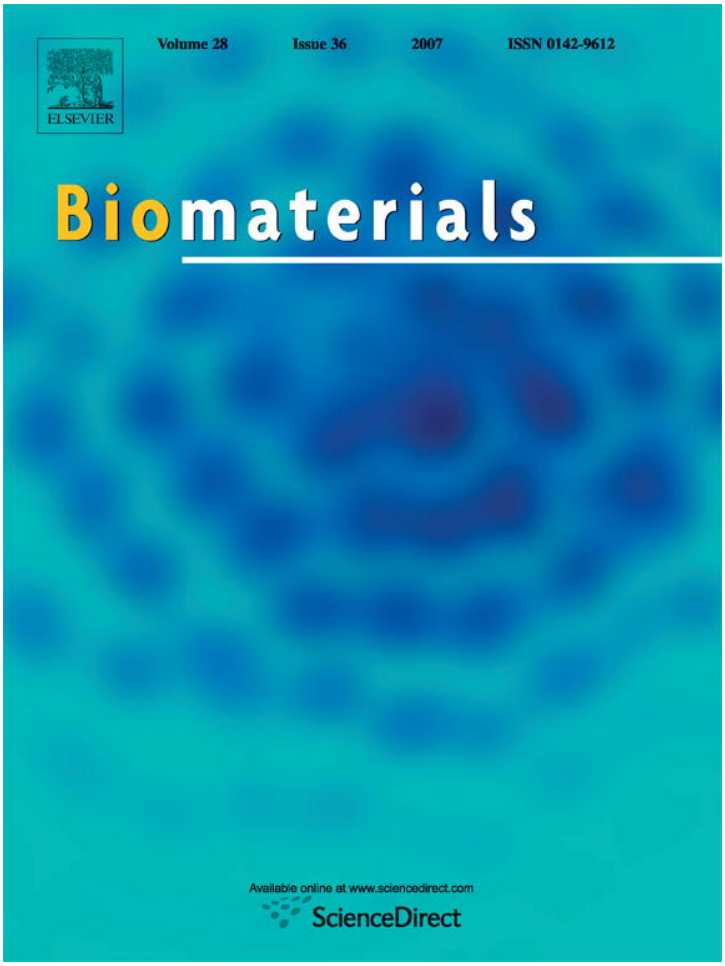

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Scaffolds based on degradable alginate hydrogels and poly(lactide-*co*-glycolide) microspheres for stem cell culture

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Abstract

We describe a method for creating alginate hydrogels with adjustable degradation rates that can be used as scaffolds for stem cells. Alginate hydrogels have been widely tested as three-dimensional constructs for cell culture, cell carriers for implantation, and in tissue regeneration applications; however, alginate hydrogel implants can take months to disappear from implantation sites because mammals do not produce endogenous alginases. By incorporating poly(lactide-*co*-glycolide) (PLGA) microspheres loaded with alginate lyase into alginate hydrogels, we demonstrate that alginate hydrogels can be enzymatically degraded in a controlled and tunable fashion. We demonstrate that neural progenitor cells (NPCs) can be cultured and expanded *in vitro* in this degradable alginate hydrogel system. Moreover, we observe a significant increase in the expansion rate of NPCs cultured in degrading alginate hydrogels versus NPCs cultured in standard, i.e. non-degrading, alginate hydrogels. Degradable alginate hydrogels encapsulating stem cells may be widely applied to develop novel therapies for tissue regeneration.

Keywords: Alginate; Stem cells; Hydrogel; Degradation; Controlled release

1. Introduction

Neural stem cells (NSCs) are characterized by the hallmark abilities to self-renew and to differentiate into the three major neural lineages—neurons, astrocytes, and oligodendrocytes [1,2]. These cells have attracted great interest because of their potential for neural regeneration; specifically, implantation of NSCs at the site of diseased or injured neural tissue may contribute to therapies for Parkinson's disease, Huntington's disease, and spinal cord injury [2–7]. A critical requirement for the development of neural stem cell therapies is devising methods to control NSC survival and fate post-implantation. A promising approach is to engineer biomimetic three-dimensional (3-D) scaffolds that can encapsulate and localize NSCs at an injury site, promote proliferation or instruct lineage-specific differentiation of NSCs, and gradually degrade to allow integration of newly formed NSC-derived tissue [8]. Currently, the development of biomaterials for neural tissue regeneration and neural stem cell implantation is a prominent research focus in regenerative medicine [3,5,9–11].

Alginate is a promising hydrogel material for neural stem cell implantation because it has demonstrated excellent *in vivo* compatibility with central nervous system tissue [11–13]. Alginate is a polysaccharide composed of (1–4)-linked β-D-mannuronic acid and α-L-guluronic acid blocks [14,15]. It is found naturally in brown seaweed and can be synthesized by bacteria [16]. Alginate hydrogels have been extensively investigated as 3-D scaffolds for cell culture due to their facile production and ability to encapsulate cells under mild conditions [2,12,15,17–20]. Alginate polymers can be ionically cross-linked to form a hydrogel by the addition of divalent cations such as calcium or barium [3,15,21,22]. A number of cell

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types including osteoblasts [18,23], chondrocytes [18,24], pancreatic islets [21], mesenchymal [22], and neural stem cells [2,3] have been encapsulated, cultured, and expanded in alginate hydrogels both *in vitro* and *in vivo*. Since alginate is not naturally enzymatically degraded in mammals, months can pass before alginate hydrogels are completely removed from implantation sites [3,12]. Even after hydrogel degradation, high molecular weight alginate polymers may not be easily removed from the body [25]. Utilization of degradable hydrogels for cell implantation enhances the rate of tissue regeneration by expediting disintegration of the hydrogel at the implantation site, thereby allowing for vascularization and subsequent tissue integration [19,26–28].

