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Research article

Nanofiber-based hydrogels with extracellular matrix-based synthetic peptides for the prevention of capsular opacification

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ABSTRACT

Nanofiber-based hydrogels (nanogels) with different, covalently bound peptides were used as an extracellular environment for lens epithelial cells (LECs) in order to modulate the capsular opacification (CO) response after lens surgery in a porcine eye model. Lenses were divided into 15 groups (n = 4 per group), the lens content was removed and the empty capsules were refilled with nanogel without peptides and nanogels with 13 combinations of 5 different peptides: two laminin-derived, two fibronectin-derived, and one collagen IV-derived peptide representing cell adhesion motifs. A control group of 4 lenses was refilled with hyaluronan. After refilling, lenses were extracted from the porcine eye and cultured for three weeks. LECs were assessed for morphology and alpha smooth muscle actin (αSMA) expression using confocal laser scanning microscopy. Compared to hyaluronan controls, lenses filled with nanogel had less CO formation, indicated by a lower αSMA expression (P = 0.004). Microscopy showed differences in morphological cell response within the nanogel refilled groups. αSMA expression in these groups was highest in lenses refilled with nanogel without peptides (9.54 ± 11.29%). Overall, LEC transformation is reduced by the presence of nanogels and the response is improved even further by incorporation of extracellular matrix peptides representing adhesion motifs. Thus, nanomaterials targeting biological pathways, in our case interactions with integrin signaling, are a promising avenue toward reduction of CO. Further research is needed to optimize nanogel-peptide combinations that fully prevent CO.

1. Introduction

Accommodative lens refilling (Koopmans et al., 2003, 2006, 2014) involves replacement of the lens with a silicone polymer in an otherwise intact lens capsule, enabling accommodation in presbyopic lenses. For the development of these injectable accommodating lenses, capsular opacification (CO) is a major problem, since these lenses require an intact and clear lens capsule (van Kooten et al., 2006). Nanogels, directing cell differentiation, could form a layer between the inserted lens refilling material and the surface of the capsular bag for the prevention of CO.

Capsular opacification is a common complication of cataract surgery and results in a diminished visual acuity as a consequence of proliferation, migration, and transdifferentiation of lens epithelial cells (LECs) in the visual axis (Marcantonio and Vrensen, 1999). This process of transdifferentiation of LECs to myofibroblasts is also known as epithelial to mesenchymal transformation (EMT) (de

Abbreviations: CO, capsular opacification; LECs, lens epithelial cells; EMT, epithelial to mesenchymal transformation; ECM, extracellular matrix; LMWG, low molecular weight hydrogelator; αSMA, alpha smooth muscle actin; PBS, phosphate-buffered saline; PBSA, phosphate-buffered saline containing 1% bovine serum albumin; BSA, bovine serum albumin; FBS, fetal bovine serum; ILK, integrin linked kinase.

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Iongh et al., 2005). TGF-β signaling pathways play important roles in EMT and CO development, but over the past decades many other signaling molecules have been found to influence LEC behavior (Nibourg et al., 2015b; Wormstone et al., 2009). Knowledge of underlying biological processes of the development of CO has changed the focus in research aimed at CO prevention. Nowadays this focus is more on agents that can interfere with these cellular processes. Rapid advances in material sciences also provide novel opportunities in research for CO prevention. Nanotechnology has recently been used in various ocular applications, including studies on CO prevention (Guha et al., 2013; Huang et al., 2013; Wang et al., 2013; Zhang et al., 2013).

In this study we developed an organ-culture model using porcine eye lenses in which, after surgical removal of the natural lens fibers, nanofiber-based hydrogels (nanogels) with attached signaling peptides were injected that by interacting with LECs should prevent EMT and therewith the formation of CO. The peptides were chosen according to their potential to influence integrin signaling pathways in LECs and were derived from the extracellular matrix (ECM) components laminin (IKVAV and YIGSR), fibronectin (RGDS and PHSRN), and collagen IV (DGEA) (Linnola et al., 2000; Olivero and Furcht, 1993). Binding of cells to components of the ECM is mediated by integrin receptors in the cell membrane. Integrin signaling is a cellular process involved in the development of CO (Mamuya et al., 2014; Walker and Menko, 2009). Most members of the integrin family have been identified in LECs (McLean et al., 2005; Worthington et al., 2011; Zhang et al., 2000). Integrins can bind a large variety of ligands on ECM components and other molecules involved in cellular signaling (Walker and Menko, 2009; Wederell and de Iongh, 2006). After cataract surgery, the extracellular environment of LECs is changed. Changes in integrin-ECM interactions are associated with transdifferentiation of LECs, and integrins were found to be activators for TGF-β, which is present in the latent form in the ECM (Dawes et al., 2007; Mamuya et al., 2014; Worthington et al., 2011). These processes result in the development of CO (Mamuya et al., 2014; Walker and Menko, 2009).

