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Physical modelling of transdermal drug delivery through non-invasive electroporation

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ABSTRACT – We are researching a method for non-invasive, transdermal drug delivery. An alternative to needle injections for the delivery of therapeutic macromolecules through the skin. The skin's outer layer, the stratum corneum, is impermeable to big and/or lipophilic molecules. To overcome this barrier, we are based on electroporation, a biological technique where high-voltage, short-duration electric pulses temporarily permeabilize lipid bilayers. Freshly-isolated skin from hairless mice is used to test the macromolecule delivery. A generator applies a pulsed electric field on the skin while we evaluate the electroporation extent through skin impedance changes and fluorescence microscopy. Our first results show that the application of a pulsed electric field rapidly decreases skin impedance, an effect attributed to the electrical breakdown of lipid bilayers. Depending on the pulse characteristics (intensity and duration), the changes observed may be reversible or permanent.

KEYWORDS – Electroporation, drug delivery, pulsed electric field, skin impedance

1. Introduction

The skin presents an accessible and convenient route for non-invasive drug delivery. Transdermal delivery platforms, such as nicotine patches, can effectively administer drugs through the epidermis in a controlled manner. Advantages include increased bioavailability, sustained steady-state blood concentration levels, painless self-administration and reduced frequency of dosing, which in turn improve patient compliance and quality of life. However, the outermost layer of the skin (stratum corneum) presents a barrier to macromolecule delivery. Passive diffusion of drugs is only achieved for low molecular weight (MW<400-500 Da), relatively lipophilic molecules (logP around 2 to 3) [1]. Electroporation can temporarily increase the permeability of the skin allowing bigger or more hydrophilic molecules to overcome the skin barrier [2].

Our goal is to create a platform that will function as both a reservoir for medicine and an electrode for the application of electrical pulses on the skin. For this, we have selected to work on an agarose hydrogel, reinforced with carbon nanotubes to increase its electrical conductivity (figure 1). The hydrogel can be dried and then reswollen in a liquid solution with a molecule of interest (e.g. a therapeutic macromolecule or a fluorescent dye). The hydrogels, loaded with a molecule of interest are placed on isolated mouse skin and a pulsed electric field is applied through a generator. We measure the electrical response of the system through a digital oscilloscope and we evaluate the macromolecule delivery through fluorescence microscopy.

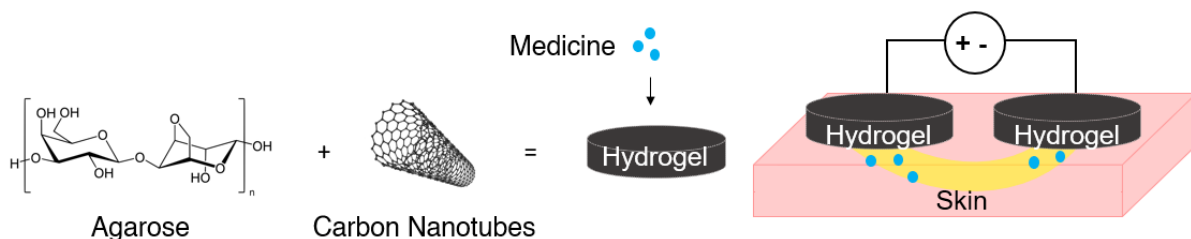


Figure 1: Transdermal delivery platform that functions as a drug reservoir and an electrode.

Lipid bilayers can be transiently permeabilized through the exposure to high-voltage, short-duration electric pulses, a technique called electroporation or electroporeabilization. Depending on the characteristics of the electrical pulses

(strength, duration, frequency, size) the permeabilization may be reversible, lasting few seconds up to minutes, or irreversible, and in the case of cell membranes eventually leading to leakage of cell components and cell death. Both versions have found numerous applications. Reversible electroporation can be used to introduce molecules such as exogenous DNA into cells (electrotransformation), to insert membrane-impermeant drugs into cancer cells (electrochemotherapy) or to extract biomolecules of interest, such as proteins from recombinant cells (electroextraction). Irreversible electroporation can be used in food technology to deactivate microbes in food and water and in medicine to ablate tissue in a non-thermal way [3]. We are interested in skin electroporation for non-invasive drug delivery, a technique that has been researched to some extent but has not yet found its way into clinical practice [2].