The degradation kinetics of alginate hydrogels have been previously controlled by varying alginate molecular weight, chemical structure, and by covalent cross-linking. Ionically cross-linked, high molecular weight alginate hydrogels degrade in an uncontrolled fashion due to the slow loss of cross-linking cations [15,17,25]. Alginate hydrogels composed of low molecular weight alginate polymers, synthesized via exposure of high molecular weight alginate to gamma irradiation, demonstrated increased degradation rates *in vivo*; however, these hydrogels also possess lower mechanical stability and still rely on the gradual exchange of cationic ions for degradation [26–28]. Alginate hydrogels composed of partially oxidized alginates [25], alginate polymers with hydrolytic covalent cross-linkers [23], or both [27] demonstrate controlled degradation kinetics that can be tuned by varying the density of hydrolytically liable bonds within the hydrogel. While these hydrogels degrade in a controlled fashion, the alginate oligomers that constitute the hydrogel are not actually being broken down [25]. Even after a month of degradation, hydrogels composed of partially oxidized alginates still possess alginate polymers with a molecular weight of 20,000 g/mol [27].

Here, we describe an alternative approach to design degradable alginate hydrogels based on the enzymatic degradation of alginate polymers. Alginate lyase (AL) digests alginate polysaccharides (tetramers or higher) in an endo-type manner at the β-1,4-glycosidic linkage via a β-elimination reaction to produce mono-, di-, and trisaccharide products [16]. We incorporate poly(lactide-*co*-glycolide) (PLGA) microspheres loaded with the enzyme alginate lyase (PLGA-AL) into alginate hydrogels; controlling the amount of incorporated enzyme, and its rate of release from the PLGA microspheres, enables the rate of enzymatic degradation of the alginate hydrogels to be tuned. Furthermore, these degradable scaffolds are compatible with the culture of neural progenitor cells (NPCs). We observe that NPCs co-encapsulated with PLGA-AL microspheres in alginate hydrogels proliferate at a higher rate as compared to NPCs cultured in standard alginate hydrogels. These findings could be applied to develop improved cell-based therapies for diseased or injured CNS tissue.

2. Materials and methods

2.1. Materials

AL (18170 U/g), alginic acid (sodium salt, 20–40 cps), polyvinyl alcohol (PVA) (MW 30,000–70,000, 80% hydrolyzed), trehalose, and magnesium hydroxide (SigmaUltra) were purchased from Sigma Aldrich. The 50:50 poly (lactic-*co*-glycolide) (PLGA, MW 40,000) was obtained from Durect Corporation (AL, USA). Sterile high molecular weight (200–300 kDa MW, 70% guluronate content) PRONOVA SLG 100 sodium alginate was purchased from FMC BioPolymer (PA, USA). Costar® Transwell plates (0.3 μm polyester membrane, 24-well plates) were purchased from Corning Inc. (New York, USA). Micro BCA protein assay kit was purchased from Pierce (IL, USA).

2.2. Microsphere preparation

PLGA microspheres were prepared aseptically using the double emulsion method at room temperature [29,30]. We prepared microspheres containing a 1:10 and a 1:100 ratio of AL to PLGA (w/w), and control PLGA microspheres without any incorporated enzyme. In brief, 50 μL of an aqueous solution of AL containing 2% (w/w) trehalose and 2% magnesium hydroxide (w/w) was suspended in 1 mL of PLGA dissolved in methylene chloride. This solution was sonicated for 3 s at 20% amplitude in an ice bath using a Vibra Cell high-intensity ultrasonic liquid processor (Sonics) to form a water/oil emulsion. This emulsion was then dispersed and stabilized in 20 ml of 0.5% (w/v) aqueous PVA, and mixed at high speed (5000 rpm) with a Silverson L4RT high shear laboratory mixer, $\frac{3}{4}$in tip, for 20 s to produce the second water/oil/water emulsion. This emulsion was stirred for 1–2 h at room temperature allowing the microspheres to form by evaporation of methylene chloride. The microspheres were then isolated by centrifugation (1500 rpm, 3 min) and subsequently washed four times with distilled deionized water to remove adsorbed PVA. To remove water, the microspheres were collected by centrifugation, frozen in liquid nitrogen, and lyophilized. The dried microspheres were stored in a sealed glass vial and placed in a desiccator at −20 °C.

2.3. Controlled release studies

The amount of AL protein released from PLGA microspheres encapsulating alginate lyase (PLGA-AL) was determined using a Micro BCA Protein Assay Kit. PLGA-AL microspheres were resuspended in 1.5 mL each of Dulbecco's phosphate-buffered saline (PBS) for 7 days at 37 °C. Each day and in triplicate for microspheres of each type, an aliquot of 1 mL was taken from each sample to measure the protein content. An additional 1 mL of fresh PBS was placed back into each sample to keep the incubation volume constant at 1.5 mL. The amount of protein released was measured at 562 nm using a Shimadzu UV-2401PC spectrophotometer.

The activity of released AL was determined by its ability to degrade sodium alginate (Sigma). As described by Preiss et al., the absorption of the unsaturated uronate portions of enzymatically degraded alginate oligosaccharides can be measured at 230 nm [31,32]. Enough microspheres were synthesized to create samples of each microsphere type, in triplicate, for each day of the 7-day experiment. PLGA-AL microspheres were resuspended in 200 μL of PBS and incubated at 37 °C. Each day 200 μL of supernatant was withdrawn from samples and mixed with 3 mL of 0.1% alginate (w/v). Enzyme in the supernatants was allowed to degrade the alginate for 1 h at 37 °C, and absorbance was measured at 230 nm.

To test the stability of AL enzyme, we made a stock solution of 50 mU/mL of AL in PBS and stored it at 37 °C over a 7-day period. Each day and in triplicate, 200 μL was withdrawn from the stock and mixed with 3 mL of 0.1% alginate (w/v). Enzyme in the samples was allowed to degrade the alginate for 1 h at 37 °C, and absorbance was measured at 230 nm.

