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Adapting Biodegradable Oligo(Poly(Ethylene Glycol) Fumarate) Hydrogels for Pigment Epithelial Cell Encapsulation and Lens Regeneration

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This study investigated the encapsulation of newt iris pigment epithelial cells (PECs), which have the ability to regenerate a lens by trans-differentiation *in vivo*, within a biodegradable hydrogel of oligo(poly(ethylene glycol) fumarate) crosslinked with poly(ethylene glycol)-diacrylate. Hydrogel beads of initial diameter of 1 mm were fabricated by a molding technique. The swelling ratio and degradation rate of the hydrogel beads decreased with increasing crosslinking ratios. Confocal microscopy confirmed the cytocompatibility of crosslinking hydrogel formulations as evidenced by the viability of an encapsulated model cell line within a crosslinked hydrogel bead. Hydrogel beads encapsulating iris PECs were also implanted into lentectomized newts *in vivo*; histological evaluation of explants after 30 days revealed a regenerated lens, thus demonstrating that the presence of degrading hydrogel did not adversely affect lens regeneration. The results of this study suggest the potential of a method for lens regeneration involving oligo(poly(ethylene glycol) fumarate) hydrogels for iris PEC encapsulation and transplantation.

Introduction

Many organisms boast regenerative properties.[1] However, among vertebrates, newts are the champions of regeneration; even adult newts can regenerate whole organs or body parts such as the limbs, tail (with spinal cord), brain, heart, jaws, and eye tissues.[2,3] Regeneration is achieved via the process of trans-differentiation from terminally differentiated cells at the site of injury or tissue removal. The process of trans-differentiation is quite striking in the case of lens regeneration. Upon lentectomy, the pigment epithelial cells (PECs) comprising the tip of the dorsal iris dedifferentiate by losing their pigments and subsequently differentiate into a lens.[4] The resulting lens is fully functional with the correct structure, complete with lens epithelium, and an anterior–posterior polarity. The process takes approximately 3–4 weeks.[5,6]

The lens regeneration process occurs *in vivo* only from PECs in the dorsal iris, never the ventral iris. However, PECs of the newt ventral iris can trans-differentiate into lens cells *in vitro* after prolonged culture. Interestingly, even PECs from other animals, including adult humans, have this capacity.[7] When cultured *in vitro*, the PECs trans-differentiate to form amorphous lentoids rather than well-rounded lenses. However, cultured newt PECs that are aggregated and subsequently transplanted into a newt eye, or even a limb, are able to produce a very well formed normal lens. The same is true for iris pieces as well.[8,9] Such a property of dissociated newt PECs implies that if PECs are placed in an appropriate scaffold that mimics the spherical shape of the lens, a whole lens may be formed while the scaffold is degraded.

Injectable, *in situ*–forming hydrogels hold great promise as tissue engineering scaffolds for cell encapsulation and transplantation.[10] Recently, an injectable macromer, oligo(poly (ethylene glycol) fumarate) (OPF), was developed in our laboratory.[11] Previous research has shown that OPF-based formulations can be crosslinked in the presence of radical initiators under physiological conditions to form a hydrogel,[12] suggesting the feasibility of fabricating implants of different shapes. Studies have also demonstrated the degradation of OPF hydrogels both *in vitro* and *in vivo* through the hydrolysis of ester linkages.[11,13] In addition, the encapsulation of cell populations and particulate drug delivery systems within the hydrogels demonstrated the potential of injectable hydrogel formulations for cartilage and bone tissue engineering applications.[13–16] Moreover, the swelling and degradation properties of the hydrogels have been shown to influence the chondrogenic and osteogenic differentiation of encapsulated marrow mesenchymal stem cells *in vitro*.[17,18]

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In this work, we investigated the encapsulation of iris PECs within OPF hydrogels for lens regeneration. Specifically, we asked the following questions: (1) can hydrogels be fabricated in the form of beads of diameter 1 mm with controllable swelling properties and degradation characteristics? (2) do encapsulated cell populations in hydrogel beads retain their viability? and (3) does the transplantation of hydrogel beads encapsulating iris PECs affect lens regeneration in a newt model?

Materials and Methods

Hydrogel bead fabrication and characterization

Silicone molds made from silver beads of diameter 1 mm were produced for hydrogel fabrication. OPF macromers were synthesized from poly(ethylene glycol) (PEG) of nominal molecular weight of 10,000 g/mol by established procedures.[11,12] After synthesis, the powdered macromers were lyophilized and stored at −20°C under nitrogen until use.[16]

To fabricate hydrogel beads, OPF (0.1 g) was combined with 0.02 g, 0.01 g, 0.0066 g, or 0.005 g of the crosslinker poly(ethylene glycol)-diacrylate (PEG-DA; nominal molecular weight 4000; Monomer-Polymer & Dajac Labs, Feasterville, PA) for crosslinking ratios (w/w) of PEG-DA to OPF of 1:5, 1:10, 1:15, or 1:20, respectively, and dissolved in 577 μL of phosphate-buffered saline (PBS; Invitrogen, Carlsbad, CA). A 0.3 M ammonium persulfate (APS; Sigma-Aldrich, St Louis, MO) solution and 0.3 M N,N,N′,N′-tetramethylethylenediamine (TEMED; Sigma-Aldrich) solution were prepared. To initiate crosslinking, 46.8 μL of APS solution and 46.8 μL of TEMED solution were added to the polymer solution, and the resulting solution was injected into the silicone mold, which was then closed, clamped, and placed in an incubator (37°C) for 8 min. The formed hydrogel beads were transferred to a Petri dish containing 10 mL PBS (Fig. 1A).

