
Contrasting effects of collagen and bFGF-2 on neural cell function in degradable synthetic PEG hydrogels

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Received 13 February 2006; revised 24 May 2006; accepted 9 June 2006

Published online 21 November 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.30970

Abstract: Injectable biodegradable cell carriers provide a potential means to improve transplanted cell viability in the nervous system by providing physical protection from compaction, shear forces, and the acute inflammatory response that occurs following transplantation into the host brain environment. Synthetic polyethylene glycol (PEG) hydrogels are ideal candidates for this purpose, as the degradation profile and mechanical properties of the gel can be controlled. Here we introduce biological components into the synthetic gel with the goal of improving neural cell function in the inert PEG environment. In this study, it was found that (1) bFGF-2 is a survival/mitogenic factor for neural precursor cells in degradable hydrogel cultures, (2) collagen has no measurable effect on cell survival, metabolic activity, or

proliferation, and (3) co-application of collagen and bFGF-2 to hydrogel cultures targets cell survival and metabolic activity, an effect that is different than either applied individually. Because collagen and bFGF-2 support the survival and growth of neural cells and other cell types, the co-encapsulation approach and functional characterization described in this study can be extended to the development of an array of tissue engineering applications. These findings suggest the importance of understanding and developing strategies to control the chemical microenvironment surrounding cells in three-dimensional biomaterials. © 2006 Wiley Periodicals, Inc. *J Biomed Mater Res* 81A: 269–278, 2007

Key words: hydrogels; stem cells; tissue engineering

INTRODUCTION

Chronic neurodegenerative diseases afflict millions of elderly individuals. In the United States nearly 5 million individuals are afflicted with Alzheimer's Disease.¹ Approximately 500,000 elderly patients suffer from Parkinson's Disease (PD).¹ Cerebrovascular disease (stroke) is a major cause of death in middle and later life; nearly 1.5 million new cases are diagnosed per year.¹ While the etiology of each of these neurological diseases differs, each condition does involve a substantial loss of neuronal function in the central nervous system (CNS). For example, during the progression of PD, dopaminergic neurons of the substantia nigra degenerate. It is this cell loss that seriously compromises the victim's cognitive and motor function. Still, current clinical treatments for PD and other neurological disorders are largely symptomatic.

Cell transplantation offers the possibility of a curative approach towards restoring lost cell function to

the CNS. For example, in the case of PD, intrastriatal transplants of fetal dopaminergic neurons innervate the host brain, form synaptic connections, release dopamine, and improve motor deficits in animal models.^{2–6} In humans, application of fetal transplants has been met with variable but significant success.^{7–11} While promising, however, widespread application of this approach is limited by the fact that cell survival is often poor and can be as low as 5%.^{12,13} In fact, the majority of apoptotic cell death occurs during the first 24 h post-transplantation.¹⁴ Injectable biodegradable cell carriers provide means to improve cell viability acutely by providing physical protection from compaction, shear forces, and the inflammatory response that occur during and immediately following transplantation into the host brain environment. Long-term survival may also be improved by incorporating chemical cues that promote neuronal cell survival into the cell carrier environment.

Most materials that are being developed for this purpose are based on networks of synthetic and naturally occurring polymers throughout which cells are distributed in three-dimensions.^{15–22} Photopolymerizable poly(ethylene)glycol (PEG)-based hydrogels provide specific advantages as a synthetic material for

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three-dimensional cell carriers. PEG is biocompatible and nonimmunogenic. Gelation can be induced directly, in the presence of cells, resulting in uniform density throughout the three-dimensional implant. Polymer network architecture can be controlled on a spatial scale that spans angstroms to microns by changing the initial network cross-linking density and its rate of change with time, for example by introducing degradable linkages into the macromer backbone.²³

While synthetic gels offer control over the macroscopic properties and degradation mechanism, they lack any biologically active components. Ideally the matrix would go beyond a simple passive protective structure to provide a three-dimensional environment that facilitates long-term grafted cell function. Extracellular matrix molecules and growth factors have been shown to influence multiple aspects of neural cell function. For example, bFGF-2 stimulates the proliferation of neural precursor cells (NPCs).²⁴ bFGF-2 has been shown to increase the total number of cells present in monolayer culture by increasing the fraction of cells that survive in culture and by maintaining cells in a proliferative state.^{25,26} Other growth factors have been shown to increase cell metabolic activity and differentiation.^{27,28} Extracellular matrix molecules, including collagen type I have been shown to support NPC expansion and differentiation.^{26,29,30}

