
Adhesive properties of laminated alginate gels for tissue engineering of layered structures

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Abstract: A significant challenge in tissue engineering is the creation of tissues with stratified morphology or embedded microstructures. This study investigated methods to fabricate composite gels from separately deposited alginate layers and examined the effects of processing methods on the mechanics of adhesion. Laminated alginate gels were created through a three step process which included: treatment of the interfaces with citrate; annealing of the gels to allow for molecular rearrangement of the alginate chains; and exposure to a CaCl₂ to crosslink the alginate sheets. Process variables included volume and concentration of applied citrate, annealing time, incubation time in CaCl₂, and CaCl₂ concentration. Laminated sheets were tested in lap-shear geometry to characterize failure phenomena and mechanical properties. The site of failure

within the gel depended on the integrity of the interface, with weaker gels delaminating and gels with mechanical properties similar to that of bulk gels failing randomly throughout the thickness. Citrate volume, citrate concentration, CaCl₂ incubation time, and CaCl₂ concentration altered the mechanical properties of the laminated alginate sheets, while annealing time had little effect on all measured parameters. This study demonstrates the integration of separately fabricated alginate layers to create mechanically or chemically anisotropic or heterogeneous structures. © 2007 Wiley Periodicals, Inc. *J Biomed Mater Res* 85A: 611–618, 2008

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INTRODUCTION

Hydrogels are commonly used in tissue engineering, in part due to their ability to form solid constructs with homogeneous distributions of cells.¹

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This is an advantage over other scaffold types such as foams or sponges, since it ensures uniformity in cell seeding throughout a solid shaped scaffold. However, the ability to generate tissues with controlled, stratified morphology, like that of skin² and cartilage^{3,4} or tissue scaffolds with embedded microstructure,^{5,6} remains a persistent challenge.

In order to utilize hydrogels to produce either a heterogeneous distribution of cells or microstructures such as channels^{5,6} within a construct, local or regional deposition of the seeded materials through methods such as layering is required. Recent studies have investigated methods to generate stratified articular cartilage constructs by depositing multiple layers of chondrocytes⁷ or chondrocyte-seeded gels utilizing various materials including PEG⁸ and agarose.⁹ Other approaches to tissue engineering include the creation of microfluidic channels within the scaffold by layering micropatterned alginate sheets together to form a sealed channel system.⁵ While these efforts show great promise, the properties of the interface between the layers limit the mechanical function of these constructs. This limitation is

particularly important with hydrogels, which are mechanically weaker than other scaffold materials currently used for tissue engineering. To date, there has been little investigation into understanding the phenomena related to adhesion between successively deposited hydrogel layers.

Alginate, an anionic linear polysaccharide, has been used as a scaffold material for cartilage tissue engineering due to its support of the chondrocyte phenotype,¹⁰ ability to be molded in desired shapes,^{11,12} support of chondrogenesis in large animal models,¹³ and biocompatibility in cell delivery in human trials.¹⁴ In addition to chondrocytes,¹¹ cell types delivered using alginate include fibroblasts,¹⁵ osteoblasts,^{16,17} hepatocytes,^{18,19} and pancreatic islets.^{20,21} The mechanical properties of bulk alginate gels can be controlled with the type,^{22,23} molecular weight,^{1,24,25} and concentration^{1,22,24,25} of alginate in addition to the chemistry,^{1,24,26} delivery,^{1,26} and concentration^{1,24,26} of crosslinker. Similarly, the removal of ionic crosslinks from alginate has also been studied in great detail,¹ such as the use of chelators including sodium citrate enabling the dissolution of alginate gels while maintaining viability of embedded cells.^{7,9,10,27} In these studies, cells retrieved from alginate gels by exposure to sodium citrate demonstrated the ability to form new tissue,^{7,9,10,27} suggesting that sodium citrate exposure had a minimal effect on cell metabolism.

While complete dissolution of alginate gels using chelators is employed commonly for cell retrieval, it is possible that controlled or focused application of such chelators could increase the mobility of the polymer in the gel state sufficiently to enhance adhesion of successively deposited gel layers. This possibility motivates the hypothesis that the interfacial mechanics of layered alginate gels can be enhanced through controlled application of crosslinking and chelating agents. Therefore the objectives of this study were the following: (1) to develop methods to fabricate and evaluate the adhesion of separately deposited alginate layers; and 2) to examine the effects of processing methods on the mechanics of adhesion.

METHODS

Laminated alginate gel formation

The protocol for casting alginate gels was based on that described previously for injection molding.¹¹ Briefly, two hydrogel sheets with a total volume of 4 mL were formed by mixing 20 mg/mL of low viscosity, high G content alginate (Protanal LF 10/60, FMC Biopolymer, Drammen, Norway) in Dulbecco's Phosphate Buffered Saline (Gibco, Auckland, New Zealand) with 20 mg/mL CaSO₄ (Mallinckrodt Baker, Phillipsburg, NJ) at a 2:1 volume ratio. The alginate and CaSO₄

solutions were mixed in two 10-mL syringes (Becton-Dickinson, Franklin Lakes, NJ) connected via a three-way stopcock (Baxter, Deerfield, IL). Once mixed, one hydrogel sheet was cast between two glass plates lined with parafilm (Pechiney, Menasha, WI) while the second sheet was cast between a parafilm-lined glass plate and a PDMS sheet to assist in the demolding process. In both cases, casting plates were separated by one millimeter spacers and allowed to set for 7 min, resulting in a 1-mm-thick sheet of alginate.

