Preparation of Collagen-Glycosaminoglycan Scaffolds Using Ice Templates for Tissue Engineering

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Abstract:

Conventional methods of repairing or replacing lost or damaged tissues, such as transplants, surgical reconstruction, artificial prostheses, and medication, help to restore some functionality, but are rarely fully integrated into the body's processes. Tissue engineering, in contrast, implants prostheses that are seeded with cells of the appropriate type. These prostheses, typically in the form of porous scaffolds, will ideally become indistinguishable from natural tissue.

Scaffolds that can mimic the structure and chemistry of the extracellular matrix (ECM) are the most compatible with implantation. To this end, this project prepared scaffolds using an ice template technique that incorporated chondroitin-6-sulfate, a glycosaminoglycan (GAG) that is an important component of cartilage. The structure and performance of the collagen-GAG scaffolds were compared with standard collagen scaffolds.

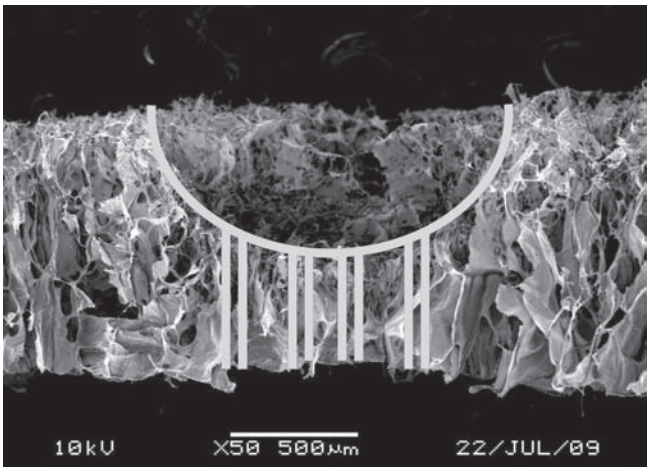

Figure 1: Cross-section of an ice template scaffold with funnel structure.

Experimental Procedure:

Scaffold Fabrication. Porous scaffolds are typically fabricated using a uniform solution of the material that will make up the structure, resulting in a uniform porous structure. The ice template technique used in this project resulted in a hierarchical funnel-like structure with large pores on the top surface and smaller pores leading to the interior of the scaffold, as in Figure 1.

This structure was created by depositing ice droplets of a uniform size on a substrate that had a silicone frame. A collagen or collagen-GAG solution was then poured over the ice droplets and frozen at -3°C for an hour, then at -80°C for several more hours, before being freeze-dried for 24 hours. The resulting porous scaffold was then crosslinked using glutaraldehyde gas, neutralized in glycine, washed, and freeze-dried again.

Results:

Collagen-GAG Solution Concentration. The optimal concentration for the collagen-GAG solution was determined by fabricating scaffolds using a fixed 1 wt% concentration of bovine collagen (Nippon Meat Packers) and varying concentrations of GAG in the form of chondroitin-6-sulfate (Sigma Life Sciences). These concentrations were 0.02, 0.05, 0.1, 0.25, and 0.5 wt%. The structure of the scaffolds was then observed using a scanning electron microscope (SEM). The 0.02 and 0.05 wt% GAG scaffolds were found to be too dense for efficient cell seeding, while the 0.25 and 0.5 wt% GAG scaffolds had a weak and inconsistent structure. The 0.1 wt% GAG to 1 wt% collagen solution was thus selected for further testing. Scaffolds fabricated using this solution will henceforth be referred to as collagen-GAG (CG) scaffolds.

Pore Structure Analysis. In order to perform comparative studies between collagen and CG scaffolds, 24 scaffolds were fabricated—12 scaffolds per solution type. The 12 scaffolds were divided into four groups, each of which used a different ice template diameter: control (no ice template used), 200 μm, 400 μm, and 800 μm. After fabrication, most of the scaffolds were separated into samples suitable for