The focus of this study was to functionalize the three dimensional environment that surrounds neural cells within the synthetic PEG hydrogel by incorporating two molecules that have been shown to impact the development of NPCs into the hydrogel, bFGF-2, and collagen type I. In this study, experiments were designed to elucidate the effect of each factor applied separately or in combination on cell function in the 3D hydrogel. NPCs isolated from the developing brain were encapsulated within PEG hydrogels. The choice to utilize NPCs was based on their demonstrated potential in cell therapies for the treatment of degenerative disorders of the CNS.^{31–36} Collagen was co-encapsulated with cells during photopolymerization of the network. bFGF-2 was exogenously added to the culture medium. Because growth factors can exert multiple effects on cell function, we monitored the survival, metabolic activity, proliferation, and differentiation of cells in hydrogel culture. A combination of direct microscopy-based assays and cell lysate assays were used to identify the role that each factor or combination of factors had on cell function in the hydrogel environment.

MATERIALS AND METHODS

Hydrogel preparation and characterization

Using previously developed procedures, poly(lactic acid) (PLA) was grafted from linear PEG chains (MW 4600) to

form triblock copolymers (2LA-PEG-2LA).²³ Macromers were end-capped with methacrylate functionalities, purified by precipitation in ether, and characterized with ¹H NMR. Hydrogels were prepared with a 10 wt % PEG macromer solution in sterile culture medium containing cells and 0.03 wt % photoinitiator (Darocur 2959). Gels were formed by exposing the solution to UV light (~4 mW/cm²) for 10 min. The compressive modulus was measured using a Materials Testing System (Synergie 100). Measurements were made in unconfined compression at room temperature. Gels were subjected to a static load that increased at a rate of 50 mN/min. The compressive modulus was determined by analyzing the linear region of the stress strain curve.

Hydrogel culture

Embryonic day 14 forebrain was dissected from rats (Sprague Dawley) and was dissociated enzymatically. Single cells were photoencapsulated in three dimensional hydrogel culture at a density of 1×10^7 cells/mL. For some studies, collagen was added to the suspension of single cells to achieve a final dose of 250 µg/mL in the gel. Culture medium was a 50:50 mixture of DMEM and F12 supplemented with N2 and penicillin streptomycin (1%). bFGF-2 was added to a subset of cultures at a concentration of 10 ng/mL. Culture medium was exchanged every 2–3 days to maintain appropriate growth factor levels. All materials were purchased from InVitrogen.

Measurement of total DNA, ATP, and caspase activity in gel cultures

On day 0 and day 7 of culture, each hydrogel was rinsed with cold PBS and was transferred to a 15-mL centrifuge tube containing cell lysis buffer. Each hydrogel was homogenized for 1 min at 40% power on ice. The supernatant was stored at –80°C until the day on which assays were performed. Total DNA content was quantified using the PicoGreen assay (Molecular Probes). ATP levels were determined using the Cell Glo Assay (Promega). The Apo-One assay (Promega) was used to quantify the activities of caspase-3 and caspase-7 in lysates as an indicator of the level of apoptosis. These members of the caspase family play key effector roles in apoptosis in cells. Data are presented as mean ± standard error of the mean (S.E.M.). Statistical significance was determined using Student's *t*-test, with *p* < 0.05 considered significant. Data was obtained from three separate experiments involving five hydrogel cultures per time point.

MTT assay

Hydrogel cultures were transferred to phenol-free medium containing 0.5 mg/mL MTT reagent (Sigma). Gels were incubated in this media for 3 h. Then, an equal volume of 50:50 1N HCl:Isopropyl Alcohol was added to gel cultures to dissolve the precipitate that was formed. Fol-

