Fabrication and Characterization of Porous Hyaluronic Acid-Collagen Composite Scaffolds

Shunqing Tang[1,2,3], Scott M. Vickers[2,4], Hu-Ping Hsu[2,3], and Myron Spector[2,3,4]
[1] Jinan University, Guangzhou, China; [2] VA Boston Healthcare System, Boston, MA; [3] Brigham and Women's Hospital, Harvard Medical School, Boston, MA; [4] MIT, Cambridge, MA

Statement of Purpose: Hyaluronic acid (HA) plays a vital role in many tissues, influencing water content and mechanical function, and it has been shown to have positive biological effects on cell behavior. To begin to determine if these benefits can be accessed if HA is incorporated into collagen-based scaffolds for tissue engineering, HA-collagen composite matrices were prepared, and selected properties evaluated.

Methods: Solutions of HA (*Streptococcus zooepidermicus*) and a type I collagen (bovine tendon microfibrillar collagen; Integra LifeSciences, Inc., Plainsboro, NJ) were prepared using different HA concentrations and pHs. Highly porous scaffolds were prepared using a freeze-drying method; 8-mm diameter discs were punched from 2-mm thick sheets. Cross-linking was performed using a water-soluble carbodiimide; 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) and N-hydroxysuccinimide at a molar ratio of 2:1 in 95% ethanol at room temperature. The HA concentration of solutions was analyzed by a carbazole method. Loss rates of HA in culture medium were evaluated. Scaffold pore structures were determined by light and scanning electron microscopy. Adult canine chondrocytes (passage 3) were grown in selected HA-collagen scaffolds to assess the effects of HA on chondrogenesis.

Results/Discussion: Homogenous HA-collagen slurries were achieved when polyionic complexes were suppressed. HA was uniformly distributed through the scaffolds, which demonstrated honeycomb-like pores, with interconnectivity among pores increasing as HA content increased. The freeze-dried HA-collagen scaffolds prepared with different HA:collagen ratios displayed differences in pore diameter (Table 1).

The amount of HA bound to collagen scaffolds varied with the method for treating the specimens (Table 1). Virtually all of the HA added to the collagen slurry was incorporated into the composite scaffolds that underwent a 7-day cross-linking protocol. Non-cross-linked specimens contained less than a third of the HA added to the slurry, compared to almost 90% of the HA bound to the EDAC-cross-linked samples (Table 1).

After 5 days in culture medium, the HA content in the scaffolds was 5-7% regardless of initial HA loading. By 10 days no HA was found remaining in the 2 groups with the highest initial HA contents. After 21 days, samples prepared with 50%HA retained 5.5% HA.

After only 2 weeks in culture cartilaginous tissue was found in the chondrocyte-seeded HA-collagen scaffolds. However, there were not noticeable differences in the tissues formed in the scaffolds with the various HA-collagen ratios.

Table 1. Percentages of Hyaluronic Acid Incorporated into the Scaffolds, and Pore Diameters; %HA column shows the amount of HA (by wt.) added to the slurry; n=3, mean±std. dev.

% HA	Percent (by wt.) of HA in the Scaffolds*				Pore Dia µm
	EDAC X-L for 7 da	EDAC X-L for 2 hr.	95% EtOH 30 min.	Non-X-L	EDAC X-L 7d
100	-	-	-	-	97 ± 4
80	82.6 ± 0.7	45.5 ± 0.2	-	-	132 ± 5
65	72.9 ± 0.7	39.3 ± 0.2	-	-	137 ± 5
50	52.4 ± 0.2	38.0 ± 0.2	18.6 ± 0.1	-	164 ± 4
35	30.6 ± 0.3	28.2 ± 0.2	17.8 ± 0.1	11.9 ± 0.1	190 ± 6
10	8.4 ± 0.2	9.3 ± 0.1	8.3 ± 0.1	8.4 ±0.1	120 ± 4
0	-	-	-	-	151 ± 5

* Scaffolds were washed 7x in water for 30 min. before analysis.

The covalent linkage of HA to collagen is critical for the fabrication of HA-collagen scaffolds. Because HA is easily damaged at temperatures exceeding 90°C, dehydrothermal treatment is not possible. The solvent used for EDAC cross-linking critical. Because the chemical cross-linking of HA proceeds at a slow rate, during the initial stage of cross-linking HA should be in a solution with little water in order to prevent its dissolution. At the same time, because the esterization process requires water as a catalyst and medium, the gradual increase of the water content of the cross-linking solution is necessary, as was done in the present investigation.

Although HA was cross-linked to the collagen in the scaffold, it was still easily lost in culture medium. Scaffolds with higher amounts of HA became fragmented after a few days in culture medium. The release of HA *in vivo* may favor certain cell behavior.

Chondrogenesis tended to be localized to ceratin areas in the scaffolds, perhaps due to the lack of interconnectivity among pores. While light microscopy revealed variation in the structure and interconnectivity of the pores of the scaffolds with varying HA content, no noticeable differences were observed in chondrogenesis of the cells in the scaffolds with the various HA-collagen ratios, in the 2-week course of this study; longer culture periods may be required to reveal differences among scaffolds.

Conclusions: This study contributes to the understanding of the effects of HA content on various properties of HA-collagen scaffolds. The demonstration that these scaffolds can be populated by chondrocytes and support *in vitro* formation of cartilaginous tissue warrants further investigation of this material system for tissue engineering.

Acknowledgments: Department of Veterans Affairs and the Hong Kong Foundation for Overseas Research (ST).