BIOINSPIRED MULTIFUNCTIONAL NANOSTRUCTURES FOR MICRO-OPTICAL IMPLANTS

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ABSTRACT

We have demonstrated, for the first time, the use of multifunctional biophotonic nanostructures on an implantable medical device to improve its optical readout performance and anti-biofouling properties. Inspired by the unique transmissive light-scattering nanostructures found on the transparent wings of the *Chorinea faunus* (*C. faunus*) butterfly, we fabricated multifunctional bioinspired nanostructures (BINS) on a freestanding Si₃N₄-membrane using a polymer phase-separation process and used the nanostructured Si₃N₄-membrane as an optomechanical sensing element in a microscale implantable intraocular pressure (IOP) sensor with remote optical readout. In a 1-month *in vivo* study using New Zealand white rabbits, BINS-integration on the resonance-based IOP sensor significantly reduced the angle dependence of the sensor's readout process and led to 10-fold and 3-fold reduction in IOP error compared to traditional tonometry and an IOP sensor without BINS, respectively. Furthermore, the use of BINS effectively suppressed biofouling and inflammation and greatly improved the sensor's biocompatibility by 12 folds.

INTRODUCTION

With increased dependence on medical implants for improving quality of life, research efforts are underway to integrate multiple functionalities in highly-miniaturized implantable packages while delivering reliable *in vivo* performance [1, 2]. Numerous multifunctional nanostructures on insect wings demonstrating omnidirectional antireflection, self-cleaning, anti-fouling and bactericidal properties have been previously studied and replicated [3, 4]. However, their incorporation into implantable biomedical devices remains unexplored.

Here, inspired by the multifunctional properties of the biophotonic nanostructures found on the wings of the *C. faunus* butterfly, we engineer bioinspired nanostructures (BINS) on a freestanding Si₃N₄-membrane. The BINS exhibit isotropic transmissive light-scattering similar to nanostructures in the inner sections of the *C. faunus* wings (indicated by blue arrows in Figure 1a) that could be beneficial to optical sensors' readout processes. Additionally, being improved on the biophysical properties of the *C. faunus* wing nanostructures, the BINS achieves antifouling through structurally-induced hydrophilicity that inhibits the adhesion of proteins, prokaryotes and eukaryotes *in vitro*. Antifouling by preventing adhesion is more advantageous when used with implants as it could minimize cell and tissue damages and avoid inflammation. Finally, we integrate the BINS Si₃N₄-membrane onto a resonance-based implantable IOP sensor and successfully demonstrated a greatly improved range of optical readout angles and biocompatibility in a month-long *in vivo* study.

THEORY

The AFM, SEM, optical, and biological characterizations have revealed that two groups of dome-shaped nanostructures with different periodicity co-exist on the transparent wings of the *C. faunus*: (1) angle-independent anti-reflective nanostructures with

periods of 140-180-nm in the postdiscal areas indicated by red arrows in Figure 1a; and (2) angle-independent transmissive light-scattering nanostructures with periods of 200-300 nm in the basal areas indicated by blue arrows in Figure 1a. The difference in structural periodicity between the two groups of nanostructures is shown in Figure 1b. This zone-dependent variation in periodicity influences the extent of light scattering on the wing. Due to its sub-wavelength structural periodicity (< 200 nm), the postdiscal area is scattering-free and anti-reflective. This phenomenon is consistent with other sub-wavelength nanostructures found in nature and can be modelled using the established effective medium theory and the transfer matrix model [3]. On the other hand, the basal area with a periodicity comparable to light wavelengths (> 200 nm) shows forward narrow-angle Mie scattering. In addition, the diffuse (non-scattered and scattered) and specular (non-scattered only) transmittance of the postdiscal areas were almost identical while the basal areas showed 20% reduction in the specular transmittance, indicating noticeable isotropic scattering effects (Figure 1c-d). Furthermore, the nanostructures in the basal areas exhibit a short-range order and consequently an angle-independent transmissive light-scattering property.