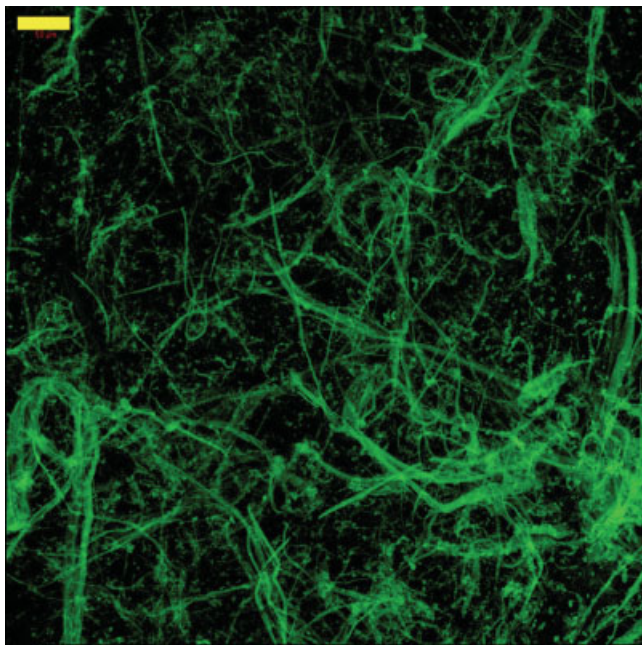


Figure 1. Distribution of collagen in PEG-PLA hydrogel cultures. The distribution of collagen fibers throughout PEG-PLA hydrogel cultures was detected by fluorescence-based immunolabeling. Images throughout thin sections of hydrogel culture were obtained by confocal microscopy. This image represents a series of 40 optical sections 2 μm thick throughout the gel. A network of collagen fibers was visible in all regions of the hydrogel. Scale bar represents 50 μm . [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

lowing dissolution of the precipitate, the absorbance of the solution was read at 560 nm.

Direct imaging of neural cells in degradable PEG hydrogel culture

Cell viability within hydrogels was assessed with a fluorescence-based membrane integrity assay (LIVE/DEAD kit, Molecular Probes). Viable neural cells were labeled with calcein-AM and dead cells were labeled with ethidium bromide. Confocal microscopy was used to spatially localize living and dead cells throughout the hydrogel. Each image represents a projection of a series of 50–60 optical sections 10 μm thick throughout the interior region of the hydrogel. Aggregate diameter was calculated by tracing the perimeter of aggregates in images obtained using confocal microscopy in the Zeiss LSM Image Examiner software program. Aggregate size data was obtained from at least 50 aggregates derived from three separate experiments.

Immunocytochemistry and Western blot

After 7 days of culture, cells were fixed in 4% paraformaldehyde. Sections of hydrogel cultures, 40 μm thick were cut with a cryostat. Using standard immunocyto-

chemical techniques, hydrogel sections were processed for staining with antibodies against β -tubulin (neuron), nestin (precursor cell), and collagen type I (Sigma). Unless otherwise noted, all antibodies were purchased from Chemicon. Secondary antibodies conjugated to fluorophores were purchased from Jackson Laboratories. These antibodies were also used to probe levels of β -tubulin and β -actin (loading control) on immunoblots of hydrogel culture supernatants. All Western blotting reagents and protocols were performed according to BIORAD Opti-4CN kit recommendations.

RESULTS

Effect of collagen on the compressive modulus and degradation rate of the hydrogel

Collagen fibers fluorescently labeled by immunohistochemistry were visualized throughout sections of PEG-PLA hydrogels after 7 days of culture by confocal microscopy. Collagen fibers were found distributed throughout the hydrogel (Fig. 1). Incorporation of collagen into PEG-PLA hydrogels did not significantly impact ($p \gg 0.05$) the compressive modulus after 7 days of culture (collagen, 266 ± 35 kPa and control or native 252 ± 30 kPa). Complete degradation of the native PEG-PLA gel occurred over the course of 2 weeks; addition of collagen did not influence the time-scale for degradation (data not shown).

Cell proliferation

As a measure of the proliferative capacity of cells in hydrogels with different chemical environments, the amount of DNA present on day 7 of culture was measured and is expressed relative to the amount present initially on day 0 of culture in Figure 2. The relative increase in total DNA content depended on the chemical environment of the hydrogel. In native PEG-PLA hydrogels, a 2.7-fold increase in total DNA content was observed. Addition of bFGF-2 to the culture medium resulted in a significantly greater increase in total DNA content; a 3.4-fold increase was observed.

In hydrogels doped with collagen after 7 days of culture, a 2.6-fold increase in total DNA content was observed. This increase was not statistically different from that observed in native PEG-PLA hydrogels in the absence of bFGF-2. Addition of bFGF-2 to gels containing collagen did not result in a further increase in DNA content. Levels of DNA in collagen containing cultures supplemented with bFGF-2 were not statistically different from levels in hydrogels without bFGF-2. This finding suggests that bFGF-2

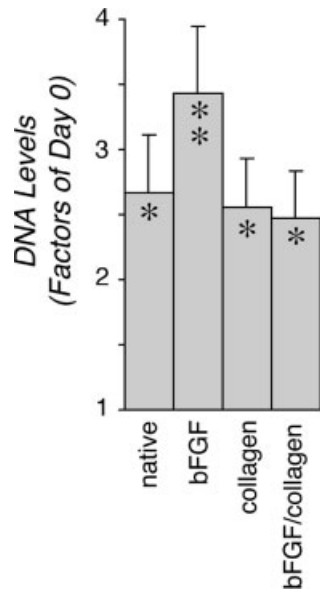


Figure 2. Total DNA content in gels with different chemical environments. Total DNA content was measured in PEG-PLA (native) gels or gels supplemented with bFGF, collagen, or bFGF/collagen. Total DNA content after 7 days of culture is expressed relative to values on day 0 of culture. Statistically significant groups are indicated by one or two asterisks ($p < 0.05$).

only exerts a proliferative effect on NPCs in PEG-PLA hydrogels in the absence of collagen.