After fabrication, hydrogel beads were immersed in PBS in an incubator at 37°C. For each group, hydrogel beads were divided into chains of four beads in order to minimize any weight measurement error. At days 0 (after fabrication), 1, 7, 14, and 21, five samples (chains of four beads) from each group were retrieved, blotted with weighing paper to remove surface water, and weighed. Then they were lyophilized for 24 h and reweighed.

The swelling ratio of hydrogels was determined from their wet and dry weights at each time point. Percent polymer loss was calculated by comparing dry weights of hydrogels to initial dry weights, as shown in the following equations:

$$\text{Swelling ratio} = \frac{W_w - W_d}{W_d}$$

$$\%\ \text{Polymer loss} = \frac{W_i - W_d}{W_i} \times 100,$$

where, W_w and W_d represent wet and dry weights of hydrogel beads at each time point, respectively, and W_i represents the dry weight of beads after fabrication (day 0). The swelling ratio represents the increase in the weight of the hydrogel due to water absorption, whereas percent polymer loss over time characterizes the extent of hydrogel degradation.

Both swelling ratio and percent polymer loss data were analyzed by analysis of variance and Tukey's test, where $p < 0.05$ pointed to a statistically significant difference. Statistical analysis was performed only on data from days 1 and 7 due to the difficulty in sample retrieval at days 14 and 21.

Cell encapsulation and evaluation of cell viability in vitro

Rat fibroblasts were used as a model cell line in place of newt PECs to assess the efficacy of cell encapsulation within crosslinked hydrogel beads *in vitro*, due to the limited number of PECs that can be harvested per newt and to minimize the number of animals required for the study. In particular, cells were encapsulated into hydrogels at a concentration of 10 million/mL in accordance with previous studies with marrow stromal cells.[16] OPF, PEG-DA, and silicone molds were sterilized by exposure to ethylene oxide, whereas the initiators, PBS, and medium were filter sterilized. OPF (0.1 g) and proper parts of PEG-DA (for 1:5, 1:10, 1:15, 1:20 PEG-DA to OPF crosslinking ratios [w/w]) were dissolved with 300 μL of PBS. Passage 8 rat fibroblasts (CRL1764; American Type Culture Collection, Manassas, VA) cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum and 1% antibiotics were lifted with trypsin–ethylenediaminetetraacetic acid (0.05%; Invitrogen). After the addition of 46.8 μL of APS and 46.8 μL of TEMED, 168 μL of the cell suspension containing 7 million cells was added, and the solution was injected into the silicone molds. After an 8-min incubation, the hydrogel beads encapsulating fibroblasts were transferred into a Petri dish containing 10 mL of DMEM with 10% fetal bovine serum.

A live/dead assay was performed at 2 and 24 h after encapsulation.[19] After rinsing with PBS, the beads were placed in dye solutions, 20 μL of 2 mM ethidium homodimer-1, and 5 μL of 4 mM calcein acetoxymethyl ester (Calcein AM) in 10 mL PBS (Invitrogen), for 30 min. Cells encapsulated in the beads were examined by confocal fluorescence microscopy (Zeiss LSM 510, Thornwood, NY). Using argon laser excitation at 488 nm, the cells were imaged under a 10× objective, and the emitted light was collected using 505–526 nm and 612–644 nm filters. A negative (dead cell) control was also prepared by placing the beads in ethanol before they were examined using the same settings.

Encapsulation and transplantation of iris PECs

Newts (*Notophthalmus viridescens*) were utilized for this study and all animals were anesthetized in 0.1% 3-aminobenzoic ethyl ester solution before surgeries. Dorsal irises were first isolated from the iris plate of the newts. Each iris piece has a dimension of approximately 300 μm in diameter and contains approximately 500–1000 PECs; no other cells (like stem cells) have been shown to play a role in trans-differentiation.[5,6] Then, hydrogel beads (1 mm in diameter) of crosslinking ratio of 1:10 (PEG-DA to OPF [w/w]) were fabricated as previously described. During the process of crosslinking, a dorsal iris piece was encapsulated. Hydrogel beads without cells were also fabricated in a similar fashion, serving as a control. After fabrication, hydrogel beads encapsulating iris PECs ($n = 11$) and beads alone ($n = 3$) were implanted bilaterally in lentectomized eyes. Thirty days later, the animals were euthanized by overexposure to the anesthetic (3-aminobenzoic ethyl ester solution) and their eyes were retrieved. Samples were

