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Review Article

A New Concept for Designing Eye Drop Providing Extended Protection to Corneal Surface

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Abstract

Based on the molecules listed in monograph and a new "cell coating" design concept, a new tear substitute is developed.

Cell coating technology applies a layer of coating directly onto the cell membrane, not cell products in tear (mucin or lipid layer), which is believed to maximize corneal contact time. PERFECT DROP™ is created for the extended protection on the foundation of a cell coating technology. The general concept of cell coating is to apply functional polymers directly onto the cell membrane by involving cell adhesion molecules on the epithelium. The binding between cell membrane and functional polymers is driven by the adhesion molecules and will not be affected by the insufficiency of the mucus or lipid layer in natural tear. As a result, coating can cover most surface areas of epithelium including those spots without natural mucus or lipid layer.

With specifically designed functionality, cell coating protects epithelium from exposure to adverse environment conditions. This function is particularly valid in the management of dry eyes and irritated eyes.

Keywords: *Designing Eye Drop; Corneal Surface; PERFECT DROP™; Lipid Layer*

General principle of cell coating

The concept of cell adhesion onto polymer substrate is the fundamental premise of cell coating

In the past few decades, *in vitro* cell migration and attachment phenomena were well-studied. It was recognized that the adhesion molecules play important roles in cell adhesion and signaling. The initial attachment of most cultured cell types, including fibroblasts, endothelial cells, normal lung epithelial cells and long-term cultured corneal epithelial cells, to polymers is based on a linkage of integrin receptors to serum-derived fibronectins (Fn) and/or vitronectin that adsorb onto the polymer surface. The adhesion process starts with saturating the substrate surface with Fn/ vitronectin which takes a matter of seconds. The α5β1 subunits on the integrin then bind with the arginine-glycine-aspartic acid (RGD) peptide region on Fn which is mediated by calcium. Cells are anchored on the polymer substrate via this linkage.

Cell coating is considered the opposite of cell adhesion

Immediately after solutions contact the cell membranes, the functional polymer ingredients bind to Fn to form polymer-Fn complex (PF). Established research has discovered that Fn coated latex beads strongly bind to retinal pigment epithelial cell surface integrins [1].

PF complex is presumed to act at the same way. As the RGD domain on PF is exposed, PF binds to the α5β1 subunits on the integrin. In consequence, the linkage of polymer-Fn-integrin (PFI) ensures functional polymers are retained on the cell membrane (Figure 1). Additional linkages on cell membranes provides more protection for the cell.

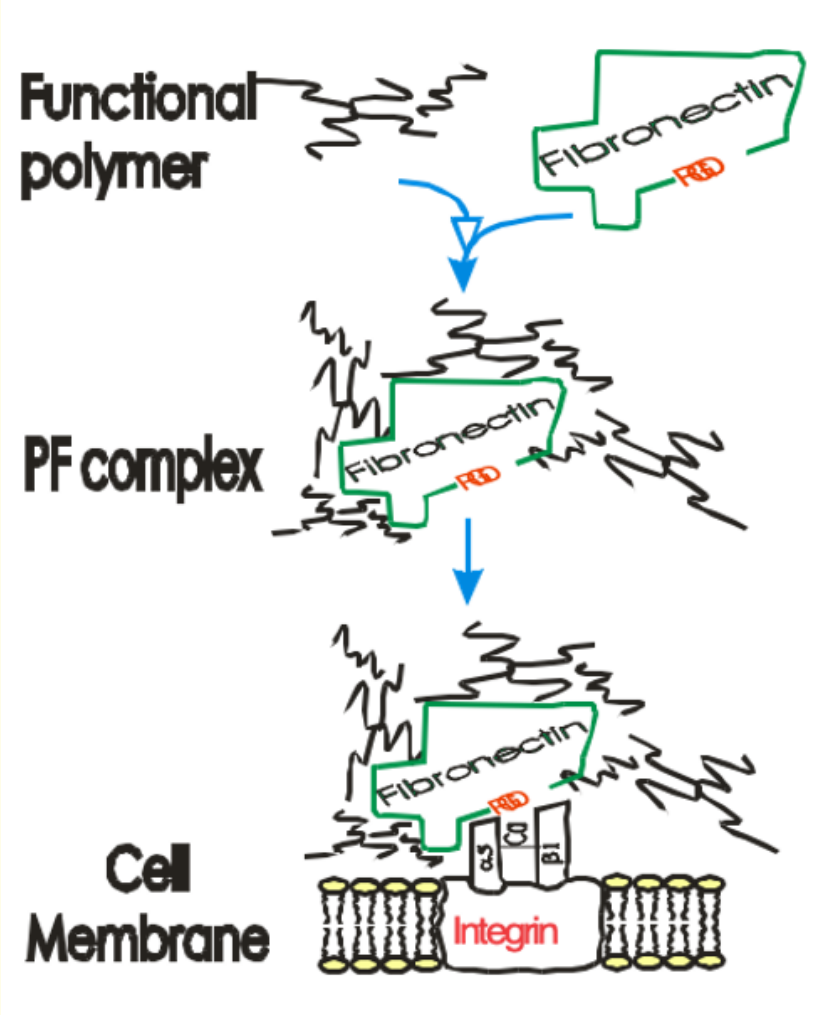

Figure 1: *Schematic of the principle of cell coating. When eye drop is instilled, functional polymers in eye drop are physically attached to tear Fns to form polymer-Fn (PF) complex. A strong linkage formed between PF and adhesion molecule integrin when the RGD region in Fn is exposed to the α5β1subunit of integrin. The binding of integrin and PF is a calcium mediated process.*

Design considerations of OTC tear substitute for extended protection

Cell coating as a protection shield holds a promising future in dry eye and irritated eye management. However, there are two theoretical concerns that need to be addressed. First, the binding between integrin and PF complex is reversible. Integrins can be internalized and degraded when cell adhesion does not occur. It is logical to suspect the disconnection between Integrin and PF occurs prior to the onset of internalization. The disconnection likely terminates the retention of functional polymers on the cell membrane. Second, probability of

the effective collision between α5β1 and RGD may be low. Formation of chemical bonds is determined by the effective collision between two active molecules. In order to form a binding, exposed RGD and α5β1 must collide into each other. This probability is doubtful since the number of cell surface integrins is limited under normal physiological conditions and the exposed of RGD peptide region might not appear on every PF complex. These concerns must be addressed in designing a new tear substitute.