2.4. Alginate hydrogel preparation

Sodium alginate (FMC Biopolymer) was dissolved in sterile deionized water to yield a 1% (w/v) sodium alginate solution. Using a PHD 2000 infuse/withdraw syringe pump (Harvard Apparatus), 1% sodium alginate solution was extruded from a standard 1 mL plastic syringe at a rate of 120 mL/h into an aqueous solution containing 100 mM $CaCl_2$/0.9% NaCl, immersed in a 4 °C water bath and stirred at 250 rpm. The alginate beads were allowed to gel for 10 min before being washed once with PBS. This method produced spherical beads approximately 4.1 ± 0.1 mm in diameter ($n = 7$) as verified via a Nikon Eclipse TS100 inverted microscope equipped with a SPOT Insight QE CCD camera (DIAGNOSTIC Instruments Inc.).

2.5. Alginate hydrogel degradation studies

To prepare alginate hydrogels containing PLGA or PLGA-AL microspheres, the desired amount of microspheres (10 or 100 μg of microsphere per mg of alginate) was added to the sodium alginate solution, which was then extruded into a solution containing calcium chloride (100 mM) and sodium chloride (0.9% w/v) as described above. Alginate beads were individually placed in an eppendorf vial with 200 μL of PBS and incubated at 37 °C. We tested 12 independent samples for each microsphere type; three independent samples were tested at each of the four time points (days 1, 3, 5, and 7). The byproducts of alginate hydrogel degradation in the supernatant of each sample were measured by spectrophotometry at 230 nm. The spectrophotometric reading for a fully degraded alginate hydrogel was obtained by adding 18 mU of AL to each of six eppendorf vials containing alginate hydrogels. After an overnight incubation, the 230 nm absorbance of the supernatant from three of the samples was measured. This measurement correlated to a completely degraded alginate hydrogel as a further overnight incubation with an additional 18 mU of enzyme in the other three samples produced no change in absorbance at 230 nm.

2.6. NPC studies

Adult NPCs were isolated from the hippocampi of 6-week-old female Fisher 344 rats and cultured as described [1]. DMEM/F12 medium with N2 supplement (Invitrogen) and FGF-2 (Promega) was changed every other day, and cells were subcultured using Accutase (Phoenix Flow Systems) upon reaching 70% confluency on poly-ornithine/laminin-coated plates. To encapsulate cells in alginate hydrogels, the cells were subcultured and mixed with the sodium alginate solution at a concentration of 40,000 cells per mL of alginate solution. Alginate beads were then constructed as previously mentioned.

All experiments to test the influence of components of degradable alginate hydrogels on NPC proliferation were performed in Costar® 96-well plates with NPCs seeded at 1000 cells per well in 200 μL of DMEM/F12 media supplemented with N2 and 20 ng/ml FGF-2. Experiments were carried out over an 8-day period and 50% of the media was changed every other day. The influence of AL was tested at 100, 10, 1, 0.1, and 0 mU of AL per 200 μL of media. The level of AL was kept constant even through media changes. The influence of PLGA microspheres was tested at 100, 50, 10, and 0 μg of microspheres per well (200 μL of media). Microspheres were added after cell seeding and settled at the bottom of the well. Special care was taken when performing media changes not to remove microspheres from the wells. To test the influence of enzymatically degraded alginate (FMC BioPolymer), 1 mL of a 20 mg/ml sodium alginate solution was incubated at 37 °C overnight with 10 mU of AL. The following day, the absorbance of an aliquot at 230 nm was measured spectrophotometrically. Further incubation of the alginate solution with additional enzyme produced no change in the absorbance at 230 nm. This solution was added to respective wells to test the cytotoxicity of 5, 2.5, 1, 0.5, and 0 mg of degraded alginate per mL of media. The level of degraded alginate was kept constant even through media changes. At the end of the 8-day culture period, the cell number in each well was quantified using Molecular Probes CyQuant™ Assay.

Costar® 24-well Transwell plates were used for all encapsulated NPC proliferation studies. In previous alginate hydrogel studies, 1–2% sodium alginate solutions were utilized for cell encapsulation [2,3,12,22]. A single 1% alginate hydrogel bead encapsulating NPCs, NPCs and PLGA microspheres, or NPCs and PLGA-AL microspheres was placed in each transwell insert/well of a 24-well plate. NPCs were seeded at approximately 2000 cell per alginate hydrogel. One milliliter of DMEM/F12 medium with N2 supplement and 20 ng/ml FGF-2 was added to the lower compartment of the transwell, and 150 μL of similar media was added to the transwell insert. The encapsulated NPCs were allowed to proliferate for 8 days with a 50% media change in the lower compartment every 4th day. On days 0, 4, and 8, the media in each transwell insert of three hydrogels from each experimental group was removed by blotting the bottom of the insert on a paper towel. To release encapsulated NPCs, the alginate hydrogel was incubated with 100 μL of 15 mM sodium citrate/150 mM NaCl solution for an hour [2,22]. Using a pipette, each hydrogel solution was mixed until a homogenous solution was obtained. The cell/alginate suspension was then pipetted from the transwell insert into a well of a 96-well plate. The 96-well plates were stored at −80 °C. At the end of the experiment, all hydrogels were assayed in parallel using Molecular Probes CyQuant™ Assay.

