Coating of bioceramic microneedles

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Abstract

Needle injections can be troublesome and painful and the oral and transdermal delivery route does not suit all types of drugs. Microneedles (MNs) are an alternative to painlessly facilitate intra- and transdermal drug delivery and expand the spectra of drug types that can be delivered through the skin. Among the various materials that can be used to fabricate MNs, bioceramics could be a good alternative. This painless delivery method can e.g. be a good way to deliver sedatives and analgesia to paediatric patients. In these cases, where a rapid onset of action is desired, coated MNs containing potent drugs can enable a rapid release of a bolus dose.

In this study coating formulations including polyethylene glycol (PEG), a polyvinyl alcohol/polyethylene glycol graft copolymer (Kollicoat®IR) and sodium carboxymethylcellulose (NaCMC) were drop-coated on to MNs made of the bioceramic material calcium sulfate to explore how the coatings settled on the MNs. Release studies of sodium salicylate (SS) were performed with the formulations including the compositions that had shown the most promising distribution on the MNs.

The distribution of the coatings on the MNs varied with different compositions. PEG diffused deepest down into the MNs. Pre-treating the MNs with PEG enabled the Kollicoat®IR and NaCMC formulations in this study to form thicker layers upon the MNs compared to non-pre-treated MNs. Pre-treating the MNs with PEG may have induced a faster average release of SS from NaCMC and Kollicoat®IR coated MNs. However, this could not be confirmed as statistically significant at an appropriate significance level, thus further research with more replicates are needed. When pipetting 20 µl of the coatings on the MNs by hand, the needle tips can remain sharp, but more suitable coating methods are still needed to enable a uniform coating located on the needles only.

Keywords: bioceramic microneedles, calcium sulfate, coating distribution, release studies
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List of abbreviations and acronyms

BC 20 µl methylene blue coloured carboxymethylcellulose sodium 0.01 g/g DI water coated on MNs
BC+P 20 µl methylene blue coloured carboxymethylcellulose sodium 0.01 g/g DI water coated on MNs pre-treated with 60 µl PEG
BK 20 µl methylene coloured Kollicoat®IR 0.05 g/g in DI water coated on MNs
BK+P 20 µl methylene blue coloured Kollicoat®IR 0.05 g/g DI water coated on MNs pre-treated with 60 µl PEG
BP 20 µl methylene blue coloured PEG coated on MNs
BP+P 20 µl methylene blue coloured PEG coated on MNs pre-treated with 60 µl PEG
DI water deionized water
MN microneedle
NaCMC sodium carboxy-methylcellulose
PEG polyethylene glycol
SC stratum corneum
SEM scanning electron microscope
SS sodium salicylate
SSC 20 µl sodium salicylate 0.1 g/ml in carboxymethylcellulose sodium 0.01 g/ml DI water solution coated on MNs
SSC+P 20 µl sodium salicylate 0.1 g/ml in carboxymethylcellulose sodium 0.01 g/ml coated no MNs pre-treated with 60 µl PEG
SSK 20 µl sodium salicylate 0.1 g/ml in Kollicoat®IR 0.05 g/ml DI water coated on MNs
SSK+P 20 µl sodium salicylate 0.1 g/ml in Kollicoat®IR 0.05 g/ml coated no MNs pre-treated with 60 µl PEG
SSP 20 µl sodium salicylate 0.1 g/g in PEG coated on MNs
SSP+P 20 µl sodium salicylate 0.1 g/g in PEG coated on MNs pre-treated with 60 µl PEG
1. Introduction and objective

1.1 Introduction

Over 80% of drugs are administered via the oral route and that is indeed a convenient way to deliver drugs. Nevertheless, sometimes it is necessary to administer drugs via e.g. injection to get a rapid onset in emergency situations or with patients that have difficulties to swallow. It can also be necessary simply because not all drugs can survive the hazardous route from the mouth through the gastrointestinal tract, the hepatic portal system, and the liver to finally reach the systemic circulation or the desired place of action. An example of this is peptides and proteins, which due to enzymatic degradation, as well as their large size and hydrophilic properties make it difficult for them to pass the lipid rich membranes in the gastrointestinal tract. Still, to give drugs by injection can produce biohazardous sharp waste, be painful (this can be extra difficult for children) as well a stressful (e.g. patients that suffer from needle phobia) why most people prefer to have their medications administered in other ways if possible (1).

A drug delivery method that offers the advantages of bypassing the hazardous route of the intestinal tract and first passage metabolism and yet avoids the pain and inconveniences associated with hypodermic needles is transdermal delivery of drugs (1, 2).