The mechanism by which electroporation permeabilizes the lipid bilayers is not yet fully understood. Various hypotheses exist for the effect of pulsed electric fields on a molecular level: (1) electrically induced aqueous pores on the lipid bilayer, where an initial unstable hydrophobic pore is followed by a reorganization of the polar heads to transiently form an aqueous pore; (2) electrically induced chemical changes to the phospholipids, including peroxidation, which deforms their tails thus rendering the membrane more permeable to small molecules; (3) electrical breakdown of membrane proteins, creating a permeable channel [4].

2. Materials and methods

2.1 Two-in-one electrode-reservoir platform

2.1.1 Carbon Nanotube preparation

The double-walled Carbon Nanotubes (CNTs) employed are prepared through catalytic chemical vapour disposition. The catalyst is $Mg_{0.99}(CO_{3/4}MO_{1/4})_{0.01}O$. During the synthesis, a flux of methane and hydrogel gas is decomposed at high temperature (1000 °C). The CNTs start to grow on the metal oxide support. At the end of the reaction a composite powder is obtained which contains around 10 % w/w CNTs [5].

The CNTs are then extracted from the composite powder through acid dissolution. An excess of HCl is added on the powder, dissolving the catalyst and the support while leaving the CNTs intact. The suspension is then filtered through a nitrate cellulose membrane and washed with deionised water to neutralize its acidity. The CNTs are recovered from the membrane while the aqueous acid solution passes through it.

2.1.2 Preparation of nanocomposite hydrogels

Nanocomposite hydrogels are prepared by mixing a suspension of CNTs in deionised (DI) water with a mixture of DI water and agarose (low EEO, Sigma Aldrich). Carboxymethyl cellulose (CMC, ultra-low viscosity, Fluka) is added to the CNT suspension to facilitate their dispersion (concentrations shown on Table 1). The mixture is heated to 90 °C to dissolve agarose in the water. It is then mechanically mixed before being cast on a silicone mould. Agarose is a linear polymer that dissolves in warm water (85 °C) and then gels fast as temperature falls to 40-50 °C. The hydrogels are cast on a cylindrical silicone mould with a 10 mm diameter and 2 mm height (figure 2). They are then stored in DI water at 4 °C.

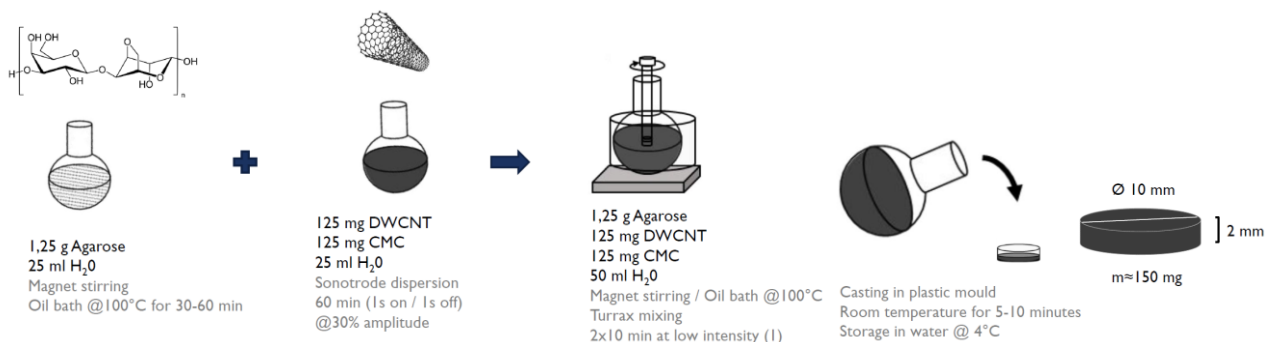


Figure 2: Preparation of nanocomposite hydrogels

Table 1: Composition of nanocomposite hydrogels

| | H ₂ O | AGR | CNT | CMC |
|------------------------------|------------------|------|------|------|
| Concentration (mg/ml) | | 25 | 2,5 | 2,5 |
| Concentration % w/w | 97,08 | 2,43 | 0,24 | 0,24 |

2.1.3 Reservoir properties of hydrogels

Agarose hydrogels dry rapidly in air. They can then be placed again in an aqueous solution and they recover approximately 20 % of their initial volume. We exploit this property to use them as drug reservoirs. The fresh hydrogels are air-dried at 30 °C for 48h, then placed in an aqueous solution with a molecule of interest. After 48h in an aqueous solution, the hydrogel has reswollen and is ready for the electroporation experiments or further characterization.

