Title: The Nature and Consequence of Vitronectin Interaction in the Non-Compromised Contact Lens Wearing Eye

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.

Keywords: Biomarkers; tears; deposition; fibroblast cells; post-lens tear film.

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Conflicts of interest: None

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 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 $5x10^4$ 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 minutes, at 37°C and 5% CO_2, and gently agitated every 10 minutes.

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 $5x10^4$ 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 minutes 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 x 1 cm which, when read under a x10 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 hours 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 specific μg/ml concentration of Vn per assay requirements. The on-lens cell attachment assay was then performed.

2.6. The effect of concentration, lens water content and lens ionicity on Vn adsorption

To study the effect of concentration polyHEMA contact lenses (n=4) were used in order to keep the lens type consistent with the initial experiment and to reduce experiment parameter variability. The four concentrations of Vn used to dope the lenses were: 1 μg/ml, 5 μg/ml, 10 μg/ml and 20 μg/ml. Control non-doped lenses (n=4) were also analysed. No antibody controls were required. Two separate assays were designed to assess the influence of the lens on the adsorption of Vn to its surface. Firstly, a water content assay was performed. The lenses used were all Vistagel non-ionic lenses (n=4) with water contents of 38%, 42%, 60%, and 75%. HEMA or HEMA/NVP lenses were chosen to evaluate the effect of lens water content on cell adhesion because the material is available in a range of water contents. Secondly, an ionicity assay using non-ionic Group II (vasurfilcon A, n=2) versus ionic Group IV (etafilcon A, n=2) lenses (both are high water content lenses) was performed. All of the lenses were doped with Vn at a concentration of 10 μg/ml for 24 hours. Only the Vn positive control and antibody control lenses were tested.

2.7. Ex vivo contact lenses assays

A range of lenses (Table 2) was worn by asymptomatic subjects in either a daily wear modality, averaging 196 hours over 2 weeks, or an extended wear modality, averaging 168 hours over 1 week, as specified with each assay. Upon removal, lenses were placed in PBS and stored at 4°C. A total of over 100 lenses was assayed in this study. Cell adhesion on the anterior and posterior surface of Group IV etafilcon A lenses was analysed. Lenses from 8 subjects were worn on a daily wear basis (n=8) and on an extended wear (n=8) basis. Centre versus edge analysis was analysed on Group II (vasurfilcon A, n=8) daily wear and Group IV (etafilcon A, n=8) daily wear lenses. The study was extended to include one conventional hydrogel lens material (etafilcon A) and three silicone hydrogel lens materials (lotrafilcon A, lotrafilcon B and balafilcon A) to assess material variation and lens modulus. Each lens type (n=4) was worn by the same asymptomatic subject on a daily wear basis and stored in saline solution after removal. ReNu® multipurpose solution (B&L) was used to clean the lenses where necessary. Work done previously in our laboratories found that levels of adsorbed Vn were only marginally reduced (<5%) by treatment with this care solution. This was also found to be the case with *ex vivo* lenses. The study and its protocol were approved by an Institutional Review Board in accordance with the tenets of the Declaration of Helsinki.

2.8. Assessment of the contributory role of fibronectin

Fn is an important adhesion protein which, in addition to Vn, has been reported in tears [43]. Both of these cell attachment-promoting proteins are immunologically unrelated and biochemically different but, due to the fact that their cell adhesive properties are similar, it was inadvisable to assume that Vn was solely responsible for both adsorption onto the contact lens and the cell-mediated adhesion. From a doubling dilution range of standards, a concentration of 12.5 μg/ml of Fn was selected - taking into account the requirement for a satisfactory cell count and cost effectiveness. This concentration is similar to those levels used for the Vn doping experiments, and slightly higher than those levels of Fn measured in tears (~ 4 μg/ml [44]) allowing for a marginal increase in tear levels. The designated, control, anti-Vn and anti-Fn, Group IV etafilcon A lenses were doped in 12.5 μg/ml of Fn for 24 hours. For all initial validation assays, an anti-Fn antibody at 1:100 in HBS, was also used as a direct comparison against the anti-Vn antibody and Vn standard controls. Again, this was to either eliminate or accept the role of Fn as a competitive adhesion molecule in contact lens wear and/or to further validate the dominant adhesion of Vn onto the contact lens.

