NanoBandage for Controlled Release of Topical Therapeutics

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ABSTRACT

Burn injuries, trauma, and surgical procedures result in complex skin wounds which can lead to inflammation, infection, and scarring. Management of complex wounds remains a critical challenge for the medical community and effective, localized treatments that provide sustained drug release, are limited. Recent advancements in nanotechnology offer a unique opportunity to deliver therapeutics to wounds and improve healing. The overall goal of our work is to combine non-toxic, biodegradable nanomaterials with therapeutics in order to design a topical drug delivery system for controlled release into skin. In this study we demonstrate the potential of combining solid lipid nanoparticles (SLNs) and bacterial cellulose into a novel system for controlled topical drug delivery.

Keywords: topical, bandage, wound healing, skin, nanotechnology

1 INTRODUCTION

The skin is a formidable barrier to applied pharmaceuticals due to its complex structure and composition, as well as the constant sloughing of corneocytes from its outer layers [1, 2]. Despite this extensive barrier function, the skin remains a desirable site for local administration of therapeutics to injured or inflamed tissue resulting from local trauma (e.g. burns) or surgical procedures.

Recent advances in nanotechnology have resulted in the development of nanometer-sized particles designed for drug-delivery applications [3], [4], [5]. SLNs are an especially promising platform for topical drug delivery due to their biocompatibility, stability, and their capacity to accept a wide range of active ingredients. SLNs are made from lipids that are solid at room temperature, typically with a mean diameter between 10-1000 nm. A decrease in particle size has been correlated with increased penetration of the skin [3], [4], [5]. We have previously synthesized ultra-small, non-toxic SLNs designed to penetrate the stratum corneum [6]. However, these nanoemulsions exhibit low viscosity and alone, are not optimal for prolonged contact with target tissue, as is desirable for long-term treatment regimens. In order to achieve extended delivery of SLN to skin, it is useful to take advantage of biocompatible bandage materials which can act as reservoirs, and promote continuous diffusion of SLNs into skin over time.

Bacterial nanocellulose, derived from *Acetobacter xylinum,* naturally exhibits several characteristics such as hydrophilicity, non-toxicity, and low immunogenicity, that make it an appropriate material for many biomedical aplications. This material has the potential to be hydrated and loaded with pharmaceuticals for controlled drug release. Bacterial cellulose has shown specific promise as a wound dressing due to its maintenance of skin moisture levels and reduction of proteolytic enzymes, inflammatory cytokines and reactive oxygen species [7, 8] The unique properties of cellulose make it an ideal candidate to serve as a reservoir for controlled delivery of skin-penetrating, drug-loaded SLNs. This paper describes our initial work to characterize skin penetration by SLNs, demonstrate the potential of bacterial cellulose as a wearable reservoir for controlled release of SLNs, and establish the biocompatibility of this system.

2 EXPERIMENTAL

2.1 Materials

Eicosane (C20, Lipid) (Aldrich), Brij 97 (C18E10) (Aldrich), Decyl Alcohol (Sigma), 9,10-is(phenylethynyl)-anthracene (TCI, Dye), Orcosolv yellow (organic Dyestuffs), phosphate buffered saline (PBS, Sigma), fetal bovine serum (FBS, Sigma), XCell Wound Dressing (Medline Industries).

2.2 Nanoparticle Preparation and Characterization

SLNs were prepared by the phase inversion temperature (PIT) method [9], [10], [11]. In this method, the composition remains constant while the temperature is changed. A modified nanoparticle preparation was used where 9,10-bis(phenylethynyl)-anthracene or orcosolv yellow, and decyl alcohol were combined into a vial. The following step was the addition of Brij 97 and eicosane. The resulting mixture was co-melted at 90 ˚C and stirred [6]. DI water was added to the mixture, heated and stirred.