Cell survival in degradable PEG-PLA hydrogels

ATP content, normalized with respect to total DNA content, was utilized as a quantitative indicator of cell viability in hydrogels on day 7 of culture. Values are expressed relative to levels in native PEG-PLA cultures on day 7 of culture in Figure 3. In the absence of bFGF-2, levels in collagen containing cultures were not statistically different from levels in native PEG-PLA cultures. Addition of bFGF-2 to native PEG-PLA gel cultures significantly increased normalized ATP content ($p \gg 0.05$). In the presence of both bFGF-2 and collagen a larger increase in normalized ATP content was observed. These values are statistically different from one another and from levels in native gels. These findings indicate that when applied separately, bFGF-2 acts as a survival factor for NPC in PEG-PLA gels; it increases the overall viability of the culture. Collagen does not exert a survival effect on cells in culture. However, when applied in combination with one another, the two factors exert a more potent effect on cell survival than either applied alone. The fact that cell survival is the highest in bFGF-2/collagen cultures, which contain the same amount of

DNA as PEG-PLA control cultures (native gels), suggests that this increase in normalized ATP content is the result of an increase in the survival of non-proliferating cells.

Metabolic activity

As a quantitative index of the level of metabolic activity of cells in hydrogels, MTT activity was assessed on day 7 of culture. Equal numbers of cells are not present in cultures with different chemical environments. To account for this, MTT activity was normalized to total DNA content and values are expressed relative to levels in native PEG-PLA cultures on day 7 of culture in Figure 4. In the absence of bFGF-2, the level of metabolic activity in native gels was not statistically different from the level of metabolic activity in gels containing collagen. This finding suggests that collagen has no effect on the metabolic activity of the culture when presented to cells in the absence of bFGF-2. Addition of bFGF-2 significantly increased the metabolic activity of cells in both native PEG-PLA hydrogels and in collagen supplemented hydrogel cultures. However, simultaneous exposure to collagen and bFGF-2 resulted in a

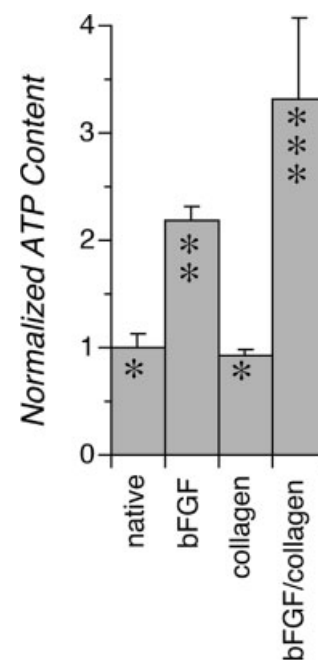


Figure 3. Normalized ATP content in gels with different chemical environments. Total ATP content was measured and normalized to total DNA content in culture, after 7 days of culture. Normalized ATP content in gels supplemented with bFGF, collagen, or bFGF/collagen is expressed relative to levels in native PEG-PLA gels after 7 days of culture. Statistically significant groups are indicated by one, two, or three asterisks ($p < 0.05$).

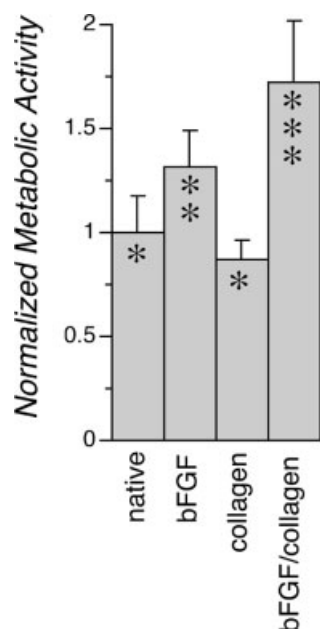


Figure 4. Normalized metabolic activity in gels with different chemical environments. MTT activity was measured and normalized to total DNA content. Normalized MTT activity in gels supplemented with bFGF, collagen, or bFGF/collagen are expressed relative to levels in native PEG-PLA gels after 7 days of culture. Statistically significant groups are indicated by one, two or three asterisks ($p < 0.05$).

more potent increase in metabolic activity relative to levels in native gels. This finding is consistent with ATP measurements. This increase in MTT activity may reflect an increase in (1) the metabolic demand of cells in the gel, and/or (2) an increase in the fraction of viable cells present in culture.

