

Bioreactors mediate the effectiveness of tissue engineering scaffolds¹

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SPECIFIC AIM

The present study tested the hypothesis that the mechanically active environment present in rotating bioreactors mediates the effectiveness of 3-dimensional (3-D) scaffolds for cartilage tissue engineering.

PRINCIPAL FINDINGS

Cartilaginous constructs were engineered by using bovine calf chondrocytes in conjunction with two scaffold materials (SM) (benzylated hyaluronan or Hyaff-11[®] and polyglycolic acid or PGA) and three scaffold structures (SS) (sponge, nonwoven mesh, or NWM and composite mesh or CM) (Fig. 1A) using two culture systems (CS) (a bioreactor system and Petri dishes). Individual and interactive effects of model system parameters (SM, SS, CS, SM*CS, and SS*CS) on construct structure, function, and molecular properties were evaluated.

1. Bioreactors improved cell seeding of 3-D scaffolds

Bioreactors yielded 3-day constructs with spatially uniform cell distributions, whereas dishes yielded 3-day constructs in which cells were located at the bases of the pores of the sponge or the bottom surface of the mesh. Bioreactor-grown 3-day constructs had significantly higher cell numbers (76–132% of the number initially seeded) than the corresponding dish-grown constructs (47–63% of the number initially seeded) due mainly to the effect of CS (Table 1).

2. Bioreactors improved in vitro chondrogenesis

Bioreactors yielded 1-month constructs that were large, thick, and contained more uniformly distributed GAG than dish-grown constructs, which were relatively small, thin, and inhomogeneous (Fig. 1B vs. C). Bioreactor-grown, 3-day constructs stained intensely and homogeneously for collagen type I and II mRNAs; 2-wk and 1-month constructs stained intensely and homogeneously for collagen-II mRNA and only weakly and peripherally for collagen-I mRNA. Type-specific colla-

gen mRNA and protein staining patterns were well correlated. Bioreactor-grown, 1-month constructs made using mesh-based scaffolds had compressive moduli that ranged from 0.40 to 0.54 MPa (Table 1), values comparable to those previously reported for native bovine calf cartilage.

3. CS was the parameter with the greatest effect on 1-month construct properties

The individual effect of CS was significant for five of the six properties assessed in 1-month constructs [cell number, wet weight (ww), amount and ww fraction of GAG, and amount of collagen] (Table 1). CS*SM interactions affected five of the six properties (ww and amounts and ww fractions of glycosaminoglycans (GAG) and collagen) and CS*SS interactions affected three of the six properties (ww and ww fractions of GAG and collagen) (Table 1). Bioreactor-grown 1-month constructs also had better molecular properties than corresponding dish-grown constructs, as shown by relatively higher levels of cartilage-specific collagen type II and lower levels of the nonspecific collagen type I.

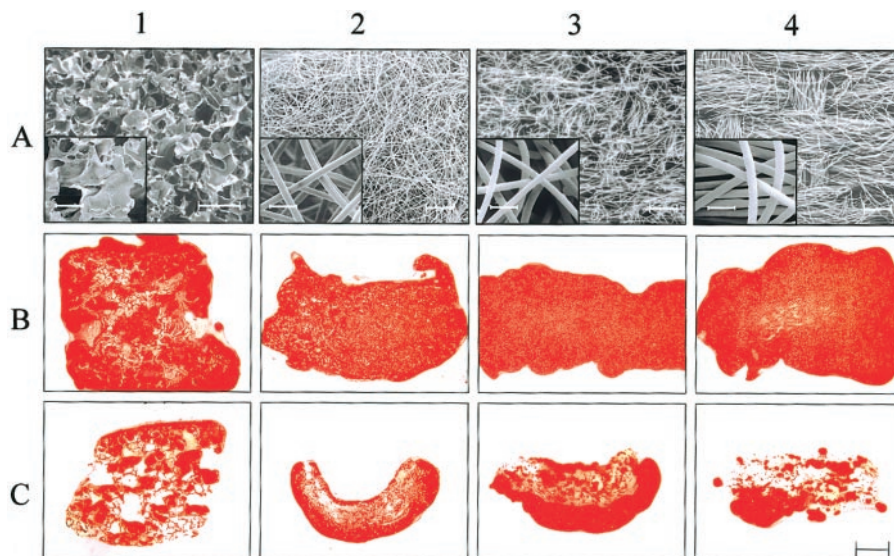
4. SS had a greater effect than SM on 1-month construct properties

