Tracking Adsorption & Loading of Target Molecules into Hydrogels

Insplorion's Localized Surface Plasmon Resonance (LSPR) technology enables the tracking and measurement of target molecules as they adsorb onto the hydrogel and allows for the calculation of the loading amount. Herein used for applications in antimicrobial coatings for implants and for improving biocompatibility.

Introduction

Hydrogels are solids that are formed by a network of cross-linked hydrophilic polymers, and up to 90% water. Their application can range from agriculture to medical devices and drug delivery. Due to their highly absorbent nature and slow diffusion kinetics, they make ideal candidates for loading with a target that requires continuous release over time. In medical applications specifically, they can be used to improve the biocompatibility and efficacy of implants or to immunomodulate the implant surface; for example by releasing anti-inflammatory, antibacterial, and pharmaceutical molecules. Here, NPS has been used to validate the loading capability and determine the loading capacity of two hydrogels.

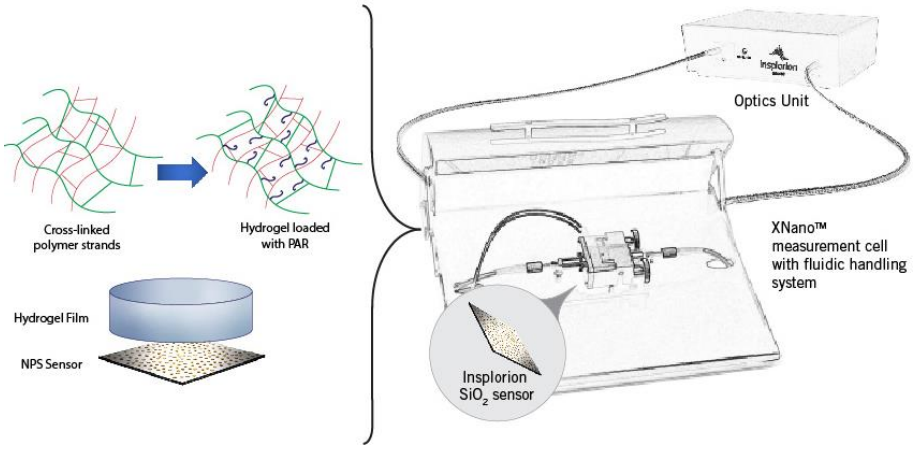

Figure 1: Insplorion system setup. The inset shows a schematic illustration of the sensor coated with the hydrogel film (bottom), and the attachment of PAR to the cross-linked hydrogel polymer strands (top)Figure is not to scale.

Experimental Procedure

In the first study, films were prepared with a solution of gelatin (14% w/v) mixed with hyaluronic acid tyramine (HA-tyramine, 1% w/v). The heated solution was then spin coated onto the sensor. Two processes were used to crosslink the films and obtain an interpenetrated network (IPN): making amide bonds between the lysine and glutamine residues using transglutaminase; and HA-tyramine crosslinking based on dimerization of tyramine to di-tyramine by horseradish peroxidase. The sensor was placed inside the XNano flow chamber, and once a stable baseline was established, a solution of the protein bovine serum albumin was flowed across. The change in plasmonic peak position was recorded and graphed.

In the second study, gelatin (5% or 4.5% w/v for Gel or Gel/HA-Ald, respectively) was mixed with HA-Aldehyde (0.5% w/v) and dissolved in Tris solution. The solutions were heated, then spin coated onto the sensors. The films were then crosslinked with transglutaminase enzyme at room temperature for 30 min. The sensors were placed in the XNano flow chamber and the poly-arginine (PAR) solution (78 nmol in PBS) was flowed over at a rate of 100 µL·min^{-1}. The centroid of the plasmonic peak was tracked in real time. The volume mass concentration of PAR was calculated using the equation below based on the assumption that the sample is uniform and significantly thicker than the NPS probe depth.

$$c_S = \frac{\Delta n_s}{dn_s/dc} = \frac{\Delta \lambda_{NPS}}{S_0 . dn_s/dc}$$