Cell death in bFGF supplemented cultures

To determine directly whether or not a difference in apoptosis is involved in the effects of bFGF-2 and collagen on cells in gel cultures, apoptotic activity was quantified after 7 days of culture. Levels of caspase activity were normalized with respect to total DNA content, and are presented in Table I, relative to levels in

native PEG-PLA gels. After 7 days of culture, levels of caspase activity were significantly higher in native gels relative to levels in bFGF-2 supplemented gels. Apoptotic activity in collagen/bFGF-2 supplemented gels was not significantly different than that in bFGF-2 supplemented gels. This finding is consistent with a role for bFGF-2 as a survival factor for NPC's in PEG-PLA gel culture. The finding that levels of apoptosis in bFGF-2 gels are not statistically different than levels in bFGF-2/collagen gels suggests that differences in MTT activity and ATP content are not due, in large part, to differences in cell survival and are more likely related to a difference in the level of metabolic activity of cells in gels.

Neurosphere formation as a function of gel chemical microenvironment

While not quantitative, the fluorescence-based membrane integrity assay is a useful tool that can be used in conjunction with confocal microscopy to visualize the spatial distribution of living and dead cells throughout the hydrogel environment. Initially on day 0 of culture, single cells were distributed throughout the hydrogel. After 7 days in culture, small aggregates of cells were visualized throughout the hydrogel (Fig. 5). Aggregate size depended on the chemical environment of the hydrogel (Table I). In the absence of bFGF, aggregate diameter was slightly lower in hydrogels supplemented with collagen (PEG-PLA $25 \pm 1 \mu\text{m}$, PEG-PLA collagen $22 \pm 1 \mu\text{m}$). In contrast, in the presence of bFGF-2 aggregate size was significantly higher in hydrogels supplemented with collagen (PEG-PLA bFGF-2 $30 \pm 1 \mu\text{m}$, PEG-PLA collagen bFGF-2 $39 \pm 1 \mu\text{m}$).

Cell differentiation in bFGF-2 supplemented gel cultures

Cell differentiation within the hydrogel environment was assessed by measuring the amount of β tubulin (a marker for differentiated neurons) present in culture by Western blot and immunocytochemistry. No significant differences in the amount of β -

TABLE I

Effect of Gel Chemical Environment on Neural Precursor Cell Growth and Apoptotic Activity in PEG-PEA Hydrogels

Gel Chemical Environment	Aggregate Diameter (μm)	Apoptotic Activity	β -Tubulin Content
Native PEG-PLA	25 ± 1	$1 \pm 0.07^*$	1 ± 0.01
bFGF	22 ± 1	$0.9 \pm 0.15^{**}$	1.1 ± 0.001
Collagen	30 ± 1	N/D	N/D
bFGF/Collagen	39 ± 1	$0.95 \pm 0.2^{**}$	1 ± 0.01

N/D: not determined.

*, ** indicate statistically significant groups.