3. Results

3.1. In vitro method validation

The average cell counts on doped lenses were greatest for the Vn positive control lenses (59±3) and for the antibody control lenses (52±3). The lenses blocked with anti-Vn demonstrated expected low cell counts (4±2) (Fig. 1). A set of non-doped blank lenses (n=3) was also assayed where an average background level of <5 cells was observed on all lenses; a similar cell count to the lenses treated with anti-Vn. The comparison of cell counts between the blank lenses and doped lenses shows a 20-fold increase in the average cell count per field. These results verify Vn dependant adhesion of cell fibroblasts to lenses and demonstrate the use of a cell-based assay to detect Vn. Fn demonstrated poor adsorption out of doping solution onto the contact lens, displaying low cell counts (10±2) in comparison to Vn-mediated adhesion (59±3). Anti-Fn antibodies which were also incorporated into the assay to negate the role of this adhesive glycoprotein in the attachment of the cells to the contact lens demonstrated no inhibitory influence. Thus, it could be concluded that Fn is clearly not involved in cell adhesion with these cell types and therefore does not adversely interfere with this Vn dominated cell-based assay.

3.2. The effect of Vn doping concentration, lens water content and ionicity on Vn adsorption

The higher the concentration of Vn in the doping solution the higher the cell counts. Increasing the Vn concentration from 1 μg/ml to 20 μg/ml gave an increase in the average cell count per field from 5±0.8 to 37±2 (Fig. 2). All subsequent lenses doped with Vn were doped with 10 μg/ml as this was found to be the optimum concentration. The effect of lens water content on cell counts showed the higher the water content of the lens, the higher the Vn-mediated cell adhesion. Average cell count per field for lenses with water contents of 38% and 75% increased from 42±0.8 to 58±1 respectively (Fig. 3). The results illustrate the fact that water content does not have a substantial effect on these non-ionic lenses, but there is a progressive increase in adsorption with water content, with an assumed tendency to reach a plateau of adsorption. Anti-Vn antibody controls were used to validate the cell-Vn adhesion and to negate cell-lens interactions. Greater cell counts on high water content ionic Group IV lenses (73±6) were observed in comparison to high water content non-ionic Group II lenses (53±9) (Fig. 4). High water content ionic lenses were found to adsorb the greatest protein, in contrast to low water

content non-ionic lenses. This may suggest that the ionicity of the lens is more important than the water content with regard to Vn adsorption.

3.3. Vn deposition on anterior and posterior surfaces of ex vivo lenses in daily wear and extended wear modalities

Higher cell counts on the posterior surface of the lenses were determined in comparison to the anterior surface, irrelevant of the modality of wear, as average cell counts per field increased by 95% from the anterior to the posterior surface in both wear modalities (Fig. 5). All further test lenses, therefore, analysed the posterior lens surface only. Greater cell counts were also detected on extended wear lenses than daily wear lenses with an average increase in cell count of 46% for both the posterior and anterior surfaces of the lenses.

3.4. Vn deposition on ex vivo daily wear lenses, center versus edge

It was noted that there was a difference in locus of Vn deposition on the posterior lens surface. Therefore, a comparison of Vn adsorption at the centre and edge of the lenses was performed. Greater Vn deposition was observed at the lens edge compared to the centre, irrespective of the lens material type. Average increases in cell counts from centre to edge (on the posterior lens surface) of 68% and 65% were observed for Group II lenses and Group IV lenses respectively **(**Fig. 6). The results revealed higher cell counts on the Group IV ionic material in comparison to the Group II non-ionic material, both for the lens centre with an average cell count increase of 30% and the lens edge with an average increase of 25%.

3.5. Material variation and the effect of lens modulus

The highest levels of Vn deposition was determined on the etafilcon A material (117±4), with lotrafilcon B, balafilcon A and lotrafilcon A showing decreasing cell counts at the lens edge (115±4, 107±5, 88±6 respectively) (Fig. 7). The relationship between vitronectin-mediated cell count and lens modulusin terms of water content is summarised in Table 3. The lower the modulus, the greater the deposition at the lens edge. Lotrafilcon A, a low water content high modulus material has a lower edge:centre ratio of 1.3:1, whereas etafilcon A, a high water content low modulus material, has a higher edge:centre ratio of 3.3:1. A comparison of two similar lenses revealed that lotrafilcon B, with a lower modulus of 1.0 MPa, had a much greater

edge:centre ratio in comparison to lotrafilcon A, with a higher modulus of 1.5 MPa. Lotrafilcon B, the softer material, allows more drape of the lens over the cornea, thus lens-tissue interaction is emphasised at the lens edge.