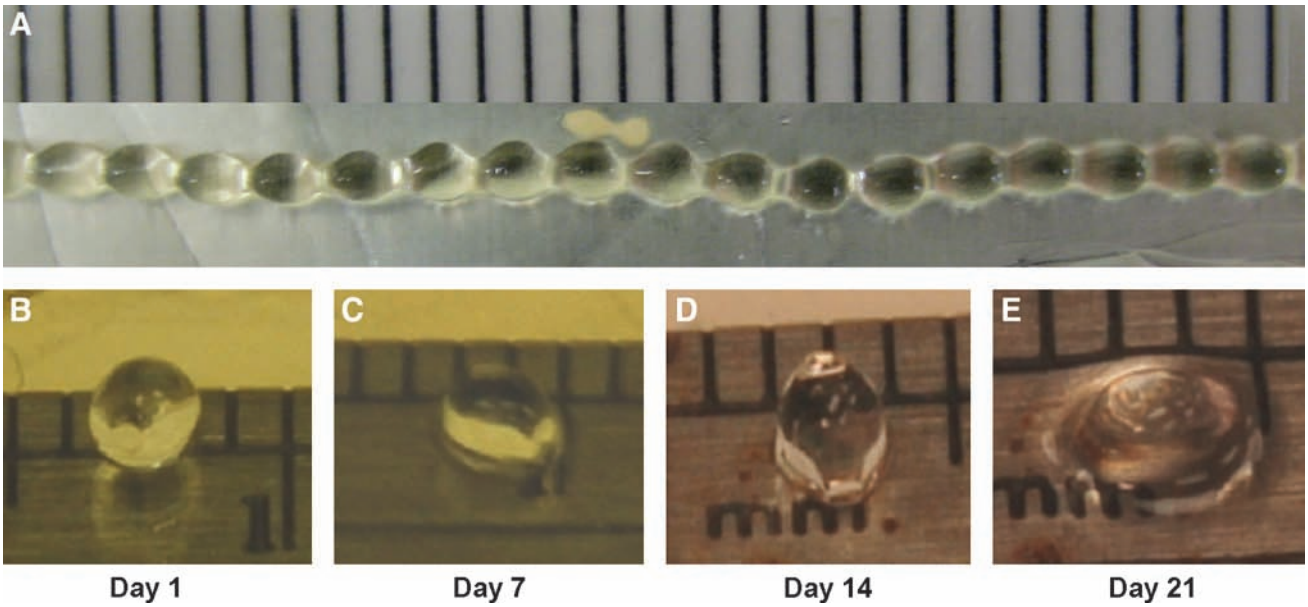

FIG. 1. Hydrogel beads of crosslinking ratio of 1:5 after fabrication (**A**) and during the course of degradation (**B–E**). Each unit in the ruler represents 1 mm.

examined histologically with hematoxylin and eosin staining. Sections were also stained with an α-crystallin antibody to verify trans-differentiation of PECs to lens cells[20]; the secondary antibody (1:100) was a goat anti-mouse IgG (Alexa 488; Invitrogen).

Results

Hydrogel bead fabrication and characterization

Using silicone molds, hydrogel beads of four crosslinking ratios were made, which had initial diameters of about 1 mm (Table 1). The swelling of hydrogel beads of crosslinking ratios of 1:5, 1:10, 1:15, and 1:20 was examined by measuring swelling ratios at days 1, 7, 14, and 21 (if the hydrogel beads were retrievable), as shown in Figure 2. A lower PEG-DA to OPF ratio resulted in a higher swelling ratio of the hydrogels at day 1, except for the crosslinking ratio of 1:20. Statistical analysis revealed that all swelling ratios at day 1 were significantly different, except for the pair of crosslinking ratios of 1:10 and 1:20. At day 7, hydrogel beads of crosslinking ratio of 1:10 still had a significantly higher swelling ratio than beads of crosslinking ratio of 1:5, and the swelling ratios of both groups significantly increased compared to day 1 values. The swelling of the hydrogel beads was also assessed by measuring the bead diameter at day 0 and 1, as shown in Table 1. After fabrication, hydrogels of all formulations had a diameter of about 1 mm. However, at day 1, there was a trend of increased equilibrium diameter with lower crosslinking ratio.

Table 1. Initial and Equilibrium Diameters of Oligo(Poly(Ethylene Glycol) Fumarate) Hydrogel Beads of Varying Crosslinking Ratios

Crosslinking ratio	*Initial diameter (mm)*	*Equilibrium diameter (mm)*
1:5	0.85 ± 0.10	1.45 ± 0.06
1:10	0.98 ± 0.05	1.50 ± 0.08
1:15	0.95 ± 0.06	1.70 ± 0.16
1:20	1.13 ± 0.10	1.90 ± 0.08

Values represent means ± standard derivation for $n = 4$.