2.2 DC conductivity of nanocomposite hydrogels

The electrical conductivity of the hydrogels is measured using an Ampere meter with an integrated electrical source. The applied tension is 1 V DC and the current is measured during 4 minutes. Tension values lower than 1.21 V are chosen to avoid electrolysis. The electrical conductivity is then calculated from the tension applied, the current measured and the geometry of the hydrogel (diameter and width).

$$\sigma = \frac{I \cdot e}{U \cdot S}$$

Where U and I the tension applied and the current measured and e and S the width and the surface area of the hydrogel, respectively. The current is obtained from an average of the 10 last measurement points, where steady state conditions are reached.

2.3 Numerical model

A Finite Element Method (FEM) simulation was run on a simplified numerical model of the electroporation system (COMSOL Multiphysics v5.6). The skin is simulated as layers with different conductivities, with values obtained from the relevant literature [6].

2.4 Electroporation

For the electroporation experiments, isolated skin of hairless mice (female, SKH1 Charles River) is used. The part of the skin used for the experiments corresponds to the back and the side of the mice, between the front and back legs. The belly skin is avoided because it is considerably thinner. Depending on the mouse and the extraction process, the skin extracted can be cut into 3 or 4 rectangular pieces measuring approximately 20 x 40 mm.

The isolated skins are placed on a gauze wetted by phosphate buffer solution (PBS, Eurobio), to keep them humidified. Two hydrogels are placed on top of the skin sample, with a distance of 15 mm (centre to centre) between them. Two cylindrical metal electrodes are then placed on top of the hydrogels. The system is connected to the generator (ELECTRO cell B10 HVLV Betatech) through a custom-made shell that keeps everything in place and has a dedicated connection for the voltage and the current probes of the oscilloscope (figure 2).

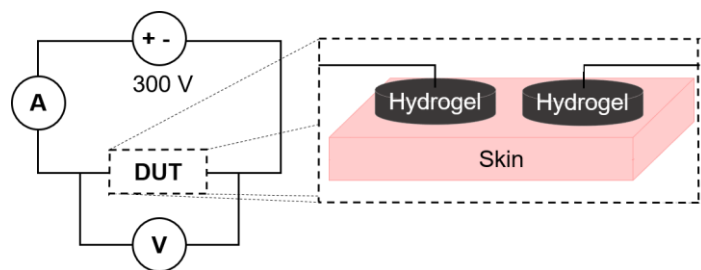


Figure 3: Electroporation configuration with voltage and current probes

The electric pulses applied are 20 ms long, with a frequency of 1 Hz, for a total of 8 pulses. The tension applied varies from 50 to 400 V. A digital oscilloscope (Tektronix 4 series mixed signal oscilloscope) measures the voltage applied and the current passing through the system during the application of the pulsed electric field. By dividing them we also get the dynamic resistance of the system.

$$R_{dy}(t) = \frac{U(t)}{I(t)}$$

3. Results and discussion

3.1 DC conductivity of nanocomposite hydrogels

We found that the percolation threshold is between CNT concentration of 1.25 and 2.5 mg/ml (figure 4). The measurements also highlight the role of the dispersant, since high concentrations of CNTs alone do not lead to a conductivity increase (not shown here). CNTs are highly hydrophobic and tend to form big aggregates in aqueous solutions. Carboxymethyl cellulose is added to make the surface of the CNTs more hydrophilic and facilitate their dispersion.

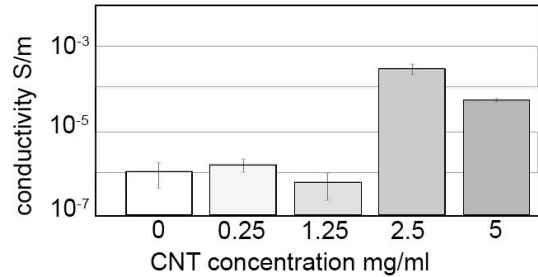


Figure 4: Nanocomposite hydrogel electrical conductivity (measured at 1 V DC) with increasing DWCNT content

3.2 Numerical model

The FEM simulations show that the system's electrical and geometrical properties play an important role in the distribution of the electric field through the skin. The current density passes through the skin and the substrate (a wet gauze) and exits through the second hydrogel/electrode (Figure 5). For a hydrogel conductivity $\geq 10^{-4}$ S/m, an electric field of 570 V/cm penetrates into the stratum corneum. This value is within the range of values for reversible tissue permeabilization, evaluated at 400-600 V/cm [6].