4. Discussion

The adhesive nature of Vn and its role in fibroblast cell attachment and movement was exploited to demonstrate the interaction of this glycoprotein with contact lens surfaces and the influence of the contact lens on Vn deposition out of tears and/or the corneal tissue bed was established. *In vitro* doping of contact lenses with Vn at varying concentrations showed a direct correlation between an increase in Vn concentration and its adsorption onto the contact lens. Although a plateau was not reached within the concentration levels studied, it can be expected that a limiting plateau would ultimately be achieved after a gradual increase. However, as previously stated, Vn concentrations in the eye have been reported at 0.75±0.32 μg/ml in the open eye [1]. It is therefore extremely unlikely for Vn concentrations to exceed the *in vitro* experimental limits of 20 μg/ml, in tears. These results demonstrate that cell adhesion is dependent upon Vn concentration in solution. However, concentration curves cannot be used to calculate Vn concentration against cell counts as each assay must be treated separately. It was also observed that the percentage water content of materials directly affected Vn adsorption; an increase in water content clearly exhibited a rise in the adsorbed levels of Vn. In addition, the ionicity of the lens also greatly influenced the adsorption of Vn with greater accumulation of Vn on the ionic (Group IV) versus the non-ionic (Group II) lenses.

Deposition of Vn was significantly greater on *ex vivo* lenses in comparison with lenses doped with Vn *in vitro*. Centre versus the edge of the lens analysis revealed interesting differences in Vn levels. There was a marked increase in Vn accumulation at the edge of the lens in contrast to the centre of the lens. This phenomenon was evident in both the daily wear and extended wear regimes. The fact that this preferential adsorption was evident for both wear modalities is very significant. As the name suggests, daily wear lenses are inserted and removed on a daily basis. The effect of daily handling of the lens, varying insertion and removal parameters and cleaning would be expected to change the characteristics of the deposition of Vn. However, this was not the case and the deposition profiles resembled that of the extended wear modality. The use of the multipurpose solution had little effect on Vn levels. Both wear modalities

appeared to create a Vn rich micro-climate at edge of the lens, which may be the site of localised inflammatory mediation.

Vn deposition was predominantly found to occur on the posterior surface of the lens in accordance with those results established in a preliminary study [45] where significantly greater levels of Vn were observed on the back surface of a worn lens in comparison with the front surface irrespective of wear modality or lens material properties. The accumulation at the posterior lens surface may reflect Vn moving out of the corneal tissue bed due to lens-tissue interactions, as opposed to simply reflecting tear derived Vn. Aggregation of Vn on the posterior surface is thought to create a particular micro climate, which due to its multifunctional nature and ability to bind to numerous ligands and proteins, could lead to the initiation of, or role in, a number of effector systems. A sliding motion of the eyelid over the lens surface will bring about fricto-mechanical stimulation [46]. This movement might be expected to stimulate a biochemical response with the potential to generate an upregulation of particular components or to activate additional pathways in response to the physical trauma of the eyelid-lens interaction – modest though that might be. Diminished tear flow at the posterior surface of the lens, in addition to a reduction in the dynamic interaction with the tissue bed, would be expected to produce a quite different level of response with the potential adsorption of specific biochemical components from the more 'closed' environment. The importance of anterior versus posterior lens differential in terms of biochemical interactions and physical parameters cannot be under estimated and its significance has been reviewed elsewhere [47, 48].

Greater Vn deposition was also detected on extended wear lenses compared with daily wear lenses. Taking the total wear time and lens-tissue bed contact into consideration, the cell attachment was always greater for the extended wear lenses than the daily wear lenses. The cell attachment observed at the *in vitro* concentrations equivalent to those found in tears are much lower than those found on posterior surfaces of worn lenses. This may be explained by the interaction between the ocular tissue and contact lens, with the posterior microclimate favouring Vn attachment. The investigation was extended to include Group II and Group IV material types and a comparison of Vn levels for the central and peripheral regions of the lenses was carried out on the posterior surface of the lenses. Greater Vn deposition on Group II materials in contrast with Group IV materials demonstrated that cell attachment is markedly dependent upon material type, highlighting the potential for materials effects in influencing Vn deposition and the nature of the post-lens microclimate. Again, an evaluation of the centre

versus the edge of the lens revealed that a greater Vn concentration resulted on the edge. The locus of Vn deposition is significant as this may influence the locus of plasmin regulation. Localised plasmin at the edge has the potential to escape into the tear film, whereas plasmin regulated at the centre of the lens may become 'trapped' in that region.

The edge:centre cell count ratio was clearly material dependent and it appears that the influence of modulus on cell adhesion overrides any effect of lens water content and ionicity one. The modulus of a lens describes its resistance to deformation, where modulus is equal to stress/strain. In general, it was observed that the lower the modulus, the greater the deposition of Vn at the lens edge and a low water content high modulus material, such as lotrafilcon A, has a lower edge:centre ratio compared with a high water content low modulus material, such as etafilcon A, which has a higher edge:centre ratio. Although the morphology of silicone hydrogel lenses complicates matters further, the lens modulus is a significant if not dominant factor.