Under continued stirring, cooling the sample caused inversion of the water-in-oil emulsion to an oil-in-water emulsion, creating a nanoemulsion in the process. A further decrease in temperature below the melting point of the lipid produces SLNs from the emulsified droplets. The particle size and the polydispersity of the SLNs were measured using dynamic light scattering (DLS). The samples were measured as prepared, using Nanotrac Ultra (serial number U1985IS). The particle diameter was determined to be 8.95 nm [6].

2.3 *Ex vivo* Human Skin Penetration

Human skin explants were obtained from the Cooperative Human Tissue Network (CHTN), Mid Atlantic Region (University of Virginia). Skin explants from the breast/chest region, shipped overnight, were rinsed briefly with PBS upon arrival. SLN solution (200 μl) was pipeted onto the surface of the skin. Skin was incubated at 37°C for 19 hrs with humidity. Following incubation, skin was wiped with PBS, rinsed with distilled water, and snap frozen in OCT (Ted Pella). Frozen sections were made (AML Laboratories, Baltimore) and then counterstained with propidium iodide. Sections were visualized by confocal fluorescence microscopy. Three sections were examined per treatment and representative images are shown in the results section.

2.4 Bacterial Cellulose Characterization

Bacterial cellulose (XCell) was dried using a supercritical point dryer (Samdri-795, Tousimis Research Corporation). The morphology and pore size were examined using a Hitachi S4700 cold field emission gun scanning electron microscope (SEM).

2.5 Nanoparticle Diffusion

A Franz diffusion cell was use to determine the kinetics of particle diffusion from cellulose. Before experimentation, identically-sized pieces of cellulose (XCell) were mounted on a benchtop Franz diffusion chamber (37˚C water jacket). Nanoparticle solutions (150 μl), containing 1 mg/ml dye, were slowly applied to the surface. Each opening of the Franz chamber was then covered with three layers of parafilm. The receptor solution (4.5% FBS in PBS) was sampled at various time points and analyzed by high performance liquid chromatography (HPLC).

2.6 Tape Stripping *in vitro*

For tape stripping experiments, Franz chambers were set up as described, only with porcine ear skin mounted underneath the SLN-loaded cellulose. Fresh, intact porcine ear skin was collected from a local slaughterhouse, cleaned with 70% ethanol and rinsed in distilled water. (Skin was stored frozen at -80˚C and used within three months.) Before experimentation, the skin was examined for any damage and similar sections from each ear were cut and utilized for samples run in parallel. Skin was then mounted on a benchtop Franz diffusion chamber (37˚C water jacket). Particles were added to the cellulose as described and samples were incubated for 24 hrs, after which the cellulose was removed and the skin blotted gently, followed by air drying. Tape strips were applied with uniform pressure for 10 seconds, carefully removed, and collected in clean glass vials. Particles were extracted from the tape strips using a minimum of 0.5 ml acetonitrile. An aliquot was diluted with an equal volume of water and injected onto a Dionex Ultimate 3000 high performance liquid chromatography (HPLC) system. The sample was chromatographed using a Water/Acetonitrile (30/70) mobile phase on a 2.1 mm X 50 mm C18 column at a flow of 0.2 mL/min. The separation was monitored at 420 nm and the orcosolv peak response was quantified with an external standard.

2.7 *In vivo* Irritancy

Male Lewis rats were prepared by applying a professional depilliatory cream for sensitivive skin to an area on the back large enough to accommodate 4 cm^2 X-cell dressing. After 7 days, X-cell loaded with the nanoparticle suspension (800 μl) was applied to the back and wrapped with an elastic bandage. Bandages were stiched at 4 corners to be easily secured. After 24 hrs, bandages were removed, images were taken of the animals, and punch biopsies were collected. The biopsies were fixed in 10% formalin and embedded with paraffin for histological analysis. Tissue sections were then stained with hematoxyline and eosin (H&E), and analyzed for inflammation. All animal protocols were approved by The Johns Hopkins University Animal Care and Use Committee.