Mechanisms to deliver drugs transdermal are complex and the skin is designed to act as a self-repairing barrier against external agents, such as chemicals and drugs. The utmost part of the skin is called stratum corneum (SC) and it is the primary barrier and is generally the rate-limiting layer for drugs to penetrate the skin. It consists of several layers of differentiated keratinocytes called corneocytes, which are flattened, keratin filled, nucleus free cells closely packed into lipid bilayers. These layers are constantly shed and replaced with new ones and the thickness and number of layers of the SC varies in different areas of the body. The SC swells when it is wet and is commonly around 10 μm in a dry state. This creates an intact barrier through which drugs must cross before traveling through the aqueous viable epidermis, and then finally reach the systemic circulation. To pass the SC the molecules can diffuse either through the fatty layers around and then through the aqueous environment inside of the corneocytes (transcellular) or wriggle between the bilayers of the cells (intercellular). Imbedded in the corneocytes lipid bilayers are hair follicles and glands that can act as faster alternative routes for molecules to pass through the SC compared to the earlier mentioned transcellular and intercellular diffusion. Nevertheless, these shunt routes preoccupy a relatively small area compared to the rest of the SC and often there is an excreting or secreting flow that inhibits drug delivery. Dissolved drug molecules that are able to penetrate the skin could also encounter a number of metabolically active components when in the skin. This puts certain demands on the physiochemical properties of drugs that are administered transdermal. An intact SC barrier typically inhibits molecules larger than 500 Da (e.g. larger peptides and proteins) and/or hydrophilic molecules (desirable logP 1-4) from diffusing through it, and ideally the drugs should function in relatively low doses, desirably less than 10mg/day (1).

There are many ways to interact with the SC and increase skin permeability e.g. occlusion (1). Yet another way that have been demonstrated to painlessly increase skin permeability to a range of different substances like small molecules, DNA, vaccines, peptides and proteins are microneedles (MNs) (1-3).

With lengths that can differ between tens to hundreds of μm and the possibility to fit hundreds of MNs inside a one cm² area, the use of MNs for intradermal as well as transdermal delivery of small and large drugs molecules is presently attracting a lot of attention (1). In simple terms MNs facilitate drug delivery by piercing micro-scale pores through the SC, thus creating pathways in the main barrier of the skin (1, 3). As the needles can be designed in small sizes, which make them penetrate only through the SC, the nociceptors of the skin will not be
stimulated. This in turn offers a painless delivery of drugs, and the drugs can be incorporated into the MNs in different ways (1). There have been human trials that concluded that MNs to a large extent caused little to no pain (4, 5), when designed in appropriate manners (6). Even though MNs can reach down deeper into the part of the skin where nerves appear, the reported lack of pain can be assumed to relate to the lesser risk of hitting nociceptors and providing sufficient stimulation to them (3). MNs are also estimated to offer not more or even lesser risk of infection compared to hypodermic needles (7, 8) and studies have shown that the skin recovers its barrier properties within 2 hours after MNs removal when no occlusion was applied to the skin (9).

There is a growing trend of publications in this area (10-12) and a continuing progress has been made over the past few years. This indicates that microneedles could be a promising way of delivering several different substances, such as vaccines and proteins (11). Although a variety of medical applications have been studied, focus has mainly been on dermal delivery of drugs. In vivo studies have confirmed successful administration of a number of drugs ranging from small molecules like lidocaine and phenylephrine to lager molecules like insulin (12). Nevertheless, there are still obstacles that require continues work e.g. it takes substantial work to develop proper MN coating processes (11).

In terms of approved MNs so far there are some cosmetic as well as medical products with various designs for e.g. treating scars, acne, and hyperpigmentation, and even a few influenza vaccinations. The first commercial MN product was the Dermaroller®, that is e.g. used to treat wrinkles and started to sell in the European market in 1999 and can now also be found in more places of the world (12).

MN have been fabricated in various shapes and sizes (3, 12) and as the designs and applications of MNs have been developed over the years, several materials like metals, polymers, glass, silicon and ceramics have been used to manufacture them (12). However, the MNs need to possess proper shapes as well as proper physical properties to be able to penetrate the skin without breaking (3).

Some properties of ceramic materials, such as being easy to work with, possessing sufficient mechanical strength and being biocompatible, may make them promising materials for the making of MNs (13).

In general, ceramic materials can be described as inorganic, non-metallic materials, often compounds consisting of metal and non-metal (14, 15). The properties of different ceramics vary, but they are overall hard, heat resistant, solid, brittle materials with relatively low density, and exhibit a high tolerance to severe chemical environments (14). The type of ceramics that are used for medical applications such as implants for repair, replacements or reconstruction of tissue is called bioceramics and are used for many different medical applications (16-18). Some examples of these applications are bone- and dental implants (18). Depending on what kind of bioceramics that are used, and how the tissue reacts to them, they can be divided in bioinert or bioactive. The ones that are bioactive can also be divided in resorbable or non-resorbable (17).