3. Results and discussion

3.1. Controlled degradation of alginate hydrogels

We incorporated AL into PLGA microspheres (PLGA-AL) to enable its release at a controlled rate [33]. We monitored the rate of release of AL from PLGA-AL microspheres incorporating two different loadings of AL (1:10 and 1:100 ratios of AL to PLGA w/w). As seen in Fig. 1a, both types of PLGA-AL microspheres released AL in a controlled fashion over a period of 7 days. As expected, the rate of AL release could be tuned by varying the enzyme loading in the microsphere (Fig. 1a), and was proportional to the amount of enzyme incorporated into the microsphere (Fig. 1b). While the amount of protein released increased approximately linearly with time, the activity of the released enzyme reached a plateau within the 7-day period (Fig. 1c). Control experiments suggested that this plateau in the activity of released enzyme was due to a gradual decline in AL activity at 37 °C; the activity of AL was observed to decrease significantly after 48 h of incubation at 37 °C (data not shown). Importantly, significant "absolute" enzymatic activity was obtained, even after 7 days. Further increase in enzyme stability at 37 °C could be obtained by using a thermostable variant of AL [34].

Having demonstrated our ability to control the rate of AL release from PLGA-AL microspheres, we next demonstrated the ability to tune the rate of degradation of alginate hydrogels by incorporating PLGA-AL microspheres (Fig. 2). We first tested the influence of AL loading in the microspheres on the rate of hydrogel degradation. To that end, we incorporated into alginate hydrogels 100 μg/mg of either control PLGA microspheres or PLGA-AL microspheres containing 1:10 or 1:100 ratios of AL to PLGA (w/w), and monitored the rate of hydrogel

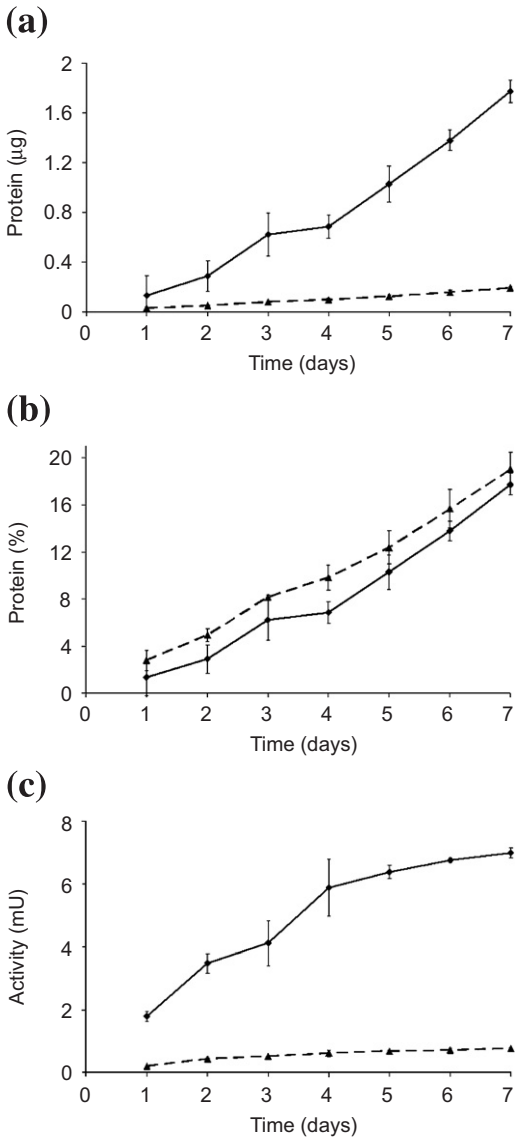

Fig. 1. Controlled release of alginate lyase from PLGA microspheres. Data for (a) absolute protein released, (b) percent protein released, and (c) released enzyme activity as a function of time are presented on a 0.1 mg microsphere basis for PLGA-AL microspheres synthesized at 5000 rpm loaded with 1:10 (♦) and 1:100 (▲) alginate lyase. Error bars represent standard deviation; $n = 3$ for each data point.

degradation (see Section 2). As seen in Fig. 2a, the extent of hydrogel degradation could be tuned by varying the AL loading in the microspheres; however, a 10-fold increase in AL loading resulted in only a 2-fold increase in the extent of alginate hydrogel degradation. Negligible degradation was seen for microspheres incorporating control PLGA microspheres. Next, we investigated the ability to tune alginate hydrogel degradation by varying the concentration of encapsulated microspheres (Figs. 2b and c). An increase in the concentration of PLGA-AL microspheres having 1:10 (Fig. 2b) or 1:100 (Fig. 2c) ratios of AL to PLGA (w/w) resulted in an increase in the extent and rate of hydrogel degradation. Collectively, these results indicate that the rate of hydrogel degradation can be tuned over a

