Cellulose based thin films as a platform for drug release studies to mimick wound dressing materials

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Abstract

In this paper, the use of ultrathin cellulose supports as platform for the incorporation of analgesic drugs in wound dressings is proposed. As a model drug, diclofenac (DCF) is chosen, which is commonly used in pain easing medical treatments. The DCF containing cellulose films are prepared by mixing solutions of trimethylsilyl cellulose (DS_{Si}:2.5) with diclofenac dissolved in THF. After depositing the material on a solid surface by spin-coating, the films are subjected to vapor-phase hydrolysis using 3 M HCl in order to achieve regeneration of cellulose. The release of DCF from these films over time is studied by UV-VIS. Upon deposition of additional layers of cellulose that do not contain DCF, the release from these films can be decelerated significantly. The release kinetics from these films is very similar to those of viscose fibers impregnated with DCF solutions. These studies indicate a potential use of cellulose thin films as model platform for viscose based wound dressings.

Keywords: cellulose, model platform, wound dressings, TMSC, diclofenac, release studies

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Introduction

In the design of new healthcare materials, an important issue is how to comply with extremely high quality standards while considering appropriate safety precautions. Especially, if human use is envisaged such efforts are particularly challenging as they are usually quite expensive (White 2011; World Wound Care Markets 2011 2011). Currently, wound care is one of the fastest growing markets for medical materials (World Wound Care Markets 2011 2011). Novel approaches in wound dressing design aim at either enhancing the healing efficiency of defective tissues or at decreasing the sensation of pain that accompanies many different wound types through inclusion of analgesic or anesthetic drugs (Davies and White 2011; Kitchener 2010; Benbow 2010; Bell and McCarthy 2010; Romanelli et al. 2009). One of the most promising approaches to achieve the latter comprises the incorporation of desired drugs into polysaccharides (and their derivatives), which already have an important position in the wound dressing market. Polysaccharides exhibit a wide range of beneficial properties for wound healing, since they are biocompatible, mostly biodegradable, and often feature a high water uptake capacity (Thomas 2008). Materials with a high water uptake capacity and long release times are able to enhance healing rates and limit the extent of wound scaring since the wound is kept wet/humid (Gantwerker and Hom 2012). Additionally, wound excretions can be soaked into such polysaccharide matrices, which contributes to enhanced regeneration of damaged tissues (Widgerow 2011; Richetta et al. 2011; Hurlow and Bowler 2009). In particular, cellulose, one of the most abundant organic biopolymers on earth, exhibits many interesting properties that make it an ideal candidate in wound care, as well as for other healthcare applications (Czaja et al. 2006). Cellulose features high biocompatibility and mechanical strength, while its water uptake capacity can be easily tuned using different pretreatments (Pivec et al. 2013; Peršin et al. 2014; Stana-Kleinschek et al. 2011).

In dependence of wound type and size, wound dressings are designed to bear different characteristics. Besides the stoppage of blood loss, wound dressings maintain a moist environment at the tissue-dressing interface, remove excess exudate, provide thermal insulation and mechanical protection, act as barriers for micro-organisms, allow suitable gaseous exchange and should be easily removed without causing additional trauma accompanied with sensation of severe pain (Patton et al. 2013; Klode et al. 2011; Jurgens et al. 1995). While all mentioned characteristics are thought important in advanced wound treatment (and are treated as such), many recent studies have been conducted to assess the influence of pain on the wound healing process. Based on scientific and clinical evidence, pain can significantly slow down the healing process (mostly through stress induced release of hormones like cortisol and norepinephrine), which results in decreased patient quality of life as well as in exponentially increased personal and public expenditures (Gantwerker and Hom 2012; Solowiej et al. 2010; Petrulyte 2008). Effective and safe pain reduction is therefore very important in the course of the healing process for different types of wounds. Non-steroid anti-inflammatory pain-killing drugs (NSAIDs) have been proven very effective in pain reduction as they feature anti-inflammatory activity as well, which is of particular importance for the treatment of chronic wounds. Since the risk of unwanted side effects with NSAIDs systemic administration is relatively high, their direct delivery to the wound and surrounding tissue could significantly increase the safety and efficiency of related therapies (Gaufberg et al. 2007; Alessandri et al. 2006; Jorgensen et al. 2006). The drug can be incorporated into wound dressing materials *in situ* or *ex situ* (after host

material preparation). Whatever the approach, a desired drug release profile has to be achieved in terms of the biological activity, frequency of the wound dressing exchange, and of course treatment costs.

