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The nature and consequence of vitronectin interaction in the non-compromised contact lens wearing eye

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ABSTRACT

Purpose: The aim of this work was to investigate the locus and extent of vitronectin (Vn) deposition on *ex vivo* contact lenses and to determine the influence of wear modality together with surface and bulk characteristics of the lens material.
Methods: The quantity and location of Vn deposition on the surfaces of contact lens materials was investigated using a novel on-lens cell attachment assay technique.
Results: Vn mapping showed that deposition resulted from lens-corneal interaction rather than solely from the tear film. Higher cell counts on the posterior surface of the lenses were determined in comparison to the anterior surface. Overall gross Vn deposition was greater for high water content-low modulus materials (117 ± 4 average cell count per field) than low water content-high modulus materials (88 ± 6 average cell count per field).
Conclusions: The role of Vn in plasmin regulation and upregulation is widely recognised. The findings in this paper suggest that the locus of Vn on the contact lens surface, which is affected by material properties such as modulus, is potentially an important factor in the generation of plasmin in the posterior tear film. Consequently, the potential for materials to affect Vn deposition will influence lens-induced inflammatory processes.

1. Introduction

There is a growing interest in biomarkers related to inflammation and immunoregulation of the ocular environment in contact lens wear. One interesting candidate biomarker is vitronectin (Vn), a prominent inflammatory regulatory glycoprotein and adhesion molecule, which has been detected in tears [1,2]. Vn is a relatively small glycoprotein of 459 amino acid residues [3] and is commonly detected in two forms; a single 75 kDa polypeptide chain and a 65 kDa subunit linked to a 10 kDa fragment by a disulphide bond [4]. This glycoprotein is involved in many physiological processes, a number of which are summarised in Table 1. Its functions are dependent on its binding to various matrix and cellular components, which in turns stabilises or activates a variety of biological macromolecules.

One of the primary functions of Vn is to regulate the spreading and attachment of a wide variety and range of cells [32,33]. Both Vn has an Arg–Gly–Asp (RGD) cell binding sequence that enables the individual proteins to bind integrins. The integrin family of heterodimeric proteins, which directly regulates cellular differentiation, proliferation, and migration - can influence processes such as wound healing, inflammation, and cancer [34]. Vn also inhibits complement activity [5,35]. The concentration of Vn in tears has been shown to be dependent on the tear state; the levels in reflex, open-eye, and closed-eye tear samples have been reported to be 0.08 ± 0.3 μg/ml, 0.75 ± 0.32 μg/ml and 3.7 ± 2.2 μg/ml respectively [1,2]. In terms of contact lens wear bandage lenses doped with recombinant human Vn have been shown to be effective in the enhancement of corneal epithelial wound healing on excised donor human corneas [36,37].

Initially it was suggested that the majority of Vn came from conjunctival blood vessels [2], but Vn has since been found within the basement membrane of the corneal epithelium, which may indicate a possible endogenous source [38]. It has also been proposed that the overall elevation of protein levels during eye closure is due to an increase in vascular permeability in combination with the accumulation of leakage products resulting from a reduced tear turnover [1,39]. The effect of vascular leakage on the ocular surface and the potential consequences of the altered overnight ocular environment are at present poorly understood. Although the parallel influx of certain components, including plasmin and complement proteins [40], are likely to be related, the

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Table 1
A summary of the diverse functions of vitronectin.

Process	Description	Consequences
Complement regulation [5–7]	Inhibition of MAC (C5b-9)	Protection of bystander cells
T-cell cytolysis [8,9]	Inhibition of cell lysis by perforin	Protection of bystander cells
Cellular adhesion [10–14]	Integrin binding	Migration, attachment and aids healing
Thrombosis [15–17]	Binds thrombin-antithrombin III	Inhibits thrombin inactivation, regulating blood coagulation
Fibrinolysis [18–21]	Stabilizes PAI-1	Anti-proteolytic activity
Inflammation [22]	Binds β-endorphin	Pain suppressor
Binds structural macromolecules [23–26]	Heparin, collagen, heparin sulphate	For activation or adhesion to surfaces. Healing?
Growth factor interaction [27–29]	Vn-GF complexes	Promote wounding healing and cell regulation
Anti-bacterial [30,31]	Cell-bacteria mediated interaction	Enhanced intracellular killing of surface bound bacteria

anti-inflammatory properties of Vn, such as the inhibition of complement lysis and plasmin-mediated inflammation, may be extremely important in controlling or co-controlling the closed eye environment.