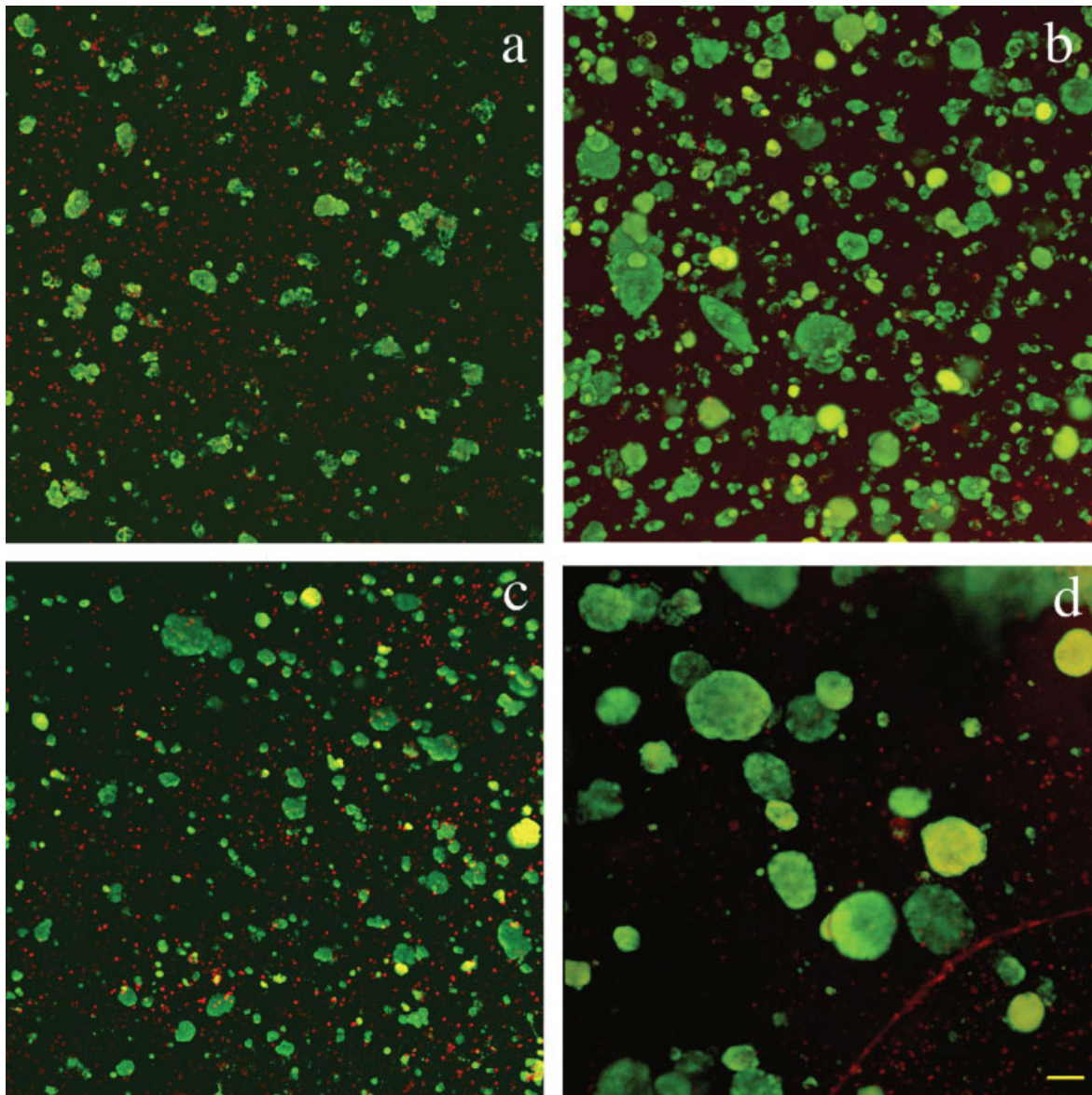


Figure 5. Effect of chemical environment on neural tissue development in PEG-PLA hydrogels. Neural cells in degradable hydrogels were fluorescently labeled with a dye that fluoresces green upon de-esterification in living cells (calcein-AM) and a dye that fluoresces red when bound to DNA in dead membrane compromised cells. Living and dead cells were directly visualized using confocal microscopy. Over the course of 7 days multicellular aggregates or neurospheres are visible throughout the culture. Aggregate diameter and density depended on the chemical environment surrounding cells: (a) native gels (no bFGF), (b) bFGF, (c) collagen, and (d) bFGF and collagen. Images represent a projection of 50–60 optical sections 2 μm thick throughout the gel. Aggregate size data was obtained from greater than 50 aggregates derived from three separate experiments. Scale bar represents 30 μm . [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

tubulin, relative to β -actin content, were detected by Western blot in gels with different chemical microenvironments [Table I, Fig. 6(a)]. However, when assessed by immunocytochemistry, in collagen/bFGF-2 containing gels, a fraction of β -tubulin positive cells appeared more differentiated [Fig. 6(b)]. These cells possessed long processes that emerged from aggregates to penetrate and grow through the hydrogel environment.

DISCUSSION

bFGF-2 exerts effects on NPCs by binding to their receptors, which are expressed on the surface of developing neural cells.³⁷ Similarly, integrin receptors present on the surface of NPCs can bind to cell adhesion sequences present within extracellular matrix molecules to impact cell function.^{38–41} Here we characterize the impact of one extracellular matrix