In this paper, we address these issues by a model approach, which involves the use of cellulose ultrathin films as active drug carrying materials. As a model drug, diclofenac is chosen, which is commonly used in pain easing medical treatments. In the first part of the manuscript, we will describe how to prepare and characterize diclofenac containing cellulose ultrathin films starting from trimethylsilyl cellulose, while in the second part the release of diclofenac from these films is studied under various conditions and compared with those of viscose fibers. Surprisingly, the results indicate that the model approach on cellulose thin films is highly comparable to release from cellulosic fibers (e.g. viscose) allowing for direct comparisons in terms of release kinetics. Therefore, we propose the model approach as a fast and cheap alternative to current testing systems available on the market for studying drug release. In addition to its use as a platform for release testing of new drugs from cellulose materials, this approach may also serve as a component in actual wound dressings which allows to tune drug release kinetics by simple additional spin coating steps that are capable to introduce additional functional layers (e.g. antimicrobial activity) acting additionally as physical barriers.

Materials and methods

2.1. Materials

Trimethylsilylcellulose (TMSC) with a DS value of 2.5 (from TITK, Rudolstadt, Germany; Mw= 50000) and diclofenac sodium (DCF) obtained from Sigma-Aldrich, were used as the starting material for film preparation. Regenerated cellulose fibers were studied in their non-woven form, i.e. viscose (CV), as produced by KEMEX, The Netherlands. The specific surface area of the used viscose fabrics was 175 g/m^2. Tetrahydrofuran (THF) (99.9%) was purchased from Carlo Erba. For contact angle measurements and as acceptor medium in release studies Milli-Q water (resistivity = 18.2 Ω^{-1} cm^{-1}) from a Milli-Q-water system (Millipore, USA) was used. Quartz crystal microbalance (QCM-D) sensors coated with a gold layer (QSX303) were purchased from LOT-Oriel (Germany).

2.2. Film and fibers preparation

Silicon wafers (Topsil, Germany) were used as base-substrates for film preparation. Prior to spin-coating, the silicon wafers were cut into pieces of 1.5 × 1.5 cm^2, subjected to piranha treatment (soaking in an H_2O_2(30%)/H_2SO_4 (conc.) mixture (1:7 v/v)), rinsed thoroughly with MQ water and finally dried in a stream of dry nitrogen of high purity (5.0). For the preparation of the spin coating solution, TMSC was dissolved in THF with 0,33 wt% for pure TMSC films and in 1 wt% for films with incorporated drug. The latter was prepared as follows. The 1 wt% TMSC solution was added to a THF solution of DCF (1.0 wt%) in a ratio of 1:2 (TMSC : DCF), resulting again in 0,33 wt% TMSC. For spin coating, 200 µl of the respective solution was deposited on the static substrate and subjected to spin coating (v = 4000 rpm, a = 2500 rpm s^{-1}, t = 60s). Afterwards, the films were regenerated for 15 minutes at room temperature in a petri dish, containing vapors of HCl. This process yielded layers with thickness of approximately 50 nm. For some experiments an additional layer of cellulose was prepared on top of these regenerated cellulose surfaces by spin coating a 0.33wt% TMSC solution onto the as-prepared samples, followed by regeneration as described above.

Viscose fibers (1 cm^2) were impregnated with 1 mg/ml solution of DCF (Sigma Aldrich, Munich, Germany) in Milli-Q water (≈18.2 MΩ·cm at 25 °C) from a Milli-Q-water system (Millipore, USA). This process was performed at room temperature (25°C) for 30 minutes.

2.3. Contact angle measurements

Static contact angles (SCA) of water were measured by using OCA15Pro contact angle measurement system Dataphysics (Germany) with the sessile drop method. All measurements were conducted at room temperature with a drop volume of 3 µl. On each surface (pure TMSC, TMSC with DCF and both regenerated surfaces) at least three drops were deposited. Determination of the SCA was performed with the software provided by the manufacturer (software version SCA 20.2.0).

2.4. Infrared spectroscopy

FTIR-ATR spectra were recorded using a PerkinElmer Spectrum GX Series-73565 FTIR-spectrometer at a scan range of 4000–650 cm^{-1}. QCM-D quartz crystals coated with a gold layer were used as substrates for FTIR-ATR measurements. The spin coating and regeneration of TMSC were performed in the same way as with silicon wafers. The scans were performed on three different places of each sample.

2.5. Atomic force microscopy (AFM)

Atomic force microscopy (AFM) was used for surface roughness determination, examination of the film porosity and thickness. As-prepared samples were dried under high grade nitrogen (5.0). The samples were attached onto round shaped metal disc sample holders and mounted on the Nanosurf FlexAFM (Switzerland).Topography images and film thicknesses were acquired in tapping operation mode. For the film thickness analysis the film was partially removed mechanically and the cross section was measured. Silicon AFM tips (Budget Sensors Tap190Al-G, Innovative Solutions, Bulgaria) with a nominal spring constant of k= 48 N/m and a nominal resonance frequency of 190 kHz were used for imaging purposes for all samples. Images of 2.5 × 2.5 μm^2 were recorded with a resolution of 512 × 512 pixels. All images were processed and the corresponding roughness was calculated using Gwyddion 2.31 software.