The emerging patterns are clear - the presence of a contact lens concentrates Vn near the ocular tissue bed, and thus the preferential adsorption of Vn, particularly towards the edge, on to the lens surface reveals a Vn rich post-lens micro climate that can influence localised inflammation and upregulation of plasmin. Vn is involved in the regulation of fibrinolysis, a consequence of the fact that it binds and stabilises plasminogen activator inhibitor-1 (PAI-1), which thereby allows PAI-1 to inhibit the action of tissue plasminogen activator (tPA) and the fibrinolysis process as a whole [20]. If Vn is localised on a surface adjacent to the cellular site, the somatomedin B homology region of the protein binds to the active site of PAI-1, thereby generating plasminogen activator and resulting in the upregulation of plasmin [49]. Tear plasmin activity has been observed in tear fluid of subjects with corneal disorders, for example an increase in tear plasmin in particular in corneal ulcers has been demonstrated [50], leading to the implication that elevated plasmin levels are important in the pathogenesis of ocular infection and dysfunction. The view is that it is an important potential trigger of additional events leading, in extreme cases, to pathology in an otherwise healthy tissue. Plasmin, for example, can cleave Fn (an important adhesion molecule involved in corneal re-epithelisation), which is significant as Fn degradation is associated with impaired wound healing [51, 52].

Levels of tear plasmin have been shown to increase progressively in the sequence: no lens control group, daily wear soft lens group, extended wear soft lens group [53], and it has been

proposed that the proteolytic activity of plasmin may contribute to corneal epithelial abnormalities associated with lens wear [49, 53]. Vn localised on the lens surface adjacent to the corneal surface may remove PAI-1 leading to local upregulation of plasmin in the posterior tear film. If Vn is localised on a (synthetic) surface adjacent to the cellular site (such as a chronic wound) it removes PAI-1 from the reaction by fixing it, creating an imbalance in favour of plasminogen activator. This can result in a local upregulation of active plasmin formation, thereby controlling an important regulatory mechanism in wound repair; an increase in degradative proteolysis which may then result in a state of non-healing and excessive inflammation.

It remains unclear whether Vn binds to the lens surface to form a monolayer or whether constant protein binding continues to occur. Is there an initial layer of Vn formed which is always retained on the surface or is there constant competitive binding through some form of proteolysis or denaturation?

5. Conclusions

The potential for materials to affect Vn deposition will affect the locus of plasmin generation and thus influence lens-induced inflammatory processes. Clinical implications of this are yet to be determined, and further studies are required in order to comment on the ideal properties of a lens when considering the connection between contact lenses and the consequential inflammatory process. It can be said that an ideal lens is one that minimises protein interaction at the surface and that allows more exchange of protein at the edge. The unique conformational flexibility and multidomain structure of Vn which allows it to bind to a large repertoire of ligands makes its potential interactions with biomaterials all the more intriguing. Its versatile binding capabilities and receptor functions could, in the future, be used in the characterisation of structure-function properties and importantly in the design of new biomaterials.

The significance of this body of research must lie in the area of wound healing and the compromised anterior eye. Bandage contact lenses (BCLs) are regularly used to protect the compromised cornea from further ocular insult, to enable the relief of pain and to improve the ability of the corneal epithelium to heal. However, the use of contact lenses in therapeutics as bandage lenses is in its infancy. Lens choice appears to be influenced by convenience and availability rather than specific knowledge of their biochemistry of the healing process. Little

370 is known about the interaction of specific materials with corneal conditions and the way in
371 which materials can promote/inhibit the healing process. The importance of lens choice is
372 therefore apparent when evaluating the effect of material on the inflammatory process. *Ex vivo*
373 lenses worn in the uncompromised eye only were analysed in this study. The study is currently
374 being expanded to include lenses worn for bandage use in the compromised eye.

6. References

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Figure Legends

Figure 1. Cell counts on Vn doped (20 µg/ml) polyHEMA lenses in vitro. The greatest cell counts were found to be on Vn positive control lenses (59±3.3) and similar levels were found as expected on the antibody control lenses (52±3). Lenses treated with the anti-Vn negative control were found to have the lowest cell counts (4±2), validating the role of Vn for cell adhesion.

Figure 2. Cell counts with varying concentrations of vitronectin on polyHEMA lenses in vitro. The higher the doping concentration of Vn, the higher the cell counts; increasing the Vn concentration from 1 µg/ml to 20 µg/ml gave an increase in the average cell count per field from 5±0 to 37±1.7.