As the PEG-DA to OPF crosslinking ratio decreased, hydrogel beads showed a faster degradation rate (Fig. 3). At day 1, hydrogels of lower crosslinking ratios exhibited greater values in percent polymer loss. At day 7, hydrogel beads of crosslinking ratios of 1:15 and 1:20 were irretrievable or completely degraded, whereas beads of crosslinking ratios of 1:5 and 1:10 were still retrievable and had greater values of percent polymer loss compared to day 1. Beads of crosslinking ratio of 1:10 became irretrievable on day 14 and fully degraded by day 21, whereas beads of crosslinking ratio of 1:5 were still retrievable at day 21. Photos of the progression of degradation of hydrogel beads are shown in Figure 1B–E. The hydrogel beads were spherical initially and flattened with time.

Cell encapsulation and evaluation of cell viability in vitro

Figure 4 shows confocal images of hydrogel beads of different crosslinking ratios encapsulating fibroblasts at 2 and 24 h after encapsulation. Calcein AM stains live cells with green fluorescence, and ethidium homodimer-1 stains dead cells with red fluorescence. More than 99% of fibroblasts in view were alive for all formulations of the hydrogel beads (Fig. 4A–H). The negative control identified dead cells (Fig. 4I), validating the confocal fluorescence microscopy technique. Figure 4J, taken at the border of a hydrogel bead, confirms that viability was not position dependent.

Encapsulation and transplantation of iris PECs

OPF hydrogel beads with or without encapsulated iris PECs were implanted in newt lentectomized eyes for the evaluation of lens regeneration. For animals receiving a

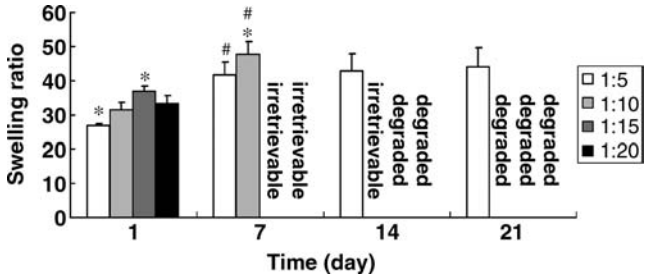

FIG. 2. Swelling ratio of 1 mm diameter hydrogel beads as a function of the crosslinking ratio (1:5, 1:10, 1:15, 1:20). Error bars represent means ± standard deviation for $n = 5$. * indicates a significant difference in swelling ratio compared to the other groups at the same time point based on pairwise comparisons. # indicates a significant difference in swelling ratio compared to the previous time point in a group.

hydrogel bead alone, regeneration of the host lens from the dorsal iris was observed in all three animals 30 days after transplantation, as exemplified in Figure 5D. Of the 11 eyes that received the encapsulated implant, the host lens regenerated in 10. The host regenerated lenses were observed in the dorsal area of the eyes (Fig. 5C and E, above), and they were connected with the dorsal iris, as indicated by checking serial sections (data not shown). Cells encapsulated in the hydrogel beads trans-differentiated into lens tissue in 5 out of the 11 cases, as shown in Figure 5A. In these cases, a host regenerating lens in the dorsal area and an induced lens in the ventral area (indicated with an arrow) were observed (Fig. 5C and E). The trans-differentiation of the cells was confirmed by the formation of characteristic lens fiber tissue (indicated with an arrow in Fig. 5A) as well as by immunostaining with an α-crystallin antibody (Fig. 5B). Cells in the induced lens were depigmented (indicated with an arrow in Fig. 5C), and they formed a lens of a spherical shape and of similar size to the host regenerating lens (approximately 150–300 μm in diameter, at top in Fig. 5C and E). Additionally, both lenses showed a positive staining for the α-crystallin antibody, indicative of cell trans-differentiation (Fig. 5E).

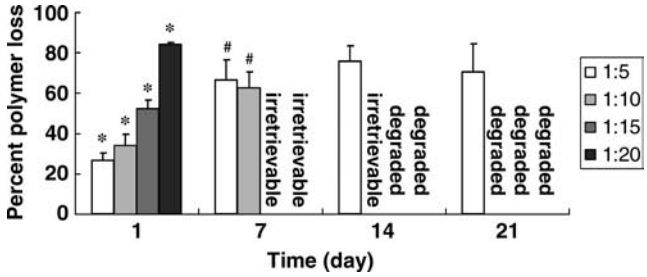

FIG. 3. Percent polymer loss of 1 mm diameter hydrogel beads as a function of the crosslinking ratio (1:5, 1:10, 1:15, 1:20). Error bars represent means ± standard deviation for $n = 5$. * indicates a significant difference in percent polymer loss compared to the other groups at the same time point based on pairwise comparisons. # indicates a significant difference in percent polymer loss compared to the previous time point in a group.