One alginate sheet cast between glass plates was cut into 8.5 mm by 13 mm rectangles. The second sheet of alginate was partially demolded with the removal of the glass plate. The resulting exposed sheet was then treated with sodium citrate (Fig. 1, step 1) with the application of an 85 mm by 70 mm paper wipe (Kimwipe, Kimberly-Clark, Roswell, GA) to evenly distribute the solution. Concentrations ranging from 0 to 30 mg/mL and volumes ranging from 1 to 4 mL of sodium citrate were dripped onto the paper wipe with a 10-mL syringe while it was in contact with the alginate sheet. The paper wipe was carefully removed after an exposure time of 2 min. The treated alginate sheet was then lowered onto the cut alginate rectangles [Fig. 2(A)] producing a laminated structure of two separate alginate gels (Fig. 1, step 2). The PDMS sheet of the mold was retained on the alginate gel to assist with lowering the alginate sheet onto the rectangles, after which point the PDMS sheet was removed. The layered sheets were placed into a mold consisting of 2 parafilm lined glass plates separated by 2 mm spacers and an 800 g weight was added on the top plate to ensure contact between the layers. The laminated gels were kept in contact for time periods ranging from 1 to 16 min, which is defined as the annealing time, allowing for molecular rearrangement and interdigitation at the gel-gel interface.

Following annealing, samples were transferred into a 200 mL bath of CaCl₂ (Sigma, St. Louis, MO) with concentrations ranging from 5 mg/mL to 40 mg/mL on a rotary mixer set at 40 RPM (Fig. 1, step 3). Samples were maintained in the bath for time periods ranging from 30 s to 1 h to reverse the effects of the calcium chelator. Upon removal from the CaCl₂ bath 8.5 mm × 13 mm samples of the laminated alginate were cut out with a scalpel using the original alginate rectangles as a guide.

A total of five experiments were performed to test variables in the chemistry to produce the laminated alginate gels (Fig. 1). Bulk 2-mm-thick gels were created identically to the 1-mm-thick gels, soaked for 8 min in a 20 mg/mL CaCl₂ bath and mechanically tested in the same manner to act as a reference to compare to the data generated from the laminated gels. Experiment 1 varied the concentration of sodium citrate applied to the gel from 0 to 30 mg/mL using 2 mL of sodium citrate with 8 minutes annealing time and 8 min in a 20 mg/mL CaCl₂ bath. Experiment 2 used a 15 mg/mL sodium citrate solution in varying volumes from 1 to 4 mL with 8 min annealing time and 8 min in a 20 mg/mL CaCl₂ bath. Experiment 3 varied annealing time from 1 to 16 min and used 2 mL of 15 mg/mL sodium citrate and 8 min in a 20 mg/mL CaCl₂ bath. CaCl₂ concentration was investigated in experiment 4 varying from 5 to 40 mg/mL with other parameters including 2 mL of 15 mg/mL sodium citrate, 8 min annealing time and 8 min in the CaCl₂ bath. Lastly,

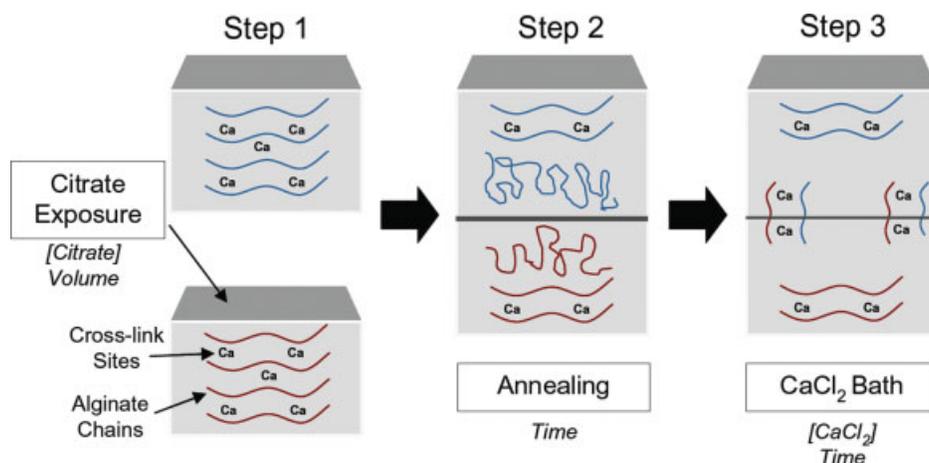


Figure 1. Schematic representation of the three-step method to fabricate layered alginate constructs. Step 1 involved the surface exposure of one alginate sheet to sodium citrate at variable citrate concentrations and citrate volumes to chelate Ca^{++} ions. An annealing step (Step 2) allowed un-crosslinked alginate chains to interdigitate from the two alginate sheets. Lastly, Step 3 involved immersing the layered construct in a CaCl_2 bath to reverse the chelating effects of the sodium citrate and cross link the interface between the two alginate sheets. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Experiment 5 utilized 2 mL of 15 mg/mL sodium citrate, 8 min annealing time, 20 mg/mL CaCl_2 bath and expose to CaCl_2 was varied from 30 s to 1 h.