3 RESULTS AND DISCUSSION

3.1 SLN Penetration of Human Skin

The SLNs used in this study were prepared as described previously and measured to be an average diameter of 8.95 nm [6]. In order to initially characterize the interactions of this SLN formulation with skin, we utilized a human skin explant model. This tissue model was derived from freshly isolated human skin following surgical procedures. For these experiments, a fluorescent dye was added to the SLN during synthesis, to allow their visualization within the skin after topical application. Figure 1 shows a comparison of the skin penetration of 9,10-bis(phenylethynyl)-anthracene dye alone vs. dye-loaded SLN (green). Following topical administration and overnight incubation, dyed particles were observed to have deposited in the dermal layer of the skin. Though penetration was not uniform across the entire surface of the skin, we observed multiple areas where particles had completely crossed the stratum corneum.

These results indicate the potential of SLN to cross through the outer layers of intact human skin. However, due to the fact that the nanoemulsions were mainly comprised of water, with very low viscosity, they were difficult to administer topically due to uneven distribution of the liquid on skin. Evaporation of the nanoemulsion from skin was also problematic and did not allow a large quantity of particles to maintain contact with the skin surface for prolonged periods of time.

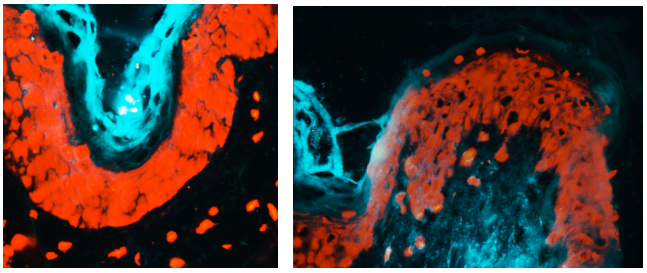

Figure 1: Penetration of dye alone (left) vs. dye-loaded SLN (right) into human skin explants. Dye and dyed particles are shown in green, counter-stained nuclei are shown in red.

3.2 Bacterial Cellulose

In order to simplify the administration of SLNs to skin and promote their continuous topical delivery, we next chose to explore the use of bacterial cellulose as a wearable SLN reservoir. For these experiments, we utilized a commercially available source of bacterial cellulose (XCell) which is partially hydrated. We first explored two methods of drying the cellulose prior to loading. Air drying of the cellulose resulted in a collapse of its structure as seen by SEM, whereas use of a critical point dryer maintained integrity and porosity of the cellulose (Fig 2). We next examined the loading potential of the material using aqueous solutions to mimic a nanoemulsion of SLN. We found that, on average, we were able to load approximately 140 μl/cm^2 water to packaged XCell and 250 μl/cm^2 water to cellulose after drying (data not shown).

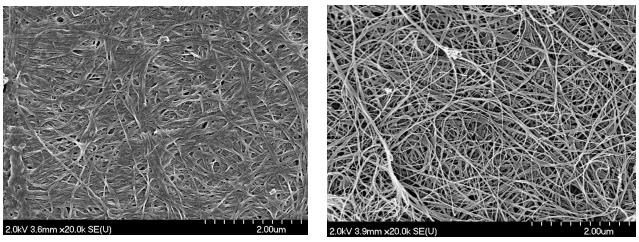

Figure 2: Bacterial cellulose after air-drying (left) vs. critical point drying (right).

3.3 SLN Diffusion from Cellulose

In order for bacterial cellulose to serve as an effective reservoir for controlled drug release, it must not only have the potential to absorb a given quantity of SLNs in suspension, but also release them slowly, over time. In order to measure the kinetics of particle release from the cellulose, we first synthesized SLN loaded with a lipophilic visible dye, orcosolv yellow. We then loaded this nanoemulsion into the Xcell, to saturation, and carried out diffusion studies, using a benchtop Franz diffusion chamber. Over time, aliquots from the receptor solution were analyzed for the visible dye by HPLC. After a measurement period of four days, approximately 85% of the dye-loaded particles had diffused into the receptor solution (Fig 3A), indicating the potential of this system for prolonged drug delivery.

