

A Titania Nanotube Membrane for Extended Zero-Order Macromolecule Delivery

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ABSTRACT SUMMARY

We have developed a robust, biocompatible, nanoporous titania membrane that produces zero-order release rates of macromolecules, potentially improving efficacy and reducing side effects of chronic disease treatments.

INTRODUCTION

Most macromolecule therapeutic delivery options for patients are limited to injections and infusions. In addition to the pain and compliance issues associated with these delivery techniques, the delivery profile is often non-optimal, spiking and dropping with each injection. Many therapeutics would benefit from a more stable, zero-order release rate, potentially reducing side effects and improving efficacy.

Recently, constant rates of delivery have been achieved by confining diffusion of therapeutic molecules through nanoporous membranes¹. For example, in some pore geometries, having a nanopore that is roughly twice the Stokes radius of a molecule is sufficient to produce constant rates of diffusion, rather than concentration-driven Fickian diffusion¹. The materials used in most studies involving nanoporous membranes are silicon and alumina, neither of which is commonly used as a tissue-contacting surface in FDA-approved implantable devices.

We have produced highly uniform, titanium oxide nanotubes, with pore diameters that are appropriate for confined diffusion with zero-order kinetics. These nanotubes have been integrated into a robust membrane and coupled with an implantable device, tested with several molecules, and shown to be biocompatible in rats.

EXPERIMENTAL METHODS

Titanium oxide (titania) nanotubes were grown electrochemically on titanium following the protocol outlined in Paulose, et al². After nanotube growth, the backs of the tubes were opened in pre-patterned areas, permitting the remainder of the nanotubes to maintain contact with the underlying titanium support structure. The nanotube backs were opened either using a dilute hydrofluoric acid solution or using an inductively couple plasma etch, similar to Parker, et al³.

After fabrication, membranes were imaged using scanning electron microscopy (Hitachi S-5000 and FEI Nova NanoSEM 650) and by optical stereo microscope (Amscope).

To test diffusion kinetics, membranes were assembled into capsules and loaded with fluorescein isothiocyanate IgG Fab₂ (henceforth, FITC-Fab₂, from Rockland Immunochemicals) or fluorescein labeled dextran 3000 (henceforth, Dextran 3000, from Invitrogen) in phosphate buffered saline. Loaded capsules were immersed in phosphate buffered saline and incubated in closed vials at 37°C, with agitation. Samples were taken and read on a fluorescent plate reader (Cytofluor 4000).

To test biocompatibility, membranes were implanted in the dorsal subcutaneous tissue on three rats. Control animals were implanted with titanium membranes without nanotubes. The animals were euthanized at 1 week and at 3 months. Tissue samples were analyzed by histopathology (hematoxylin and eosin stain) and blood samples were analyzed with a completed blood count.

RESULTS AND DISCUSSION

Vertically aligned titania nanotubes were grown on the membrane and were 50 microns in length with pores roughly 30-75 nanometers in diameter, depending on growth conditions (see Figure 1). Both ends of the nanotubes were open

inside the windows (Figure 1a), whereas only one end was open where the other end attached to titanium.

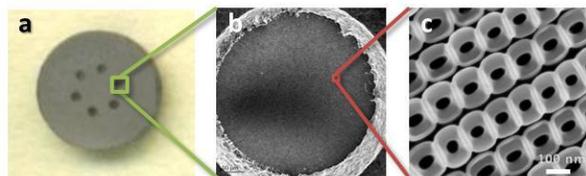


Figure 1. Images of nanotube membranes: a) under stereo-microscope, b) and c) under scanning electron microscope.

When the nanotube membranes are assembled into a capsule loaded with test molecules, we find that a larger molecular weight results in non-Fickian behavior (see Figure 2). FITC-Fab₂ (roughly 110 kDa) diffuses at a linear rate beyond 70% of complete elution, whereas Dextran 3000 (roughly 3 kDa) diffuses in a Fickian manner (non-linear).

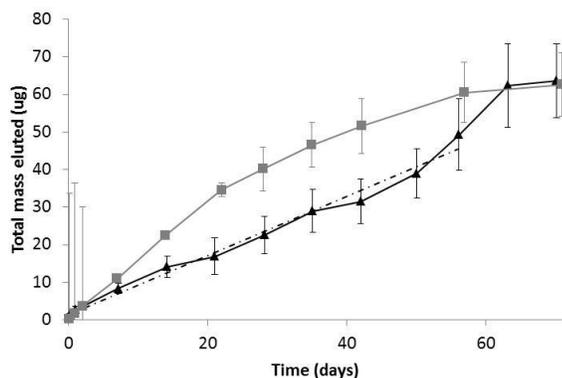


Figure 2. Diffusion of FITC-Fab₂ (black triangle, 110 kDa) and Dextran 3000 (gray square, 3 kDa) through nanotube membranes. The black dotted line shows a linear regression through day 56 of the FITC-Fab₂ data ($R^2 = 0.99$).

When implanted in rats, the nanotube membranes produced no significant differences in behavior compared to control titanium discs. At three months, mature fibrous capsules with relatively thin walls (4 to 5 layers thick) and rare mononuclear cells surrounded both membranes and control discs (see Figure 3). Furthermore, a complete blood count and blood smear showed no significant difference between

the nanotube membranes and control discs at any time point.

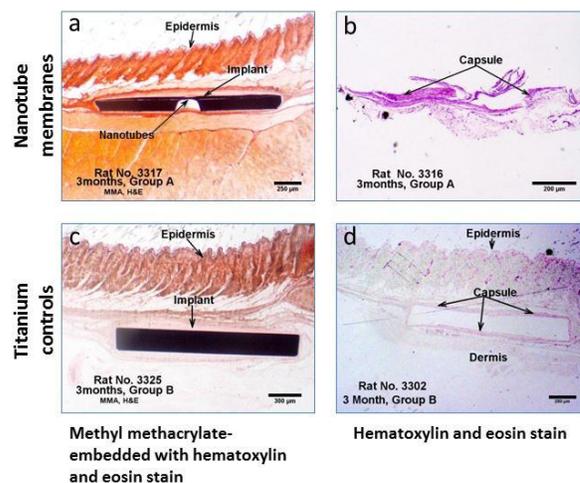


Figure 3. Histopathology of the tissue with implants at 3 months. a) and c) show nanotube and control implants in place. b) and d) show tissue around the implants.

CONCLUSION

Thus, using electrochemical anodization and inductively coupled plasma etching, we have developed a vertically-oriented nanotube membrane. Due to the nanometer-scale pores, diffusion is confined for a larger molecule, producing non-Fickian, linear diffusion rates. Furthermore, in vivo studies demonstrate the nanotube membranes to be biocompatible. As part of an implant, these membranes may allow for delivery of macromolecular drugs at a linear rate, thereby reducing side effects and improving efficacy for chronic disease treatments.

REFERENCES

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