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Sustained Delivery of Proteins to the Ocular Surface *via* a Mucoadhesive Delivery System, NanoM™

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Protein Therapeutics to Treat Ocular Surface Disorders: The Issue

Protein therapeutics have been shown to have immense promise in treating ocular disorders, such as dry eye, corneal wound healing, etc. This is due to the high receptor specificity of biological molecules, promising targeted therapy without off-target side effects common to small molecule therapeutics. Protein therapeutics are highly water soluble and of high molecular weight, and thus can spread evenly on the ocular surface. However, although these molecules have varied structure and properties, they have potential to oxidize, hydrolyze, undergo deamidation, or degrade due to *in-vivo* proteolysis. The synergistic result of these characteristics and rapid turnover of the tear film imparts short half lives and fleeting residence time on the ocular surface, compromising their therapeutic effect.

We have developed a platform nanostructured ocular insert **(NanoM™)** that is placed into the lower conjunctival fornix in a dry form. Rapid absorption of water results in a "hydrogel-like" delivery system, aligning to underlying tissue. This characteristic of the delivery system contributes to the biocompatibility and minimal immunogenicity at the target site.

One added feature of this product is the necessity of a **hydrating solution** that is applied on the dry wafer after it is suitably placed on the tissue surface. The hydrating solution "wets" the wafer, establishes a "contact surface" of the drug delivery system with the tissue surface and prevents drying out of the surface locally.

We present physico-chemical characterization and delivery of two proteins IgG (MW 120,000 Da) and BSA (MW 62,500 Da) via the NanoM wafer. This work demonstrates feasibility of sustained release of proteins.

NanoM Wafer: Applied without Clinical Intervention, Biocompatible

- Dimensions : 8-10 mm length x0.150-0.200mm thickness x 1-2 mm width
- Placement: In lower conjunctival fornix, then hydrated with two drops of a buffering solution.
- Translucent hydrogel upon hydration
- Fluids permeable (O_2, CO_2, H_2O)
- >90% water in hydrogel
- ~2% loading

Method of Placement

Materials & Methods

○**Fabrication :** NanoM Wafer with IgG was fabricated using hydrophilic components like PEGs and PVA, using a proprietary process that generates an intercalated woven structure of interstitial pore size ~50-100 nm and containing ~2.2% IgG. Post-fabrication, the wafer was dried under vacuum to remove residual solvents. IgG was purchased from Sigma Aldrich, and had a molecular weight of 120,000 –150,000 Daltons. NanoM Wafer with Bovine Serum Albumin (BSA) was fabricated in a similar composition as above. BSA was purchased from Sigma Aldrich, and had a molecular weight of 62,500 Daltons.

○**Encapsulation (µg/mg):** 10-20 mg of the dry wafer was dissolved in an appropriate solvent, to dissolve the wafer and the encapsulated protein. Ethanol was added to precipitate the polymer, and the slurry was centrifuged. The supernatant was removed for SEC analysis and analyzed for protein concentration. BSA and IgG were separated on Size-Exclusion Chromatography (SEC).

○**Hydrating Solution**: A hydrating solution was developed, to apply the wafer after the wafer is positioned. A drop of the hydrating solution is adequate to wet the wafer and form good contact with the ocular surface.

The hydrating surface was characterized for osmolality, pH and viscosity.

○***In-Vitro* Release (%):** (20-40 mg) samples were placed in 1 mL Float-a-lyzer tubes, suspended in 40 g of buffer and rotated at 1 RPM at 37ºC. Time point samples were taken over the duration of study and analyzed by HPLC for protein released.

○**Thickness:** Fabricated NanoM wafers were measured (n=3) by a Mitutoyo Screw Gauge.

○**Quantification of Mechanical Adhesive Strength of NanoWafer to Tissue:** Measurement of Mechanical Adhesive Strength to conjunctival tissue was assessed using a Shimadzu Mechanical Tensile tester. A representative sample of the NanoM wafer was applied to hydrated bovine conjunctival tissue. The force required to pull apart the wafer from the tissue was recorded in N.

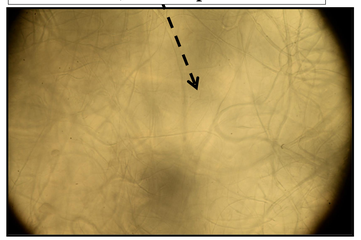
Results : Encapsulation, Thickness

	Thickness	Encapsulation
NanoM-IgG	~120 microns	~22 µg/mg
NanoM-BSA	~120 microns	~20 µg/mg

Results: Rapidly Hydrating and Translucent due to Nano-architecture

NanoM Wafer rapidly hydrates into a flexible hydrogel that adheres to conjunctival mucosa. % hydration = ~75%

Biomimetic, Biocompatible Insert



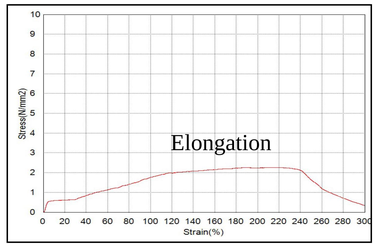
Porous Nano-architecture allows high uptake of water (Optical Micrograph, 40X)

Results: Hydrated NanoM Demonstrates Strong Bonding to Tissue

Bonding occurs ~30 minutes after application of wafer and hydrating buffer. This allows removal of wafer if applied incorrectly.

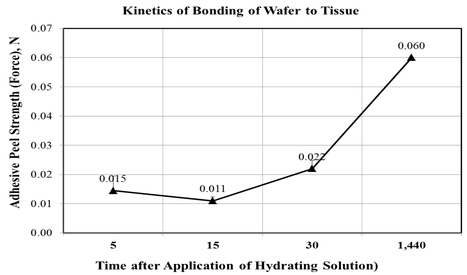
Failure during Peel Test is not adhesive failure—but **cohesive failure**

Results: Wafer Demonstrates Elastomeric Elongation upon Applied Stress



Elastomeric Elongation allows mechanical pliability with underlying tissue

Kinetics of Bonding of Wafer to Tissue after Placement



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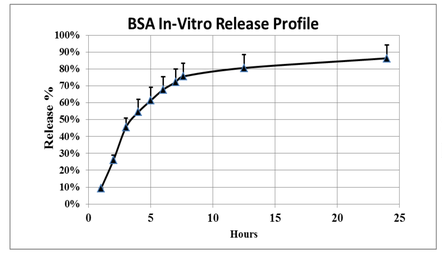
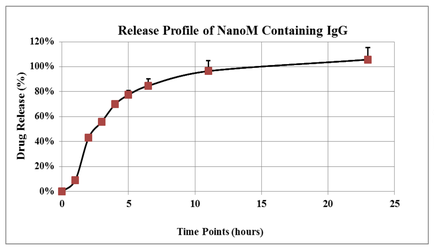
Sustained Release of Proteins



Sustained release of BSA



Sustained release of IgG

Conclusions and Future Work

- Early feasibility data presented herein, demonstrates that proteins can be released on the ocular surface in a sustained manner.
- The adhesion of the NanoM insert to the conjunctival surface has been optimized to last at least a week in-vivo.
- The hydration of the NanoM insert with the hydration solution has been concluded to be an important and critical parameter.

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