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Decellularized retinal matrix: Natural platforms for human retinal progenitor cell culture

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ABSTRACT

Tissue decellularization strategies have enabled engineering of scaffolds that preserve native extracellular matrix (ECM) structure and composition. In this study, we developed decellularized retina (decell-retina) thin films. We hypothesized that these films, mimicking the retina niche, would promote human retinal progenitor cell (hRPC) attachment, proliferation and differentiation. Retinas isolated from bovine eyes were decellularized using 1% w/v sodium dodecyl sulfate (SDS) and pepsin digested. The resulting decell-retina was biochemically assayed for composition and cast dried to develop thin films. Attachment, viability, morphology, proliferation and gene expression of hRPC cultured on the films were studied *in vitro*. Biochemical analyses of decell-retina compared to native retina indicated the bulk of DNA (94%) was removed, while the majority of sulfated GAGs (55%), collagen (83%), hyaluronic acid (87%), and key growth factors were retained. The decell-retina films supported hRPC attachment and growth, with cell number increasing 1.5-fold over a week. RT-PCR analysis revealed hRPC expression of rhodopsin, rod outer membrane, neural retina-specific leucine zipper neural and cone-rod homeobox gene on decell-retina films, indicating photoreceptor development. In conclusion, novel decell-retina films show promise as potential substrates for culture and/or transplantation of retinal progenitor cells to treat retinal degenerative disorders.

Statement of significance

In this study, we report the development of a novel biomaterial, based on decellularized retina (decell-retina) that mimics the retina niche and promotes human retinal progenitor cell (hRPC) attachment, proliferation and differentiation. We estimated, for the first time, the amounts of collagen I, GAGs and HA present in native retina, as well as the decell-retina. We demonstrated that retinas can be decellularized using ionic detergents and can be processed into mechanically stable thin films, which can act as substrates for culturing hRPCs. Rhodopsin, ROM1, NRL and CRX gene expression on the decell-retina films indicated photoreceptor development from RPCs. These results support the potential of decell-retina as a cell delivery platform to treat and manage retinal degenerative disease like AMD.

1. Introduction

Retinal degeneration and associated photoreceptor loss is the leading cause of blindness in the world. Approximately 25–30 million people worldwide suffer from age-related macular degeneration (AMD) alone [1]. Unlike lower vertebrates, which can regenerate retina, adult mammalian retina does not possess the ability to self-repair following injury or disease [2]. Current treatment strategies including gene therapy, anti-angiogenic therapy, and growth factor treatment focus mainly on slowing down the degeneration process [3]. Photoreceptor replacement holds great promise for recovery of vision. Recent reports indicate that retinal precursor cells implanted into the subretinal space of the eye can survive, differentiate into photoreceptors, integrate into retinal

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tissue and improve visual function [1,4–13]. The retina is a fairly easily accessible compartment of the central nervous system, and is considered immune privileged, making it a prime candidate for cell-based therapies and observation with relative ease [1,13]. A particularly promising approach has been the transplantation of retinal progenitor cells (RPCs) into the subretinal space [3,4,14]. Researchers have demonstrated that these cells survive, differentiate into photoreceptors, integrate into the retinal structure, promote host photoreceptor rescue, and improve visual function in a model of retinal degeneration [4,15].

Significant limitations to the success of cell-based retinal regeneration are cell loss and low integration within the host retina. Subretinal cell injection results in high (>90%) cell loss due to death and efflux of precursor cells during and post-transplantation [16]. Many cells that survive remain in the subretinal space rather than integrating within the retinal tissue in a manner appropriate for enhancing visual function. Implantation of RPCs on scaffolds decreased cell loss and enhanced differentiation into photoreceptors. Young and his co-workers demonstrated that delivery of RPCs on polymer scaffolds resulted in enhanced cell survival (up to 10-fold increase in survival following transplantation compared to cell suspension grafts) with associated increases in RPC integration [15–19]. Attachment on polymers (e.g., poly (L-lactic acid) (PLLA), poly (lactic-co-glycolic acid) (PLGA), polyglycerol sebacate (PGS), poly (methylmethacrylate) (PMMA), polycaprolactone (PCL)) has also been shown to enhance RPC differentiation [15,17,18]. However, current polymer-based cell delivery approaches still result in extremely limited overall integration (<2%).

Decellularized tissue is a promising scaffold for tissue regeneration that can present precise tissue architecture and chemistry [20]. Recently, decellularized extracellular matrix has been utilized as a scaffold material with considerable success for liver, cornea, adipose, nerve, dermal, musculofascial, cartilage, lung and cardiovascular tissue [20–36]. It is recognized that regenerative and developmental processes depend in an extremely complex fashion on structural as well as insoluble and soluble chemical cues. Acellular ECM offers the advantage of precise structural features and chemical cues that promote cell behaviors including attachment, migration, and signaling [37]. In addition, the clinical feasibility of this approach is supported by the conservation of ECM components among species and general toleration by xenogeneic recipients [34]. These facts motivate the exploration of a cell delivery vehicle derived from native retinal ECM to facilitate RPC survival and integration.

