CULTURE OF CHONDROCYTES IN RGD-ALGINATE: EFFECTS ON MECHANICAL AND BIOSYNTHETIC PROPERTIES

*Genes, N G. (A-University of Massachusetts); **Rowley, J A. (A-NIH); **Mooney, D J. (A-NIH); +*Bonassar, L J. (A-University of Massachusetts)
+*University of Massachusetts, Worcester, MA. Tissue Engineering Laboratory/55 Lake Avenue North/Worcester MA 01655, 508-856-5657, Fax: 508-856-3921, lawrence.bonassar@umassmed.edu

INTRODUCTION Hydrogels such as alginate have been used for culture of anchorage-independent cells like chondrocytes. Alginate, an anionic polysaccharide capable of reversible gelation, has been shown to support the differentiated phenotype and expression of cartilage-specific proteins by chondrocytes [1]. Chondrocytes suspended in alginate do not appear to attach to the polymer as cells attach to cartilage matrix *in situ.* A polymer matrix that encouraged cell attachment via integrins while maintaining the preferred spherical shape would be of great advantage for cartilage tissue engineering as well as for investigating mechanisms of chondrocyte mechanotransduction. The objectives of this study were 1) to characterize the interaction of chondrocytes with normal and RGD-modified alginate by scanning electron microscopy; 2) to examine the effect of cell attachment to alginate on the mechanical properties of the hydrogel; and 3) determine the effect of the RGD-alginate on chondrocyte matrix assembly.

MATERIALS AND METHODS

Chondrocyte Cell Culture Articular chondrocytes were isolated from the cartilage of gleno-humeral joints of 1-2 week-old calves by digestion in 0.2% collagenase for 16 hours at 37°C. Cell suspension was poured through a 125 μm filter, washed 3 times with PBS, resuspended in DMEM and counted with a hemocytometer. Cells were resuspended in 2% alginate or 2% RGD-alginate in phosphate buffered saline (PBS). RGD-alginate was produced as described previously [2]. Briefly, the peptide sequence GGGGRGDY was covalently bonded to medium viscosity alginate through condensation reaction with manuronic or guluronic acid residues by carbodiimide chemistry.

Chondrocyte-alginate culture discs were constructed by adding 80 μl of well-mixed 0.266 mg/ml $CaSO_4$ to 2 ml of 2% alginate or 2% RGD-alginate containing 0 to 150×10^6 chondrocytes/ml. After thorough mixing, the gel was quickly spread between plates separated by 1 mm spacers, producing a sheet of crosslinked alginate within 10 minutes. Using a biopsy punch, 6 mm diameter discs were cut from the sheet, and each disc was placed into 2 ml DMEM with 10% FBS, 25 mg/ml ascorbic acid, antibiotics and antimycotics in the presence of absence of 10 μM cytochalasin D at 37°C, 5% CO_2. The media was replaced every 48 hours.

Scanning Electron Microscopy Alginate disks were fixed in 2.5% gluteraldehyde in PBS for 10 min, followed by immersion in 1% OsO_4 for 30 minutes. Samples were critical point dried and cut to expose interior surfaces. Surfaces of interest were sputter-coated with Au/Pd for 5 minutes, then examined on a Hitachi model 2000 scanning electron microscope.

Mechanical Testing At 4 hr and 24 hr after casting, 3 samples from each condition were placed in uniaxially confined compression. A series of ten steps in displacement were imposed on each disc, up to a maximum of 30% total strain. Resultant forces were measured at a rate of 5 Hz for 30 sec/relaxation, with displacements and loads normalized to sample geometry to yield strain and stress. Material properties were calculated using a poroelastic model of hydrogel behavior [3].