One resorbable bioceramic that has been demonstrated to yield MNs with the mechanical strength to penetrate the SC in vitro is calcium sulfate (CaS). To mix CaS alpha hemihydrate and water, as done in previous publications, creates a self-setting paste that can be moulded into a mechanical strong, yet biodegradable MN (13, 19).

Including the biodegradable type, MNs can overall be divided into four groups; solid MNs, hollow MNs, dissolving/biodegradable MNs and coated MNs. Solid MNs can be used to pierce micro-scale channels in e.g. skin as a pre-treatment to increase permeability. After the solid MNs are removed the holes allow for transdermal diffusion of drugs. With hollow MNs the drug can be infused or diffused trough the holes of the needles. With dissolving/biodegradable
MNs the drug can be incorporated into the needles which, when in the skin, will release the drug as they are degraded or dissolved. Since the needles are degraded no sharp waste will be left behind. The MNs may also be coated with drug formulations that can dissolve in the hydrophilic environment of the skin before the MNs are removed (12).

Coated MNs made out of biomaterial could provide benefits like simple one-step administrations and not having to worry about complications arising from breakage of needles in the skin (11). Coating the MNs or incorporating substances into the MNs also give the possibility to achieve rapid or controlled release of the drug (2) and among MNs, coated MNs are indeed an attractive alternative for rapid delivery of certain compounds. However, since the amount of active substance that can be administered via coated MNs are limited to the surface of the combined amount of MNs on the MN array, and the drug solubility in the coating solution, coated MNs are more appropriate when the drugs are potent (20).

In recent studies, where the aim was to deliver lidocaine for local analgesia, with the use of coated MNs, the results showed rapid intradermal delivery of therapeutic levels within approximately 1-minute insertion time on swine in vivo (21, 22). Another publication demonstrated complete coating dissolution within 20 seconds of one single MN, and later rapid delivery of several µg (90% of 7.86±0.3 µg) vitamin B dermally within 5 minutes via rows of coated MNs in vitro was established (20).

It is well known that children sometimes struggle with anxiety over upcoming clinical procedures and hypodermic injections can also be trying for these individuals. Therefore, to rapidly and painlessly administer systemic drugs e.g. as premedication to treat anxiety before minor procedures via coated MNs could prove beneficial in paediatric clinical practice.

Yet, to make a proper coating for MNs is difficult and involves substantial research, and the coating will need to be optimised depending on the drug of choice (11). Coating materials usual consist of formulations that have a high water-solubility (12) and the physical properties of the coating material, can affect the amount of drug settling on the MNs (21, 23), e.g. increased viscosity of the coating agent can increase the amount of coating settling on the MNs when dip-coating (23). Previous studies when applying drugs upon CaS MNs indicated that some of the substance tended to penetrate into the pores of the needles, thereby slowing down the release rate (13, 19).

To achieve fast release of a large fraction of the coated drug, it would be desirable to have a coating that did not penetrate into the pores of the ceramic to a large extent. It would also be desirable to avoid high temperatures during manufacturing to keep the coating procedure suitable for larger spectra of active compounds.

1.2 Objective
The aim of this study was to investigate different coating formulations applied on MNs made out of CaS. It was preferable to find a formulation that did not get absorbed by the ceramic material, thus creating a top layer upon the surface. Observations of how the coating settles on the MNs was made with the aim to find a composition that could create a uniform load over the needles, while still maintaining the tips sharp enough to pierce the skin. Release studies of active substance from the MNs coatings were made to find a composition with fast dissolving properties.
2. Materials and Method

2.1 Materials
The materials used in this study will be discussed in this section. The MNs were moulded using CaS, otherwise known as dried gypsum (CaSO$_4$·½H$_2$O), and is an odour-free, slightly water soluble, tasteless, hygroscopic powder. When this powder is combined with water it takes the form of a hard mass and can be used for making certain orthopaedic casts, as well as having a number of applications e.g. in the building industry (24, 25).

One of the coating formulation excipients used was polyethylene glycol (PEG) and it is a hydrophilic, stable polymer that generally does not irritate the skin. The PEG polymers can be chosen with different molecular weights, one of the lower weights being PEG 200 (and is the PEG used in this study), which is a viscous hygroscopic liquid. PEGs has many applications in e.g. the cosmetic, food and pharmaceutical industry, one of the applications being as a pharmaceutical coating agent (25, 26). PEGs have good dissolving properties and does not penetrate the SC easily (26). The next one used was Kollicoat®IR, also known as Ethylene Glycol and Vinyl Alcohol Grafted Copolymer, which is also a hydrophilic polymer with good dissolving properties. The material is slightly hygroscopic and mostly used in tablet coatings, but have other uses as well. Its good solubility is not dependent of pH value (27). The last one used in this study was sodium carboxymethylcellulose (NaCMC) and it is a tasteless and fragrance-free, white like granular powder that has hygroscopic properties when dried. It has many uses in pharmaceuticals as well as other industries. It is mainly used as a viscosity-enhancing agent in formulations, but it is also used as e.g. emulsifying agent, tablet and capsule disintegrate and binder (28).