Mechanical testing

Immediately after creation, the laminated alginate gels were mechanically tested using a lap-shear test to measure the interfacial material properties.²⁸ Custom grips were fabricated consisting of strips of aluminum (8.5 mm wide) with a 1 mm offset bend [Fig. 2(C)]. The resulting test

geometry caused the direction of force to go through the interface of the gel ensuring that only shear and no moments were imposed at the gel-gel interface.

The layered samples were attached to the grips using cyanoacrylate glue. After both grips were attached to the samples, a small clamp was added around the grips in the sample region to add stability to the construct in order to move the sample to the test frame [Fig. 2(B)]. The clamped grip-gel-grip assembly was loaded into an EnduraTEC ELF3200 mechanical test frame. Once the grips were secured in the test frame, the clamp was removed from the assembly. Samples were pulled to failure at a displace-

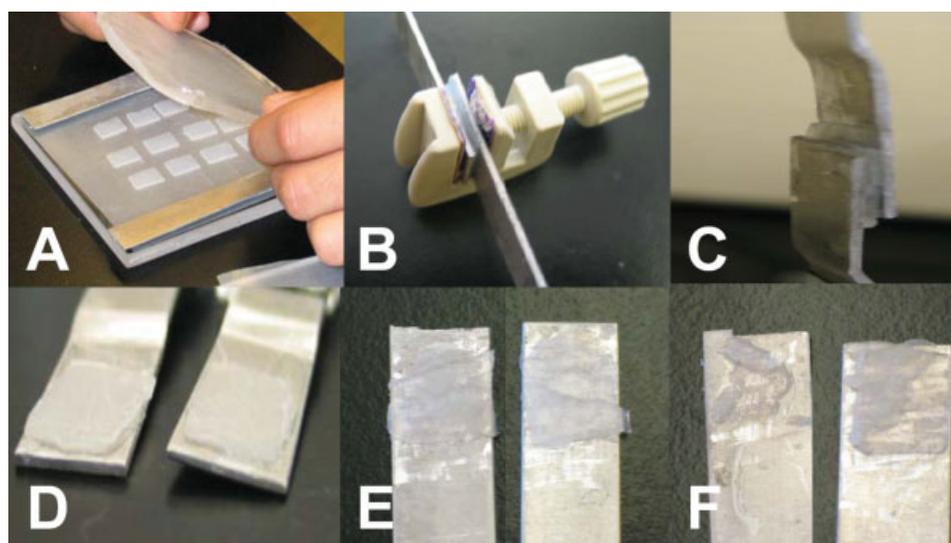


Figure 2. Formation of the laminated alginate constructs (A). Mechanical testing procedure which included a clamp to hold the grips together (B) while loading the sample into the mechanical test-frame and the resulting grip alignment (C) as the samples were pulled to failure. Representative samples of a laminated construct with weak mechanical properties delaminating at the interface (D) and a laminated construct with mechanical properties similar to the properties of a bulk 2-mm gel (E). Failure was seen randomly throughout the bulk 2-mm gel (F) which is similar to E. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

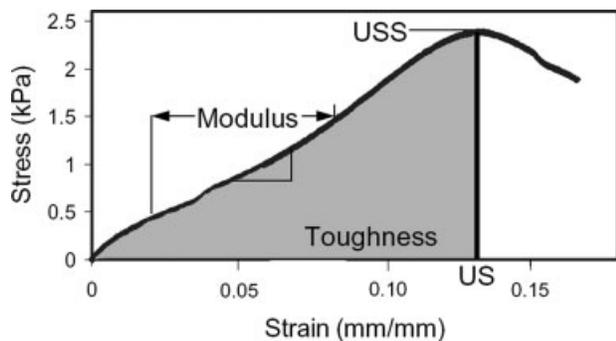


Figure 3. Sample stress-strain curve produced from lap shear testing of a laminated gel construct to illustrate ultimate shear stress (USS), ultimate strain (US), modulus taken in the linear region, and toughness indicated as the shaded region.

ment rate of 0.025 mm/s, with load measured to within 1 g at a sampling frequency of 10 Hz [Fig. 2(C)]. A low strain rate (1.25% strain/s) was deliberately chosen to approach a quasi-static limit to minimize any viscoelastic effects of the hydrogel. This was confirmed by additional tests demonstrating similar properties at rates slightly higher (2% strain/s) and lower (0.75% strain/s) strain rates.

Using sample geometry, displacements and loads were converted to strains and stresses. The resulting stress-

strain curves enabled the calculation of the ultimate shear strength (USS), shear strain at failure or ultimate strain (US), shear modulus, and toughness (Fig. 3). The USS is calculated as the maximum value of shear stress the sample was able withstand prior to failure and the strain at that point is denoted as the US. The modulus was determined as the slope of the linear elastic region between 2% and 8% strain of the stress-strain curve. Toughness is the area under the stress-strain curve bounded by zero strain and the ultimate strain calculated with a Riemann sum technique.^{29,30} Throughout mechanical testing, the location of failure between the layered constructs was documented.