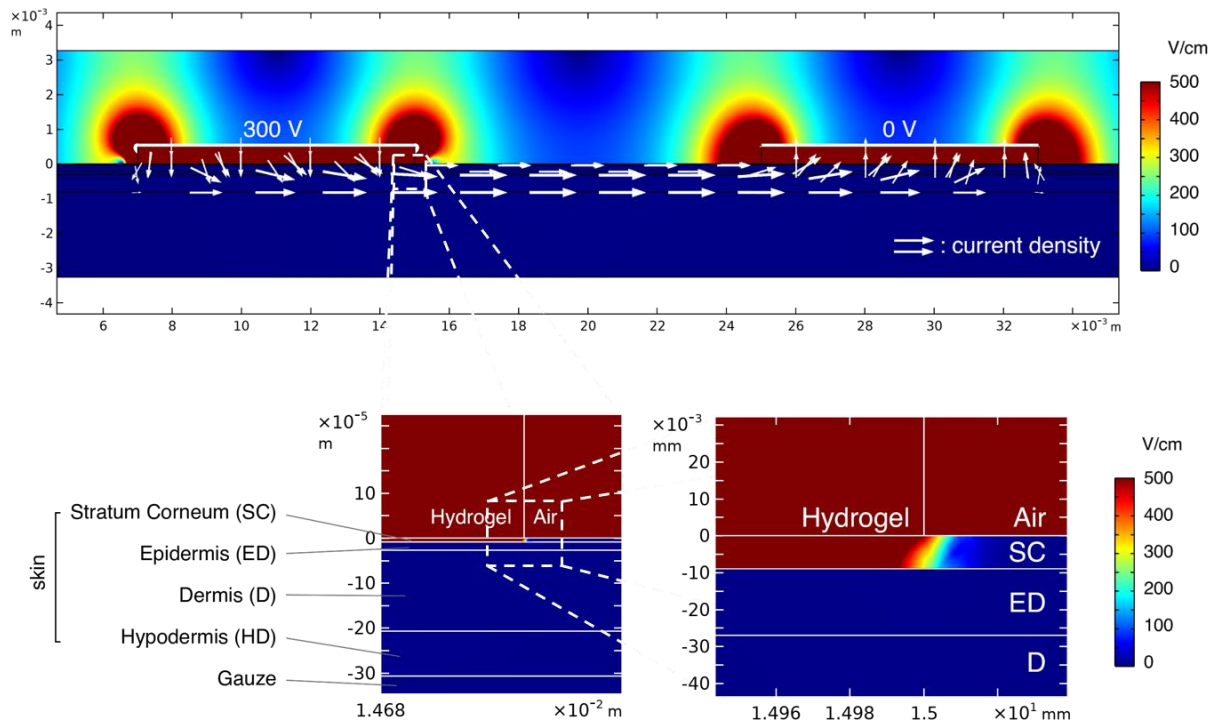


Figure 5: FEM simulation of electric field and current density distribution in hydrogels and mouse skin during electroporation (steady state). An electrical field ≥ 500 V/cm reaches the Stratum Corneum. Applied tension is 300 V.

3.3 Skin resistance during electroporation

The electrical properties measured can give us some information about the permeabilization of the skin. The dynamic resistance ($R_{dy} = U/I$) of the system is decreasing during the application of an electric pulse (figure 6). This decrease is attributed to the electroporation of the lipid bilayers of the skin [7].