Sodium salicylate (SS) was chosen as a model drug in this study due to its high solubility (125g/100ml (25)) and the fact that it is easy to measure with UV spectrophotometry. SS is an anti-inflammatory drug with analgesic and antipyretic properties. It is used to treat fever, pain and arthritic conditions, as well as being a constituent in a number of other medications (29).

The materials were obtained from different distributors; calcium sulfate alpha hemihydrate (CaSO$_4$·½H$_2$O; CaS) was obtained from Bo Ehrlander Eftr. AB (Gothenburg, Sweden), Kollicoat®IR was obtained from BASF (Germany), PEG 200 was obtained from Sigma Aldrich (Sweden), sodium carboxymethylcellulose was obtained from Apoteket Produktion & Laboratorier (Sweden) while sodium salicylate was obtained from Sigma Aldrich (Sweden).

2.2 Microneedles
The MNs were prepared by using a mild conditions micro moulding method already described in other studies (13, 19), with the difference that in this study the MNs was not prepared under vacuum. The used CaS powder was mixed with deionized (DI) water with a ratio of water/CaS ratio of 0.4, and then filled in silicone moulds by hand and left to cure for at least 16 hours. The CaS used to manufacture the MNs was sieved to a particle size ≤ 125 µm using a sieve shaker (AS200 Retsch, Germany) with a subsequent sieve analysis performed on the sieved powder. During the sieve analysis 100 g of the powder was sieved through 90 µm and 63 µm sieves for periods of 10 minutes running for six hours.

The MNs were characterised by using a scanning electron microscope (SEM) (Zeiss EVOLS15, Germany). The finished MNs consisted of 25 needles with the height 600 µm, which were evenly distributed on a bottom plate as seen in Figure 1. The area of each needle was 0.41 mm$^2$, giving a total needle area of 10.25 mm$^2$ and the total area including the needles and bottom plate was 58.4 mm$^2$. 
2.3 Coating formulations
To facilitate ocular inspections of MNs coatings a number of different formulations (without SS) was coloured with methylene blue. 20 µl of methylene blue coloured coating solutions were dripped with a pipette on to the needle arrays and set to dry under ambient conditions for various amounts of time depending on the coating. In some cases, a pre-treatment of 60 µl coating solution was dripped on the MNs, set to dry for 24 - 72 hours. The components used when preparing the aqueous formulations were different concentrations of Kollicoat®IR (5%, 10% and 20% in DI water), PEG (50% in DI water and 100 %) and NaCMC (1% and 2% in DI water).

The dried coated MNs were examined with stereomicroscope, and photographed using a Canon EOS 600D camera (Japan) with macro lens. The coating thickness and uniformity on the arrays was investigated. The coated MNs was also split in two to investigate the amount of methylene blue coating diffusing down into the material. If two concentrations of the same excipients appeared to have similar distribution patterns on the MN arrays, the lowest concentration of excipients was chosen. The coating concentrations and combinations that seemed to have the most uniform distribution on the arrays were chosen for release studies and these where: methylene blue coloured Kollicoat®IR at a concentration of 0.05 g/g in DI water (BK); methylene blue coloured NaCMC at a concentration of 0.01 g/g in DI water (BC); and methylene blue coloured PEG (BP). These compositions are listed in Table 1.
Table 1. Initial coating formulations.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Composition</th>
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<tbody>
<tr>
<td>BC</td>
<td>20 µl methylene blue coloured sodium carboxymethylcellulose 0.01 g/g DI water coated on MNs</td>
</tr>
<tr>
<td>BK</td>
<td>20 µl methylene coloured Kollicoat®IR 0.05 g/g in DI water coated on MNs</td>
</tr>
<tr>
<td>BP</td>
<td>20 µl methylene coloured PEG coated on MNs</td>
</tr>
<tr>
<td>BC+P</td>
<td>20 µl methylene blue coloured sodium carboxymethylcellulose 0.01 g/g DI water coated on MNs pre-treated with 60 µl PEG</td>
</tr>
<tr>
<td>BK+P</td>
<td>20 µl methylene blue coloured Kollicoat®IR 0.05 g/g DI water coated on MNs pre-treated with 60 µl PEG</td>
</tr>
<tr>
<td>BP+P</td>
<td>20 µl methylene blue coloured PEG coated on MNs pre-treated with 60 µl PEG</td>
</tr>
</tbody>
</table>

For the release studies, formulations (without methylene blue) were prepared and SS was added to the formulations, which are listed in Table 2. The solutions prepared for release studies consisted of SS that was dissolved to a concentration of 0.1g/ml in DI water containing 0.01g/ml (1%) C (SSC); SS that was dissolved to a concentration of 0.1g/ml in DI water containing 0.05g/ml (5%) Kollicoat®IR (SSK) (earlier the Kollicoat®IR formulation had been mixed with a magnetic stirrer for approximately 10 h and it should be mentioned the liquid was still not quite clear after 10 h); SS that was dissolved to a concentration of 0.1g/g in PEG (SSP). The SS release studies were performed on MNs pre-treated with 60 µl PEG as well as MNs without pre-treatment.