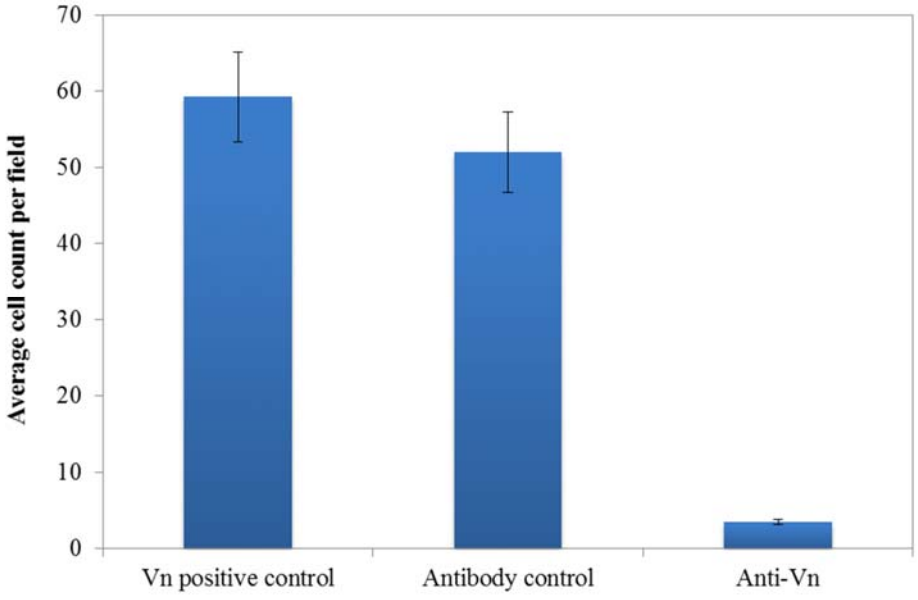
Figure 3. The relationship between Vn-mediated cell counts and lens water content. The higher the water content of the lens, the higher the Vn-mediated cell adhesion; the average cell count per field for lenses with water contents of 38% and 75% increased from 42±0.8 to 58±0.96 respectively.

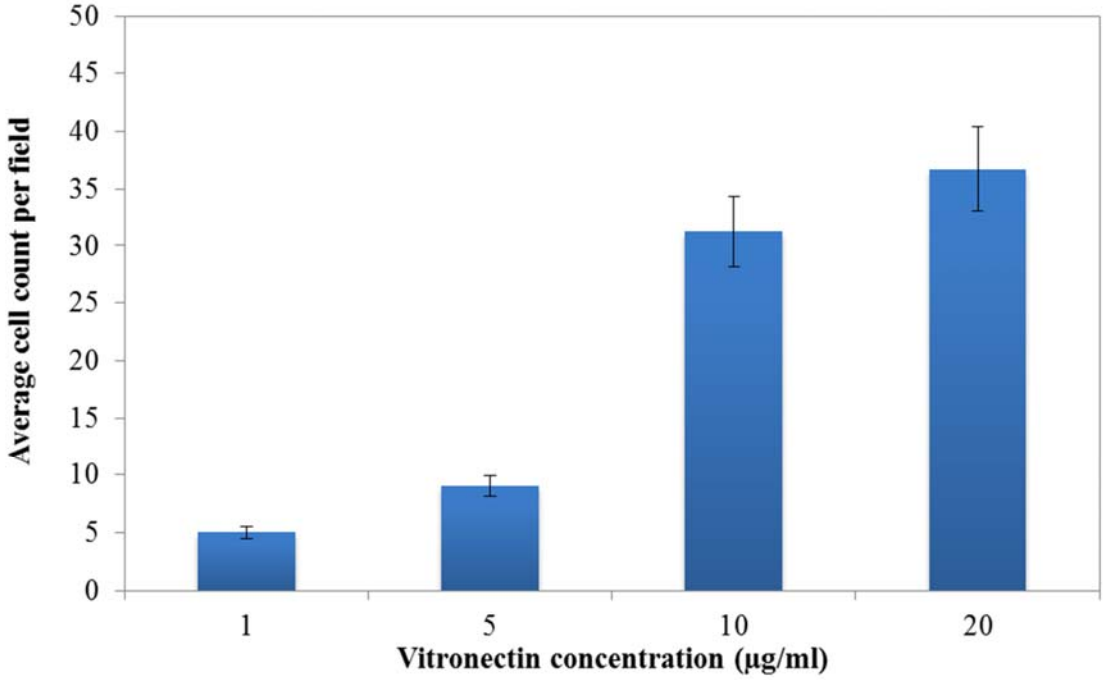
Figure 4. The relationship between Vn-mediated cell counts and lens ionicity. High water content ionic Group IV lenses had greater cell counts (etafilcon A 73±6) compared with high water content non-ionic Group II lenses (vasurfilcon A 53±9).