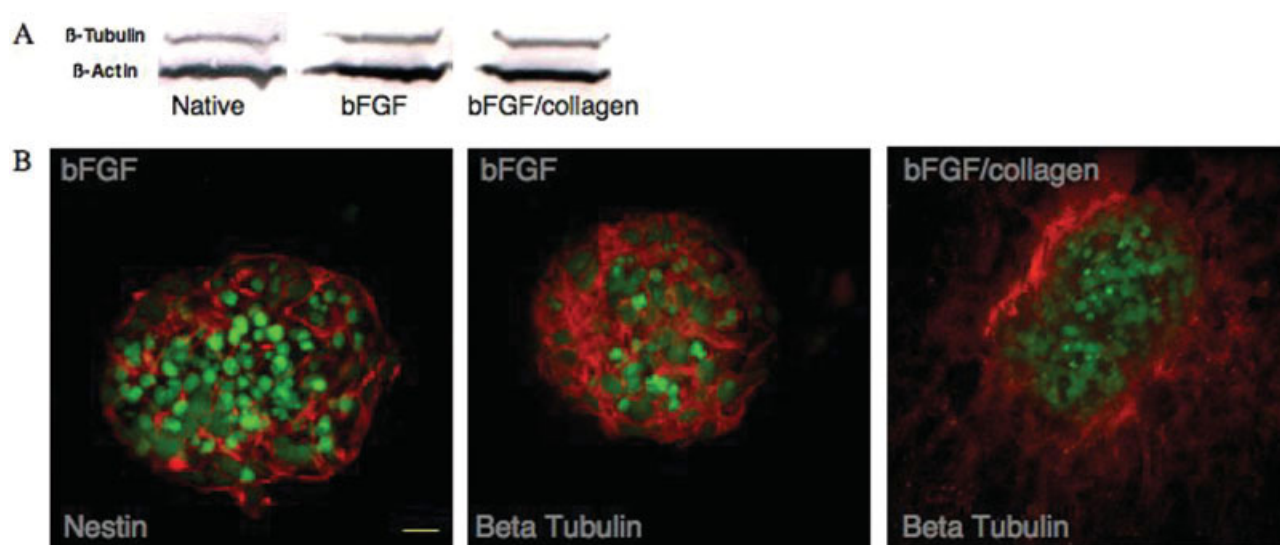


Figure 6. β -tubulin expression in PEG-PLA hydrogel culture. (a) β tubulin expression in native gels was compared to bFGF supplemented hydrogels with or without collagen by Western blot analysis. (b) Immunofluorescence analysis of cells in PEG hydrogel culture using antibodies against β -tubulin (neurons) and nestin (precursors). Scale bar represents 15 μ m. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

molecule, collagen, and bFGF-2 on NPC function in three dimensional degradable PEG hydrogels.

This study is different from previous work in two important ways. First in this study cells and collagen are co-suspended throughout a three-dimensional photopolymerized PEG network. While a weakly cross-linked hydrogel is formed when collagen is in solution at 250 μ g/mL, in this study, addition of collagen did not impact the mechanical properties of the hydrogel. This is reasonable given that the mass of collagen in the gel accounts for less than 0.25% of the weight of the gel. The physical properties of the gel are dominated by the PEG polymer. Second, while in other studies collagen served as the infrastructure of the 3D scaffold; here the PEG network serves as the support for cell growth.^{16,29,42,43} This enabled us to compare NPC growth in 3D culture in the presence and absence of collagen. We extended this work to clarify the individual contribution of bFGF, collagen, and the two supplied in combination to elucidate possible combinatorial effects of these molecules on NPC development. Each of these factors, when presented separately or in combination with one another, had a unique impact on neural cell function. The importance of these findings is described below.

bFGF is a mitogen and a survival factor for NPCs in PEG-PLA hydrogel cultures

In monolayer culture, bFGF-2 has been shown to impact both NPC survival and proliferation.^{24,25} To

identify a similar role for bFGF-2 in degradable PEG-PLA culture, cell survival and proliferation were measured after 7 days in culture. Cell survival was quantified using three measurements namely: ATP content, MTT activity, and apoptosis level. One week following encapsulation, cell survival was significantly greater in bFGF-2 supplemented cultures (Fig. 3). This result coupled with the fact that apoptosis is slightly reduced after 7 days in bFGF-2 supplemented cultures (Table I) indicates that bFGF-2 is a survival factor for NPC in degradable PEG hydrogel culture; the overall viability of the culture is improved.

When encapsulated in PEG-PLA gels, cells proliferate to form neurospheres (Fig. 5). Over the course of 1 week of culture, a larger increase in total DNA content (relative to day 0 of culture) is observed in bFGF-2 supplemented cultures (Fig. 2). This increase in total DNA content may be the direct effect of an increase in cell survival. The increase in DNA content is also likely a mitogenic effect of bFGF-2 on NPC. Cells also differentiate to form post-mitotic neurons during 7 days of PEG-PLA culture. Addition of bFGF-2 may increase the fraction of cells that remain in the proliferative state or the rate of NPC division over the course of 1 week of hydrogel culture.

