intense investigation into optimizing biomaterials.[1] Material(s) and architectural design must allow and/or promote cellular integration, provide mechanical strength and stability, and address perfusion demands in order to functionally become incorporated. To address these issues, we chose an accepted material with known osteoconductivity, hydroxyapatite (HA), and used a novel architectural design.[2] Mimicking natural long bones, the scaffold was designed with a trabecular like inner core with large interconnecting pores and an outer cortical shell with fewer and smaller interconnecting pores. It was hypothesized that the inner core would promote fluid dynamics, while the outer core would provide the majority of the mechanical strength. The scaffold was evaluated using an *in vivo* rabbit radius segmental defect model.

METHODS: Using a template coating process, bilayered HA scaffolds were prepared with an outer dense shell of pore size 200µm and an inner porous core of pore size 450µm. Porosity measurements of the scaffolds were measured by helium pycnometry. The ability to regenerate bone in *in vivo* was evaluated using a 10-mm radius defect capable of healing in rabbits (mean weight 4.849Kg). At 8 weeks post implantation, bony in growth was examined using micro-CT and histology. Scaffold mechanical functionality was measured *ex vivo* by 4-point flexural testing using the contralateral limb as a control (n=8).

RESULTS: With specific gravity of HA computed at 3.04 g/cm^3, helium pycnometry indicated a mean scaffold porosity of 65.32 ± 3.99%. Using micro-CT analysis, the scaffolds had an average surface to volume ratio of 5.71mm^{-1} and a mean volume fraction of 37 and 62% for the 450 and 200µm pore size scaffolds, respectively. Radiographic and histological evaluations 8 weeks following implantation demonstrated integrated bone-scaffold interfaces with connective tissue surrounding and within scaffold pores, along with bony infiltrates throughout the scaffold, Fig. 1. Connective tissues were stained pink/purple with Paragon, and the HA scaffolds were stained blue/black. The proximal interface had an increased bone volume to total volume ratio compared to the distal interface (p=0.04), Table 1. Flexural evaluation of the scaffolds showed that the strength of the scaffold recovered to 95.5 ± 5.7% and the flexural modulus to 71.1 ± 12.3% of intact bone over the 8 week *in vivo* implant period.

DISCUSSION: Taking an organizational cue from human bone, a novel bilayered HA scaffold with distinct cortical and trabecular like architecture was investigated. Histology revealed vascular connective tissue both encapsulating and within interconnecting pores, along with occasional lamellar organization of collagen fibrils oriented around struts. These findings, along with the observation of new bone found throughout the entire scaffold, as seen by micro-CT, suggest the bilayer design promotes tissue integration. The micro-CT data suggests the new bone potential is favored from proximal to distal. Furthermore, the incorporated scaffold, defined by new bone bridging the bone-scaffold interfaces, provided adequate strength to closely match natural bone. The significant connective tissue encapsulation provided necessary stability during mechanical testing.

	Proximal Interface	Midshaft	Distal Interface	
Bone Volume/Total Volume	44.5± 7.5	41.1± 5.7	39.3± 6.0	(%)
Bone Surface/Total Volume	40.2± 9.0	35.2± 8.0	43.0± 11.5	(mm^{-1})
Bone Surface/Bone Volume	17.6± 3.5	14.2± 2.5	16.6± 4.0	(mm^{-1})
Trabecular Number	4.0± 1.0	3.2± 0.7	3.4± 0.8	(mm^{-1})
Trabecular Thickness	116.0±22.6	132.0± 31.6	120.2± 33.2	(µm)
Trabecular Spacing	129.3±27.7	153.7± 27.1	141.1± 34.3	(µm)

Table 1. Micro-CT analysis of scaffolds 8 weeks following implantation. (All data as means ±1 SEM, n=12)

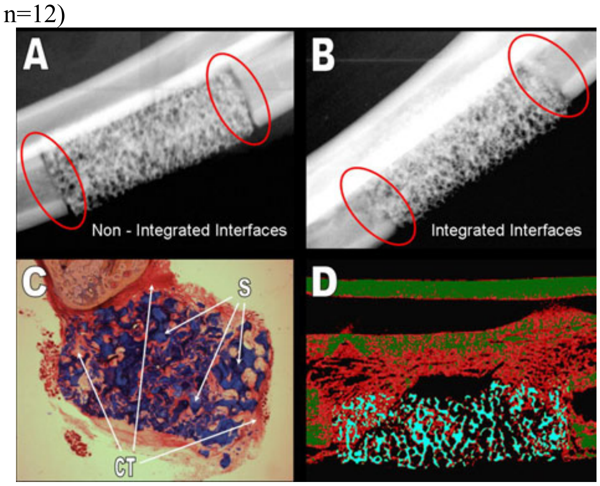

Figure 1. (a) Plain film of implanted scaffold immediately following and (b) 8 weeks post surgery; (c) Connective tissue (CT) encapsulating and infiltrating the scaffold (S), 2X; (d) Micro-CT demonstrating new bone (red) throughout scaffold (blue) and adjacent to host bone (green).

CONCLUSIONS:

- The implanted scaffolds promoted tissue integration with new bone present throughout the scaffold.
- Connective tissue encapsulation provided good scaffold stability.
- Flexural strength of the scaffolds following implantation was similar to intact bone.

REFERENCES:

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