# Nanoscale Constrained Delivery: A Novel Technology for Subdermal Implants

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**Background**

Beyond general dislike of needles, standard injection therapy has several limitations. Poor pharmacodynamic responses, including side effects deriving from high concentrations immediately after injection and reduced efficacy owing to subtherapeutic concentrations between injections, can limit tolerability and efficacy of treatment. Pressure from payers for improved compliance and, thus, convenience is driving the need to innovate novel solutions in drug delivery. Extended-release, constant-rate implants address all of these issues and may even improve patient outcomes.[1] In addition to producing constant-rate delivery, an optimal device would be small, easy to implant, and most importantly, safe over the duration of the implantation.

Recent developments have enabled the creation of a titania nanoporous membrane that can enable a small implant to provide passive, long-term, constant-rate drug delivery (Figure 1). Under standard conditions, the diffusion rate is proportionate to the concentration gradient, according to Fick's laws. Pore size reduction decreases the effective coefficient of diffusivity, extending the release while maintaining a correlation between rate of diffusion and concentration gradient. Polymer implants, such as Nexplanon, utilize the aforementioned phenomena to extend the release of many drugs, including synthetic hormones, for which the therapeutic window is large enough that first-order release kinetics do not adversely impact side effects or efficacy. When the pore size approaches the size of a molecule, the concentration-driven diffusion regime is no longer relevant, and molecules diffuse through the membrane pores at a linear rate that no longer correlates with the concentration gradient across the membrane.[2–4]

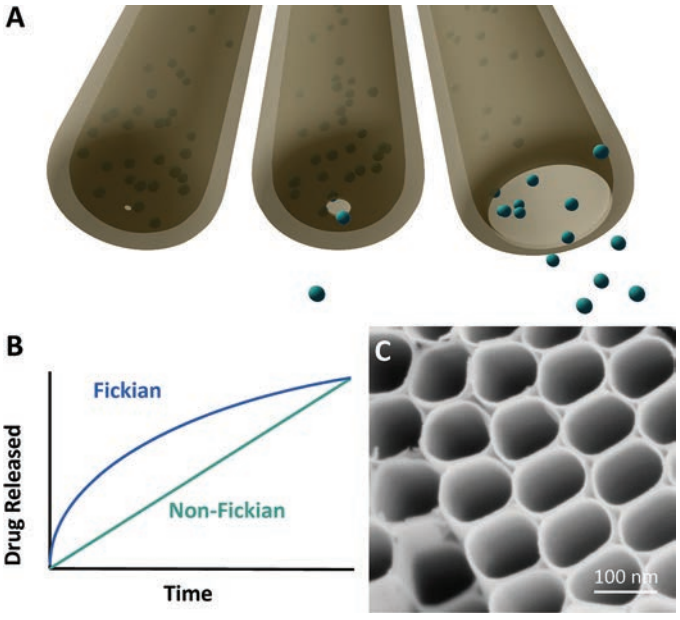


**Figure 1.** *(A) Molecules are approximately the same size as the pores for non-Fickian, constrained, linear diffusion (middle), whereas they are significantly smaller than the pore size in Fickian, nonlinear diffusion (right). (B) Schematic showing differences in diffusion curves for the two types of diffusion. (C) Scanning electron micrograph of titania nanotubes.*

Numerous materials have been used to constrain diffusion, including aluminum, aluminum oxide (alumina), silicon/silicon oxide (silica), and titanium/titanium oxide (titania). Because the desired duration for an implant represents a period of months to years, it must be both stable and biocompatible (nontoxic, noncarcinogenic, nonantigenic, and nonmutagenic). Additionally, nanoporous membranes, because of their extensive surface area, are more susceptible to degradation processes. Furthermore, the materials must not adsorb sufficient material to clog membranes. Unmodified alumina membranes can be prone to surface fouling and pore clogging and can induce mild to moderate inflammation.[5] Silicon/silica has not shown significant toxicity *in vitro* or in animal studies; however, there are only limited studies of its interaction with human tissue. Nanoporous polymer systems are in development in the academic laboratory and show significant promise for degradable delivery systems,[6] but long-term safety still needs to be demonstrated.