The SM individually affected only one property assessed in 1-month constructs (ww fraction of total collagen), whereas the SS individually affected all seven of the assessed properties (cell number, ww, amounts and ww fractions of GAG and collagen and modulus) (Table 1). In bioreactors, PGA CM was superior to PGA NWM with respect to construct size and cell number, and Hyaff-11[®] NWM was superior to Hyaff-11[®] sponge with respect to construct cell number, ww, and ww GAG fraction (Table 1). Bioreactor-grown constructs based on Hyaff-11[®] NWM had fourfold higher compressive moduli than corresponding constructs based on

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Figure 1. Scaffold structures and histological appearances of constructs cultured in bioreactors or Petri dishes. *A*) Scanning electron micrographs of 1) sponge made of Hyaff-11[®]; 2) nonwoven mesh (NWM) made of Hyaff-11[®]; 3) nonwoven mesh made of PGA; and 4) composite mesh made of PGA (a composite with one piece of PGA woven fabric on each side of a piece of PGA NWM). Scale bars: 500 μ m; inset scale bars: 50 μ m. *B*, *C*) Size and distribution of glycosaminoglycans in 1-month constructs made using the four scaffolds in bioreactors (*B*) or Petri dishes (*C*). Representative cross sections of constructs based on scaffolds 1–4. Stain: safranin-O/fast green; counterstain: hematoxylin. Scale bar: 1 mm.



Hyaff-11[®] sponge, which had relatively higher levels of collagen type I.

CONCLUSIONS

We have shown that the mechanically active environment present in rotating bioreactors mediated the effectiveness of 3-D scaffolds for cartilage tissue engineering, which implies that the judicious selection of model system parameters (i.e., CS, SM, and SS) can be used to improve *in vitro* chondrogenesis and yield larger cartilaginous constructs with better structure, function, and molecular properties.

Type-specific collagen gene expression varied temporospatially during *in vitro* chondrogenesis in a manner that implied a switch from type I to type II collagen synthesis. The predominant fraction of total collagen protein in 1-month constructs was the cartilage-specific type II collagen, consistent with previous studies. Type-specific collagen protein levels depended on model system parameters such that collagen-type II levels were higher in bioreactor than in dish cultures, collagen-type I levels were lower in bioreactors than dishes, and collagen-type I was lowest in constructs based on scaffolds with mesh structures.

One-month constructs made by using scaffolds with mesh structures and cultured in bioreactors had compressive moduli that ranged from 0.40 to 0.54 MPa (Table 1), values comparable with those previously reported for native bovine calf articular cartilage. One-month bioreactor-grown, sponge-based constructs had low moduli (0.13 MPa) and high levels of collagen-type I, consistent with previous reports of lower moduli of fibro- than hyaline articular cartilage. Three-day constructs, which consist mainly of cells and PGA mesh, had been found to be too fragile to allow measurement of mechanical properties.

The CS affected cell seeding of 3-D scaffolds such that a higher yield of more spatially uniform cells was

obtained in bioreactor- vs. dish-grown 3-day constructs and affected subsequent chondrogenesis such that higher cell numbers, wet weights, wet weight fractions of GAG, and levels of collagen-type II were observed in bioreactor- than in dish-grown 1-month constructs. These data were consistent with reports showing a positive correlation between chondrocyte density and extracellular matrix (ECM) deposition in monolayers and 3-D constructs. Our experimental design involved the use of either a bioreactor system or conventional Petri dishes for cell seeding and tissue cultivation such that all reported effects of CS represent the cumulative effect of these two processes.

