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Replenishable dendrimer-nanoparticle hybrid membranes for sustained release of therapeutics

Myoung-Hwan Park^{1,2,†}, Sung Tae Kim^{1,†}, Subinoy Rana¹, David Solfiell¹, Youngdo Jeong¹, Bradley Duncan¹, Bo Yan¹, Bü ra Aksoy¹, and Vincent M. Rotello¹

Vincent M. Rotello: rotello@chem.umass.edu

¹Department of Chemistry, University of Massachusetts 710 North Pleasant St., Amherst, MA 01003 (USA)

²Department of Chemistry, Sahmyook University, Seoul 139-742, South Korea, Fax: 1 413 545 4490; Tel: 1 413 545 2058

Abstract

We report a versatile hybrid membrane for sustained release therapeutic delivery systems. Chemically-directed assembly of a hybrid membrane of nanoparticles and dendrimers was integrated with a fluidic delivery device and a refillable drug reservoir, providing continuous sustained release.

Surface-mediated delivery technologies provide platforms for controlled release of bioactive molecules.¹ Maintaining an effective therapeutic dosage of drugs over time is a critical determinant of concentration-dependent drug properties, providing an important means of regulating pharmacokinetics and drug dose regimens.² Various strategies have been employed for sustained release, including degradation of encapsulating polyelectrolyte matrices³ and systems that release their payload upon exposure to external stimuli such as pH,⁴ temperature,⁵ and applied electrical⁶ and magnetic fields.⁷ Porous materials provide an alternative platform for controlled release applications.

Porous materials have a number of benefits relative to continuous films, including tunability of surface properties and porosity.⁸ Traditional porous delivery systems that depend on simple diffusion to mediate release, however, can face several challenges including high initial rates of release (bursts), low payload capacities (especially for water soluble therapeutics), and, most importantly, challenges in generating desired sustained release profiles for different applications.

The development of synthetic biomimetic membranes that can regulate the release of small molecule drugs and biologics provides a potential tool for enhancing the utility of porous

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Correspondence to: Vincent M. Rotello, rotello@chem.umass.edu.

[†]These authors contributed equally to the work.

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delivery systems.⁹ Artificial biomimetic membranes can serve as barriers between drug reservoirs and surrounding tissue environments. In this way, membranes can buffer initial release bursts to achieve a constant release rate and lengthen the time over which optimum drug concentrations are present within target regions. Such technologies have the potential to provide numerous benefits in areas of therapeutic design and clinical dosing schedules, as well as improving patient compliance and quality of life.¹⁰

In our previous studies, we described a dendrimer-nanoparticle (Den-NP) composite material with controllable porosity. These Den-NP composites employ the strong covalent dithiocarbamate (DTC) bond to couple amine groups of the poly (amido amine) (PAMAM) dendrimer to the gold surfaces of NPs.¹¹ This composite material provides a reimbibable therapeutic platform,¹² a feature with important implications in controlled delivery.¹³

A fundamental challenge in the application of porous delivery systems is loading capacity. We hypothesized that Den-NP composite membranes could serve as “gatekeepers” between the drug reservoir and tissue environments, providing extended controlled release. To this end, we fabricated a hybrid membrane consisting of an anodic aluminum oxide (AAO) disk coated with chemically crosslinked Den-NP composites. The porous AAO disk provides a reservoir for drug loading for increased release times. The membrane also provides a scaffold that can be incorporated into microfluidic¹⁴ and microchip devices¹⁵ for eventual use in implantable medical devices.¹⁶

Hybrid membranes were fabricated through precipitation of the Den-NP composite onto either silica,¹¹ or for extended release applications onto AAO. For the AAO system the amine-functionalized AAO was immersed in a solution of gold NP (AuNP), dendrimer, and carbon disulfide (CS₂). The composite materials precipitated onto the AAO surface, and the coating procedure was then repeated on the reverse side of the AAO. The scanning electron microscope (SEM) image indicates the formation of a hybrid Den-NP membrane with a smooth surface (Figure 1). These membranes were quite stable, with less than 0.01% leaching observed after 1 week incubation in media supplemented with 10% serum.