Normally distributed data was analyzed with a one-way analysis of variance ($p < 0.05$) with a *post hoc* Student-Newman-Keuls test for pairwise comparison. Data with a non-normal distribution was analyzed with a Kruskal-Wallis one-way analysis of variance on ranks with Dunn's method utilized for pairwise comparison.

RESULTS

The stress-strain behavior of bonded alginate gels was qualitatively similar to that of the bulk 2-mm-thick gels. Bonded gels exhibited an extended linear region, after which the samples either yielded or failed directly. The ultimate strain achieved for both

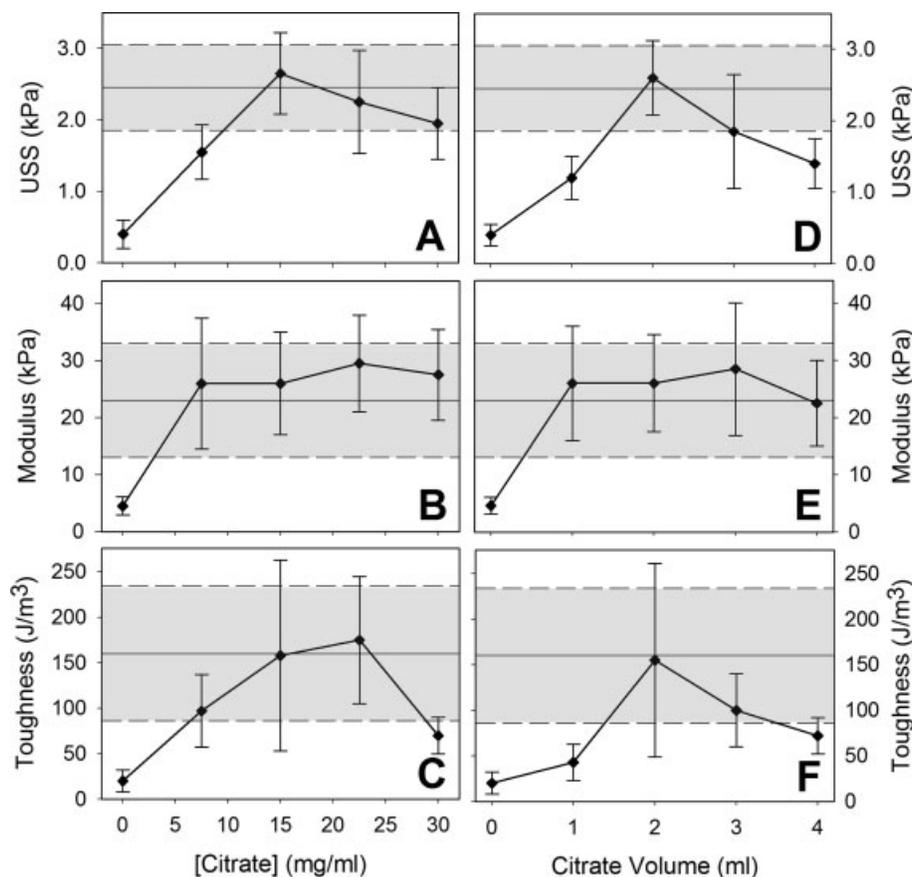


Figure 4. Effect of citrate concentration (A–C) for $n = 6$ –11 samples and citrate volume (D–F) for $n = 6$ –8 samples \pm SD on an individual alginate sheet. Overlaid gray box represents mean \pm SD of a 2-mm-thick injection molded alginate sheet for ultimate shear strength (A,D), modulus (B,E), and toughness (C,F).

laminated gels and the bulk 2 mm gel was 0.13 ± 0.08 and did not vary significantly due to variations in processing techniques (data not shown).

The site of failure within the gel depended on the chemistry used to adhere the two alginate layers. The mechanically weaker layered gels delaminated at the adhesive interface [Fig. 2(D)], while bulk 2mm gels [Fig. 2(F)] and layered gels with comparable properties to the 2-mm bulk gels [Fig. 2(E)] failed randomly throughout the thickness when subjected to lap shear.

Citrate exposure

USS increased with citrate concentration and volume up to 15 mg/mL and 2 mL, respectively, where a peak value of 2.7 kPa was reached and then dropped for both higher amounts of citrate [Fig. 4(A)] and volume [Fig. 4(D)]. Shear modulus increased with the addition of 7.5 mg/mL and 1 mL of citrate and did not change at higher concentrations [Fig. 4(B)] and volumes [Fig. 4(E)]. Toughness, similarly to USS, generally increased with increasing citrate concentration and volume, achieved a peak of 173.8 J/m^3 for 22.5 mg/mL and 156.3 J/m^3 for 2 mL, then decreased with higher citrate concentrations [Fig. 4(C)] and volumes [Fig. 4(F)]. Statistically significant increases were noted with the application of citrate in USS [15–30 mg/mL, 2 and 3 mL], modulus [7.5–30 mg/mL, 1–4 mL], and toughness [15–22.5 mg/mL, 2 mL]. For all parameters there was a statistical difference ($p < 0.001$) between properties generated with no citrate treatment to the laminated gels and the bulk 2-mm gels.