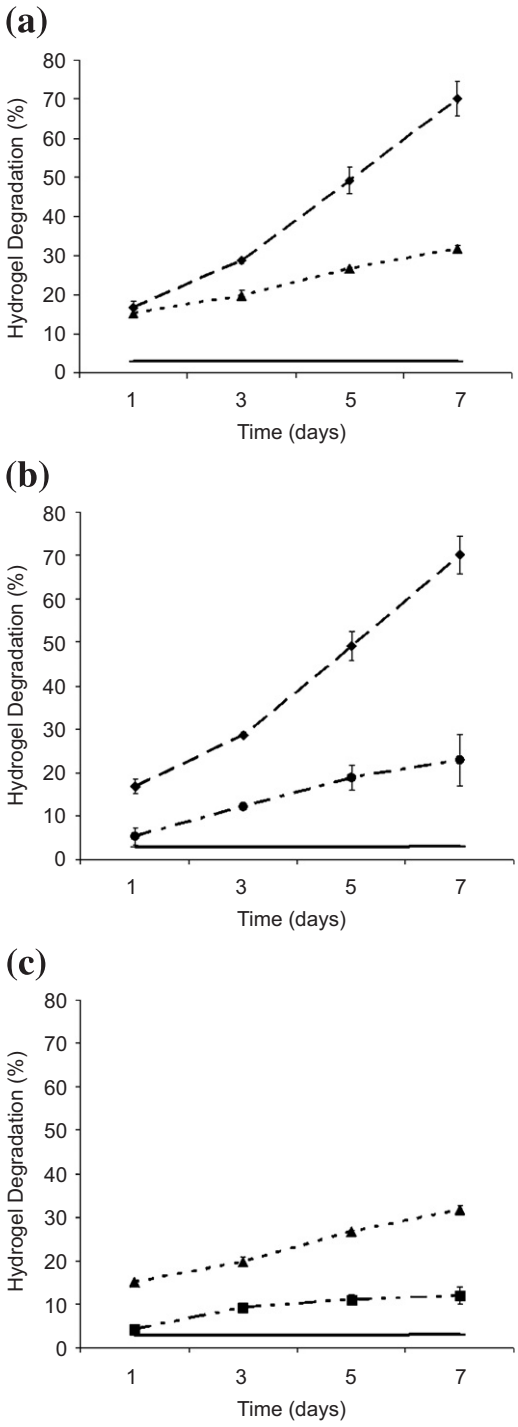

Fig. 2. Controlled degradation of alginate hydrogels. (a) Percent degradation of alginate hydrogels encapsulating 100 μg/mg of PLGA microspheres with (♦) 1:10, (▲) 1:100, and (—) 0 alginate lyase loadings. (b) Percent degradation of alginate hydrogels encapsulating (◆)100 μg/mg of 1:10 loaded PLGA-AL, (●) 10 μg/mg of 1:10 loaded PLGA-AL, and (—) 100 μg/mg of PLGA microspheres. (c) Percent degradation of alginate hydrogels encapsulating (▲) 100 μg/mg of 1:100 loaded PLGA-AL, (■) 10 μg/mg of 1:100 loaded PLGA-AL, and (—) 100 μg/mg of PLGA microspheres. All microspheres were prepared at 5000 rpm, and experiments were repeated twice. Error bars represent standard deviation; $n = 6$ for each data point.

wide range (0–70% degradation after 7 days) by varying the alginate loading and the concentration of PLGA-AL microspheres in alginate hydrogels.

3.2. NPC studies: NPC proliferation in hydrogels

Next, we tested the ability to influence NPC proliferation in alginate hydrogels by controlling the rate of hydrogel degradation. We encapsulated NPCs in 1% alginate hydrogels without PLGA microspheres (control) and in 1% alginate hydrogels incorporating 10 μg/mg of PLGA microspheres with either 0, 1:100, or 1:10 ratios of AL to PLGA (w/w). The rate of hydrogel degradation had a significant effect on NPC proliferation (Fig. 3). Over an 8-day culture period, we observed a 30-fold increase in NPC cell number within alginate hydrogels incorporating 10 μg/mg of PLGA-AL microspheres with 1:10 ratios of AL to PLGA (w/w); a 30-fold increase in NPC cell number over an 8-day period is similar to the rate of NPC proliferation in standard monolayer cultures [35]. The rate of proliferation decreased with decreasing rate of hydrogel degradation, and only a 2-fold increase in NPC cell number was observed in alginate hydrogels containing no microspheres or 10 μg/mg of control PLGA microspheres (Fig. 3). Li et al. [2] observed a similar 2-fold increase in the cell number of embryonic hippocampus-derived mouse neural stem cells cultured in 1.5% alginate hydrogels over a 7–9-day period.

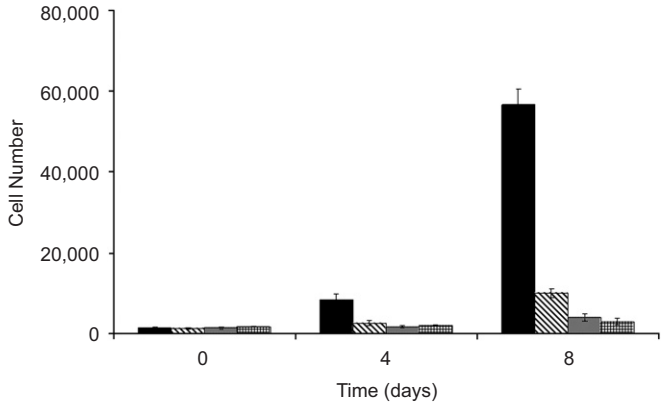

Fig. 3. Proliferation of NPCs in degradable alginate hydrogels. The proliferation of NPCs encapsulated in hydrogels made from a 1% alginate solution with either no PLGA microspheres (■) (control) or 10 μg/mg of PLGA-AL microspheres loaded with (▦) 0, (▨) 1:100, or (■) 1:10 alginate lyase was monitored over 8 days. Error bars represent standard deviation; $n = 3$ for each data point.

These proliferation experiments were complemented by microscopic observations of the morphologies of the proliferation NPC populations in hydrogels (Fig. 4). Similar to observations by Li et al. [2], NPCs cultured in non-degrading alginate hydrogels formed elongated growth cones (Fig. 4a and b) burrowing through cross-linked alginate hydrogels. In contrast, NPCs cultured in degrading alginate hydrogels, e.g., those containing 10 μg/mg of 1:10 and 1:100 AL-loaded microspheres, proliferated as neurospheres, similar to NPCs grown in on 2-D non-adhesive substrates (Fig. 4c and d) [36]. Presumably, the growth was less constrained (Fig. 4c and d) due to the partial degradation of the alginate hydrogel. These results demonstrate the ability to control the rate of proliferation and morphology of NPCs