Biochemical Analysis Five weeks after casting the chondrocyte-alginate discs, the media was supplemented with 10 uCi/ml of [^{35}S] SO_4 ions for 18 hours. Each disc was then serially washed six times in 1 hr to remove free radiolabel [4]. Discs were digested with papain for 24 hr at 60°C, and aliquots were mixed with scintillation fluid for radioactivity measurement. DNA content of digests was quantified by reaction with Hoescht 33258 dye [5]. Sulfated GAG content of digests was quantified by reaction with DMB dye at pH 1.5 to prevent cross reaction with alginate mannuronic acid and guluronic acid moeties [6].

RESULTS

Scanning Electron Microscopy Chondrocytes cultured in alginate remained suspended in the material with no apparent attachment to the polymer (Fig 1A 900X). In contrast, large clumps of polymer decorated the surface of cells cultured in RGD-alginate (Fig 1B, 1400X). Chondrocytes cultured in RGD-alginate and exposed to cytochalaisin D did not interact with the polymer (Fig 1C, 2000X).

Mechanical Properties Seeding of cells into alginate resulted in a slight decrease in equilibrium modulus (H_a) at 4 hr which remained at 24hr (Fig 2). RGD-alginate constructs seeded with chondrocytes showed a time and cell concentration dependedent increase in equilibrium modulus, with the largest increase at 150×10^6 cells/ml at 24 hr. RGD-alginate constructs seeded with cells showed a decrease in equilibrium modulus after treatment with cytochalaisin D, resulting in mechanical properties similar to seeded constructs of control alginate.

Biochemical Analysis Sulfate incorporation of cells in RGD-alginate disks was significantly ($p<0.05$, 2-factor ANOVA) lower than that of cells in control alginate (Table 1). Similarly, GAG content of control alginate specimens was significantly ($p<0.05$) more than RGD-alginate samples and approached ~25% that of normal cartilage by 5 weeks in culture (Table 2).

DISCUSSION The SEM images indicate the presence of RGD peptide enables cells to adhere to alginate. Disruption of the adhesion by cytochalaisin D suggess that cell attachment to RGD-alginate is mediated by the cytoskeleton. The increase in modulus of RGD-alginate discs is consistent with the hypothesis that the chondrocyte cytoskeleton contributes to the mechanical integrity of the cell/alginate constructs. This effect is similarly inhibited in the presence of cytochalaisin D.

Decreased of biosynthetic activity and proteoglycan content in RGD-alginate discs may indicate that attachment inhibits matrix biosynthesis by a negative feedback mechanism. This is consistent with studies documenting increased matrix synthesis by chondrocytes in hydrogels after removal of pericellular matrix [7].

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Figure 1A

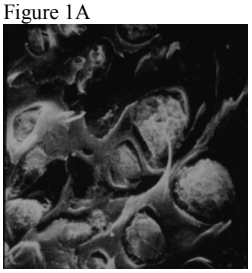


Figure 1B

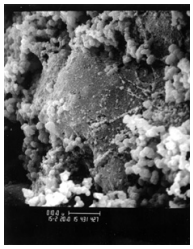


Figure 1C

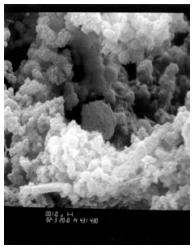
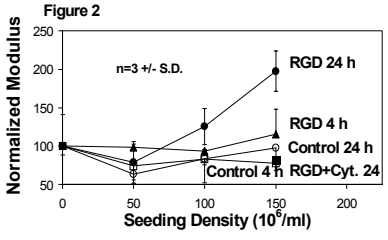


Figure 2



Tables 1 & 2

Cells/ml x10^6	50	100	150
CPM / ug DNA	1.78	2.23	2.15
Std. Dev.	0.315	0.771	0.553
Cells/ml x10^6	100 RGD	150 RGD	
CPM / ug DNA	1.12	2.03	
Std. Dev.	0.221	0.391	

Cells/ml x10^6	50	100	150
Avg. GAG (ug)	236	351	313
Std. Dev.	98.36	108.27	14.51
Cells/ml x10^6	RGD 100	RGD 150	
Avg. GAG (ug)	191	161	
Std. Dev.	48.05	16.20	

**University of Michigan, Ann Arbor, MI.