Annealing time

Annealing time had little effect on all calculated parameters. No statistical difference was found in USS [Fig. 5(A)], modulus [Fig. 5(B)], and toughness [Fig. 5(C)] with longer annealing times, nor the properties of the laminated gels compared to the 2-mm solid gels.

Calcium chloride exposure

Laminated gels exposed to 0 mg/mL CaCl_2 and those not exposed to CaCl_2 could not be tested due to extreme fragility that resulted in failure during the mounting process. Generally all mechanical properties increased with increasing CaCl_2 concentration (Fig. 6). Exposure to 40 mg/mL CaCl_2 increased shear strength relative to 5 mg/mL ($p < 0.039$) and produced laminated gels with properties similar to 2-mm bulk gels [Fig. 6(A)]. A bath concentration of 40 mg/mL did produce a laminated construct which had a significantly higher modulus

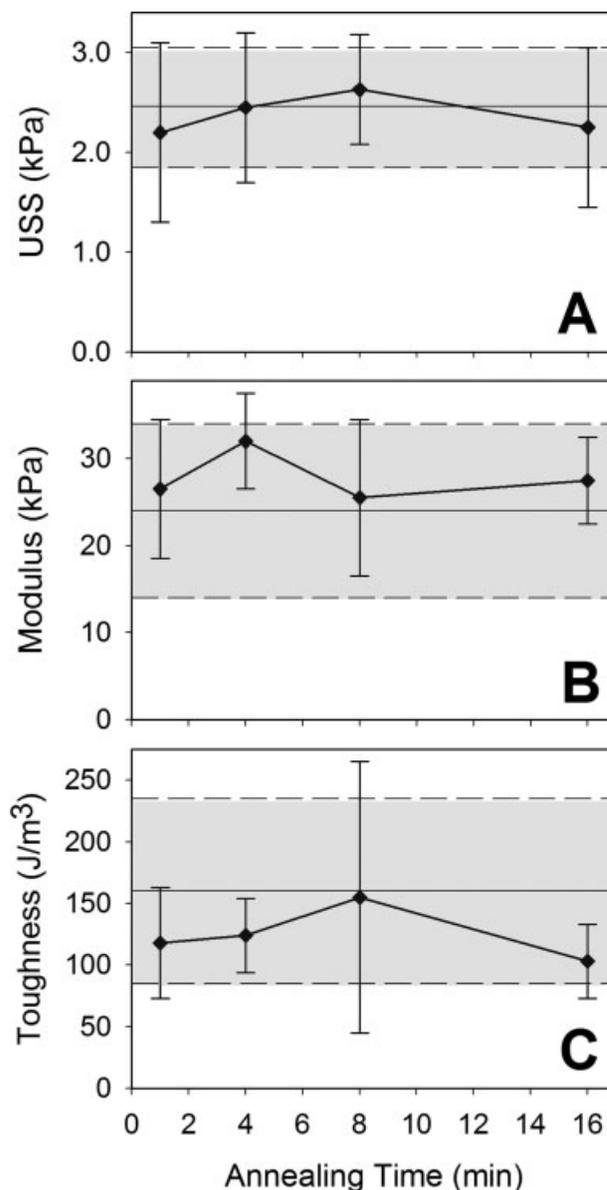


Figure 5. Effect of annealing time for $n = 6-8$ samples \pm SD on ultimate shear strength (A), modulus (B), and toughness (C). Overlaid gray box represents mean \pm SD of a 2-mm thick injection molded alginate sheet.

[47.6 kPa, $p < 0.012$] than both laminated gels in a 5 mg/mL CaCl_2 bath and the bulk 2 mm gels [Fig. 6(B)]. Toughness likewise increased with CaCl_2 concentration producing a maximum value of 255.9 J/m^3 at 40 mg/mL [Fig. 6(C)]; however this difference was not statistically significant.

Similarly, time of CaCl_2 exposure was found to have an effect on all mechanical properties evaluated. USS increased with time of CaCl_2 exposure [Fig. 6(D)], although there was no significant difference found after 2 minutes or compared to 2 mm bulk gels. One hour in CaCl_2 bath produced laminated gels which had moduli that were significantly higher [478.3 kPa, $p < 0.012$] than gels immersed in