Table 2. Coating formulations used for release studies.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Composition</th>
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<tbody>
<tr>
<td>SSC</td>
<td>20 µl sodium salicylate 0.1 g/ml in sodium carboxymethylcellulose 0.01 g/ml DI water solution coated on MNs</td>
</tr>
<tr>
<td>SSK</td>
<td>20 µl sodium salicylate 0.1 g/ml in Kollicoat®IR 0.05 g/ml DI water coated on MNs</td>
</tr>
<tr>
<td>SSP</td>
<td>20 µl sodium salicylate 0.1 g/g in PEG coated on MNs</td>
</tr>
<tr>
<td>SSC+P</td>
<td>20 µl sodium salicylate 0.1 g/ml in sodium carboxymethylcellulose 0.01 g/ml coated no MNs pre-treated with 60 µl PEG</td>
</tr>
<tr>
<td>SSK+P</td>
<td>20 µl sodium salicylate 0.1 g/ml in Kollicoat®IR 0.05 g/ml coated no MNs pre-treated with 60 µl PEG</td>
</tr>
<tr>
<td>SSP+P</td>
<td>20 µl sodium salicylate 0.1 g/g in PEG coated on MNs pre-treated with 60 µl PEG</td>
</tr>
</tbody>
</table>

2.4 Release studies
The release studies were conducted by using a 50 ml glass beaker containing 25 ml of DI water which was stirred with a magnetic stirrer at roughly 100 revolutions per minute. A MN array, which had been coated with 20µl of the SS solution, was positioned in a basket that was placed in the water. Samples of 1 ml were gathered and replaced with 1 ml of DI water at 10, 20, 30, 60 and 180 minutes. To enable analysis additional 2 ml of DI water was added to each of the samples (this dilution was later compensated for in the calculations of SS concentration and mass) whereupon the samples were analysed in a spectrophotometer (Cecil 3041 UV-Visible, Scanning Spectrophotometer, UK) at 295 nm. To determine the concentration a standard curve was composed by measuring the absorbance at different SS concentrations. By using this standard curve, shown in Figure 2, the concentration and mass of released SS in the samples could be calculated. These values could then be used to calculate the total amount of released SS at the times when the samples were collected. Three replicates were conducted.
with each formulation. The coating formulations used in the release studies can be found in Table 2.

![Figure 2. Standard curve used to calculate sodium salicylate concentrations and mass.](image)

### 3. Results and discussion

#### 3.1 Microneedles

Particle size in the CaS powder can affect the properties of the curing paste (30) and therefore affect the needle properties, which is why a sieve analysis was conducted. The sieve analysis of the powder used to manufacture the needles showed that after 6 hours (36 x 10 minutes) the total mass of the powder sample was divided into different fractions; 26% was found to be ≤125 µm, 32% was ≤90 µm and 42% was ≤63 µm.

Initial attempts of micro moulding MNs proved to be quite difficult and the yield of sharp needles was poor. **Figure 3** illustrates pictures taken with the use of scanning electron microscopy (SEM) of one of the sharper MN arrays produced in this study. As can be seen, the CaS material exhibits a porous structure.

![Figure 3. Pictures of the microneedle array (left) and a zoomed in microneedle tip (right) taken with scanning electron microscopy.](image)

#### 3.2 Coating formulations

Distribution of 20 µl coatings that was set to dry upon the MNs, were observed with a stereomicroscope and photographed. As can be seen in **Figure 4**, the coatings (without 60 µl PEG pre-treatment) were distributed as a thin layer upon the MN arrays and bottom substrate. BC and BK created an even blue layer upon the MN arrays, whilst BP appeared to create a somewhat uneven colouring on the bottom substrate.
To examine the coating diffusion into the MN material the needles were split and inspected. Splitting of the needles showed that some of the methylene blue coatings entered into the material as demonstrated in Figure 5. For BC and BK the blue colour has penetrated slightly into the ceramic material. In a previous publication that explored release from coated CaS MNs, the coating material was suggested to have diffused into the pores of the bioceramic (19) which was assumed also to be occurring in this study. This diffusion may not be optimal, since a rapid release of drugs is desirable in this scenario, and to have the drug diffusing into the ceramic material could mean a longer distance that the drug would have to diffuse (before the ceramic degrades) to finally get released into the skin. Other factors such as molecular interactions between the drug and the MNs material, as well as concentration gradients could possibly also hinder a fast release if the drug were to diffuse too far into the CaS.