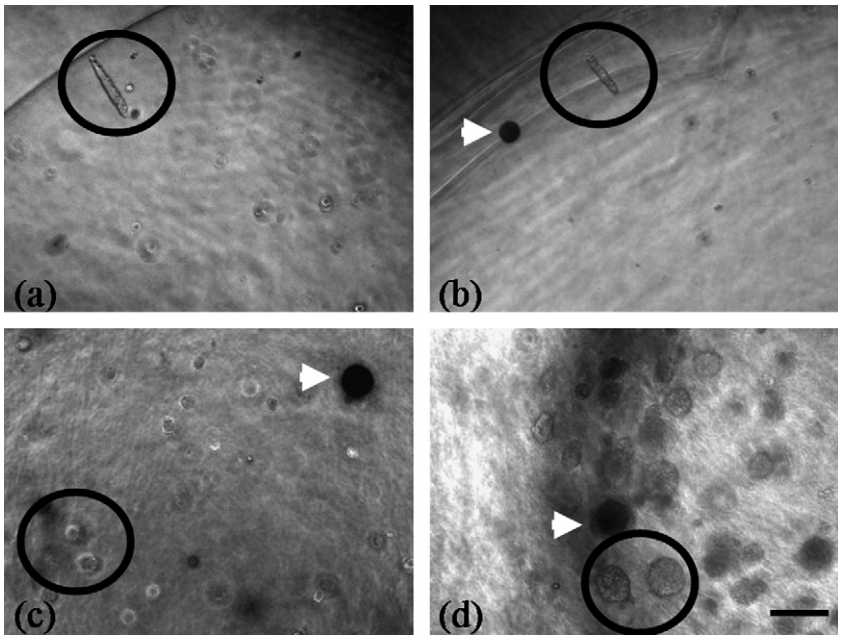

Fig. 4. Micrographs for NPCs proliferating in degradable alginate hydrogels (day 6 of culture). Alginate hydrogels were formed encapsulating (a) NPCs, (b) NPCs and PLGA microspheres, (c) NPCs and PLGA-AL loaded with 1:100 alginate lyase, and (d) NPCs and PLGA-AL loaded with 1:10 alginate lyase. All hydrogels with microspheres contain 10 μg microspheres/mg of alginate. Circles outline NPC clusters, and arrows point to microspheres. Scale bar, 100 μm.

in alginate hydrogels, by tuning the rate of hydrogel degradation.

3.3. NPC studies: influence of concentrations of PLGA microspheres, AL, and degraded alginate

While the enhanced proliferation observed in hydrogels incorporating PLGA-AL microspheres is promising, to better define the appropriate conditions for NPC expansion in degradable alginate hydrogel scaffolds, we evaluated NPC proliferation in media containing various concentrations of PLGA microspheres, AL, and degraded alginate byproducts over an 8-day period (Fig. 5). The previous NPC proliferation experiments (Figs. 3 and 4) exposed NPCs to concentrations of PLGA microspheres as high as 50 μg/mL (for a 100 μg/mg alginate loading), AL activities as high as 3.5 mU/mL, and a maximum theoretical concentration of alginate degradation byproducts of 0.5 mg/mL. These values were taken into consideration when designing the concentrations at which to test the effect of the components of degradable alginate hydrogels.

The presence of PLGA microspheres did not influence NPC proliferation at concentrations as high as 500 μg/mL (Fig. 5a). No cell death was observed in response to the presence of AL, PLGA microspheres, or degraded alginate byproduct. Similarly, AL did not influence NPC