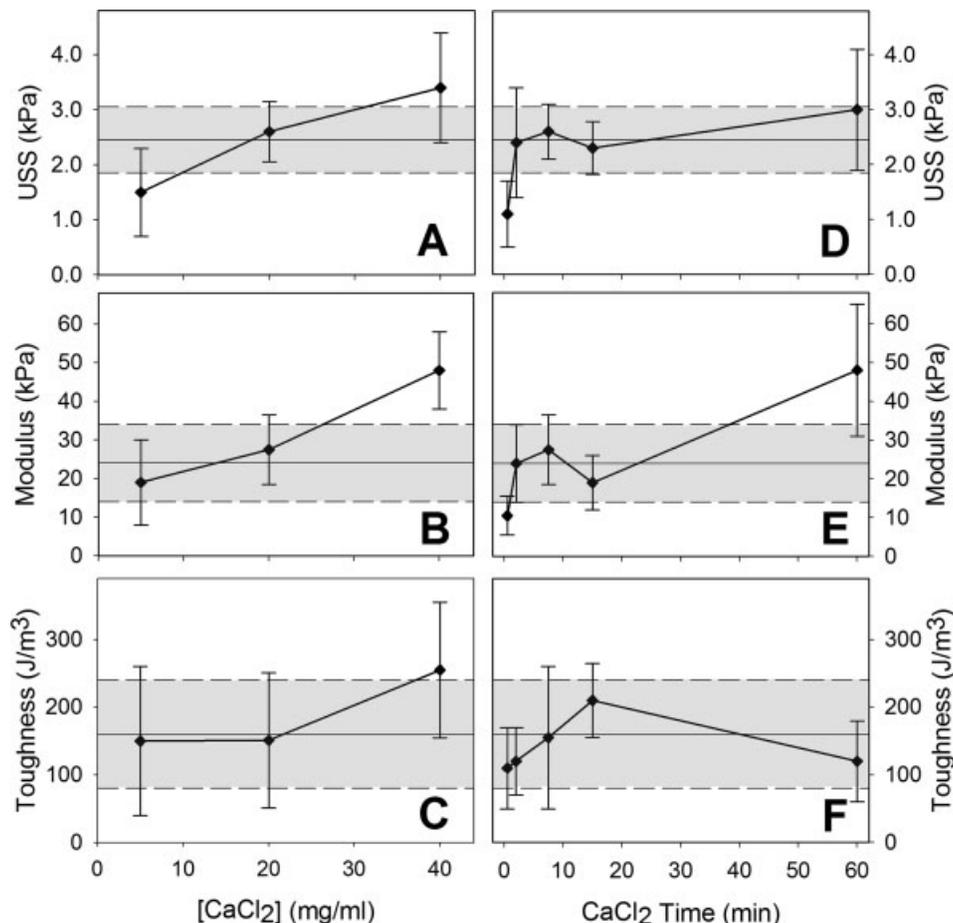


Figure 6. Effect of CaCl_2 concentration (A–C) for $n = 6$ –7 samples, and time in CaCl_2 (D–F) for $n = 5$ –6 samples \pm SD of the laminated alginate gels. Overlaid gray box represents mean \pm SD of a 2-mm-thick injection molded alginate sheet for ultimate shear strength (A,D), modulus (B,E), and toughness (C,F).

a bath for 30 s and 15 min [Fig. 6(E)]. Toughness increased then decreased with a peak of 210.3 J/m^3 at 16 min [Fig. 6(F)] with no significant difference over time in CaCl_2 or compared to bulk 2 mm gels.

DISCUSSION

This study demonstrates the integration of separately fabricated alginate layers for the purpose of assembling multilayered engineered tissues. Alginate sheets were held in apposition for short periods of time, mounted in grips, and pulled apart in a lap-shear geometry. Some integration of alginate sheets was observed in overlapping sheets after exposure to CaCl_2 , producing mechanical properties similar to that of bulk 2 mm thick gels.

The adhesive properties of alginate layers were enhanced by the controlled application of sodium citrate, a chelating agent typically used to retrieve cells from alginate cultures.^{7,10,27} It was hypothesized that controlled citrate exposure would remove calcium from the surfaces of alginate gels, so that subsequent

exposure to CaCl_2 would augment bonding between sheets. Citrate exposure did enhance alginate adhesion as indicated most directly by the shear strength and toughness of the layered gels. Maximal enhancement was observed at 15 and 22.5 mg/mL citrate, but higher amounts of citrate weakened the gels. This phenomenon is possibly due to excessive reduction in cross link density throughout the sample rather than localizing the chelation effects to the interface of the alginate sheets.

The annealing time had little effect on any of the mechanical parameters that were investigated, suggesting that the molecular mobility of the polymer chains at the interface was very high. Once the Ca^{++} ions were chelated at the interface, interdigitation between polymer chains from the two gel layers was not enhanced with longer annealing time. The laminated gels achieved properties similar to that of solid 2 mm gels within 1 min of annealing time.

Calcium chloride treatment is an important step required to put Ca^{++} at the interface after initial gelation. Samples not treated with citrate but with CaCl_2 had minimal interfacial strength, but those

without any CaCl_2 treatment resulted in no interfacial connection between the sheets. These data suggest that the key to forming laminated alginate gels is replacing intralaminar crosslinks with interlaminar crosslinks. The removal of intralaminar crosslinks at the gel surface is accomplished via citrate exposure, while interlaminar crosslinks are generated via CaCl_2 exposure. The increase in interfacial properties in cases of CaCl_2 treatment without citrate exposure suggests the presence of a small but finite density of potential crosslinking sites with sufficient mobility to cross the gel-gel interface. In contrast, the lack of interfacial adhesion in the absence of CaCl_2 exposure suggests that existing ionic crosslinks formed by Ca^{++} within the gel layers are not sufficiently mobile to form interlaminar crosslinks.

The maximum shear strength observed in the laminated gels is 10–70% that reported for intact alginate gels,^{22,23,31} although the composition and testing methods for the reported gels differed from the current study. The ultimate shear strains reported here are much lower than those seen in tensile testing of alginate gels. Modulus and toughness however, are similar to those reported for alginate gels in tension and shear.^{22,23,31} The laminated gels were able to be fabricated under various chemical conditions to produce values that were not statistically different from solid 2-mm thick gels tested in our system. This study validates our procedure to produce laminated alginate gels with similar properties to those reported for bulk gels made with other methods.