To quantify the sustained release features, doxorubicin hydrochloride (Dox)-loaded hybrid membranes were fitted into a phosphate buffered saline (PBS, pH 7.4) flow system consisting of a sealed polydimethylsiloxane (PDMS) channel and a peristaltic pump, as shown in Figure 2a. We first studied the film on a planar Si substrate, observing approximately 4 h of sustained Dox release without a significant initial discharge using the Den-NP composite (Figure 2b). The steep decrease in release concentrations at approximately 4 h presumably arises from depletion of drug from the film. We next incorporated an AAO disk as an internal reservoir to prolong sustained drug release (Figure 1), providing an additional 6 h of sustained release to the hybrid material, bringing the total sustained release time for a single loading up to 10 h (Figure 2b).

We next investigated the ability of the Den-NP membrane to regulate the release of a payload from an external reservoir that could be refilled with drugs.^{13c, 17} This strategy would lend itself well to the design of minimally invasive “rechargeable” medical devices for the continuous delivery of a drug during long term treatments.¹⁸ To this end, a fluidic

delivery device was fabricated possessing a refillable reservoir. First, an injection line was fitted to a reservoir that was made to contact with surface of a refillable membrane (Figure 3a). This injection line provided a means by which drug-depleted media contained within the reservoir could be easily replaced by fresh media via external injection. A continuous flow of PBS was maintained at the opposite surface of the membrane, and the collected PBS was analyzed for drug content as a function of time. Dox release following a single loading of the reservoir revealed sustained release of the drug over a period of 12 h, compared to a control experiment using unmodified AAO membrane without composites (Figure 3b). Additional injections of fresh Dox solution to the reservoir allowed the drug to be released from the film in a sustained manner over a period of 24 h (Figure 3c). It is expected that such a delivery profile could be maintained for much longer periods of time through continuous injections. Buffering payload concentrations within target tissues in this way would essentially represent a process of continuous replenishment and exhaustion of the delivery material *in situ*. These results demonstrate the potential utility for this hybrid membrane to act as a biocompatible barrier between a drug reservoir and the external environment in biomedical applications.

As a proof of concept study for therapeutic delivery we investigated the ability of the hybrid membrane to continuously deliver therapeutics in a sustained manner to cultured cells *in vitro*. As shown in Figure 4a, cell culture media was continuously circulated past a Dox-loaded membrane and reservoir, and flowed over HeLa cells. Cell viability assays revealed that the delivery system significantly decreased cell viability while no decrease in viability was observed in the absence of drug loading (in the presence of the apparatus alone) (Figure 4b). To further analyze cellular uptake of Dox, cells were observed with an inverted fluorescence microscope after a 4 h incubation within the fluid delivery system. A strong red fluorescence image was observed in the presence of a drug-loaded membrane (Figure 4b inset). These results demonstrate successful delivery of a drug by the hybrid membrane and reservoir to cultured cells, as well as the biocompatibility of the Den-NP scaffold.

Conclusions

Den-NP hybrid membranes represent a versatile and robust platform for sustained release of therapeutics. Incorporation of a reservoir extended the sustained drug release from 4 to 10 h. By placing this membrane between a refillable reservoir and a flow channel, sustained delivery was further extended to a period of 24 h following a single replenishment of the system. Finally, the system was used to deliver a drug to cultured cells in contact with circulating media. The combined micro- and nanoscale technologies presented by this system provide a versatile delivery platform with potential utility in the future design of implantable medical devices.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.

Formation of hybrid Den-NP membranes via DTC crosslinking of AuNPs onto the surface of porous AAO disks and loading of payload into the reservoir. The SEM micrograph shows the cross section of the hybrid film, with the Den-NP layer being ~800 nm determined by SEM and verified through profilometry.

Figure 2.

(a) Schematic illustration of the fluidic device (b) sustained fluorescence release profile of Dox ($\lambda_{\text{ex}}/\lambda_{\text{em}}$: 485/590 nm) collected after having passed through the PDMS channel containing the loaded Den-NP hybrid films with and without an AAO reservoir layer.

Figure 3.

(a) Schematic illustration for the hybrid membrane in the presence with refillable reservoir and their sustained fluorescence release profiles (b) of one time injected Dox ($\lambda_{\text{ex}}/\lambda_{\text{em}}$: 485/590 nm) and (c) repeatedly injected Dox that are collected after having passed through the fluidic channel.

Figure 4.

(a) Schematic illustration of an *in vitro* apparatus using HeLa cells of incubation in a fluidic channel and (b) *in vitro* cytotoxicity of with and without a Dox-loaded membrane after 20 h. Inset is bright-field and fluorescence images of HeLa cells with a Dox-loaded membrane after 4 h of incubation.