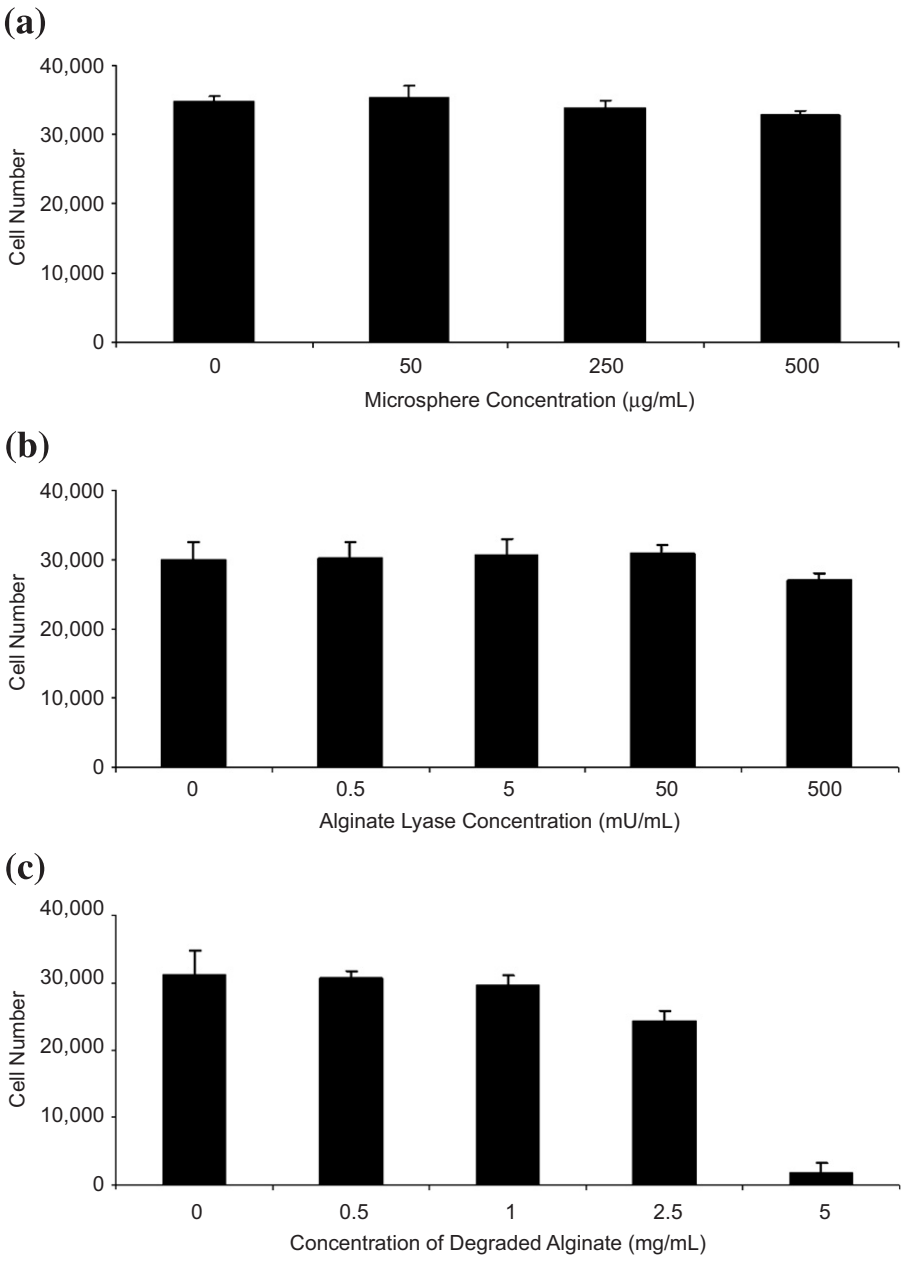

Fig. 5. Evaluating influence of degradable alginate hydrogel components on NPC proliferation. NPCs seeded at 1000 cells per well in 200 μL of media were allowed to proliferate in culture for 8 days in the presence of different concentrations of (a) PLGA microspheres, (b) alginate lyase, and (c) degraded alginate byproducts. Total cell number was quantified on the 8th day. Error bars represent standard deviation; $n = 4$ for each data point.

proliferation at concentrations as high as 50 mU/mL, and only a slight inhibition of NPC proliferation was observed at 500 mU/mL (Fig. 5b); these concentrations far exceed those required for the experiments described previously (Figs. 3 and 4). In contrast, NPC proliferation can be significantly inhibited by high levels of degraded alginate byproducts (Fig. 5c). However, no toxic effects were seen at concentrations that would be present during the previously described proliferation experiments (Figs. 3 and 4). Collectively, these results indicate that the incorporation of PLGA-AL microspheres is compatible with NPC culture in alginate hydrogels.

4. Conclusions

Although numerous cell types have been cultured in alginate hydrogels, limited cell proliferation is often observed within unmodified alginate hydrogels [22,37,38]. We demonstrate a facile approach for degrading alginate hydrogels, based on the incorporation of PLGA-AL microspheres. The rate of hydrogel degradation can be tuned over a wide range by varying several parameters. We have also demonstrated that degradable alginate hydrogels can be used to enhance NPC expansion rates *in vitro*. The use of PEGylated forms of AL [39] should further improve the suitability of this method for applications *in vivo*. Finally, we have shown that PLGA microspheres do not influence NPC proliferation at concentrations far greater than those required for hydrogel degradation. Consequently, one could incorporate within the same hydrogel both PLGA-AL and PLGA microspheres incorporating other bioactive molecules including growth factors [19,40], cytokines, and other therapeutic molecules. Such 3-D platforms will be crucial for future cell biology studies that are clearly moving away from standard 2-D culture conditions. A degradable and biocompatible 3-D scaffold that can localize stem cells at an injury site for a desired period of time while providing factors that control stem cell fate would also be valuable for applications of stem cells in regenerative medicine.

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References

[1] Lai K, Kaspar B, Gage FH, Schaffer DV. Sonic hedgehog regulates adult neural progenitor proliferation *in vitro* and *in vivo*. Nat Neurosci 2003;6(1):21–7.

[2] Li X, Liu T, Song K, Yao L, Ge D, Bao C, et al. Culture of neural stem cells in calcium alginate beads. Biotechnol Prog 2006;22(6):1683–9.

[3] Prang P, Muller R, Eljaouhari A, Heckmann K, Kunz W, Weber T, et al. The promotion of oriented axonal regrowth in the injured spinal cord by alginate-based anisotropic capillary hydrogels. Biomaterials 2006;27:3560–9.

[4] Thuret S, Moon LD, Gage FH. Therapeutic interventions after spinal cord injury. Nat Rev Neurosci 2006;7(8):628–43.

[5] Pfeifer K, Vroemen M, Caioni M, Aigner L, Bogdahn U, Weidner N. Autologous adult rodent neural progenitor cell transplantation represents a feasible strategy to promote structural repair in the chronically injured spinal cord. Regen Med 2006;1(2):255–66.

[6] Pfeifer K, Vroemen M, Blesch A, Weidner N. Adult neural progenitor cells provide a permissive guiding substrate for corticospinal axon growth following spinal cord injury. Eur J Neurosci 2004;20(7):1695–704.

[7] Vroemen M, Aigner L, Winkler J, Weidner N. Adult neural progenitor cell grafts survive after acute spinal cord injury and integrate along axonal pathways. Eur J Neurosci 2003;18(4):743–51.

[8] Griffith LG, Naughton G. Tissue engineering—current challenges and expanding opportunities. Science 2002;295(5557):1009–14.

[9] Wu S, Suzuki Y, Kitada M, Kitaura M, Kataoka K, Takahashi J, et al. Migration, integration, and differentiation of hippocampus-derived neurosphere cells after transplantation into injured rat spinal cord. Neurosci Lett 2001;312(3):173–6.

[10] Lin PW, Wu CC, Chen CH, Ho HO, Chen YC, Sheu MT. Characterization of cortical neuron outgrowth in two- and three-dimensional culture systems. J Biomed Mater Res B Appl Biomater 2005;75(1):146–57.

[11] Kataoka K, Suzuki Y, Kitada M, Hashimoto T, Chou H, Bai H, et al. Alginate enhances elongation of early regenerating axons in spinal cord of young rats. Tissue Eng 2004;10(3-4):493–504.

[12] Read TA, Stensvaag V, Vindenes H, Ulvestad E, Bjerkvig R, Thorsen F. Cells encapsulated in alginate: a potential system for delivery of recombinant proteins to malignant brain tumours. Int J Dev Neurosci 1999;17(5-6):653–63.