Based on data for mechanical properties as a function of the concentration and volume of citrate exposure, it is apparent that it is advantageous to minimize exposure of the bulk of the gel to chelators while ensuring exposure at the adhesive interface. While investigation into local mechanical properties was not performed, the effects of citrate application were thought to be confined to the interface surface since the bulk mechanical shear properties were not affected. In this study a paper wipe was used to uniformly distribute the sodium citrate solution, limiting citrate exposure to one surface of the gel. However, contact methods used to apply citrate may remove some of the un-crosslinked alginate. As a result, other techniques to reliably deliver a specified volume of a citrate solution to the surface of the alginate sheet, such as spraying, need to be developed.

These studies represent a novel technique and exploration of a large variable space to characterize the adhesion of separately deposited alginate sheets that enables future studies to be focused on cell response to these environmental variables. Levels of citrate and calcium equal to or higher than those used in the current study have previously been shown to have minimal effect on ECM synthesis.^{27,32–35} As such, utilization of the materials processing techni-

ques developed here should enable culture of layered cellular gels with minimal effect on cell viability or metabolism. Further, the use of CaSO_4 as a crosslinker via injection molding^{11–13} generates constructs that do not weaken over culture time as noted by others,²² suggesting that layered constructs will maintain mechanical integrity during culture.

The ability to deposit successive adhesive layers of alginate has many potential applications for tissue engineering. In addition to the prospect of creating heterogeneous cell populations by layer, this technique can also be applied to create mechanically or chemically anisotropic or heterogeneous structures that more effectively match native tissues. Further, this technique can be used with other fabrication and lithography techniques to embed topographic features in layers to create microfluidic systems to engineer vascular tissues.

References

1. Lee KY, Mooney DJ. Hydrogels for tissue engineering. *Chem Rev* 2001;101:1869–1879.
2. Wang HJ, Bertrand-de Haas M, van Blitterswijk CA, Lamme EN. Engineering of a dermal equivalent: Seeding and culturing fibroblasts in PEGT/PBT copolymer scaffolds. *Tissue Eng* 2003;9:909–917.
3. Wong M, Wuethrich P, Eggli P, Hunziker E. Zone-specific cell biosynthetic activity in mature bovine articular cartilage: A new method using confocal microscopic stereology and quantitative autoradiography. *J Orthop Res* 1996;14:424–432.
4. Hunziker EB, Quinn TM, Hauselmann HJ. Quantitative structural organization of normal adult human articular cartilage. *Osteoarthritis Cartilage* 2002;10:564–572.
5. Cabodi M, Choi NW, Gleghorn JP, Lee CS, Bonassar LJ, Stroock AD. A microfluidic biomaterial. *J Am Chem Soc* 2005;127:13788–13789.
6. Chrobak KM, Potter DR, Tien J. Formation of perfused, functional microvascular tubes in vitro. *Microvasc Res* 2006;71:185–196.
7. Klein TJ, Schumacher BL, Schmidt TA, Li KW, Voegtline MS, Masuda K, Thonar EJ, Sah RL. Tissue engineering of stratified articular cartilage from chondrocyte subpopulations. *Osteoarthritis Cartilage* 2003;11:595–602.
8. Kim TK, Sharma B, Williams CG, Ruffner MA, Malik A, McFarland EG, Elisseeff JH. Experimental model for cartilage tissue engineering to regenerate the zonal organization of articular cartilage. *Osteoarthritis Cartilage* 2003;11:653–664.
9. Ng KW, Wang CC, Mauck RL, Kelly TA, Chahine NO, Costa KD, Ateshian GA, Hung CT. A layered agarose approach to fabricate depth-dependent inhomogeneity in chondrocyte-seeded constructs. *J Orthop Res* 2005;23:134–141.
10. Hauselmann HJ, Fernandes RJ, Mok SS, Schmid TM, Block JA, Aydelotte MB, Kuettner KE, Thonar EJ. Phenotypic stability of bovine articular chondrocytes after long-term culture in alginate beads. *J Cell Sci* 1994;107 (Part 1):17–27.
11. Chang SC, Rowley JA, Tobias G, Genes NG, Roy AK, Mooney DJ, Vacanti CA, Bonassar LJ. Injection molding of chondrocyte/alginate constructs in the shape of facial implants. *J Biomed Mater Res* 2001;55:503–511.
12. Hott ME, Megerian CA, Beane R, Bonassar LJ. Fabrication of tissue engineered tympanic membrane patches using computer-aided design and injection molding. *Laryngoscope* 2004;114:1290–1295.

