MOLECULAR DIFFUSION IN TISSUE-ENGINEERED CARTILAGE CONSTRUCTS: EFFECTS OF TIME AND CULTURE CONDITIONS

Holly A. Leddy, Hani A. Awad, M. Quinn Wickham, and Farshid Guilak

Orthopaedic Research Laboratories
Department of Surgery
Duke University Medical Center
Durham, NC

INTRODUCTION

Articular cartilage is an avascular and alymphatic tissue, suggesting that the primary mode of transport for nutrients, oxygen, wastes, signaling molecules, and matrix remodeling molecules is diffusion. Diffusive transport may play the same crucial role in tissue engineered cartilage constructs both in vitro and in vivo. Understanding diffusive transport in tissue-engineered cartilage constructs is important because adequate nutrient supply via diffusion may be necessary for cell proliferation and extracellular matrix production. For example, collagen and glycosaminoglycan production is increased in mixed versus static cultures of tissue-engineered cartilage constructs, potentially due to altered molecular transport conditions in the mixed cultures [1]. In addition, providing the appropriate diffusive environment may be another biophysical cue, similar to mechanical stress [2], that can promote cartilage formation in a construct. Furthermore, the diffusivity in a tissue-engineered construct can regulate the retention and assembly of newly synthesized matrix within a construct.

The diffusivity of a tissue-engineered construct represents the net sum of a number of different factors. Initially the diffusion coefficient will primarily be a function of the scaffold material; however, over time there will be a number of processes occurring that can affect the diffusivity. For example, the type and amount of new extracellular matrix molecules produced will depend upon the specific culture conditions. These matrix molecules may be retained within the construct and therefore influence the diffusivity, or they may diffuse out of the construct and thus have little or no effect on diffusivity. Furthermore, certain cells may exert significant contractile forces on some scaffolds [3], potentially consolidating the scaffold material in a manner that may decrease the diffusivity. Finally, the scaffold material itself may degrade over time, leading to an increase in diffusivity.

The goal of this study was to determine how the diffusive properties of tissue-engineered cartilage constructs vary with scaffold biomaterial, culture conditions, and time in culture. Acellular scaffolds were compared to tissue-engineered cartilage constructs seeded with human adipose derived stromal cells.

MATERIALS AND METHODS

Human adipose derived adult stromal cells were isolated from subcutaneous adipose tissue from three donors. Cells were seeded into all scaffolds at approximately 10^7 cells/ml, and all scaffolds consisted of 5-8mm diameter disks. Four different scaffold materials were used: Surgifoam™ porous gelatin scaffold (Johnson & Johnson), fibrin (from the Tisseel™ two component fibrin sealant kit, Baxter), 2% weight/volume low viscosity sodium alginate, and 2% weight/volume low melting point agarose. Scaffolds with cells were cultured for 1 or 28 days at 37ºC and 5% CO_2 in control media (DMEM-HG, 10% FBS, 1% penicillin/streptomycin) or chondrogenic media (control media plus 1x insulin-tranferrin-selenium supplement, 37.5μg/ml ascorbate, 10ng/ml TGF-β1, 10nM dexamethasone). This medium has been shown previously to induce chondrogenesis in this cell type [4]. In addition, a set of acellular scaffolds (blanks) was cultured for the same duration in control media.

After culture, constructs were incubated overnight in solutions of 3, 70, or 500kDa fluorescein-conjugated dextran suspended in phosphate buffered saline. Diffusion coefficients of these fluorescently labeled molecules in the constructs were measured using fluorescence recovery after photobleaching (FRAP). Diffusion coefficients were calculated from the size of the bleached area and the time to half-recovery during the FRAP experiment [5].

RESULTS

The diffusion coefficients of all sizes of dextran in tissue engineered cartilage constructs were at least twice those for the same molecules in native cartilage [6] (Figure 1). In addition, the diffusivity of the fibrin scaffolds tended to be lowest, with alginate and agarose higher, and Surgifoam had the highest. This trend was consistent across all size dextrans. Three main trends were observed with respect to how the diffusion coefficients changed from day 1 to day 28 within each culture condition (Table 1):

- Diffusivity tended to increase in the acellular constructs.
- Diffusivity tended to remain constant in the control constructs.
- Diffusivity tended to decrease in the chondrogenic constructs.

In most cases the presence of cells, whether in control or chondrogenic conditions, caused the diffusivity in the constructs to decrease or remain constant while the diffusivity in blank scaffolds increased (Figure 2a). In addition, in most cases the diffusion coefficient of the construct in chondrogenic conditions decreased beyond the control (Figure 2b). Only the two hydrogel scaffolds with the smallest dextran showed no consistent effect of the presence of cells on the diffusivity over time.

