FUNCTIONAL PROPERTIES OF BIOMATERIALS FOR CARTILAGE TISSUE ENGINEERING USING ADIPOSE DERIVED ADULT STEM CELLS

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INTRODUCTION

Tissue engineering promises novel alternatives to conventional repair techniques by combining cells, biomaterials, and biofactors into constructs that can be surgically implanted into cartilage lesions [1]. Previous studies have shown that human adipose-derived adult stem (*h*ADAS) cells can be differentiated into a chondrocyte phenotype [2]. For effective utilization in cartilage tissue engineering applications, *h*ADAS cells must be seeded onto biomaterial scaffolds that should meet minimum biological and biomechanical functional criteria [3]. The goal of this study was to assess the functional characteristics of a gelatin (Surgifoam, denatured collagen type I) sponge as a biomaterial for cartilage tissue engineering compared to two materials frequently used in 3D chondrocyte culture studies; alginate and agarose. We hypothesized that *h*ADAS cells seeded on Surgifoam and cultured in chondrogenic conditions will grow a functional cartilage-like tissue.

METHODS

*h*ADAS cells, isolated from subcutaneous adipose tissue (n=3 donors), were suspended directly in a 2% (w/v) low viscosity alginate and low melting point agarose gels at 10^7 cells/ml. The cell suspensions were cast in custom molds and allowed to gel. Smaller disks (6 mm diameter) were then created with a biopsy punch. Porous gelatin (SURGIFOAM, J&J) disks (8 mm diameter), pre-wetted in culture medium in flat bottom tubes, were populated with *h*ADAS cells at a comparable concentration (10^7 cells/ml) by centrifugal force-induced flow. All disks were cultured in either control media (DMEM-hg + 10% FBS + 1% antibiotics) or in chondrogenic media (Control media + 1X insulin-transferrin-selenium, 37.5 μg/ml ascorbate, 10 ng/ml TGF-β1, and 100 nM dexamethasone [2]).

Biological Properties: Cell viability and cellular morphology were examined *in situ* on days 1, 7, 14, and 28 using a confocal laser scanning microscope and the fluorescent Live-Dead probes (Calcein AM and Ethidium homodimer, Molecular Probes, Eugene, OR). To assess cell synthesis rates, constructs were dual-labeled with 5 μCi/ml $^{35}SO_4$ and 10 μCi/ml ^{3}H-proline for 24 hours on days 1, 7, 14 and 28. Afterwards, the constructs were rinsed to remove unincorporated free label and then digested in a 50μg/ml papain solution at 65°C overnight. The digests were then analyzed on a Tri-Carb Scintillation Analyzer after sampling aliquots for DNA content determination as described below.

Biochemical Analysis: Constructs, digested with Papain, were analyzed to determine the DNA content using the picoGreen DNA quantification kit (Molecular Probes). Sulfated glycosaminoglycan (S-GAG) and hydroxyproline content were determined using the DMB and chloramine-T DMBA assays, respectively, at days 7, 14 and 28.

Immunohistochemistry: Frozen (agarose and alginate) and paraffin embedded (Surgifoam) sections were prepared from constructs cultured for 28 days using routine techniques and then stained for a primary antibody against the 2B6 epitope of chondroitin sulfate using the Histostain-SP kit (Zymed Laboratories, Inc.).

Biomechanical Properties: The elastic compressive modulus was determined from equilibrium stress-strain data from stepwise stress relaxation tests in unconfined compression configuration at strains of 4, 8, 12, and 16%. Likewise, the elastic shear modulus was determined from equilibrium stress-strain data from stepwise shear stress-relaxation (pure torsion) experiments at shear strains of (0.03, 0.04, and 0.05 radian). Following the stress-relaxation tests, the rheological properties of the constructs were determined by subjecting the samples to oscillatory shear strain $\gamma(t)= \gamma_o.\sin(\omega t)$ of a fixed amplitude (γ_o=0.05 radian) and varying frequency (1-100 rad/sec). The resultant oscillatory shear stress $\sigma(t)=\sigma_o.\sin(\omega t+\delta)$ was recorded and the rheological properties such as the complex shear modulus $|G^*(\omega)|^2=[G'(\omega)]^2+[G''(\omega)]^2$ and the loss angle δ were determined for each of the applied frequencies; where G is the storage modulus $[G'(\omega)=\sigma_o.\cos(\delta(\omega))/\gamma_o]$ and G'' is the loss modulus $[G''(\omega)=\sigma_o.\sin(\delta(\omega))/\gamma_o]$. Biomechanical tests were performed in a bath of DMEM-hg at room temperature using an ARES Rheometrics System on days 1, 14, and 28.

Statistical Analysis: Analysis of variance with Student-Newman-Keuls (SNK) multiple ranges tests were used to compare the different biomaterials and culture conditions (α=0.05).

RESULTS

All constructs showed relatively uniform distributions of cells with viability greater than 95% at all time points. Cells in alginate and agarose maintained a spherical morphology whereas cells in Surgifoam displayed mixed morphologies with a significant population having elongated "fibroblastic" shape.

Protein and proteoglycan synthesis rates, normalized by DNA content, were significantly greater for all disks cultured in chondrogenic conditions compared to those cultured in control conditions ($p<0.05$). However, in general there were no significant differences between the synthesis rates in the different biomaterials (Figure 1).