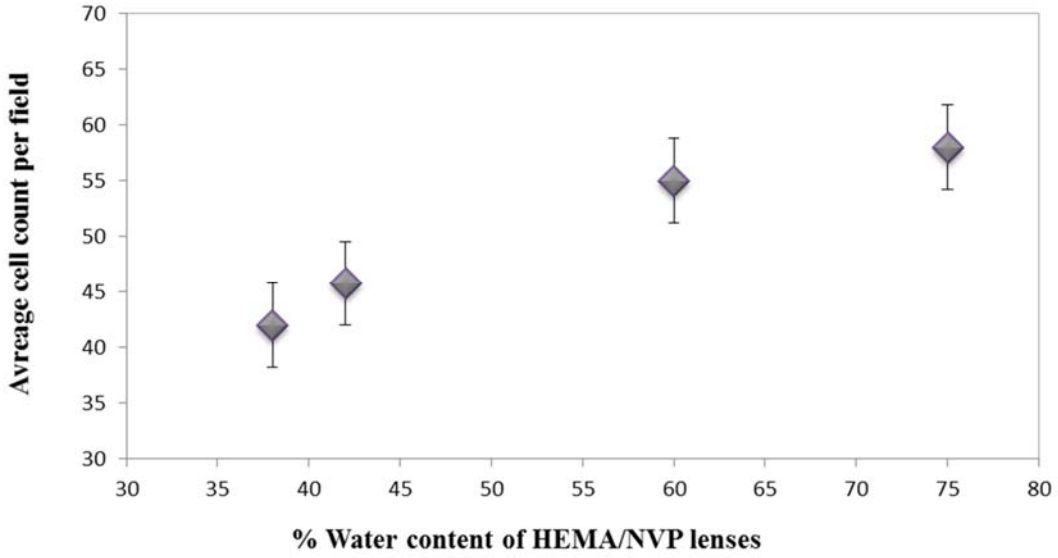
Figure 5. Comparative vitronectin-dependent cell counts on daily wear and extended wear etafilcon A lenses: anterior versus posterior. Higher cell counts were always detected on the posterior lens surface (daily wear 144±15, extended wear 265±26) than on the anterior lens surface (daily wear 7±0.3, extended wear 13±1). Greater cell counts overall were also detected on extended wear (198 hours wear) lenses in comparison to daily wear (168 hours wear) lenses.

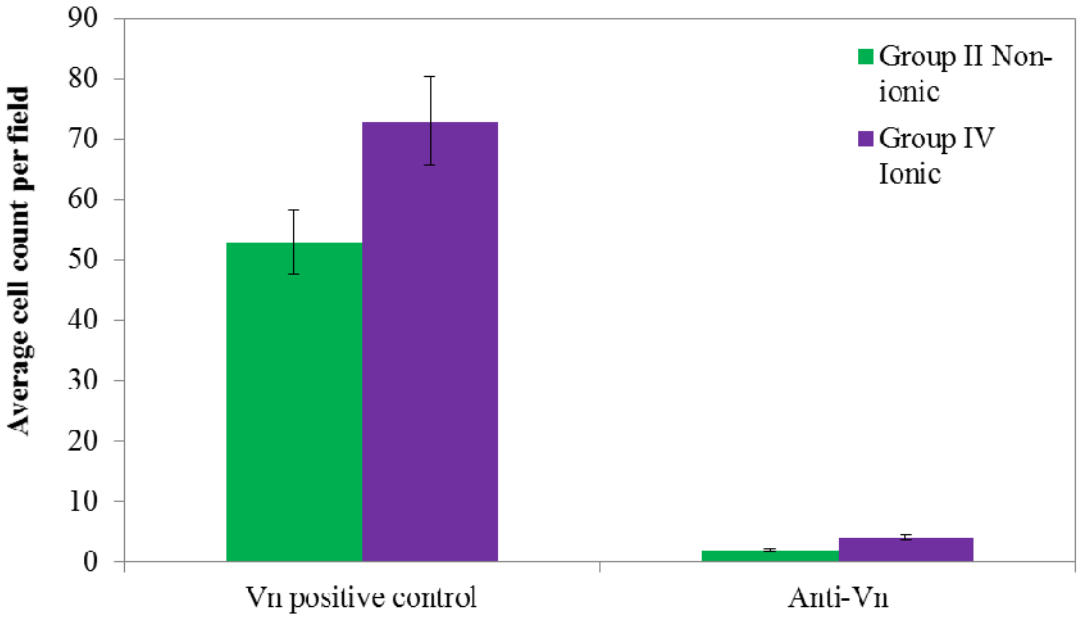
Figure 6. Comparative vitronectin-dependent cell counts on the posterior surface of ex vivo Group II (vasurfilcon A) and Group IV (etafilcon A) contact lenses worn on a daily wear basis: centre versus edge. Cell counts on the centre and edge areas. Greater cell attachment was observed at the lens edge compared to the centre: cell counts increased from centre to edge for Group II and Group IV lenses by 68% and 65% respectively. The ionic Group IV material showed greater cell counts over the entire lens surface in comparison to the non-ionic Group II material.