The hydrodynamic conditions present in rotating bioreactors include 1) efficient mixing of the bulk medium, 2) laminar flow, and 3) low flow velocity and shear stress (<0.01 cm/s and <1 dyn/cm², respectively) in the vicinity of the construct, whereas mixed Petri dishes provide only limited convection during construct cultivation. The finding that bioreactor cultivation improved certain construct properties is consistent with earlier studies showing beneficial effects of hydrodynamic and direct mechanical stimulation. Chondrocytes are known to respond to fluid flow-related phenomena, and flow-induced shear stress at the construct surfaces can potentially affect chondrocyte proliferation, ECM production, and/or the release of regulatory factors, which in turn can affect cells in the interior of the constructs.

The individual effect of SM on construct properties was lower than that of SS, possibly because all SMs studied were biocompatible and supportive of chondrogenesis. The SS individually affected all of the properties assessed in 1-month constructs, including cell number and compressive modulus. In bioreactors, scaffolds with the CM structure yielded 1-month constructs that were larger and contained more cells than scaffolds with the NWM structure, which themselves yielded constructs with higher moduli and lower collagen-type I levels than the scaffold formed as a sponge. These

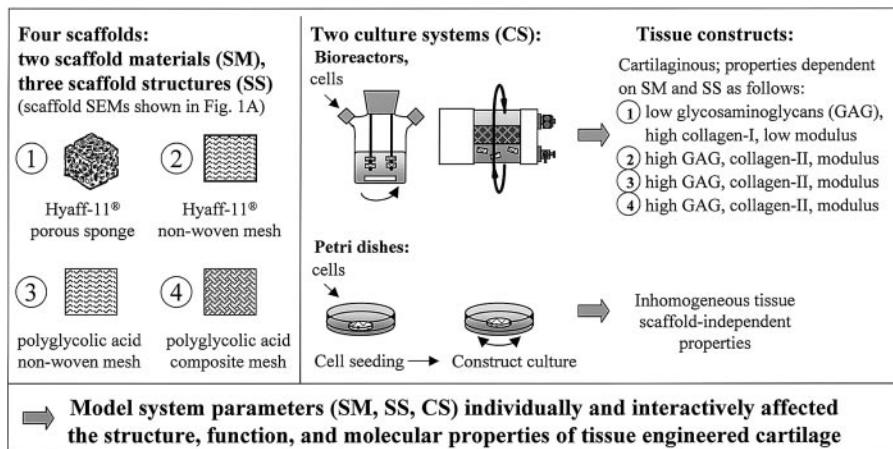
TABLE 1. Individual and interactive effects of model system parameters on construct properties^a