The purpose of our study was to develop decellularized retina (decell-retina) based substrates and observe their impact on hRPC. Decell-retina solutions were biochemically characterized and cast dried to develop thin films, and their surface topology was analyzed using scanning electron microscopy (SEM). hRPCs were seeded onto decell-retina films and cell responses (cell attachment, viability, morphology, proliferation and gene expression) on the developed matrices were studied as a first step in assessing this novel biomaterials' potential for enabling treatment for retinal degeneration disease like AMD.

2. Materials and methods

2.1. Retina isolation, decellularization and fabrication of substrates

2.1.1. Isolation of retina from bovine eyes

The retina was isolated from adult bovine eyes collected fresh from the abattoir (Research 87 Inc., Boston) within 1 h after slaughter. Briefly, the muscle surrounding the eye was removed and the eyeball was hemisected with fine scissors to remove the cornea and the vitreous humour. The hemisected eye-cup was flooded with phosphate buffer saline (PBS, Sigma–Aldrich) and the retina was peeled gently from the retinal pigment epithelium using a microspatula. The isolated retina was transferred into deionized (DI) water using a transfer pipette and shaken on an orbital shaker (TECHNE Mini Orbital Shaker, TSSM1) at 75 rpm for 3 min at room temperature. The retinas were collected using transfer pipet and processed further for decellularization (Fig. 1a).

2.1.2. Decellularization of the retina

Retinas isolated from bovine eyes were decellularized with 1% sodium dodecyl sulfate (SDS). In brief, 8 isolated retinas were transferred into a conical flask containing 120 ml of 1% SDS solution at room temperature. The flask was then placed on the orbital shaker at 120 rpm for 3 h. The retina was then harvested using a transfer pipet, minimizing the amount of transferred SDS, transferred into DI water and centrifuged at 25 °C at 10,000 rpm for 15 min. The retinas were harvested again using transfer pipette, resuspended in DI water and purified further using dialysis. The residual/bound SDS was removed by loading the retina suspension within dialysis tubing (MWCO 12,500, Sigma Aldrich) and dialyzing against DI water with several changes (8–10 times over a span of 48 h) to obtain the decell-retina suspension (Fig. 1b).

2.1.3. Lyophilization and digestion of retina

Decell-retina suspension was frozen in 50 ml falcon tubes in −80 °C freezer (Thermo Electron) overnight and then subjected to lyophilization using a freeze drier (Flexi-Dry MP, Kinetics thermal systems) with the temperature maintained within the sample chamber (−55 to −85 °C) (Fig. 1c). The decell-retina samples were dried for 48–72 h. The lyophilized decell-retina was snap-frozen in liquid nitrogen, and frozen pieces were pulverized using a mortar and pestle to obtain lyophilized powder. The lyophilized decell-retina powder was digested using Pepsin-HCl. In brief, 10–30 mg of the lyophilized decell-retina was digested with 1 mg of pepsin (Sigma–Aldrich) in 0.01 N HCl (Sigma–Aldrich) for 48 h at room temperature under constant stirring. The partially digested decell-retina was diluted with 10× PBS and 0.1 N NaOH [38].

2.1.4. Fabrication of decellularized retina (decell-retina) films

Decell-retina films were fabricated from the dialyzed and purified decell-retina. In brief, the isolated decell-retina solution was cast onto weighing boats/tissue culture plate surface (TCPS) at room temperature and left overnight for drying (Fig. 1c). The dried films were peeled from the weighing boats and sterilized using 70% ethanol/UV light for an hour. The decell-retina films on the TCPS were sterilized using UV light for an hour followed by treatment with 70% ethanol. The ethanol treated samples were rinsed three times with sterile PBS and then were used as substrates to study the hRPC response to the matrix.

2.2. Biochemical characterization

2.2.1. Sulfated glycosaminoglycan

Sulfated glycosaminoglycan concentrations of native and decell-retina were quantified via spectrophotometry with 1,9-dimethylmethylene blue chloride (Sigma–Aldrich) [39]. Pepsin digested samples were assayed and the concentrations were calculated based on a standard curve generated using chondroitin sulfate (Sigma–Aldrich). The absorbance at 492 nm was measured using a plate reader (Biotek, Powerwave XS, USA). The assay was performed in triplicate three times, and the concentrations were represented as μg/mg dry weight.

2.2.2. Hyaluronic acid

The hyaluronic acid (HA) contents of the pepsin digested native and decell-retina were measured using a hyaluronan enzyme-linked