Cellular Response to the Structure of Semi-degradable Hydrogels Based on Poly(vinyl alcohol)

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Statement of Purpose: Articular cartilage has a limited capacity for spontaneous repair, but tissue engineering strategies employing cell-seeded scaffolds and hydrogels present a promising approach. Poly(vinyl alcohol) (PVA) hydrogels have been well-studied as implants to replace damaged cartilage because of their mechanical properties and water content, but their lack of cell adhesiveness limits their ability to promote cell migration and integration with surrounding tissue (Schmedlen RH. Biomat. 2002;23:4325-4332). This study examines the effects of incorporating a phase of poy(lactic-co-glycolic acid) (PLGA) into a PVA hydrogel in order to increase cell adhesion. In addition, very few studies have specifically addressed how a hydrogel's material properties affect cellular response and tissue formation. It is generally accepted that scaffolds must be degradable, but fully degradable scaffolds often result in a loss of long-term stability (Bryant SJ. JBMR. 2002;59:63-72). It is also assumed that high porosity is better for cell migration, but higher porosity leads to lower mechanical properties, reducing a biomaterial's chance of success (Frenkel SR. Ann. Biomed. Eng. 2004;32:26-34). The addition of a degradable PLGA phase dispersed within the nondegradable PVA phase results in a semi-degradable hydrogel whose porosity and relative degradability can be varied, so that the effects of material properties of hydrogels on cellular response and cartilage tissue formation can be examined. This study specifically addresses the effects of structure of the PLGA phase as well as the relative amount of the PLGA phase on cell adhesion.

Methods: PLGA was incorporated into the PVA hydrogel solution, which was subsequently crosslinked by freeze-thaw cycling. The effect of the structure of the hydrogels on cell adhesion was investigated by seeding cells on PVA hydrogels with a phase of either PLGA microparticles or dispersed fibrous PLGA. The PLGA microparticles were prepared by a double emulsion technique and physically mixed into the hydrogel solution prior to crosslinking, or fibrous PLGA was dispersed throughout the hydrogels by creating an emulsion of PLGA in dichloromethane with the aqueous PVA solution before crosslinking. Fibroblast-like cells derived from late-passage rat bone marrow stromal cells were seeded on the hydrogels and allowed to attach overnight in low-glucose media at 37ºC. The following day, the hydrogels were washed twice with PBS to remove nonadherent cells and trypsin was added. The number of cells that detached from the hydrogels after trypsinization was determined by counting. The effects of the relative content of PLGA on cell adhesion was determined by seeding cells on hydrogels with increasing amounts of fibrous PLGA dispersed throughout the hydrogels.

Results: Figure 1 shows environmental scanning electron microscopy (ESEM) images of cross-sections of hydrogels prepared with PLGA microparticles (Fig. 1a) or in the emulsion method, with fibrous PLGA dispersed throughout the matrix (Fig. 1b).

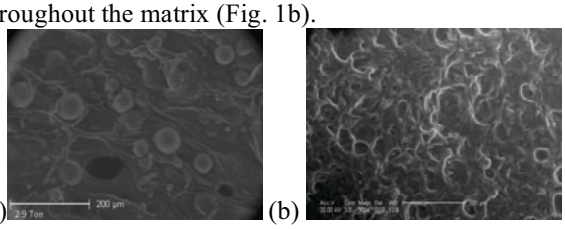

Figure 1. ESEM images of hydrogels prepared with (a) PLGA microparticles or (b) dispersed PLGA.

Despite having the same content of PLGA (40wt% of the polymer phase) and similar porosity (around 33%), cells did not adhere to the hydrogels made with microparticles, but 59 (±12)% of the cells adhered to the hydrogels made with dispersed PLGA. The increased cell adhesion on the latter formulation may be due to the increased surface area of PLGA available for attachment, since the microparticles in the other formulation are embedded within the PVA matrix, or its closer similarity to the native extracellular matrix. A cell adhesion study was also performed on hydrogels of the formulation with dispersed PLGA in order to determine the optimal PLGA content. The water content of all the hydrogels was around 67% and the porosity was around 33%. The fraction of cells that adhered to these hydrogels, presented as mean ± standard deviation, is shown in Fig. 2. The content of PLGA is expressed as a percentage of the total polymer phase of the hydrogel.

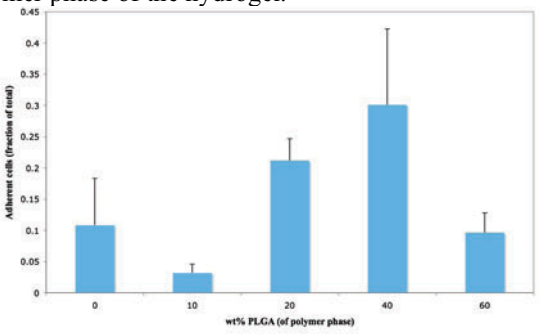

Figure 2. Cell adhesion to hydrogels with increasing amounts of PLGA.

In general, more cells adhered to hydrogels prepared with more PLGA, except for the formulation prepared with 60wt% PLGA, which had no more cell adhesion than the control hydrogels. The increased cell adhesion is a result of an increase in the hydrophobic area for the cells to attach, until a threshold was reached at 60wt% PLGA.

Conclusions: The addition of PLGA to these hydrogels renders them adhesive for cells, but also allows their relative degradability and porosity to be easily varied in order to study the optimal properties for cartilage repair. This study focused on the beneficial effects of the PLGA phase on cell adhesion. Ongoing studies will investigate how the degradation of the PLGA phase and the porosity of the hydrogels affect tissue formation.