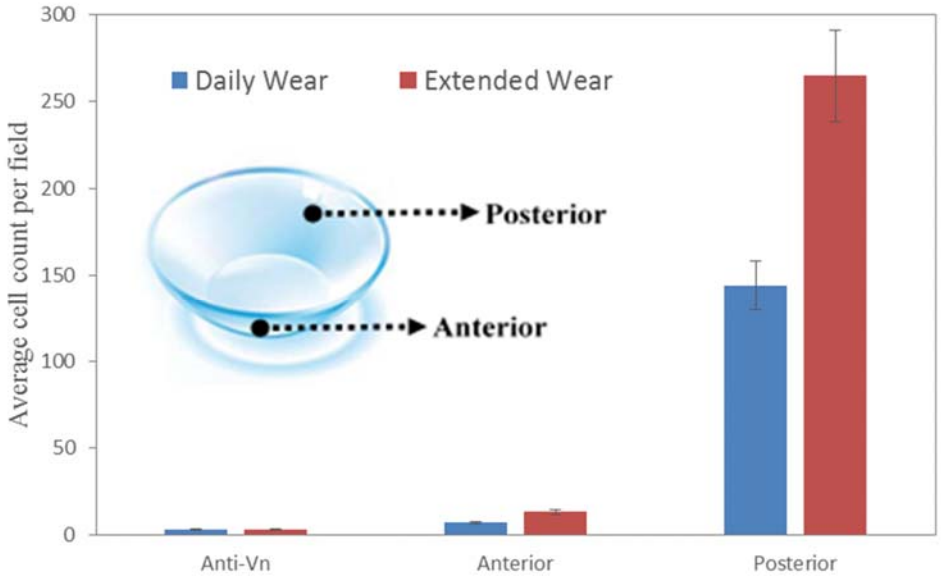
Figure 7. The influence of lens material, worn on a daily wear basis, on vitronectin-mediated cell count: centre versus edge. The highest cell counts levels were found on the etafilcon A material, with lotrafilcon B balafilcon A and lotrafilcon A showing decreasing cell counts respectively at the lens edge.

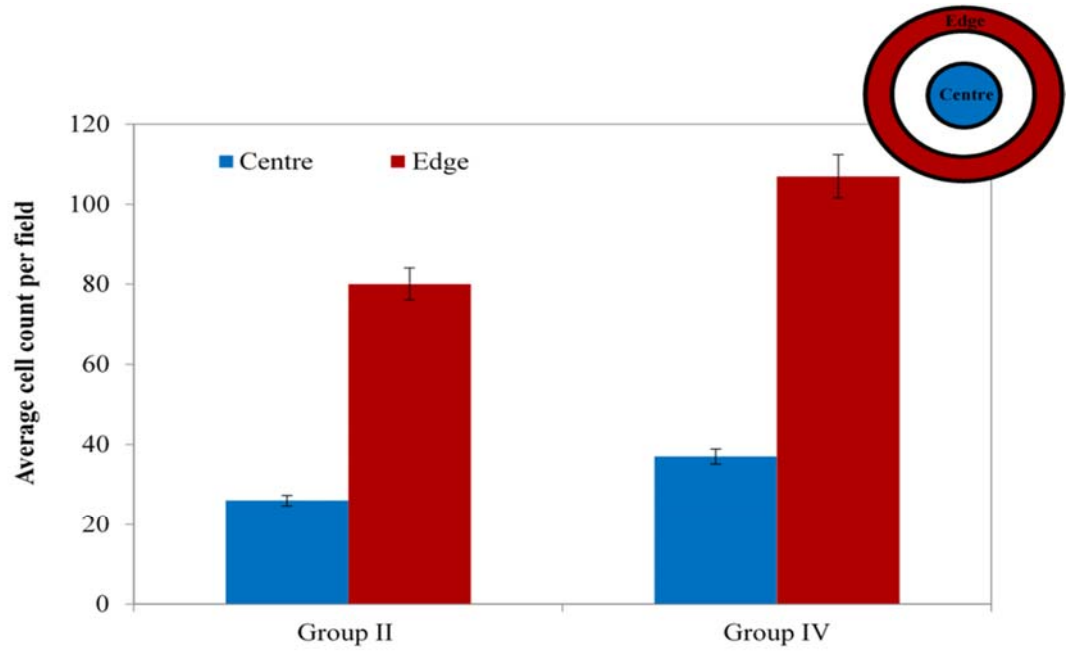

70
60
50
40
30
20
10
0
Average cell count per field
Vn positive control
Antibody control
Anti-Vn