Discussion

In this work, we investigated the encapsulation of iris PECs within OPF hydrogel beads for lens regeneration. Specifically, we investigated (1) whether we could fabricate hydrogel beads of dimensions similar to newt lens and control their swelling and degradation characteristics; (2) whether cells encapsulated in hydrogel beads would retain their viability; and (3) whether the transplantation of hydrogel beads encapsulating iris PECs would affect lens regeneration in a newt model.

In previous studies, OPF scaffolds in the form of disks, cylinders, or layered structures have been fabricated and used for cartilage and bone tissue engineering.[12,16,21] In the present study, OPF hydrogels were fabricated in a spherical shape with a diameter of 1 mm using silicone molds, in order to approximate the dimensions of a newt lens.

Previous research has shown that the swelling and degradation properties of OPF hydrogels can be tailored by factors such as the molecular weight of the PEG block of the OPF macromer and the concentration of the macromer.[12,16] Additionally, studies have shown that the swelling properties of the hydrogels influence the proliferation and differentiation of the encapsulated cells.[17,18] The swelling ratio of a hydrogel is related to the hydrogel mesh size, which affects nutrient transport and drug delivery throughout the hydrogels and may consequently influence cell behavior. Therefore, the present study examined the effect of the crosslinker (PEG-DA) to OPF macromer crosslinking ratio on the swelling and degradation properties of OPF hydrogel beads *in vitro*. The results from hydrogel swelling and degradation studies (Figs. 2 and 3) show that the crosslinking ratio significantly influenced the swelling ratio and percent polymer loss of the hydrogel beads. On day 1, higher swelling ratios and higher extent of polymer loss were observed for hydrogels of lower crosslinking ratios. Combined with the results from a previous study, which showed that OPF hydrogels of 1:2 crosslinking ratio had a swelling ratio of 17.5 ± 0.2,[16] this study demonstrated a trend that lower crosslinking ratios result in higher equilibrium swelling ratios. Compared to day 1, the day 7 swelling ratio and percent polymer loss of hydrogel beads of crosslinking ratios of 1:5 and 1:10 were greater, indicating hydrogel degradation during that time period. By day 7, beads of crosslinking ratios of 1:15 and 1:20 had become irretrievable and completely degraded by day 14. Beads of crosslinking ratio of 1:10 did not degrade completely until day 21 and beads of crosslinking ratio of 1:5 did not degrade completely over the course of this study. Lower crosslinking ratios resulted in faster degradation because of fewer crosslinks within the hydrogel network. Previous studies have demonstrated that the mechanical properties of OPF hydrogels are generally related to the swelling properties of the hydrogels, with hydrogels of lower swelling ratios typically exhibiting greater tensile strength.[12]

In order to assess the efficacy of cell encapsulation within hydrogel beads, rat fibroblasts were encapsulated and cell viability was determined via a live/dead assay. The *in vitro* study was performed with fibroblasts in place of newt PECs in an effort to minimize the use of live animals according to Institutional Animal Care and Use Committee guidelines. The live/dead assay was performed at 2 and 24 h for each of