Figure 1: (a) *C. faunus* butterfly. Postdiscal and basal areas indicated with red and blue arrows, respectively. Scale: 1 cm. (b) SEM images reveal the difference in structural periodicity between the postdiscal and basal areas. Scale: 1 μ m. Measured diffuse and specular transmittance of (c) the postdiscal and (d) the basal areas.

RESULTS

Multifunctional BINS Fabrication and Characterization

Modeled after the nanostructures observed in the basal areas of the *C. faunus* wings, we developed short-range ordered BINS on a Si₃N₄-membrane using a highly-scalable polymer-phase-separation process involving poly (methyl methacrylate) (PMMA)

and polystyrene (PS) in methyl ethyl ketone (MEK) [5]. The polymer template was then transferred to Si_3N_4 -membranes through reactive ion etching (RIE). The periodicity of BINS ranged from 385 to 505 nm and had a height of 150 nm as seen in the SEM and AFM characterizations of the surface in Figure 2a and 2b, respectively. Angle-resolved transmission spectroscopy showed that the BINS-integrated membrane closely replicated the transmissive light-scattering property of the nanostructures found in the basal areas of the *C. faunus* wing, and its light transmission was twice more angle-independent than a flat Si_3N_4 -membrane (Figure 2c-d).

Figure 2: (a) SEM image and (b) AFM image of BINS-integrated Si_3N_4 -membrane. (c) Angle-resolved transmission of flat Si_3N_4 -membrane showing 30 nm blue-shift at 40° incident angle. (d) BINS-integrated Si_3N_4 -membrane showing only 15 nm shift at 40° incident angle.

BINS In Vitro Biophysical Properties

The adhesion of representative proteins (albumin and streptavidin), prokaryotes (*E. coli*) and eukaryotes (HeLa cells) on the BINS-integrated Si_3N_4 surface was compared with that on flat Si_3N_4 and control (glass) surfaces in *in vitro* testing. Due to the surface nanostructuring, the BINS-integrated Si_3N_4 surface displays strong hydrophilicity (contact angle: $<20^\circ$) compared to the flat Si_3N_4 surface which is moderately hydrophilic (contact angle: $35\text{--}40^\circ$). Due to increased adsorption of proteins, moderately hydrophilic surfaces such as flat Si_3N_4 strongly promote cellular adhesion when compared to strongly hydrophilic surfaces [6]. Additionally, the presence of BINS on the Si_3N_4 surface leads to a reduction in cellular-adhesion points on the surface [7]. As a result, the BINS-integrated Si_3N_4 surface displayed anti-biofouling properties through anti-adhesion against proteins (albumin and streptavidin, $***P \leq 0.001$), prokaryotes (*E. coli*, $**P \leq 0.01$), and eukaryotes (HeLa cells, $***P \leq 0.001$) when compared to flat Si_3N_4 and control (glass) surfaces (Figure 3).

BINS-Integrated IOP Sensor In Vivo Performance

We used the BINS-integrated Si_3N_4 -membrane as a sensing element of a recently developed optical resonance-based IOP sensor (Figure 4a). The resonance of a Fabry-Perot (FP) cavity changes as a function of ambient pressure due to the deflection of the Si_3N_4 -membrane (Figure 4b). While the IOP sensor with a flat Si_3N_4 -membrane successfully demonstrated *in vivo* IOP monitoring in lab environments [8, 9], but its practical applications were limited by its narrow readout angle inherent to FP-resonators and infrequent but severe biofouling observed after long-term

implantation. The BINS integration onto the IOP sensor led to a 2.5-fold improvement in readout angle, or up to 30° , over an IOP sensor without BINS in benchtop testing (Figure 4c). In a one-month *in vivo* study conducted in New Zealand white rabbits (Figure 4d), the BINS-integrated IOP sensor showed a 10-fold and 3-fold reduction in IOP error when compared to conventional rebound tonometry and an IOP sensor without BINS, respectively (Figure 4e).