50
45
40
35
30
25
20
15
10
5
0
Average cell count per field
1
5
10
20
Vitronectin concentration (µg/ml)


Avreage cell count per field
70
65
60
55
50
45
40
35
30
30
35
40
45
50
55
60
65
70
75
80
% Water content of HEMA/NVP lenses


Average cell count per field
90
80
70
60
50
40
30
20
10
0
Group II Non-ionic
Group IV Ionic
Vu positive control
Anti-Vu


300
250
200
150
100
50
0
Average cell count per field
Daily Wear
Extended Wear
Posterior
Anterior
Anti-Vn
Anterior
Posterior


Edge
Centre
120
100
80
60
40
20
0
Centre
Edge
Average cell count per field
Group II
Group IV

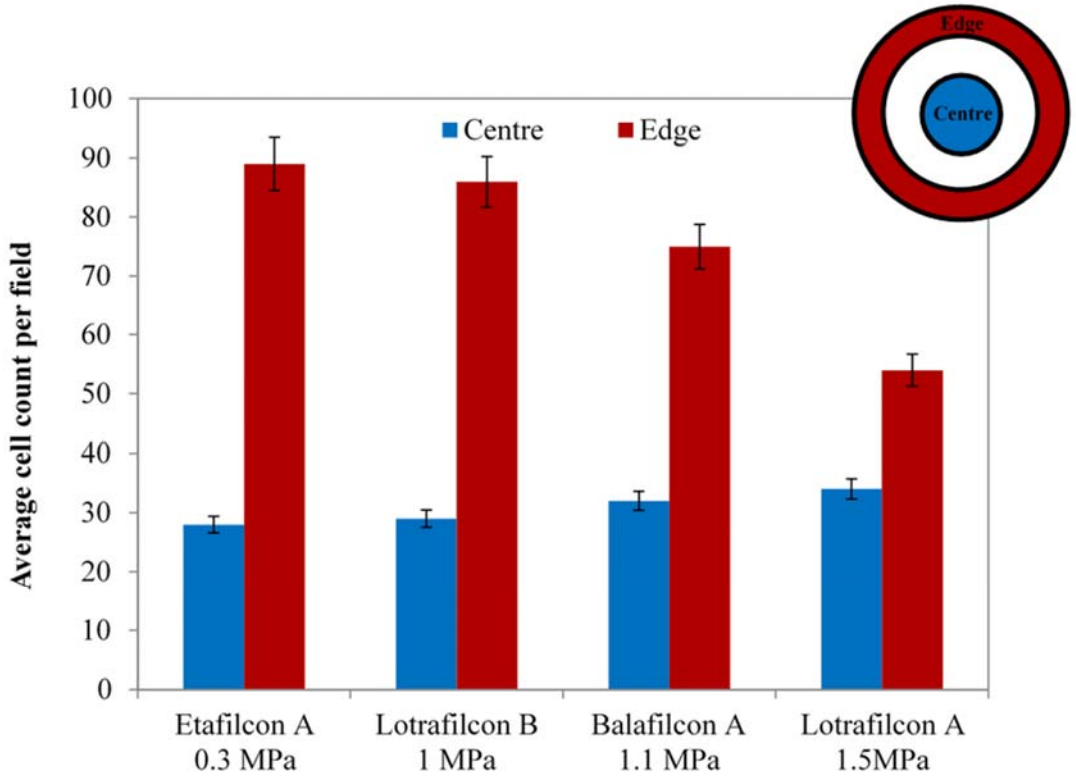

Edge
Centre
100
90
80
70
60
50
40
30
20
10
0
Centre
Edge
Average cell count per field
Etafilcon A
0.3 MPa
Lotrafilcon B
1 MPa
Balafilcon A
1.1 MPa
Lotrafilcon A
1.5MPa

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□□-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

Table 3. The relationship between lens modulus, edge:centre ratio and water content.

Material	Etafilcon A	Lotrafilcon B	Balafilcon A	Lotrafilcon A
Modulus (MPa)	0.3	1	1.1	1.5
Edge : Centre Ratio	3.3 : 1	3.0 : 1	2.5 : 1	1.3 : 1
Water Content (%)	58	33	36	24

Table 3. The relationship between lens modulus, edge:centre ratio and water content.

Material	Etafilcon A	Lotrafilcon B	Balafilcon A	Lotrafilcon A
Modulus (MPa)	0.3	1	1.1	1.5
Edge : Centre Ratio	3.3 : 1	3.0 : 1	2.5 : 1	1.3 : 1
Water Content (%)	58	33	36	24