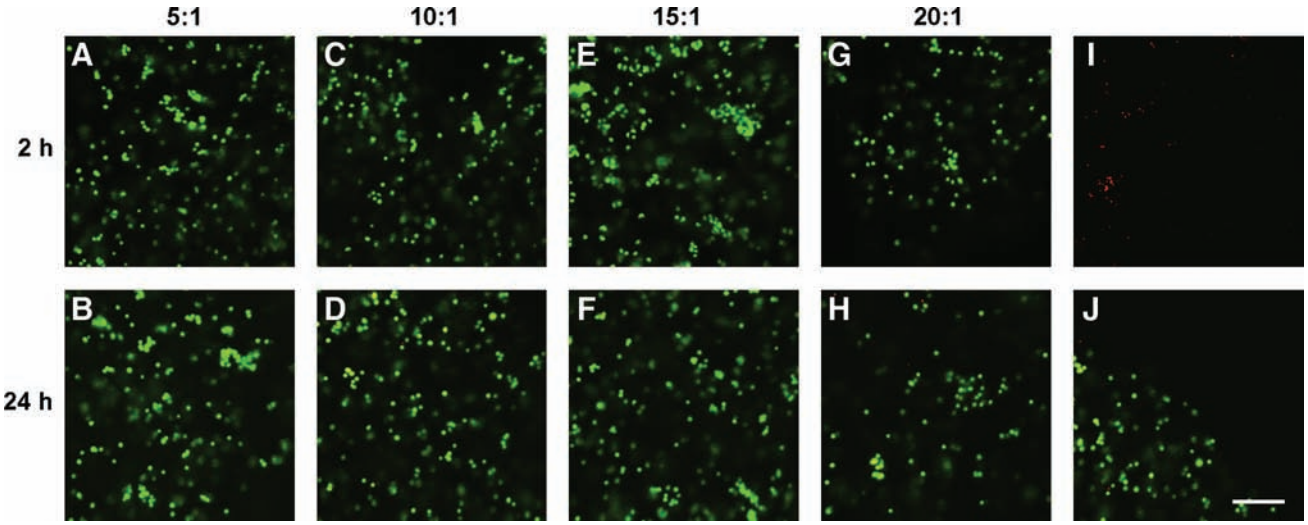

FIG. 4. Confocal fluorescence microscopy images of fibroblasts encapsulated in hydrogel beads at 2 h (**A, C, E, G**) and 24 h (**B, D, F, H**) with live/dead reagents. A green fluorescence designates live cells, whereas a red fluorescence indicates dead cells. Hydrogel beads were fabricated with four different crosslinking ratios: 1:5 (**A, B**), 1:10 (**C, D**), 1:15 (**E, F**), and 1:20 (**G, H**). A dead (negative) control (**I**) was utilized to confirm the microscopy settings. Image (**J**) shows the edge of a hydrogel bead. Scale bar represents 100 μm.

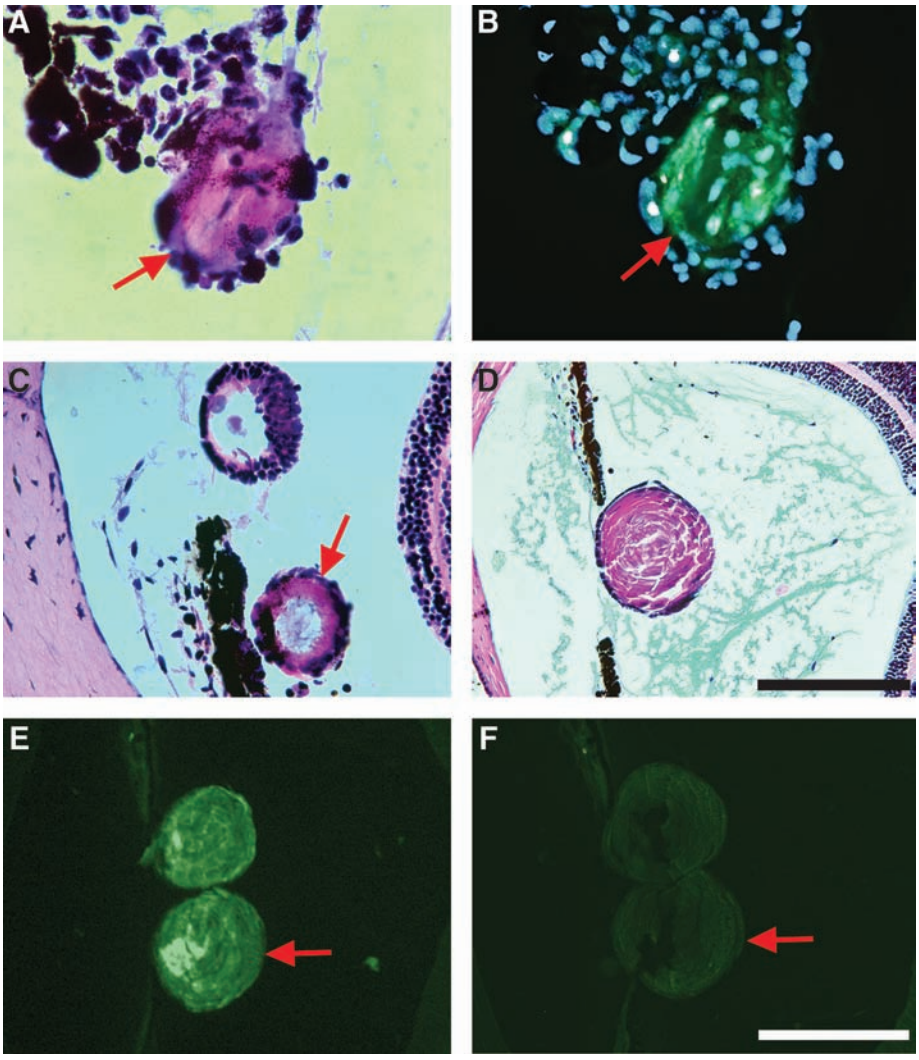

FIG. 5. Histological sections showing lens trans-differentiation of representative implants of hydrogel bead with or without iris pigment epithelial cells 30 days after lentectomy. Sections stained with hematoxylin and eosin (**A** and **C**) display regenerated lens after implantation of a hydrogel bead encapsulating dorsal iris. The arrow in (**A**) indicates the formation of characteristic lens fiber tissue. Immunostaining with an α-crystallin antibody (**B**) shows the presence of crystalline, confirming the trans-differentiation of encapsulated iris to lens. Encapsulated iris (**C**) had a spherical shape and was depigmented (indicated by an arrow in **C**), with a similar size and shape to the host regenerating lens (above). In another case (**E**), both host regenerated lens and induced lens (indicated with an arrow) show a positive staining of α-crystallin antibody, indicating trans-differentiation of cells from an encapsulated iris implant. (**F**) shows a negative control using a section from the same eye as in (**E**) and with the omission of the primary antibody. A section stained with hematoxylin and eosin (**D**) displays host lens regeneration from the dorsal iris after implantation of a hydrogel bead alone. Bar is 100 μm for (**A**) and (**B**), 200 μm for (**C**), 400 μm for (**D**), and 250 μm for (**E**) and (**F**).