DISCUSSION

Our findings suggest that the diffusion of uncharged dextran molecules in engineered tissue constructs depends on the biomaterial composition of the construct, the presence of cells, the culture conditions, and the culture time. Overall, the presence of cells grown in a chondrogenic medium led to decreased diffusivity over time, while acellular scaffolds generally showed increased diffusivity over time. These findings most likely reflect a combination of factors that include the synthesis, assembly, and retention of matrix macromolecules, cellular contraction of the matrix, and degradation of the scaffold. Furthermore, the higher diffusivity of all of the constructs as compared to native cartilage suggests that the transport of nutrients and metabolites to cells within the constructs will not be hindered in the early stages of tissue generation.

The increase in diffusivity of the acellular constructs over time probably indicates degradation of the scaffolds. As the scaffolds are broken down both a loss of connectivity between molecules, as well as a decrease in the total number of molecules, could contribute to an increase in diffusivity.

We observed significant contraction of the fibrin and Surgifoam constructs. The diameter of the constructs shrank as much as 60% from the original size. Those fibrin and Surgifoam constructs without cells, as well as the alginate and agarose constructs, did not change size during the 28 days of culture. This contraction of the constructs is likely to pack the molecules comprising the scaffold closer together. In native cartilage, a 25% volumetric compressive strain can halve the diffusion coefficient [7]. Therefore, it is likely that contraction of the cellular Surgifoam and fibrin scaffolds may have contributed in part to any observed decreases in diffusivity.

Concurrent work with the same set of scaffolds and cells has shown that the cells are producing cartilage extracellular matrix molecules under both control and chondrogenic conditions. In addition, the rates of proteoglycan and protein synthesis are greater under chondrogenic conditions than those under control conditions [8]. Thus, any decreases in diffusivity not attributable to contraction are likely due to matrix accumulation, particularly under chondrogenic conditions.

ACKNOWLEDGMENTS

Supported by Artecel Sciences, Inc., NIH grants AG15768, AR48182, and GM08555.

REFERENCES

1. Gooch, K.J., et al., 2001, Biotech. Bioeng., 72, pp. 402-407.
2. Butler, D.L. et al., 2000, J. Biomech. Eng., 122, pp. 570-575.
3. Awad, H.A., et al., 2000, J. Biom. Mat. Res., 51, pp. 233-40.
4. Erickson, G.R., et al., 2002, Biochem. Biophys. Res. Com., 290, pp.763-9.
5. Axelrod, D., et al., 1976, Biophys. J., 16, pp.1055-1069.
6. Leddy, H.A. and F. Guilak, in press, Ann. Biomed. Eng.
7. Quinn, T.M., et al., 2000, Arch. Biochem. Biophys., 384, pp.327-34.
8. Awad, H.A., et al., submitted, ASME 2003 Summer Bioengineering Conference.

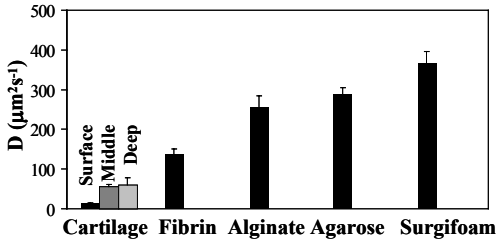

Figure 1. Diffusion coefficients of 70kDa dextran in the three zones of native cartilage [6] are less than those in tissue-engineered cartilage constructs. Means(+s.e.) for each scaffold type averaged across all times and conditions.

Figure 2. Diffusion coefficients of A. 500kDa dextran in alginate constructs and B. 70kDa dextran in fibrin constructs at days 1 (dark bars) and 28 (light bars) under blank, control, and chondrogenic conditions (mean+s.e.). *p<0.05, between day 1 and day 28.

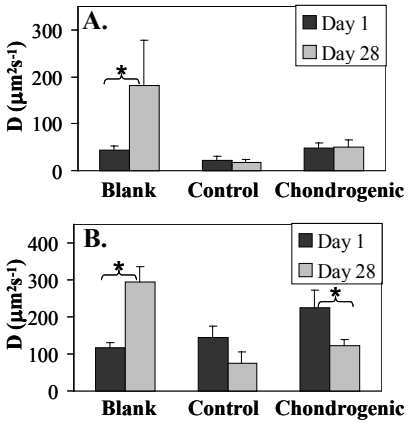

Table 1. Change in diffusivity between day 1 and day 28 for each size dextran in tissue-engineered cartilage constructs under different culture conditions. Arrows indicate the direction of significant (black arrows, t-test, p<0.05) or near significant (gray arrows, t-test, p<0.08) change in the diffusion coefficient; equal signs indicate no significant change (t-test, p>0.08).

Agarose	Blank	Control	Chondrogenic
3kDa	=	=	=
70kDa	=	↓	↓
500kDa	↑	=	↓
Alginate	**Blank**	**Control**	**Chondrogenic**
3kDa	=	=	↑
70kDa	=	=	↓
500kDa	↑	=	=
Fibrin	**Blank**	**Control**	**Chondrogenic**
3kDa	↑	=	↓
70kDa	↑	=	↓
500kDa	↑	↓	↓
Surgifoam	**Blank**	**Control**	**Chondrogenic**
3kDa	↑	↓	↓
70kDa	↑	=	↓
500kDa	↑	=	↓