Cell-Seeded Injectable Gelatin-Hydroxyphenylpropionic Acid Hydrogel for the Regeneration of Retina

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Statement of purpose: Age-related macular degeneration (AMD) and retinitis pigmentosa (RP), the leading causes of blindness, involve degenerating retinal tissue that includes retinal pigment epithelium (RPE) and photoreceptor damage and/or loss. Mammals, unlike amphibians, lack the capacity to repair/replace such tissue, necessitating cell therapy. Our supposition is that the delivery vehicle, in addition to the cell type, can play a critical role in retinal tissue regeneration. In this regard cell-seeded injectable hydrogels offer advantages over aqueous suspensions of cells[1] and pre-formed scaffolds seeded with cells which are not injectable. One such natural biopolymer conjugate commended for central nervous system applications, gelatin (Gtn)-hydroxyphenylpropionic acid (HPA), can be injected as a cell-bearing liquid and undergo covalent cross-linking *in vivo*. Covalent cross-linking enables greater control of gelation rate, physical properties, and degradation rate compared to other types of gel (*e.g*., collagen). In addition to supporting neural stem cell (NSC) viability, adhesion, proliferation, and differentiation to neurons, Gtn-HPA gels were also found to impart a high degree of oxidative stress resistance to NSCs,[2] which may be of particular importance because oxidative stress plays a role in the majority of retinal diseases including, AMD and RP. Hence with the ultimate goal of injecting a cell-seeded Gtn-HPA gel into the sub-retinal space for retinal regeneration, we investigated the Gtn-HPA hydrogel as a carrier and matrix for RPE cells. We evaluated the viability of RPE cells in Gtn-HPA gels, and the influence of the gel on adhesion and proliferation.

Methods: RPE cells were isolated from adult Sprague Dawley rat eyes as previously described.[3] Isolated RPE cells were cultured in F12 medium containing 10% FBS at a density of 10,000 cells/cm^2. Second passage cells were used for all experiments. The cell type isolated from the rat eyes was verified using the RPE-specific markers, RPE65 and CRALBP. Gtn-HPA hydrogels were prepared by sequentially mixing horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2).[2] The RPE cell response to the Gtn-HPA gel (2wt%, 0.1U/ml HRP and 1mM H_2O_2) was compared to the response to a hydrogel control (soluble rat tail type I collagen at 1mg/ml), and a 2D control (the tissue culture polystyrene surface).

Viability: RPE cell-seeded gels (1M/ml) cast at a thickness of 0.1mm, based on the thickness of sub-retinal space, underwent Live/Dead cell staining. The 2D control comprised 20,000 cells/cm^2.

Adhesion: Cells were seeded on top of the hydrogels and the control polystyrene surface at a density of 100,000 cells/cm^2 and incubated overnight. Samples were washed twice with PBS, and the cells harvested, using trypsin for polystyrene and 1000U/ml of collagenase I for gels, for DNA quantification using the PicoGreen assay.

Proliferation: Because cell proliferation may be important in the repair/regeneration process, it was quantified to ensure that the hydrogel did not impede cell division. Cells were seeded at a density of 10,000 cells/cm^2 in 2D and at 100,000 cells/ml in the gels, and cultured for 10 days. Samples were collected at days 0, 2, and 6 for determination of the cell number reflected in the DNA content using the PicoGreen assay.

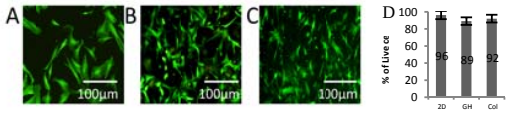
Results: Immunofluorescent staining of the isolated cells showed reactivity with RPE65 and CRALBP, thus confirming the appropriate cell type.

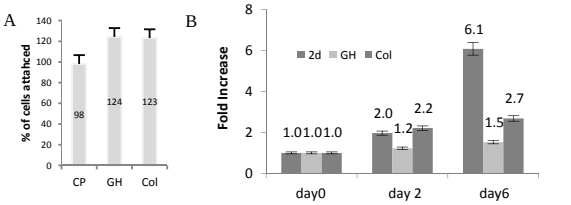
Viability: Viability of RPE cells within Gtn-HPA hydrogels remained at ~90% (Fig. 1), indicating that cross-linking of Gtn-HPA with the amounts of HRP and H_2O_2 used was highly cytocompatible.

Adhesion: Virtually all of the RPE cells were adherent to the Gtn-HPA gel as they were to the tissue culture polystyrene control surface (Fig. 2A), showing that the hydrogel provided a suitable substratum for cell adhesion.

Proliferation:. A 1.5-fold increase in cell number in the Gtn-HPA by day 6, while less than 2.7-fold increase in the collagen gel and 6.1-fold increase in 2D (Fig. 2B), indicated that Gtn-HPA is permissive of cell proliferation.

Conclusions: Covalently cross-linked Gtn-HPA hydrogel displays a favorable environment for RPE cell survival, adhesion, and proliferation. Considering the advantages of Gtn-HPA,[2] and the necessity for an injectable gel carrier to ensure uniform cell distribution in the sub-retinal space,[1] the injection of an RPE cell-seeded Gtn-HPA hydrogel into the sub-retinal space may result in an improvement in the regeneration of damaged/lost tissue. Our data compel further investigation *in vitro* and *in vivo*.





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References: (1) Ballios BG, Biomat 31;255:2010). (2) Lim TC, Biomat 2012;33:3446. (3) Maminishkis A, J Vis Exp 2010;45.