the four crosslinking ratios of OPF hydrogels, and the results showed that the majority of fibroblasts remained viable following encapsulation in the hydrogel beads (Fig. 4). These results agree with previous findings since the macromer, crosslinker, and initiators have all been shown to be cytocompatible.[16,19] Moreover, there were no spacial effects within the 1 mm hydrogel beads. Cell viability at the hydrogel boundary was the same as in the interior (Fig. 4J). The hydrogels tested had a high degree of swelling, thus allowing for ample exchange of metabolites, ensuring high cell viability. Although the cell viability observed with the model fibroblast cells indicates that the majority of encapsulated cells remain viable, the results might not directly reflect the viability of encapsulated PECs. However, the trans-differentiation observed with encapsulated PECs *in vivo* indicates that a sufficient number of PECs remain viable in OPF hydrogels to effect lens regeneration.

Based on the results of the *in vitro* studies, OPF hydrogel beads with or without iris PECs were fabricated and transplanted in newts for evaluation of their effects on lens regeneration. A crosslinking ratio of 1:10 was chosen for this *in vivo* study, because hydrogel beads of this ratio degraded fully in 21 days *in vitro*; lens regeneration takes place in 3–4 weeks in newts.[5,6] OPF hydrogel beads alone were first implanted to examine scaffold degradation and its effect on the host lens regeneration. Additionally, hydrogel beads encapsulating iris PECs were implanted to further investigate the trans-differentiation of encapsulated PECs in the beads. The design was based on previous reports that dorsal iris implanted in a lentectomized eye has the ability to transdifferentiate to lens, and this process does not affect the host lens regeneration from the dorsal iris.[8,9]

For all three newts that received hydrogel beads alone, it was found that the hydrogels degraded after 30 days, and host lenses were regenerated, as shown in Figure 5D. Although the *in vitro* and *in vivo* environments were different, hydrogel beads showed a similar degradation rate, as would be expected for degradation arising mainly from nonenzymatic hydrolysis of ester linkages.[11,13] The results also indicate that the implantation of the hydrogel beads of crosslinking ratio of 1:10 did not interfere with lens regeneration from the iris.

The implantation of hydrogel beads encapsulating dorsal iris PECs further attested to the lack of interference of lens regeneration by the presence of the hydrogel or transplanted cells. In 10 out of the 11 cases involving encapsulated implants, the host lenses regenerated from the dorsal iris, as shown in the top of Figure 5C and E. Moreover, in 5 out of the 11 cases, encapsulated iris PECs were seen to transdifferentiate into lens, as evidenced by histology and immunohistochemisty (indicated with an arrow in Fig. 5A–C and E). Compared to a previous study, where 70% transdifferentiation was achieved when an unencapsulated dorsal iris was implanted in a similar model,[8,9] the present study showed trans-differentiation of the cells in nearly 50% of the cases. The difference could be because the encapsulation of the iris piece with a hydrogel enlarged the size of an implant, and the implant was therefore placed at the posterior area of an eye. Previous research has shown that the anterior area is more advantageous than the posterior area for transdifferentiation of implanted irises.[9]

The results from this study suggest the potential of OPF hydrogels together with iris PECs for engineering lens. This method may also be applied to mammals for lens regeneration. Ideally, through the implantation of hydrogels encapsulating cells and bioactive molecules that can induce trans-differentiation, a lens with proper functions may be regenerated.

Conclusions

This study investigated the encapsulation of newt iris PECs in a biodegradable hydrogel for transplantation and lens regeneration in a newt lens model. Hydrogel beads of 1 mm in diameter were fabricated from an OPF macromer crosslinked with PEG-DA. The effect of PEG-DA to OPF crosslinking ratio (1:5, 1:10, 1:15, and 1:20) on the swelling and degradation properties of hydrogel beads was assessed *in vitro.* The results demonstrated that OPF hydrogel beads of lower crosslinking ratios had greater swelling ratios and faster degradation. Cells encapsulated in the hydrogel beads retained viability, suggesting the cytocompatibility of the crosslinked hydrogels. The implantation of OPF hydrogel beads of optimized degradation rate into newt lentectomized eyes, either alone or encapsulating PECs, resulted in host lens regeneration. The results indicate that OPF hydrogels did not interfere with cell trans-differentiation and lens regeneration. The study suggests that OPF hydrogels of tailored swelling and degradation characteristics can be used as part of a novel strategy for lens regeneration in other animals.

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Disclosure Statement

No competing financial interests exist.