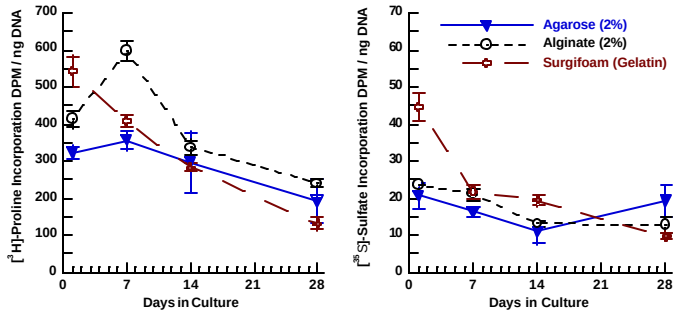

Figure 1. Normalized protein and proteoglycan synthesis rates in the tissue constructs (chondrogenic culture)

The DNA content (normalized by the wet weight) in Surgifoam disks significantly increased by more than 50% between days 7 and 28 ($p<0.05$), whereas alginate and agarose disks showed only a mild increase between days 7 and 14. The S-GAG and hydroxyproline content in all constructs (normalized by the wet weight) increased between days 7 and 28 ($p<0.05$) and were greater in chondrogenic conditions than in control conditions ($p<0.05$). The rate of increase in normalized S-GAG and hydroxyproline content between days 7 and 28 was comparable for all constructs.

Cartilage matrix formation in the disks cultured in chondrogenic media was also evident by the positive immunohistochemical staining against the 2B6 epitope of chondroitin sulfate (Figure 2), mostly around cells in cartilage-characteristics lacunae, albeit some nuclei in the Surgifoam disks lacked that distinct morphology.

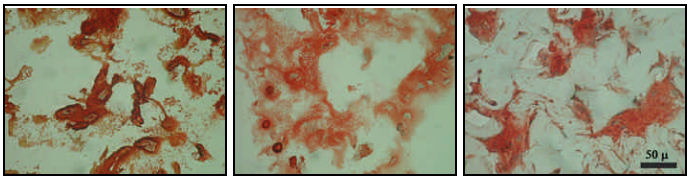

Figure 2. Immunohistology staining against chondroitin sulfate in representative sections of the *h*ADAS cell-seeded agarose, alginate, and Surgifoam disks (from let to right, 20X)

Surgifoam disks progressively contracted to an average of 70% and 87% their initial diameters under chondrogenic and control culture conditions, respectively, whereas alginate and agarose gels maintained their initial dimensions. Concomitant with the dimensional contraction, the elastic compressive and shear elastic moduli of the Surgifoam increased progressively over time, reaching values comparable to agarose by day 28. On the other hand, alginate constructs had the lowest compressive and shear elastic moduli at all time points (Figure 3). Similar results were obtained from the frequency sweep tests. The complex shear modulus of the Surgifoam disks was higher than alginate at all frequency decades (Figure 4, $p<0.05$). Further, at lower frequencies, there were no differences in the complex shear modulus between the agarose and Surgifoam. The loss angle (δ) indicated that all constructs behaved as viscoelastic solids.

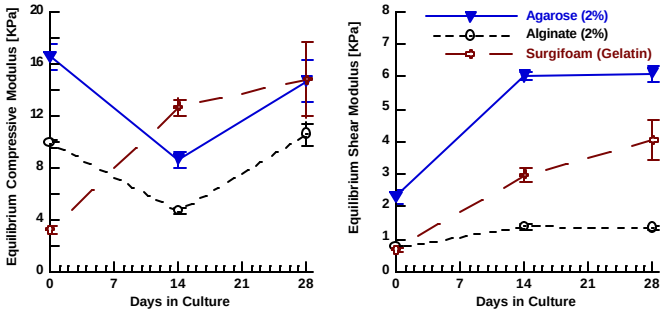

Figure 3. Equilibrium compressive and shear elastic moduli of the tissue constructs (chondrogenic culture)

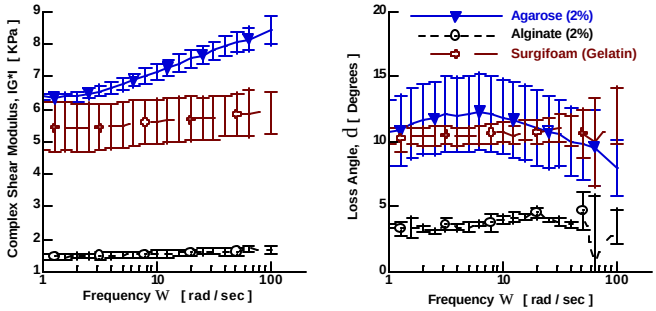

Figure 4. Dynamic frequency shear response (γ_o=0.05) of the tissue constructs after 28 days of chondrogenic culture

DISCUSSION

Our findings indicate that porous Surgifoam scaffolds have biological and mechanical functional properties that exceed those of alginate and are nearly comparable with agarose when combined with *h*ADAS cells and grown under appropriate chondrogenic conditions. The chondrogenic differentiation of the *h*ADAS cells was manifested by S-GAG accumulation and immunohistochemical detection of proteoglycans in the newly formed matrix around the cells. These results are similar to previous reports that demonstrated that mesenchymal stem cells from bone marrow readily populate Gelfoam sponges and produce cartilage matrix when cultured in chondrogenic conditions (TGF-β3) [4]. Such biodegradable Surgifoam sponges are promising for tissue engineering applications since they are approved for implantation in vivo and induce minimal immune responses [4].

REFERENCES

1. Awad et al (2002), in Tissue Engineering and Biodegradable Equivalents: Scientific and Clinical Applications. Lewandrowski et al (Eds), Marcel Decker, New York, pp. 267-299
2. Erickson et al (2002), Biochem Biophys Res Comm. 260:763-769
3. Butler et al (2000), J Biomech Eng. (122): 570-575
4. Ponticiello et al, (2000), J Biomed Mater Res. (52): 246-255

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