The aim of this work was to investigate the locus and extent of Vn deposition on ex vivo contact lenses and to determine the influence of wear modality together with surface and bulk characteristics of the lens material. To detect the adsorption of Vn onto the contact lens surface, a probe to visualise and trace its presence was required. This was achieved by using fibroblast cells as a probe, taking advantage of the adhesive nature and the cell binding domain of Vn. The importance of vitronectin (and fibronectin) in the contraction process in vitro corneal wound healing studies with fibroblasts (and myofibroblasts) has been demonstrated and highlights its influence in cell interaction and movement in the ocular environment [41,42]. The value of a cell-based assay for the study of Vn in the post-lens ocular environment is demonstrated. Such studies provide a basis for understanding and potentially modulating the upregulation of plasmin in the post-lens tear film.

2. Materials and methods

2.1. Reagents and immunochemicals

Rabbit IgG (polyclonal); rabbit anti-human fibronectin (polyclonal); human vitronectin; human fibronectin–all Sigma Aldrich, Gillingham, UK. Rabbit anti-human vitronectin (polyclonal) (Gibco, BRL). All other reagents were obtained from Sigma Aldrich, Gillingham, UK, unless otherwise stated.

2.2. Cell line

Mouse 3T3 Swiss Albino embryo fibroblasts cells were purchased from European Collection of Cell Cultures (ECACC; Salisbury, UK) and grown in Dulbecco's Modified Eagles Medium–high glucose (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% 200 mM L-glutamine solution. Cells were incubated at 37 °C in 5% CO_2, and maintained for up to 10 passages. 1 ml of resuspended cells was added to 19 ml HBS prior to each assay at a concentration of 5×10^4 cell/ml. Cell viability was determined by the standard trypan blue dye exclusion test.

2.3. On-lens cell attachment assay

All solutions and analytes were pre-heated to 37 °C. A magnesium chloride stock solution ($MgCl_2$, 50 mM), a rabbit IgG solution (300 μg/ml in HBS) and a 1% solution of glutaraldehyde in HBS were prepared. The cell assay involves the selective inhibition of the adhesion molecules, using antibody blocking techniques, to validate the significance of Vn on the lens surface. An anti-Vn antibody control diluted to 1:100 in HBS was incorporated into the assay to block the action of Vn and used as a comparison against the Vn standard control wells to assess the adhesion of Vn onto the lens. The use of an IgG antibody, at 300 μg/ml, as a control was to negate the action of an arbitrary antibody in the system. The lenses, removed from either doping solution (control lenses and unworn lenses) or saline (worn lenses), were placed individually into the appropriate test wells of a 24-well plate. Each well was rinsed with 1 ml HBS (x3). 1 ml HBS was added to the Vn positive control well, 1 ml of the prepared IgG solution was added to the antibody control well and 100 μl anti-human Vn diluted with 900 μl HBS was added to the anti-Vn control well. Each 24-well plate was incubated for 60 min, at 37 °C and 5% CO_2, and gently agitated every 10 min.

After incubation, the solution from each lens was aspirated by means of a pipette, with care taken not to touch the lens. 1 ml of the cell solution in HBS at a final cell count of 5×10^4 cells/ml was added to each well, in addition to 100 μl of $MgCl_2$ stock. The 24-well plate was then incubated for a further 60 min at 37 °C and 5% CO_2. Following this second incubation period, the cell solution from each well was removed and each well and lens was rinsed with 1 ml HBS three times. All lenses were moved to a new 24-well plate and rinsed once with 1 ml HBS. The cells were fixed in 1 ml of 1% glutaraldehyde stock solution ready for counting.

2.4. Cell counts

To count the surface-located cells, each *ex vivo* lens was notionally divided into two areas; the edge and the centre. The field of vision in which each individual count was taken was defined by an internal graticule in the eye piece of the microscope. The eye piece graticule measures 1 cm × 1 cm which, when read under a ×10 magnification, allowed a field of vision of 1 mm^2. An average of the four counts at the edge and single count in centre zone are presented.

2.5. In vitro control experiments

For the on-lens cell attachment assay, lenses ($n = 3$) were doped with Vn to illustrate that it adsorbs out of solution on to the contact lens surface. The assay relies on the basis that Vn adheres to contact lens surfaces and that fibroblast cells with integrin receptors for Vn adhere to the contact lens using Vn as the binding ligand. An initial experiment was performed to prove that Vn adsorbed out of solution onto the contact lens surface and that it could be detected using the fibroblast cell assay. Four Group I unworn polyHEMA lenses were doped for 24 h with a 20 μg/ml solution of Vn. A duplicate set of unworn, non-doped polyHEMA lenses were assayed as a control. The choice of a Group I set of lenses in this assay was used to take the assay to the limits. Group I lenses are known to display lower levels of spoilation over various wear regimes compared to Groups II and IV and thus could be classed as the lowest threshold for all lenses to be analysed. For *in vitro* assays, human Vn at various concentrations diluted in PBS was used as the positive Vn control (Vn positive control). Prior to *in vitro* doping assays, the appropriate lenses were incubated in

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