Since BP entered the material the most, 60 µl of this coating was dripped on the MNs and set to dry before the potential drug coating was applied. See Figure 6 to compare a not pre-treated MN array to a MN array pre-treated with 60 µl PEG. The meaning of this was to investigate if PEG could saturate the pores of the bioceramic thus preventing the outer drug coatings from
penetrating too deep in to the needles. However, when the BP was applied to the pre-treated MNs (BP+P) the blue colour still diffused deep down into the material.

Figure 6. Comparison between non pre-treated microneedles (left) and microneedles pre-treated with 60 µl PEG (right).

On the other hand, as shown in Figure 7, BC that was applied to MNs pre-treated with 60 µl PEG (BC+P) as well as the BK that was applied to MNs pre-treated with 60 µl PEG (BK+P) were evenly distributed as thicker layers upon the MN arrays. Thus, giving the impression of the coatings not diffusing down to the same extent into the ceramic material as with MNs not pre-treated with 60 µl PEG. Nevertheless, interactions between the pre-treatment PEG and the top coatings cannot be excluded as diffusion of the top coating as well as PEG may have occurred and could thereby have created a mix of them. Thus, the actual composition of the top layers cannot be guaranteed to consists of only BC and BK (if PEG that already was incorporated in the MNs was to have diffused up into the top coatings). This would have to be further investigated. To be able to add a thicker layer upon the needles could be a good way to incorporate more drug onto the MNs compared to when using a thinner layer. However, the layer should not be too thick, because of the risk of making the tips of the needles not sharp enough to push through the SC.

Figure 7. Microneedles pre-treated with 60 µl PEG. Coated with BC+P (left) and BK+P (right). BC+P = 20 µl methylene blue coloured sodium carboxymethylcellulose 0.01 g/g DI water coated on microneedles pre-treated with 60 µl PEG. BK+P = 20 µl methylene blue coloured Kollicoat®IR 0.05g/g DI water coated on microneedles pre-treated with 60 µl PEG.

When the formulations made for the release studies, listed in Table 2, were examined the SSC applied to MNs pre-treated with 60 µl PEG (SSC+P) demonstrated a quite uneven distribution upon the MN arrays (when dried) compared to the BC+P as shown in Figure 8. Changing the formulation from BC+P to SSC+P seems to have altered the physical properties of the coating considerably. This is a good example of how when creating a MN coating all components and amounts of them, as well as the properties of the MNs material, need to be taken into consideration. For example, a change in pH value may affect the interactions between the components of the coating and the CaS MNs. That coatings made for MNs need to be adjusted to best fit the drug of choice has already been concluded (11). Since the most noticeable difference between BC+P and SSC+P (aside from the blue colour) is the adding of SS to SSC+P,
this may be an example of how some coatings need to be adapted after the drugs they are meant to carry. One the other hand, when looking at BK+P and SSK+P the surface of the coatings appears to be quite similar, according to ocular inspections, making this an example of how formulation components may interact in different ways with different components.

Figure 8. Examples of coated microneedles pre-treated with PEG. BC+P = 20 µl methylene blue coloured sodium carboxymethylcellulose 0.01 g/g DI water coated on microneedles pre-treated with 60 µl PEG. BK+P = 20 µl methylene blue coloured Kollicoat®IR 0.05g/g DI water coated on microneedles pre-treated with 60 µl PEG. SSC+P = 20 µl sodium salicylate 0.1 g/ml in sodium carboxymethylcellulose 0.01 g/ml coated no microneedles pre-treated with 60 µl PEG. SSK+P = 20 µl sodium salicylate 0.1 g/ml in Kollicoat®IR 0.05 g/ml coated no microneedles pre-treated with 60 µl PEG.

Figure 9 gives a closer look on the distribution of coatings on the needles and it appears as if the surface of the needles becomes smoother when PEG is added. From an ocular perspective, applying 20 µl PEG does not seem to have smoothed the surface remarkably, while adding 60 µl PEG created a noticeable change in surface structure. Further analysis is needed to conclude if the material is less porous in this state, but it does seem like saturation of CaS might be done with PEG.
Figure 9. Close-ups of microneedles to compare the difference between uncoated microneedles with microneedles treated with PEG. BPEG = methylene blue coloured PEG.

When looking at the MNs coated with PEG (Figure 9) it would seem that the sharpness of the needles is still present. The same could be stated for the other coatings regarding the sharpness (Figure 10). BC seems to be creating a slightly smoother surface compared to BK. This could indicate that the NaCMC creates a slightly thicker layer upon the MNs and penetrates the ceramic material slightly less then Kollicoat®IR when the MNs are not pre-treated with 60 µl PEG.