Results

Figure 2A shows 1nm is peak shift as the BSA adsorbs and is loaded into the Gel/HA-tyramine film, thus validating its loading capabilities. The

Insplorion AB • Medicinaregatan 8A • 413 90 Gothenburg • Sweden
Tel: +46 (0)31 380 26 95
Web: www.insplorion.com
E-mail: info@insplorion.com

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authors confirm the presence of the molecule with 3D confocal microscopy and use SEM to approximate a thickness of 20 µm. The film was then used as a vehicle to deliver an anti-inflammatory cocktail to macrophage cells in vitro; it was shown to effectively aid in controlling their phenotype and improved results in the wound healing assay.

In the second study the three hydrogel films (Gelatin Type B (Gel), Gelatin with native HA (Gel/HA), and Gelatin with HA-Aldehyde (Gel/HA-Ald)) were compared using NPS for their capacity in loading PAR (Figure 2B). Gel/HA-Ald showed the highest loading capacity for PAR at 25 nmol.mL^{-1}, while Gel and Gel/HA had loading capacities of 6 nmol.mL^{-1} and 5 nmol.mL^{-1} respectively. The significant increase in the loading capacity for Gel/HA-Aldehyde can be explained by the covalent imine bond –CH=N- that forms between the aldehyde moieties of HA and the amino groups of PAR (terminal or on the backbone). The release study, quantifying the amount of fluorescently tagged PAR in the supernatant, gave similar results as that of the loading, where Gel/HA-Ald had the longer release profile as compared to Gel. The authors thus concluded that the Gel/HA-Ald film performed better as a reservoir for PAR. They then evaluated the system in two ways: in terms of viability, metabolism and morphology using cell lines, and in terms of its efficacy against bacteria. Neither Gel/HA-Ald nor PAR modified the viability or metabolism of the cells; and the release of PAR improved cell-cell contact at junctions, which is an indication of better angiogenesis (blood vessel growth). When incubated with bacteria, the sample releasing PAR over time had no visible bacteria, while the Gel/HA-Ald without PAR showed bacteria proliferation.

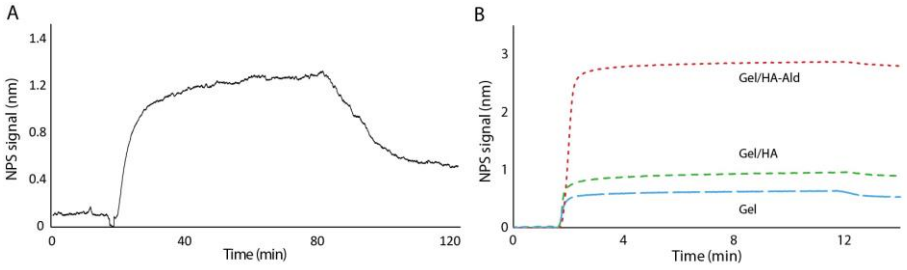

Figure 2: (A) Adsorption and loading of BSA into Gel/HA-tyramine film coated onto the sensor. (B) Comparing Gel (blue longdash), Gel/HA (green medium dash) and Gel/HA-Ald (red small dash) in their loading capacity of PAR (poly arginine).

Conclusions

Each study used NPS for the characterization of their respective hydrogel, specifically their loading capacity, before testing the hydrogels for applications in releasing anti-inflammatory, anti-bacterial, and pro-angiogenesis targets. They both show that NPS is a good method for determining and comparing the loading capacity of hydrogels. Loading and release (not shown in these studies) can be tracked in real time and under controlled conditions. NPS also offers two additional advantages: the optical mass is insensitive to the adsorbed water, thus replacement of water molecules does not lead to mass loss; and the short probe depth of the sensor (tens of nm) makes this technique insensitive to swelling and deswelling of the hydrogel.

This study was performed by Helena Knopf-Marques and Nihal Engin Vrana with co-workers at INSERM U1121, Biomaterials and Bioengineering, Strasbourg, France and PROTiP Medical, Strasbourg, France.

References

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