In addition to the porous membrane materials, one must consider the material of a reservoir and any material used to attach the membrane to the reservoir. Although the reservoir may comprise several types of biocompatible materials, nanoporous membranes often require adhesives to seal the membrane with the reservoir, increasing potential toxicity and stability issues. Titanium has been used extensively in humans and is well accepted as an implantable biomaterial; furthermore, a titanium/titania membrane may be sealed to a titanium reservoir with no other materials added.

**Methods**

Vertically aligned titania nanotubes were grown from titanium following a protocol similar to that of Paulose *et al.*[7] To produce the NanoPortal™ membrane, a portion of the titanium structure and the closed bottoms of the nanotubes were opened.[8] Pore sizes were determined with high-resolution scanning electron microscopy (FEI Nova NanoSEM 650). Membranes were attached to reservoirs temporarily using a screw-cap prototype with o-rings.

To test diffusion kinetics *in vitro*, assembled capsules were loaded with fluorescein isothiocyanate IgG $Fab_2$ (FITC-$Fab_2$, Rockland Immunochemicals) or fluorescein labeled dextran 3000 (Dextran 3000, Invitrogen) in phosphate buffered saline (PBS). Loaded

capsules were immersed in PBS and incubated in closed vials at 37°C, with agitation. Samples were read on a fluorescent plate reader (Cytofluor 4000).

To test *in vivo* release, assembled capsules were loaded with polyethylene glycol (PEG, MW 40 kDa) and implanted subcutaneously in rats. PEG was chosen as a model molecule because of its stability and ease of detection in blood plasma. Identical capsules were tested both *in vitro* (following the described protocol) and *in vivo*. To test biocompatibility, animals with implants were euthanized at 12 months for histopathology (hematoxylin and eosin stain).

**Results**

The NanoPortal membrane can be manufactured with pore sizes ranging from just a few nanometers to 100 nm, and it is made exclusively of titanium and titania (Figure 2). It can be made as small as 2 mm, thus fitting inside a 12 gauge needle for implantation, while still accommodating appropriate amounts of drug for months of release. By adjusting pore size and the number of exposed nanotubes, the NanoPortal membrane can be tailored to fit numerous molecules and applications.

*In vitro* studies with identical membranes but differently sized molecules demonstrated the impact of nanotube diameter on release rate (Figure 3). Although the FITC-Dextran 3000 had roughly linear release to 42 days, the residual errors were not random, and the overall curve was more consistent with extended Fickian release. The FITC-Fab$_2$ remained linear to 56 days, and the curve was consistent with non-Fickian, constrained release kinetics ($R^2$ = 0.99).

As expected with a molecule of 40 kDa, the release rate *in vitro* was consistent with that of extended Fickian kinetics (Figure 4). *In vitro* delivery was completed around day 14, without any significant subsequent release. The plasma concentration immediately increased from the time of implant until day 3, as the PEG released from the capsule in the subcutaneous space diffused into the bloodstream. As the capsule continued to release PEG, plasma concentrations approached equilibrium until delivery completed around day 14, at which point the PEG was eliminated.

When implanted in rats, the NanoPortal devices produced no significant differences in immune reaction compared with control titanium devices. At 12 months, mature fibrous capsules with relatively thin walls (10–20 layers thick) and rare mononuclear cells surrounded both membranes and sham implants.