Another obvious thing that can be observed in (all) the pictures in this paper is that all the coatings seem to be situated onto the substrate bottom plate to a quite large extent. This could be further determined with a method that involves dipping only the needles into a dissolution bath, this by pushing them through a membrane on top of a water reservoir. When the total amount released is measured the amount on the bottom plate could be calculated. When the MNs are pre-coated with 60 µl PEG the thicker sections of BC+P, SSC+P, BK+P and SSK+P appears mostly in the lower regions of the MN arrays. It would be desirable to have a coating formulation that created a more consistent load over the whole needle (while still maintaining the sharpness of then needle edge) thereby enabling more drug to be carried through the SC with the needle. The fact that these coatings were dripped on by hand makes the task of applying the compositions only to the needles quite difficult. An apparatus that could provide microscale precision when dripping the coating onto the MNs could facilitate in this matter. Still, the observations of the coating properties examined in this study, especially how they are distributed on the CaS MNs (as well as the release rates observed) may work as guidance when developing an appropriate coating method for these bioceramic MNs.
**Figure 10.** Close-ups of microneedles with NaCMC and Kollicoat®IR coatings in different formulations with and without PEG pre-treatment. BC = 20 µl methylene blue coloured sodium carboxymethylcellulose 0.01 g/g DI water coated on microneedles. BC+P = 20 µl methylene blue coloured sodium carboxymethylcellulose 0.01 g/g DI water coated on microneedles pre-treated with 60 µl PEG. SSC+P = 20 µl sodium salicylate 0.1 g/ml in sodium carboxymethylcellulose 0.01 g/ml coated no microneedles pre-treated with 60 µl PEG. BK = 20 µl methylene coloured Kollicoat®IR 0.05 g/g in DI water coated on microneedles. BK+P = 20 µl methylene blue coloured Kollicoat®IR 0.05 g/g DI water coated on microneedles pre-treated with 60 µl PEG. SSK+P = 20 µl sodium salicylate 0.1 g/ml in Kollicoat®IR 0.05 g/ml coated no microneedles pre-treated with 60 µl PEG.
3.3 Release studies

To compare the release patterns of different coating formulations the model substance SS was added to different coating compositions, listed in Table 2. The coating was then applied to the MNs. The amount of SS released in water was then measured at different time points. Due to lack of time and initial difficulties in fabricating the ceramic MNs the initial release studies were performed with needles that had not turned out as sharp and consistent as desired. Later, the two formulations with the most promising release rates were picked for complementary tests with the same design as the previous release studies, but with sharper MNs.

The results are illustrated in Figure 11-13 where it can be seen that all the used coatings demonstrated release of SS in a relatively rapid manner, which can be positive when a fast onset of action is desired. Also illustrated in the figures are 95% confidence intervals, these did not confirm a statistical significant difference for the release rates. Still, this data can be considered as indications that should be verified with further replicates. It tended to look like the fastest release rate was achieved with SSC+P and SSK+P coated MNs and it does appear as pre-treating with PEG could be favourable to achieve a rapid release with the SSC and SSK top coatings.

As can be seen in Figure 11 the average released fraction for SSC after 10 minutes was 31%, which can be compared to SSC+P released fraction, which was 50% in the initial tests and 51% in the complementary tests with pointy MNs. The SSC+P initial and complimentary tests exhibit a similar release pattern, which could mean that the geometry of the needles did not have a considerable impact when using this formulation. The effect of geometry of the needles has previously been shown not to have a substantial impact on the release rate when the length of the needles differed between 450 and 600 µm (19).

When pre-treating the SSK coated MNs, the average released fraction after 10 minutes for the initial tests with SSK+P was 44% and the complementary tests with pointy MNs was 54% compared to 26% from the not pre-treated MNs, which is illustrated in Figure 12. It appears as the release of SS is somewhat faster in the complimentary tests with pointy MNs. One proposed explanation could be the that higher needles tips may have contributed to a somewhat larger area that the drug could dissolve from. Nevertheless, to establish if there actually could be a significant difference between geometries, as mentioned earlier, more
replicates than the three done in this study are needed. Preferably more tests should be done with a standardised release testing method as well.

\[ \text{Figure 12. Average released fraction for SSK coatings over time with microneedles with and without PEG pre-treatment. SSK+P (s) was microneedles with sharper needles. The error bar represents a 95\% confidence interval with 3 replicates. SSK} = 20 \, \mu\text{l sodium salicylate 0.1 g/ml in Kollicoat® IR 0.05g/ml DI water coated on microneedles. SSK+P} = 20 \, \mu\text{l sodium salicylate 0.1 g/ml in Kollicoat® IR 0.05 g/ml coated no microneedles pre-treated with 60 \, \mu\text{l PEG.}} \]

On the contrary to the SSC and SSK, per-treating the MNs before the SSP coating was applied seems to have decreased the release rate, as can be seen in Figure 13. It may be speculated that the reason for this could be due to diffusion of SS through the PEG deeper into the MN. If SS has diffused into the already present PEG within the MN it could theoretically affect the concentration gradient negatively since the SS is not only concentrated in the outermost top layer, thus giving a slower release rate. A longer diffusion path to the surface of the MN could also slow down the release rate. But more information is needed to confirm if this is the actual mechanism behind the slower release rate proposed in this study for SSP+P compared to SSP.