References

1. Sanchez Alvarado, A., and Tsonis, P.A. Bridging the regeneration gap: genetic insights from diverse animal models. Nat Rev Genet **7,** 873, 2006.
2. Tsonis, P.A. Limb Regeneration. Cambridge: Cambridge University Press, 1996.
3. Tsonis, P.A. Regeneration in vertebrates. Dev Biol **221,** 273, 2000.
4. Tsonis, P.A. How to build and rebuild a lens. J Anat **209,** 433, 2006.
5. Del Rio-Tsonis, K., and Tsonis, P.A. Eye regeneration at the molecular age. Dev Dyn **226,** 211, 2003.
6. Tsonis, P.A., and Del Rio-Tsonis, K. Lens and retina regeneration: transdifferentiation, stem cells and clinical applications. Exp Eye Res **78,** 161, 2004.
7. Tsonis, P.A., Jang, W., Del Rio-Tsonis, K., and Eguchi, G. A unique aged human retinal pigmented epithelial cell line useful for studying lens differentiation *in vitro.* Int J Dev Biol **45,** 753, 2001.
8. Grogg, M.W., Call, M.K., Okamoto, M., Vergara, M.N., Del Rio-Tsonis, K., and Tsonis, P.A. BMP inhibition-driven regulation of six-3 underlies induction of newt lens regeneration. Nature **438,** 858, 2005.

9. Hayashi, T., Yamagishi, A., Kuroiwa, A., Mizuno, N., Kondoh, H., and Okamoto, M. Highly efficient transfection system for functional gene analysis in adult amphibian lens regeneration. Dev Growth Differ **43,** 361, 2001.
10. Lee, K.Y., and Mooney, D.J. Hydrogels for tissue engineering. Chem Rev **101,** 1869, 2001.
11. Jo, S., Shin, H., Shung, A.K., Fisher, J.P., and Mikos, A.G. Synthesis and characterization of oligo(poly(ethylene glycol) fumarate) macromer. Macromolecules **34,** 2839, 2001.
12. Temenoff, J.S., Athanasiou, K.A., LeBaron, R.G., and Mikos, A.G. Effect of poly(ethylene glycol) molecular weight on tensile and swelling properties of oligo(poly(ethylene glycol) fumarate) hydrogels for cartilage tissue engineering. J Biomed Mater Res **59,** 429, 2002.
13. Holland, T.A., Tabata, Y., and Mikos, A.G. Dual growth factor delivery from degradable oligo(poly(ethylene glycol) fumarate) hydrogel scaffolds for cartilage tissue engineering. J Control Release **101,** 111, 2005.
14. Park, H., Temenoff, J.S., Holland, T.A., Tabata, Y., and Mikos, A.G. Delivery of TGF-beta1 and chondrocytes via injectable, biodegradable hydrogels for cartilage tissue engineering applications. Biomaterials **26,** 7095, 2005.
15. Park, H., Temenoff, J.S., Tabata, Y., Caplan, A.I., and Mikos, A.G. Injectable biodegradable hydrogel composites for rabbit marrow mesenchymal stem cell and growth factor delivery for cartilage tissue engineering. Biomaterials **28,** 3217, 2007.
16. Temenoff, J.S., Park, H., Jabbari, E., Conway, D.E., Sheffield, T.L., Ambrose, C.G., and Mikos, A.G. Thermally crosslinked oligo(poly(ethylene glycol) fumarate) hydrogels support osteogenic differentiation of encapsulated marrow stromal cells *in vitro*. Biomacromolecules **5,** 5, 2004.
17. Park, H., Guo, X., Temenoff, J.S., Tabata, Y., Caplan, A.I., Kasper, F.K., and Mikos, A.G. Effect of swelling ratio of injectable hydrogel composites on chondrogenic differentiation of encapsulated rabbit marrow mesenchymal stem cells *in vitro*. Biomacromolecules **10,** 541, 2009.
18. Temenoff, J.S., Park, H., Jabbari, E., Sheffield, T.L., LeBaron, R.G., Ambrose, C.G., and Mikos, A.G. *In vitro* osteogenic differentiation of marrow stromal cells encapsulated in biodegradable hydrogels. J Biomed Mater Res A **70,** 235, 2004.
19. Park, H., Temenoff, J.S., Tabata, Y., Caplan, A.I., Raphael, R.M., Jansen, J.A., and Mikos, A.G. Effect of dual growth factor delivery on chondrogenic differentiation of rabbit marrow mesenchymal stem cells encapsulated in injectable hydrogel composites. J Biomed Mater Res A **88,** 889, 2009.
20. Sawada, K., Agata, K., Yoshiki, A., and Eguchi, G. A set of anti-crystallin monoclonal antibodies for detecting lens specificities: beta-crystallin as a specific marker for detecting lentoidogenesis in cultures of chicken lens epithelial cells. Jpn J Ophthalmol **37,** 355, 1993.
21. Holland, T.A., Bodde, E.W., Baggett, L.S., Tabata, Y., Mikos, A.G., and Jansen, J.A. Osteochondral repair in the rabbit model utilizing bilayered, degradable oligo(poly(ethylene glycol) fumarate) hydrogel scaffolds. J Biomed Mater Res A **75,** 156, 2005.

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