Scaffold material (SM)	Hyaff-11® sponge		Hyaff-11® NWM		PGA CM		Hyaff-11® sponge		Hyaff-11® NWM		PGA NWM		PGA CM		SM		SM	
	SS	CS*	NWM	SS	NWM	CM	NWM	SS	NWM	CM	SS	CM	SS	CM	SS	CS	SS*	CS
Culture system (CS)	Bioreactors						Petri dishes											
ANOVA	P						P						P					
3-Day constructs																		
Cells (millions/C, n=3)	3.04 ± 0.16	3.95 ± 0.74	5.29 ± 1.22	4.64 ± 1.30	1.99 ± 0.13 ^b	2.51 ± 0.34 ^b	1.88 ± 0.95 ^b	2.35 ± 0.44 ^b	NS	NS	<0.0001	0.046	NS	<0.0001	0.046	NS		
Wet weight (mg, n=3)	48.4 ± 1.5	29.6 ± 3.7	33.7 ± 2.1	47.7 ± 2.0 ^c	44.6 ± 3.1	25.4 ± 3.2	30.7 ± 4.9	43.9 ± 2.9 ^c	0.017	<0.0001	0.0097	NS	NS					
1-Month constructs																		
Cells (millions/C, n=3)	9.38 ± 0.88	12.4 ± 1.5 ^c	12.8 ± 0.6	15.6 ± 0.36 ^c	5.07 ± 0.53 ^b	6.00 ± 0.41 ^b	4.51 ± 0.82 ^b	5.91 ± 1.49 ^b	NS	0.0003	<0.0001	NS	NS					
Wet weight (mg, n=7)	144 ± 11	198 ± 29 ^c	210 ± 8	231 ± 14 ^c	75.6 ± 4.9 ^b	66.2 ± 9.0 ^b	48.5 ± 12.9 ^b	70.5 ± 16.4 ^b	NS	<0.0001	<0.0001	0.033	<0.0001					
Glycosaminoglycans (mg/C, n=3)	4.82 ± 0.74	6.72 ± 1.27	8.63 ± 0.25	9.95 ± 0.79	1.26 ± 0.15 ^b	2.51 ± 0.18 ^b	1.24 ± 0.98 ^b	1.68 ± 0.63 ^b	NS	0.0022	<0.0001	0.0016	NS					
(% wet weight, n=3)	3.29 ± 0.14	3.70 ± 0.09 ^c	4.10 ± 0.20 ^d	4.39 ± 0.22	1.58 ± 0.06 ^b	3.57 ± 0.13	2.20 ± 1.06 ^b	2.19 ± 0.45 ^b	NS	0.0007	<0.0001	0.0025	0.016					
Total collagen (mg/C, n=3)	4.90 ± 0.53	6.36 ± 0.94	7.29 ± 0.39	8.27 ± 0.72	2.37 ± 0.14 ^b	2.95 ± 0.32 ^b	1.77 ± 0.53 ^b	2.42 ± 0.58 ^b	NS	0.0040	<0.0001	0.0053	NS					
(% wet weight, n=3)	3.35 ± 0.07	3.52 ± 0.25	3.46 ± 0.15	3.65 ± 0.25	2.99 ± 0.15 ^b	4.20 ± 0.33 ^b	3.48 ± 0.13	3.20 ± 0.18	0.0042	0.0001	NS	0.014	0.0008					
Compressive moduli (MPa, n=4)	0.13 ± 0.06	0.52 ± 0.22 ^c	0.40 ± 0.08	0.54 ± 0.21	NM	NM	NM	NM	NS	0.0099	NM	NM	NM					

Construct composition was determined by assaying enzymatically digested constructs for cells (DNA), glycosaminoglycans (chondroitin sulfate), and total collagen (hydroxyproline); construct function was determined by coring discs from the constructs and measuring compressive moduli in uniaxial stress-relaxation studies.

^aAbbreviations: PGA = polyglycolic acid; NWM = nonwoven mesh; CM = composite mesh; C = construct; NM = not measured; NS = not significant. Data represent the mean ± SD of n = 3 to 7 independent samples. ^bSignificantly different (P<0.05 by Tukey test) from corresponding constructs cultured in bioreactors. ^cSignificantly different (P<0.05 by Tukey test) from corresponding constructs based on Hyaff-11® NWM. ^dSignificantly different from corresponding constructs based on Hyaff-11® sponges. ^eSignificantly different (P<0.05 by Tukey test) from corresponding constructs based on PGA NWM.

Figure 2. Schematic diagram. Mechanically active culture environments in bioreactors mediate the effectiveness of tissue engineering scaffolds. In the bioreactor system, cells were inoculated into spinner flasks in which scaffolds were fixed in place; after 48 h, constructs were transferred into rotating vessels and cultured in a dynamic laminar flow environment. In Petri dishes, cells were loaded on scaffolds and cultured with orbital mixing.



data suggest that the optimal scaffold structure provides a very high surface area for cell seeding (e.g., higher bulk density due to increased fiber entanglement may explain the advantage of the CM over the NWM) while containing a minimal amount of polymer (e.g., higher porosity and pore interconnectedness may explain the advantage of the meshes over the sponge).

Our finding that in vitro chondrogenesis was affected by SM*CS and SS*CS interactions is analogous to recently reported interactive effects of mechanical

and biochemical factors on native and engineered cartilage. Although further studies are needed to determine mechanism(s) underlying these interactions, the present study adds to a growing body of evidence that structure, function, and molecular properties of cartilaginous tissues depend on the combined effects of biochemical and physical stimuli, and suggests that bioreactors can contribute significantly to the evaluation of promising new tissue engineering scaffolds. **FJ**