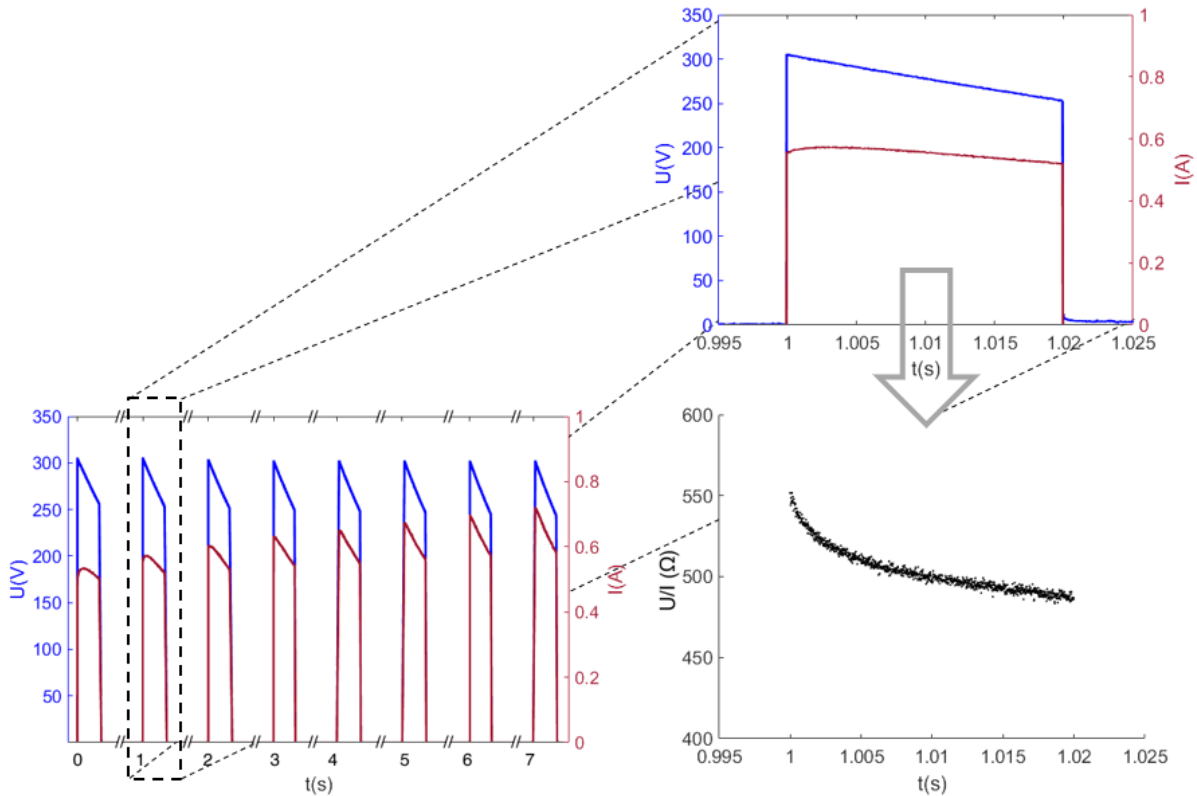


Figure 6: Electrical properties measured during skin electroporation

The application of an external electric field to a lipid bilayer increases its transmembrane potential. For a transmembrane potential over a critical value, electroporation of the membrane can be observed. Charge carriers (in this case small ions) pass through the membrane, drastically reducing its measured resistance. The skin is seen as an aggregation of multiple lipid bilayers that are affected by the electric field.

After some time, in the order of fractions of seconds and up to few minutes, the lipid bilayer recovers most of its resistance (100% recovery may last several minutes or few hours) [8]. However, if the pulsed electric field had a combination of a high strength and duration, the lipid bilayers are permanently damaged (irreversible electroporation) and the resistance does not recover.

4. Conclusion

We conceived a transdermal delivery platform that consists of an agarose hydrogel, embedded with carbon nanotubes. The hydrogel serves as a reservoir for the medicine and an electrode for the application of electrical pulses. The carbon nanotubes increased the electrical conductivity of the hydrogel by two orders of magnitude (approximately 10^{-6} to 10^{-4} S/m). The numerical model shows us that an electric field of 570 V/cm penetrates into the stratum corneum, a value consistent with the field strength for reversible electroporation.

We have set up an electroporation experiment that allows for accurate in-situ measurements of electrical properties. Additionally, we have shown that these measurements can be used to evaluate the electroporation extent. Macromolecule delivery is also being studied, through the incorporation of a fluorescent dye in the hydrogel and macrofluor microscopy of the mouse skin after the pulse application (results not shown here).

We are currently extending the electroporation experiments to a lab-made reconstructed human skin. The first results show a similar electrical behaviour as the mouse skin. These experiments highlight the potential of a non-invasive transdermal delivery platform, in the form of conductive hydrogels.

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