[13] Read TA, Sorensen DR, Mahesparan R, Enger PO, Timpl R, Olsen BR, et al. Local endostatin treatment of gliomas administered by microencapsulated producer cells. Nat Biotechnol 2001;19(1):29–34.

[14] Remminghorst U, Rehm BH. Bacterial alginates: from biosynthesis to applications. Biotechnol Lett 2006;28(21):1701–12.

[15] Rowley JA, Madlambayan G, Mooney DJ. Alginate hydrogels as synthetic extracellular matrix materials. Biomaterials 1999;20(1):45–53.

[16] Iwamoto Y, Araki R, Iriyama K, Oda T, Fukuda H, Hayashida S, et al. Purification and characterization of bifunctional alginate lyase from Alteromonas sp. strain no. 272 and its action on saturated oligomeric substrates. Biosci Biotechnol Biochem 2001;65(1):133–42.

[17] Augst AD, Kong HJ, Mooney DJ. Alginate hydrogels as biomaterials. Macromol Biosci 2006;6(8):623–33.

[18] Alsberg E, Anderson KW, Albeiruti A, Rowley JA, Mooney DJ. Engineering growing tissues. Proc Natl Acad Sci USA 2002;99(19):12025–30.

[19] Perets A, Baruch Y, Weisbuch F, Shoshany G, Neufeld G, Cohen S. Enhancing the vascularization of three-dimensional porous alginate scaffolds by incorporating controlled release basic fibroblast growth factor microspheres. J Biomed Mater Res A 2003;65(4):489–97.

[20] Drury JL, Mooney DJ. Hydrogels for tissue engineering: scaffold design variables and applications. Biomaterials 2003;24(24):4337–51.

[21] Petruzzo P, Cappai A, Ruiu G, Dessy E, Rescigno A, Brotzu G. Development of biocompatible barium alginate microcapsules. Transplant Proc 1997;29(4):2129–30.

[22] Markusen JF, Mason C, Hull DA, Town MA, Tabor AB, Clements M, et al. Behavior of adult human mesenchymal stem cells entrapped in alginate-GRGDY beads. Tissue Eng 2006;12(4):821–30.

[23] Lee KY, Alsberg E, Mooney DJ. Degradable and injectable poly(aldehyde guluronate) hydrogels for bone tissue engineering. J Biomed Mater Res 2001;56(2):228–33.

[24] Schagemann JC, Mrosek EH, Landers R, Kurz H, Erggelet C. Morphology and function of ovine articular cartilage chondrocytes in 3-d hydrogel culture. Cells Tissues Organs 2006;182(2):89–97.
[25] Bouhadir KH, Lee KY, Alsberg E, Damm KL, Anderson KW, Mooney DJ. Degradation of partially oxidized alginate and its potential application for tissue engineering. Biotechnol Prog 2001;17(5):945–50.
[26] Simmons CA, Alsberg E, Hsiong S, Kim WJ, Mooney DJ. Dual growth factor delivery and controlled scaffold degradation enhance *in vivo* bone formation by transplanted bone marrow stromal cells. Bone 2004;35(2):562–9.
[27] Kong HJ, Kaigler D, Kim K, Mooney DJ. Controlling rigidity and degradation of alginate hydrogels via molecular weight distribution. Biomacromolecules 2004;5(5):1720–7.
[28] Alsberg E, Kong HJ, Hirano Y, Smith MK, Albeiruti A, Mooney DJ. Regulating bone formation via controlled scaffold degradation. J Dent Res 2003;82(11):903–8.
[29] Shi L, Caulfield MJ, Chern RT, Wilson RA, Sanyal G, Volkin DB. Pharmaceutical and immunological evaluation of a single-shot hepatitis B vaccine formulated with PLGA microspheres. J Pharm Sci 2002;91(4):1019–35.
[30] Shastri VP, Hildgen P, Langer R. In situ pore formation in a polymer matrix by differential polymer degradation. Biomaterials 2003;24(18): 3133–7.
[31] Preiss J, Ashwell G. Alginic acid metabolism in bacteria. I. Enzymatic formation of unsaturated oligosaccharides and 4-deoxy-L-erythro-5-hexoseulose uronic acid. J Biol Chem 1962;237:309–16.
[32] Ertesvag H, Erlien F, Skjak-Braek G, Rehm BH, Valla S. Biochemical properties and substrate specificities of a recombinantly produced *Azotobacter vinelandii* alginate lyase. J Bacteriol 1998; 180(15):3779–84.
[33] Cohen S, Yoshioka T, Lucarelli M, Hwang LH, Langer R. Controlled delivery systems for proteins based on poly(lactic/glycolic acid) microspheres. Pharm Res 1991;8(6):713–20.
[34] Liu C, inventor; Novo Industri A/S assignee. Thermostable alginate lyase from *Bacillus steraothermophilus* USA, 1992.
[35] Gage FH, Coates PW, Palmer TD, Kuhn HG, Fisher LJ, Suhonen JO, et al. Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. Proc Natl Acad Sci USA 1995;92(25):11879–83.
[36] Gage FH. Mammalian neural stem cells. Science 2000;287:1433–8.
[37] Ma HL, Hung SC, Lin SY, Chen YL, Lo WH. Chondrogenesis of human mesenchymal stem cells encapsulated in alginate beads. J Biomed Mater Res A 2003;64(2):273–81.
[38] Dar A, Shachar M, Leor J, Cohen S. Optimization of cardiac cell seeding and distribution in 3D porous alginate scaffolds. Biotechnol Bioeng 2002;80(3):305–12.
[39] Sakakibara H, Tamura T, Suzuki T, Hisano T, Abe S, Murata K. Preparation and properties of alginate lyase modified with poly(ethylene glycol). J Pharm Sci 2002;91(4):1191–9.
[40] Mahoney MJ, Saltzman WM. Transplantation of brain cells assembled around a programmable synthetic microenvironment. Nat Biotechnol 2001;19(10):934–9.