# Mechanical Properties of Hydroxyapatite Reinforced Collagen Scaffolds

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**Statement of Purpose:** Hydroxyapatite (HA) reinforced collagen shows promise as a bone tissue engineering scaffold material.[1] Collagen/HA scaffolds are relatively easy to fabricate and demonstrate excellent biocompatibility.[1,2] The addition of HA to collagen dramatically improved mechanical properties of porous collagen scaffolds.[3] However, few studies have examined the effects of HA reinforcement content, and none have examined the effect of the HA reinforcement morphology. Therefore, the objective of this study was to investigate the effects of varying amounts of HA powder versus whisker reinforcement particles on the mechanical properties of freeze-dried collagen scaffolds. Additionally, the feasibility of a novel porogen leaching technique was investigated in order to further improve the mechanical properties.

**Methods:** Soluble type I collagen was extracted from fresh bovine dermis by a commonly used acid extraction process.[4] The resultant product was shown using to be nearly pure type I collagen by SDS-PAGE. HA reinforcements were either a commercially available equiaxed HA powder ~1 µm in diameter or HA whiskers with a length of ~18 µm and a aspect ratio of ~8 produced using the chelate decomposition method.[5] Freeze-dried collagen was mixed in 0.05 M acetic acid at a concentration of 60 mg/mL and homogenized for 5 min until dispersed. HA whiskers or powder were then added and mixed for another 2 min. After degassing under vacuum for 10 min, the solution was placed in silicone molds 10 mm in diameter and 8 mm in height. The solution was rapidly frozen at -80°C, resulting in a directional pore structure parallel to the eventual testing loading direction. Scaffolds were prepared by freeze-drying at 0.010 torr and -40°C for 24 h to remove all water, leaving porous open cell scaffolds with an aligned pore structure (Fig. 1). Before testing all scaffolds were crosslinked by heating to 100°C under high vacuum for 24 h.[4] Crosslinked scaffolds were re-hydrated in saline solution and tested in unconfined uniaxial compression to a strain level of 0.6. The initial elastic modulus was defined as the secant modulus at a strain of 0.2. The effects of the reinforcement morphology and fraction were analyzed using two-way ANOVA with a post-hoc *t*-test.

**Results and Discussion:** HA reinforcement increased the compressive modulus of freeze-dried collagen/HA scaffolds (Fig. 2). At a 1:1 ratio by weight (~30 vol % HA), the difference between the composite and pure collagen was not statistically significant. However, at 1:2 and 1:4 (~45 and 65 vol % HA respectively) there was a 3- and 11-fold increase in compressive modulus compared to collagen alone (Fig. 2). Overall, HA reinforcement of either morphology significantly increased ($p<0.05$, ANOVA) the compressive modulus, but only at the 1:2 weight ratio did the HA whiskers provide an increased modulus compared to the powder ($p<0.05$, *t*-test).

Note that these composites ranged from 95-99% porosity; therefore, a scaffold of similar composition with lower porosity could achieve even higher stiffness, possibly approaching that of trabecular bone. Freeze-dried scaffolds are unlikely to achieve lower porosity, but a novel porogen leaching technique was also investigated to overcome this limitation.

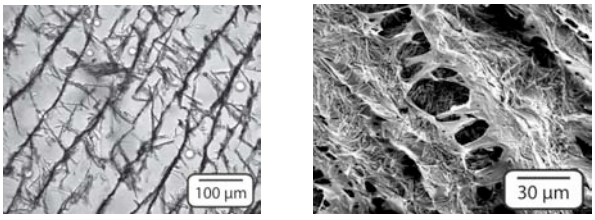


**Figure 1.** *Left:* Optical micrograph showing aligned pore structure of scaffold, and *Right:* SEM micrograph showing a freeze fractured cross section of a HA whisker reinforced collagen scaffold at 1:2 col:HA by weight.

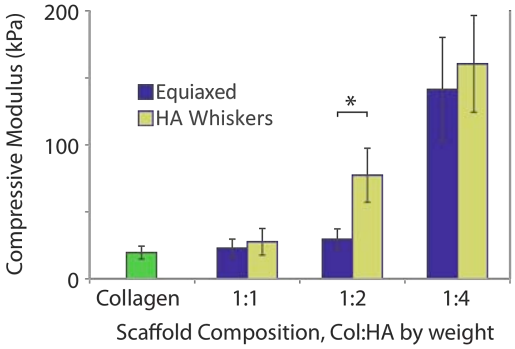


**Figure 2**. Compressive modulus of collagen-only scaffolds and scaffolds reinforced with either equiaxed or whisker shaped HA particles. Error bars span one standard deviation (* $p<0.05$).

**Conclusion:** HA reinforcement increased the compressive modulus of freeze dried collagen scaffolds by up to 1100%, and HA whiskers provided enhanced stiffness compared to the equiaxed power at the 1:2 weight fraction.

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**References:** [1]W Friess. *Europ. J. Pharm. Biopharm,* 45:113-136, 1998. [2]FJ O'Brien *et al., Biomaterials* 25:1077-1086, 2004. [3]AA Al-Munajjed *et al*., *J. Biomed. Mater. Res.* 90B:584-591, 2008. [4]CM Tierney *et al., J. Mech. Behav. Biomed. Mater.* 2:202-209, 2009. [5]RK Roeder *et al., J. Biomed. Mater. Res.* 67:801-812, 2003.