13. Chang SC, Tobias G, Roy AK, Vacanti CA, Bonassar LJ. Tissue engineering of autologous cartilage for craniofacial reconstruction by injection molding. *Plast Reconstr Surg* 2003;112:793–799; discussion 800–801.
14. Vacanti CA, Bonassar LJ, Vacanti MP, Shufflebarger J. Replacement of an avulsed phalanx with tissue-engineered bone. *N Engl J Med* 2001;344:1511–1514.
15. Ponce S, Orive G, Gascon AR, Hernandez RM, Pedraz JL. Microcapsules prepared with different biomaterials to immobilize GDNF secreting 3T3 fibroblasts. *Int J Pharm* 2005;293:1–10.
16. Park DJ, Choi BH, Zhu SJ, Huh JY, Kim BY, Lee SH. Injectable bone using chitosan-alginate gel/mesenchymal stem cells/BMP-2 composites. *J Craniomaxillofac Surg* 2005;33:50–54.
17. Li Z, Ramay HR, Hauch KD, Xiao D, Zhang M. Chitosan-alginate hybrid scaffolds for bone tissue engineering. *Biomaterials* 2005;26:3919–3928.
18. Mai G, Huy NT, Morel P, Mei J, Bosco D, Berney T, Majno P, Mentha G, Trono D, Buhler LH. Treatment of fulminant liver failure by transplantation of microencapsulated primary or immortalized xenogeneic hepatocytes. *Transplant Proc* 2005;37:527–529.
19. Lee JH, Lee DH, Son JH, Park JK, Kim SK. Optimization of chitosan-alginate encapsulation process using pig hepatocytes for development of bioartificial liver. *J Microbiol Biotechnol* 2005;15:7–13.
20. Song YC, Chen ZZ, Mukherjee N, Lightfoot FG, Taylor MJ, Brockbank KG, Sambanis A. Vitrification of tissue engineered pancreatic substitute. *Transplant Proc* 2005;37:253–255.
21. Simpson NE, Khokhlova N, Oca-Cossio JA, McFarlane SS, Simpson CP, Constantinidis I. Effects of growth regulation on conditionally-transformed alginate-entrapped insulin secreting cell lines in vitro. *Biomaterials* 2005;26:4633–4641.
22. LeRoux MA, Guilak F, Setton LA. Compressive and shear properties of alginate gel: Effects of sodium ions and alginate concentration. *J Biomed Mater Res* 1999;47:46–53.
23. Drury JL, Dennis RG, Mooney DJ. The tensile properties of alginate hydrogels. *Biomaterials* 2004;25:3187–3199.
24. Kuo CK, Ma PX. Ionically crosslinked alginate hydrogels as scaffolds for tissue engineering, Part 1: Structure, gelation rate and mechanical properties. *Biomaterials* 2001;22:511–521.
25. Kong HJ, Kaigler D, Kim K, Mooney DJ. Controlling rigidity and degradation of alginate hydrogels via molecular weight distribution. *Biomacromolecules* 2004;5:1720–1727.
26. Genes NG, Rowley JA, Mooney DJ, Bonassar LJ. Effect of substrate mechanics on chondrocyte adhesion to modified alginate surfaces. *Arch Biochem Biophys* 2004;422:161–167.
27. Chia SH, Schumacher BL, Klein TJ, Thonar EJ, Masuda K, Sah RL, Watson D. Tissue-engineered human nasal septal cartilage using the alginate-recovered-chondrocyte method. *Laryngoscope* 2004;114:38–45.
28. Matsumura K, Hyon SH, Nakajima N, Peng C, Iwata H, Tsutsumi S. Adhesion between poly(ethylene-co-vinyl alcohol) (EVA) and titanium. *J Biomed Mater Res* 2002;60:309–315.
29. Peretti GM, Bonassar LJ, Caruso EM, Randolph MA, Trahan CA, Zaleske DJ. Biomechanical analysis of a chondrocyte-based repair model of articular cartilage. *Tissue Eng* 1999;5:317–326.
30. Peretti GM, Zaporozhan V, Spangenberg KM, Randolph MA, Fellers J, Bonassar LJ. Cell-based bonding of articular cartilage: An extended study. *J Biomed Mater Res A* 2003;64:517–524.
31. Leung KC, Chow TW, Woo CW, Clark RK. Tensile, shear and cleavage bond strengths of alginate adhesive. *J Dent* 1998;26:617–622.
32. Hauselmann HJ, Aydelotte MB, Schumacher BL, Kuettner KE, Gitelis SH, Thonar EJ. Synthesis and turnover of proteoglycans by human and bovine adult articular chondrocytes cultured in alginate beads. *Matrix* 1992;12:116–129.
33. Masuda K, Sah RL, Hejna MJ, Thonar EJ. A novel two-step method for the formation of tissue-engineered cartilage by mature bovine chondrocytes: The alginate-recovered-chondrocyte (ARC) method. *J Orthop Res* 2003;21:139–148.
34. Mok SS, Masuda K, Hauselmann HJ, Aydelotte MB, Thonar EJ. Aggrecan synthesized by mature bovine chondrocytes suspended in alginate. Identification of two distinct metabolic matrix pools. *J Biol Chem* 1994;269:33021–33027.
35. Petit B, Masuda K, D'Souza AL, Otten L, Pietryla D, Hartmann DJ, Morris NP, Uebelhart D, Schmid TM, Thonar EJ. Characterization of crosslinked collagens synthesized by mature articular chondrocytes cultured in alginate beads: Comparison of two distinct matrix compartments. *Exp Cell Res* 1996;225:151–161.