Figure 3: (a) 3-fold reduction of albumin adhesion and (b) 2-fold reduction of streptavidin adhesion on BINS-integrated Si_3N_4 surface over flat Si_3N_4 surface. (c) Significant reduction of *E. coli* adhesion on BINS-integrated Si_3N_4 surface after 4 hours. (d) 7-fold reduction of HeLa cell adhesion after 72 hours on BINS-integrated Si_3N_4 surface.

Figure 4: (a) A schematic of a FP-resonator-based IOP sensor with a flat and BINS-integrated opto-mechanical Si_3N_4 -membranes that serve as a sensing element. (b) Pressure vs resonance spectra of IOP sensor: a change in the ambient pressure induces a shift in optical resonance of the FP resonator, which is detected remotely using reflected light. (c) Pressure variation of the flat and BINS-integrated IOP sensor as a function of increasing incident angle, measured at 1 atm: The flat sensor shows an IOP error of 4.5 mmHg at 12° while the BINS-integrated sensor shows an IOP error of 1.75 mmHg at 30° , displaying significant improvement in angle independence. (d) Images of the implanted flat and BINS-integrated sensors during the *in vivo* study: the flat sensor looks

hazy due to tissue build-up. (e) The BINS-integrated sensor displays 10-fold and 3-fold improvement in *in vivo* IOP-measurement errors compared to traditional tonometry and the flat sensor, respectively.

BINS-Integrated IOP Sensor *In Vivo* Biocompatibility

The IOP sensors with and without BINS integration were harvested from the rabbit eyes after 1 month to evaluate *in vivo* biocompatibility. We used confocal immunofluorescence microscopy to assess the extent of tissue growth over the surface of the two sensors at the time of retrieval. The sensors were stained with DAPI (nucleus marker), Phalloidin (F-actin marker) and MMP-2 (MMP-2 marker). DAPI was used to localize cells in adherent tissue while Phalloidin and MMP-2 were used as indicators for cellular health and inflammation, respectively. Figure 5 shows the images of the two sensors obtained using 3-channel z-stack confocal microscopy. The BINS-integrated IOP sensor (Figure 5a) displayed a 12-fold reduction in surface area covered by adherent tissue when compared to the IOP sensor without BINS (Figure 5b).

Figure 5: 3-channel z-stack confocal-microscopy images of (a) the IOP sensor without BINS and (b) IOP sensor with BINS, harvested after 1-month *in vivo* study: DAPI 405 (nucleus, in magenta), Phalloidin 488 (F-actin, in green) and MMP-2 594 (MMP-2, in red) markers were used.

CONCLUSIONS

In this work, inspired by the multifunctional basal nanostructures of the *C. faunus* butterfly wing, we develop BINS on a freestanding Si_3N_4 -membrane. The BINS-integrated Si_3N_4 -membrane was twice more angle independent than a flat Si_3N_4 -membrane. Furthermore, the BINS-integrated Si_3N_4 surface displayed strong hydrophilicity that repelled the adhesion of proteins, prokaryotes and eukaryotes *in vitro*. The BINS-integrated Si_3N_4 -membrane was then used as the sensing element on a highly-miniaturized IOP sensor implant. The BINS integration onto the IOP sensor lead to a 2.5-fold improvement in readout angle from 12° to 30° on benchtop. In a 1-month *in vivo* study, the BINS-integrated IOP sensor lowered the *in vivo* IOP error by 10-fold and 3-fold when compared to rebound tonometry and an IOP sensor without BINS, respectively. Finally, the BINS-integrated IOP sensor displayed enhanced *in vivo* biocompatibility through a 12-fold reduction in surface area covered by tissue after a 1-month implantation. With these highly promising results, we envision greater usage for the multifunctional bioinspired nanostructures in medical implant applications.

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