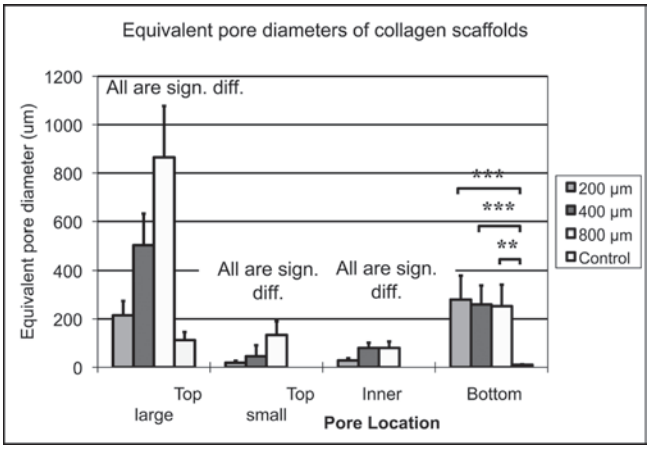

Figure 2: Mean equivalent pore diameter for collagen scaffolds.

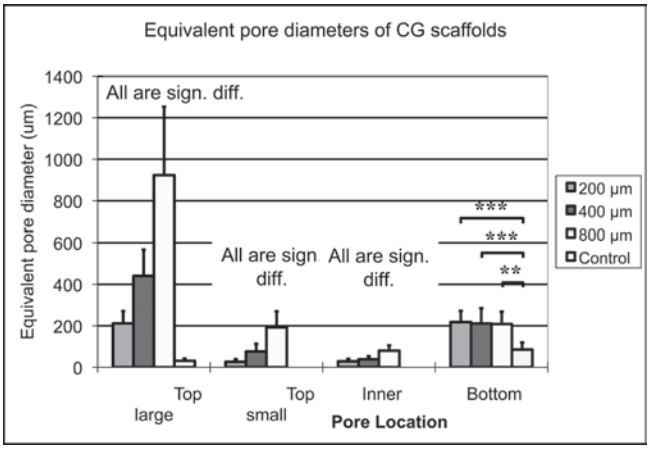

Figure 3: Mean equivalent pore diameter for collagen-GAG scaffolds.

cell culture experiments, with the remainder used to create samples for SEM imaging.

SEM images were taken of the top, bottom, and cross-section of each scaffold. The equivalent diameters of the large and small pores on the top surface, inner bulk pores, and bottom pores were sampled (n = 100) using MetaVue. The results are shown in Figures 2 and 3.

The pore equivalent diameters of the collagen and CG scaffolds were compared using a one-factor analysis of variance (ANOVA). The results are shown in Figure 3. As can be seen in Figure 3, only the 400 μm scaffolds had statistically significant differences in equivalent diameters for all pore types when comparing the collagen and CG scaffolds ($p \leq 1.22E\text{-}3$). The control scaffolds also had significantly different large top and bottom pore equivalent diameters ($p \leq 1.24E\text{-}57$), but the inner bulk and small top pores were not measured. The 200 and 800 μm scaffolds had statistically significant differences in their small top and bottom pore equivalent diameters, but their top large and inner bulk pores were not significantly different in size. (See Table 1.)

Conclusions:

From this analysis, it is clear that the addition of GAG to the scaffold has an effect on the size of the small top and bottom pores. The bottom pores of the CG scaffolds are significantly smaller than those of the collagen scaffolds, while the CG small top pores are significantly larger than their counterparts on the collagen scaffolds. There does not appear to be any pattern to the relative sizes of the top large or inner bulk pores.

Although further study is needed to determine the mechanism of the pore size differences, preliminary explanations may be formed. The significant difference between the pore sizes of the 400 μm collagen and CG scaffolds is tentatively attributed to fabrication error, as the size of the large surface pores should be mainly dependent on the size of the ice template used. The difference in the size of the small surface and bottom pores, however, is not so easily dismissed—possible mechanisms include but are not limited to the interaction of the GAG with the glutaraldehyde gas during crosslinking and differences in the thermal properties of the CG solution compared with the collagen solution.

Future Work:

The samples not used for SEM sample creation will be used in cell culture experiments to determine the effect of the addition of glycosaminoglycan on cell adhesion, growth, and differentiation.

Acknowledgments:

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Is CG Significantly Different from Collagen?

	200 µm	400 µm	800 µm	Control
Large surface	No	Yes; smaller	No	Yes; smaller
Small surface	Yes; larger	Yes; larger	Yes; larger	
Inner	No	Yes; smaller	No	
Bottom	Yes; smaller	Yes; smaller	Yes; smaller	Yes; smaller

Table 1: Results of one-factor ANOVA comparing collagen and collagen-GAG scaffolds ($\alpha = 0.05$).