As seen in the earlier mentioned figures, comparing the not pre-treated MNs, the SSC and SSK also tends to have a slightly more rapid release rate compared to SSP and could be considered as more promising top coatings for further investigations, when looking at the average release rates.

\[ \text{Figure 13. Average released fraction for SSP coatings over time with microneedles with and without PEG pre-treatment. The error bar represents a 95\% confidence interval with 3} \]
replicates. \( SSP = 20 \mu l \text{ sodium salicylate } 0.1 \text{ g/g in PEG coated on microneedles} \). \( SSP+P = 20 \mu l \text{ sodium salicylate } 0.1 \text{ g/g in PEG coated on microneedles pre-treated with } 60 \mu l \text{ PEG} \).

It should also be mentioned that the values in the release calculations is based upon estimations that 20 µl top coating contained 2 mg SS. The statement that the PEG coating applied to the MNs used in the release studies contained 2 mg SS is somewhat misleading. Since the amount distributed upon the MNs was 20 µl and the SSP was composed of weight per weight (w/w), on the contrary to the other SS-formulations that were composed of weight per volume (w/v). When the concentration is based on w/w density needs to be taken into consideration (a factor not considered in the initial studies). Nevertheless, since the density of PEG 200 alone is slightly higher than 1 (1.124 g/ml at 20°C according to Sigma-Aldrich) this would suggest the SSP coating to contain slightly more SS, but relatively close to 2 mg. Also, the release data from both SSP and SSP+P indicated the release to be slower than the other formulations. Therefore, more accurate complimentary tests were not prioritised at this time.

During the release studies the two used magnetic stirrers appeared to slowly gain some heat during the process. It was noted that the water temperature started at 21°C and ended up approximately at 24°C with one stirrer and at 28°C with the other one after three hours. This cannot be excluded as a source of error when comparing the dissolution rates. Nevertheless, when looking at the data, it seemed as though this did not affect the dissolution rate to a great extent.

Further studies are needed to refine these coated bioceramic MNs. Appropriate coating methods should be investigated more thoroughly, e.g. drop coating that probably would be facilitated by an apparatus that could provide a micro scale precision to the process. Besides micro scale precision, this kind of method may have other demands on the properties of the coating such as viscosity and surface tension. These factors can be important when applying a coating with appropriate thickness, meant to stay located on the needles, while still maintaining their sharpness.

While the method mentioned above may require certain properties of the coating material, another method e.g. dip-coating trough micro pores fitting the needles (to get a uniform coating that is located only on the needles) may require other properties of the coating. Dipping MNs through dip holes has previously been regarded as a successful method to coat e.g. solution containing NaCMC onto MNs (20).

Skin penetration, reaction and delivery data also needs to be gathered. Since the skin has properties making it an environment different to the release method used in this study e.g. pH buffer, body temperature, and degradation of CaS, different release rates in skin compared to the dissolution method used in this study is not unlikely. Potential effects on degradation of CaS, due to pre-treatment of the bioceramic material with PEG also need to be investigated. If incorporation of drugs into the bioceramic for a controlled release over time is desired, as done in other studies (13, 19), this may be of great importance. In this scenario, the coating may work as a bolus dose before the release from the degrading needles.
4. Conclusions

This study has demonstrated that when different coating formulations are applied to CaS MNs, the distribution of the coatings on the MNs varied with different compositions. Of the formulations investigated here, PEG diffuses deepest down into the bioceramic material. Pre-treating the MNs with PEG enables the Kollicoat®IR and NaCMC formulations in this study to form thicker layers upon the substrate compared to non-pre-treated MNs.

When pipetting 20 µl of any of the coatings presented in this study to these MNs by hand, the needle tips can remain sharp, but more suitable coating methods are still needed to enable a uniform coating located on the needles only.

In this study it was not possible to conclude that pre-treatment of the bioceramic with PEG saturated the pores to hinder the top coatings from diffusing down into the material to enable faster release of the drugs. Yet, when pre-treating the MNs with PEG there was a tendency towards faster release of SS from SSC and SSK coated MNs. However, more tests are needed to confirm a statistical significance at an appropriate significance level.

When composing an appropriate coating for these MNs, factors like coating viscosity and active substances may substantially affect the coating distribution on the MNs. There is still a lot of research needed before a finished coating method can be presented. Yet, the data presented in this study may be used for future coating development, hopefully being a contribution in this exciting and promising new field of bioceramic MNs.

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