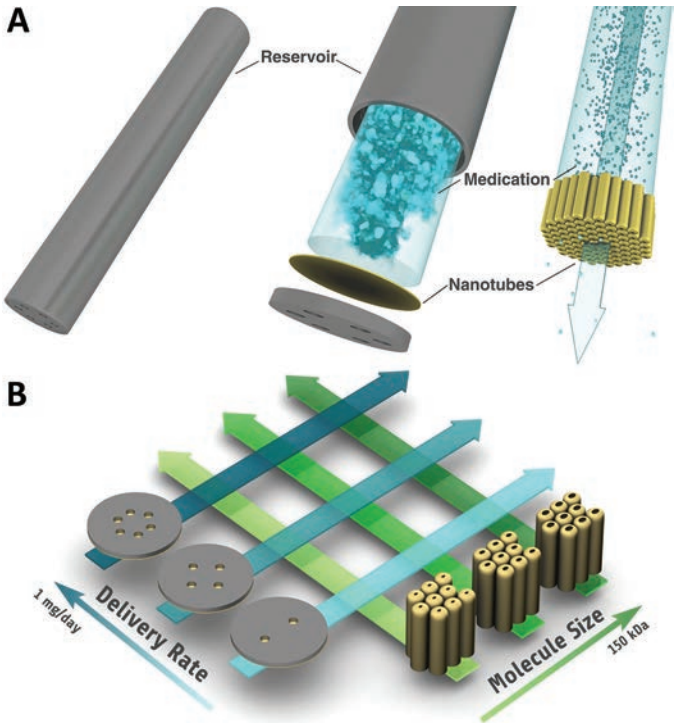


**Figure 2.** *Schematic of NanoPortal implant. (A) Overview of device assembly. (B) Constant-rate delivery for a variety of molecules can be achieved by adjusting the size of the nanopores; target delivery rates can be achieved by adjusting the number of accessible nanotubes.*

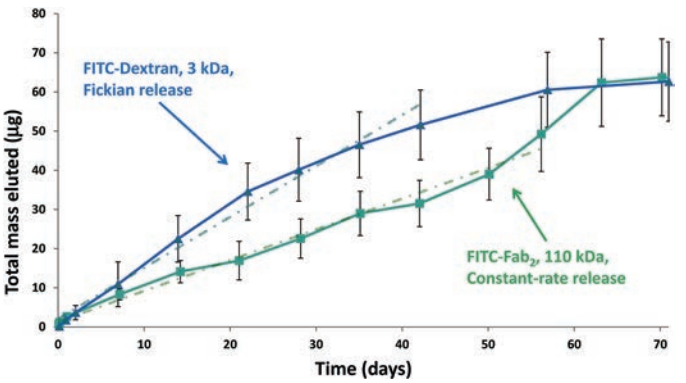


**Figure 3.** In vitro *data: with a membrane of the same pore size, FITC-Fab$_2$ diffuses at a constant rate, whereas the smaller FITC-Dextran diffuses in an extended Fickian manner.*

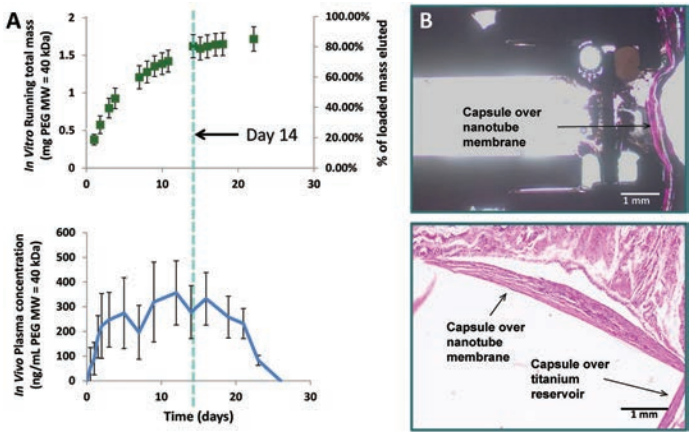


**Figure 4.** *(A)* In vitro *(top) and* in vivo *(bottom) experiments using identical devices tested in parallel (n = 5; error bars are standard deviation). Delivery is complete at day 14, when around 80–85% of the loaded mass has been released. (B)* In situ *(top) and standard (bottom) histopathology at 12 months confirms no remaining immune response and a thin fibrous capsule.*

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**Conclusions**

Constrained nanoscale diffusion offers a promising approach for extended, constant-rate delivery of a variety of molecules. By crafting a membrane from titanium and titania, it is possible to have a high degree of stability and biocompatibility, produce zero-order release *in vitro*, generate stable plasma concentrations *in vivo*, and easily integrate the membrane with a reservoir to create a functional, biocompatible system. Furthermore, the NanoPortal membrane has a range of pore sizes and device configurations, permitting its use with numerous molecules while maintaining a device that could be implanted subcutaneously in minutes with a syringe needle in an outpatient setting.

Although it has not been addressed here, significant, though not insurmountable (as demonstrated by Intarcia[9]), formulation challenges are associated with stabilizing a therapeutic for extended times *in vivo*. Nonetheless, because the devices allow a high percentage of their volume to be loaded with drug and may be used with a wide variety of molecules, nanoporous implants provide an excellent opportunity to reduce side effects and improve efficacy in the treatment of chronic disease around the world.

**References**


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