The technical challenge of this project is to establish an effective tear substitute by only employing active ingredients listed on the ophthalmic OTC monograph. Such an undertaking apparently excludes many APIs that can easily solve those theoretical concerns. PERFECT DROP™ is an attempt to put the cell coating principle into work with common biomaterials.

Design considerations are two-fold: Biochemical and physiological properties

Biochemical properties of the tear substitute primarily address the above mentioned theoretical concerns. They include increasing the probability of effective collision and stabilizing the binding between integrin and PF.

Ingredients including calcium, glycerol and magnesium are selected.	
a.	Increase of intracellular Ca^{2+} results in lacrimal gland protein secretion.
b.	Glycerol is used to stabilize fibronectin conformation, which provides more useful fibronectin molecules for forming polymer-fibronectin complex at higher probability.
c.	Magnesium should contribute to the stabilizing PF-integrin binding.

More protein molecules in tears should help increase the probability of effective collision

First potential mechanism of boosting the effective collision is to increase the expression of membrane integrins in individual cell and the number of exposed RGD in individual PF complex. Unfortunately, without direct drug stimuli, the number of integrin and exposed RGD are limited. Except for direct stimuli, available option is to increase the number of Fn in tears. More available Fns in tears creates more PF and provide sufficient RGD domains to bind integrins, which leads to increasing the probability of effective collision.

Tear secretion increases in closed eyes and Fn concentration in closed eye tears (4127 +/- 3222 ng/mL) was significantly different ($p = 0.004$) from that in open eye tears (19 +/- 24 ng/ mL). Those observations hint that, by increasing tear secretion, the Fn concentration is significantly elevated in tears. Tear secretion could be boosted by the alteration of intracellular Ca^{2+} in various excitable and nonexcitable cells. Zoukhri., *et al*. published that the increase of intracellular Ca^{2+} results in lacrimal gland protein secretion [2].

Following this thought pattern, calcium that is a trigger for boosting the quantity of tear protein is one ingredient of PERFECT DROP™.

Protein molecules in native conformation are essential for effective binding

The quality of fibronectin molecules must also be addressed. The conformation of fibronectin changes as the physiologic conditions of natural tears change. This includes osmolarity, pH, water content…etc. It can turn into the folded or aggregated conformation that decreases the exposure of RGD drastically. Vincent., *et al*'s study suggested glycerol maintains the native conformation of proteins [3]. More fibronectin molecules in native conformation increase the probability of effective binding to integrin.

Based on this information, glycerol that stabilizes tear proteins including fibronectin is one ingredient of PERFECT DROP™.

Stabilizing integrin-PF binding, magnesium is another must-have

Internalization of integrin is a normal activity on the cell membrane. A permanent covalent bond between integrin and PF and the size of PF complex may prohibit the internalization and consequently interrupt normal membrane activities although the interruption is not desired. Alternative to a permanent binding is a stabilized weak binding. A stabilized binding means the dissociation of integrin and PF complex might be decelerated and results in prolonged retention time for functional polymers. Sigurdson., *et al*'s publication indicated that Divalent Mg^{2+} enhanced the alveolar epithelial cell adherence and spreading on Fn substrata [4].

Magnesium that boosts the stabilization of integrin-PF binding, is chosen as one ingredient of PERFECT DROP™.

Physiological properties required to make a physiologically compatible tear substitute

a. The first physiological factor is the pH. Andrés group's data indicated that average pH value obtained from normal eyes was 7.52. The pH for borderline and dry eyes was higher and more basic than that of normal eyes [5]. The pH of currently market available OTC dry eye tear substitutes ranges from 4.2 to 8.5 shown in our bench tests, which indicates different tear substitutes weigh pH factor differently. To compensate the higher pH in dry eye tears and achieve neutral pH, the pH of PERFECT DROP™ is set slightly lower and defined in the range of 7.2 to 7.4.

b. The second factor to be considered is viscosity. Viscosity of currently market available OTC eye drops ranges from 3.0 to 400 centipoise (cP) in our bench tests. In general, viscous tear substitutes extended tear film break-up time (TFBUT) significantly. However, high viscosity associated blurring of vision and discomfort is a major drawback. Blurring is likely caused by the viscosity discontinuity in the native tear. Our viscosity concept is "close to dry eye tear viscosity". Viscosity of tear is around 4.4 to 8.3 and 27.1 to 31.1 cP for healthy people and dry eye patients, respectively. Viscosity of PERFECT DROP™ is defined in the range of 25 to 100 cp.

c. The third physiological factor to be considered is the functional polymer. Functional polymers function as retaining water molecules on corneal surface. On the monograph, ophthalmic demulcents include cellulose derivatives, dextran 70, gelatin, polyols, polyvinylalcohol, and povidone. Among those options, PERFECT DROP™ selects cellulose derivative as the functional polymer due to its better protection of the corneal cells [6].

Conclusion

Under the guidance of the design principle, PERFECT DROP™ is built from safe, well tolerable ingredients and expected to improve the impaired function of mucin layer in tear of dry eye sufferers. Consequently, extended protection to corneal surface is predictable.

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