For the current study we injected the whole capsule with nanogel until complete filling, since we aimed at monitoring interactions between LECs and nanogel to explore the possibilities for modulation of the capsular opacification response after lens surgery.

2. Material and methods

2.1. Experimental setup

Fresh natural porcine (*Sus domesticus*) eyes were obtained from the local slaughterhouse. All eyes were from pigs with an age of approximately six months. The lens content was removed, and the lenses were refilled with different nanogel materials or with a hyaluronan control (sodium hyaluronate, Healon 10 mg/ml; Abbot Medical Optics, Uppsala, Sweden), according to the surgical refilling procedure described below. All refilling experiments were performed at n = 4, except for the experiments with the YIGSR-nanogel (n = 3) due to material shortage at the moment of the surgery. After refilling of the lens capsule, the lenses were extracted and cultured for three weeks.

2.2. Nanofiber-based hydrogel assembly and preparation

The nanofiber-based hydrogel consists of fibers of a low molecular weight hydrogelator (LMWG) (Brizard et al., 2008; Cui et al., 2010). In Fig. 1 the structure of LMWG as well as a schematic representation of nanogel formation by self-assembly are shown. The synthesis of LMWG has previously been described by van Bommel et al. (2005). Using this process LMWG was synthesized in-house and characterized by ^{1}H NMR and HPLC-MS (+95% purity). LMWG was functionalized with a maleimide moiety using N-succinimidyl 3-maleimidopropionate (TCI, +95%) and subsequently reacted with cysteine-containing peptides (custom made at Think Peptides, >95% purity) through a process described in detail by Tuin et al. (2015; manuscript in preparation) to obtain the building blocks (characterized by ^{1}H NMR and HPLC-MS) for the different nanogel-peptide composites (next subsection). Self-assembly of LMWG and LMWG-nanopeptides into fibers and subsequent gel formation was achieved by dissolution of the appropriate mixture of LMWG and peptide derivatives (Nano Fiber Matrices B.V., Groningen, The Netherlands) in a mixture of hydrochloric acid (0.18 M HCl, prepared from 1.0 M HCl (J.T. Baker, analytical grade) diluted with distilled water (Boom)) and saline solution (NaCl (ESCO), dissolved in distilled water (Boom); solutions were sterilized prior to use by filtration through a 0.45 μm filter (20 mm Whatman)), followed by mixing by vortex of the LMWG solution with a hyaluronan containing buffer. Subsequently, the gel was quickly (within 10–30 s) injected into the empty lens capsule.

2.3. Nanogel-peptide combinations

Table 1 provides an overview of the different nanogel-peptide combinations and formulations. The peptide combinations are based on their presence within CO and their origin in the ECM (Walker and Menko, 2009; Wederell and de Iongh, 2006). IKVAV (isoleucine-lysine-valine-alanine-valine) and YIGSR (tyrosine-isoleucine-glycine-serine-arginine) are both laminin-derived peptides, RGDS (arginine-glycine-aspartic acid-serine) and PHSRN (proline-histidine-serine-arginine-asparagine) are fibronectin-derived, and DGEA (aspartic acid-glycine-glutamic acid-alanine) is a collagen IV-derived peptide.

Most nanogels consisted of a gel with mixed-in peptides at 0.5 mol percent (mol%) each. One group consisted of five peptides of which two peptides (DGEA and PHSRN) were mixed-in at 0.2 mol %, one peptide (RGDS) at 0.4 mol%, one peptide (YIGSR) at 0.5 mol%, and one peptide (IKVAV) at 0.6 mol%. The ratio's of peptides in this latter mix are based on the composition of the basement membrane (Hughes et al., 2010; Kleinman and Martin, 2005).

2.4. Surgical refilling procedure

The surgical lens refilling procedure is based on a method previously described by Koopmans et al. (2003, 2006, 2014) and Nibourg et al. (2015a). In short, the lens content was aspirated through a capsulorhexis of 1.0–1.5 mm diameter. Intentionally no efforts were made to remove the lens epithelial cell layer. Then, a 3.0 mm diameter capsular plug was inserted to close the capsular bag, and the nanogel was injected until the capsular bag was completely filled and it started to leak around the plug.

2.5. Culturing method

The lenses were extracted from the eye and were placed a in culture medium consisting of minimal essential medium (MEM) supplemented with 12% fetal bovine serum (FBS), 2 mM GlutaMAX™-I, 1 mM sodium pyruvate, and 500 Units/ml penicillin – 500 μg/ml streptomycin – 1.25 μg/ml amphotericin B (all Life Technologies Ltd, Paisley, UK). The lenses were cultured for three weeks in a 5% CO_2 incubator at 37 °C. The medium was changed twice a week.