Distinct impact of co-applied bFGF-2 and collagen on NPC function

In contrast to bFGF-2, the addition of collagen to the PEG-PLA hydrogel environment resulted in no

additional increase in total DNA content. Cell survival, metabolic activity, and apoptotic activity were not significantly different than in native PEG-PLA culture after 7 days of growth. These findings indicate that in the absence of bFGF-2, collagen does not have a distinct effect on cell growth in PEG-PLA gel cultures.

When simultaneously exposed to bFGF-2 and collagen, a distinct effect on both cell survival and proliferation was observed. This effect is unique from the effects of either factor applied separately. Total DNA content in gels containing collagen and bFGF-2 were not significantly different than levels in native hydrogels. While collagen and bFGF-2 presented together do not have an effect on total DNA content, cell survival and metabolic activity were impacted. Cell survival was significantly higher in collagen/bFGF-2 hydrogels over the course of 7 days of culture (Fig. 3). Metabolic activity was elevated on day 7 of culture (Fig. 4).

The fact that cell survival is higher while total DNA content remains constant relative to levels in native PEG-PLA gels suggests that those cells that are rescued by bFGF-2 and collagen do not continue to divide. These cells may be post-mitotic neurons. Western blot data confirm that β -tubulin positive cells are present in culture (Fig. 6). A fraction of these cells appear more differentiated in collagen/bFGF-2 cultures relative to bFGF-2 cultures when visualized by immunocytochemistry. A dense plexus of process emerged from these cells to penetrate and grow throughout the gel environment [Fig. 6(b)].

Co-presentation of collagen and bFGF-2 may activate a different set of signal transduction pathways than those activated by collagen in NPCs. Alternatively, collagen and bFGF-2 may bind to one another. Binding to collagen present within the PEG-PLA hydrogel may immobilize the growth factor within the extracellular environment. In this case, the dose of bFGF-2 that is available to bind to receptors present on the cell surface in collagen containing gels may be lower than that in native PEG-PLA hydrogels. In monolayer culture, bFGF-2 has been shown to exert an effect on cell survival at low concentrations of bFGF-2 and an effect on both survival and cell proliferation when presented with high concentrations of bFGF-2.³³ Cells in hydrogel culture that contain both bFGF-2 and collagen may respond in a similar dose-dependent manner when exposed to low doses of bFGF-2.

Multiple factors impact the metabolic activity of a cell including exposure to growth factors and cell differentiation.^{27,28} In a homogeneous population of cells, an increase in metabolic activity would simply reflect an increase in the overall metabolic activity of the culture. In this work, cultures are heterogeneous and are composed of both NPCs and differentiated

neurons. An increase in metabolic activity is likely the result of two factors (1) an increase in cell survival, and (2) an increase in the metabolic activity of the cell *per se*. This increase may reflect a shift in the fraction of the culture that is composed of differentiated neurons, a possibility that is consistent with the observation that a fraction of cells in bFGF-2/collagen cultures appear more morphologically differentiated than those in native PEG-PLA cultures.

One alternative explanation for the observed increase in metabolic activity is that cells in hydrogels are migrating throughout collagen/bFGF-2 hydrogels. While not directly observed, cells may migrate toward one another in collagen/bFGF-2 gels to produce more disperse but larger aggregates inside of the hydrogel. This hypothesis is supported by our observation that aggregate diameter was significantly greater while aggregate density was decreased in collagen/bFGF-2 supplemented cultures relative to native hydrogels.

The focus of this work was to evaluate the influence of an ECM molecule and an exogenously applied growth factor on neural cell function in degradable PEG hydrogel culture. This functionalized biomaterial is suitable for direct injection into the brain extracellular space. The improvement in cell function, observed in cell culture, may translate to a significant improvement in cell function upon transplant into the brain. Collagen gels are being used as nerve guidance materials in the spinal cord and in the peripheral nervous system.^{44,45} The functionalized PEG-PLA hydrogels described in this study may prove useful in peripheral nerve regeneration and in spinal cord regeneration as well.

To conclude: (1) bFGF-2 is a survival/mitogenic factor for NPCs in degradable PEG-PLA hydrogel cultures, (2) collagen has no measurable effect on cell survival, metabolic activity, or proliferation, (3) co-application of collagen and bFGF to hydrogel cultures targets cell survival and metabolic activity, an effect that is different than either applied individually. These findings suggest the importance of understanding and developing strategies to control the chemical microenvironment surrounding cells in three-dimensional biomaterials that are being designed as cell culture platforms.

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