We next chose to utilize the same experimental setup to examine the penetration of the particles from the SLN-loaded bandage into intact skin. These experiments were carried out by mounting the SLN-saturated cellulose onto full-thickness porcine skin, within the diffusion chamber system. Following incubation, sequential tape stripping of the skin and subsequent HPLC analysis demonstrated penetration of dye to at least the 20th tape strip collected (Fig 3B). From these measurements, we concluded that in 24 hours, the dyed particles penetrated deeply into the stratum corneum of the porcine skin model [12]. Due to the lower limit of detection of the dye by the HPLC method, it will be necessary in the future to confirm these results in a skin model using imaging techniques.

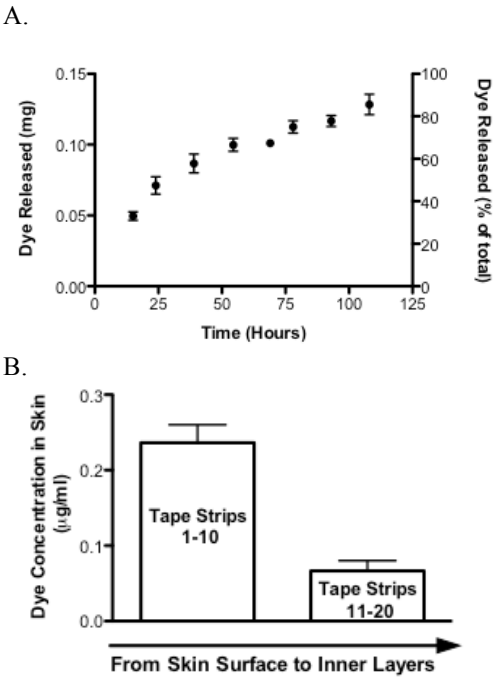

Figure 3: Kinetics of Particle Delivery in vitro. (A) Diffusion of particles from cellulose over time. (B) Distribution of dye in porcine skin layers after application. Error bars represent standard deviation.

3.4 In vivo Irritancy

We next chose to utilize an in vivo rat model to examine the potential irritancy caused by contact with SLN-loaded cellulose over a prolonged period of time. After application of the SLN-loaded cellulose to the rat model for 24 hours, the cellulose was removed and skin biopsies were collected, both at the site of administration and at a distant site. No inflammation was observed visually at the application site or at distant sites. In addition, H&E staining of this tissue showed no evidence of inflammation in any of the animals tested (Fig. 4).

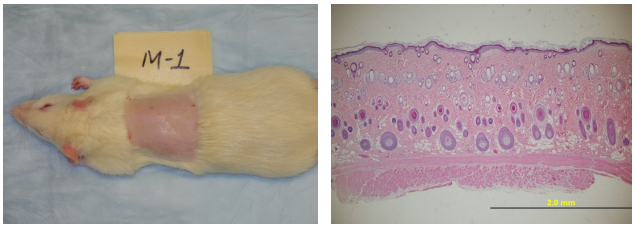

Figure 4: In vivo rat model, 24 hrs after application of SLN and cellulose (left). H&E stained cross-section of rat skin after exposure to SLN and cellulose (right).

CONCLUSIONS

In this study, we examined the potential of a commercially-available bacterial cellulose wound dressing to serve as a wearable reservoir for controlled release of SLNs into skin. We first established that the SLN formulation tested had the potential to cross the stratum corneum of human skin and reach the dermal layer. We then demonstrated that the bacterial cellulose could both absorb a significant volume of nanoparticles in aqueous suspension and slowly release them over a period of several days. We also measured the diffusion of dyed-SLNs into full-thickness porcine skin from the cellulose material. Our initial testing of the whole system in a rat model demonstrated that none of these materials was irritating in vivo after a 24 hour incubation period.

In the future, we will explore the penetration of drug-loaded SLNs as delivered via this system. By precisely controlling dosage and release kinetics, we expect to optimize the therapeutic benefit for specific skin injuries, while limiting the systemic toxicity of the incorporated drugs. This work has the potential to impact the biomedical field by optimizing localized wound treatment regimens, increasing the stability of incorporated therapeutics, and providing controlled-release of drugs over time.

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