2.6. Release studies – UV-VIS spectroscopy

The release of DCF from the as-prepared samples was studied based on the standard method for testing material performance for wound dressing applications using the Franz diffusion cell. An adaptation of the commonly used experimental setup had to be used to avoid sample loss due to withdrawal. The released drug amount was quantified by UV–VIS spectroscopy at 275 nm (Cary 50 UV-Visible Spectrophotometer, Agilent, Germany). For this purpose each film was transferred to a 15 ml glass bottle, containing 7 ml of Milli-Q water. The temperature was maintained at 37±0,2 °C. At defined time intervals the amount of released drug was quantified by transforming the measured absorbance for each sample to concentration using the Beer-Lambert law. The release measurements of at least three parallels of one layered regenerated TMSC films with DCF and three parallels of films with an additional regenerated TMSC layer were performed and evaluated. The release curves are presented either as percentage of the released amount of the incorporated drug or as the measured concentration. The incorporated amount was calculated from the concentration, measured after the release curves reached the

release end point (dc/dt=0), which was confirmed by FTIR-ATR measurement. A more thorough explanation follows in the discussion section. Finally, the first derivatives of the release data from the film samples were calculated to expose the multi-mechanism release nature.

In vitro drug release studies for viscose with incorporated DCF were performed using static Franz diffusion cells. A piece of viscose with a well-defined size (10 mm^2) was placed on top of a cellulose acetate membrane. The receptor compartment was filled with Milli-Q water and its temperature was maintained at 37±0,2 °C. During the dissolution testing the medium was stirred continuously with a magnetic bar. Samples were collected over a period of 24 hours at the same intervals as for the model films. The withdrawn sample volumes were replaced by fresh milli-Q water. During the release profile examination and calculation of concentrations from the measured absorbance, this dilution was accounted for and results were normalized. All release studies were performed in three parallels.

Results and Discussion

As already mentioned in the introduction, the development of advanced wound dressing materials exhibiting several simultaneous functions is an expensive effort. Complex products used in healthcare have to pass a multi-phase safety and efficiency testing, but also these do not always allow for quantitative characterization as their final form excludes the use of sophisticated methods devoted to the examination of more defined samples. Both arguments lead to in-company weighing of development and consecutive commercialization of even clinically already proven products (in terms of safety and efficiency). While development reaches multimillion investments, alternative methodologies on how to decrease the development costs of such advanced and often complex products that consist of several interacting components (host material, drugs etc.) have to be considered. Common approaches towards cost reduction mostly include simplification and miniaturization; therefore preparation of model films with simulated chemical composition of the final product, is a promising approach. The goal of this study is therefore to investigate to which extent ultrathin cellulose films can serve as a suitable model platform to assess the performance of viscose-based wound dressings in terms of their controlled drug release potential, and their overall performance towards application in wound treatment.

To evaluate the proposed model and our hypotheses, cellulose thin films containing a potentially therapeutic dose of the NSAID diclofenac (DCF) were prepared. Thin films are ideal for characterization purposes, since they provide a defined morphology enabling a detailed characterization of their surface properties as well as for modeling of drug release, especially for controlled release delivery systems. Although cellulose thin films and viscose fibers consist of cellulose, their different morphologies and accessibilities do not necessarily allow for a direct comparison of the two materials regarding the drug release kinetics. However, it can be expected that most of the DCF is located at accessible sites at the viscose fibers as well as for the model films. In order to explore possibilities to further tune and to control the release, another layer of cellulose was deposited on top of these films without incorporated DCF (Figure 1). This additional layer serves as a physical barrier with a significant impact on DCF diffusion from the films. By variation of the physico-chemical characteristics (layer thickness, porosity etc.) of this additional layer, tuning of the release time and rate can be achieved. The latter is highly desirable since successful analgesia in patients suffering from different wound types is not possible without proper space and time bound release control. For instance, acute trauma-induced wounds require immediate pain reduction (drug release), while for chronic wounds a long lasting pain reduction is favorable.

Synthesis and Characterization of TMSC+DCF and Cellulose+DCF films

One of the major problems in establishing efficient models that can correlate to viscose is the poor solubility of cellulose in common organic and non-toxic solvents. An elegant way to circumvent this problem is to initially use soluble cellulose derivatives, followed by their conversion to cellulose after shaping and processing. In the past decade, trimethylsilyl cellulose (TMSC) has been found to be a very suitable precursor in this respect. Its solubility can be tuned by variation of the degree of substitution (DS_{Si}) with silyl groups from rather polar, hence soluble in ethanol (DS_{Si} ca. 0.7) or DMSO (DS_{Si} ca. 1.5) to rather unpolar and soluble in solvents such as toluene and chloroform (DS_{Si} >2.5). This tunable solubility of TMSC presents a major advantage when studying drug incorporation and release, since it allows for evaluation of drug release over a wide range of polarity in a single material. Since DCF is very well soluble in THF, a THF soluble TMSC derivative (DS_{Si}: *ca*. 2.5) was chosen for our studies.

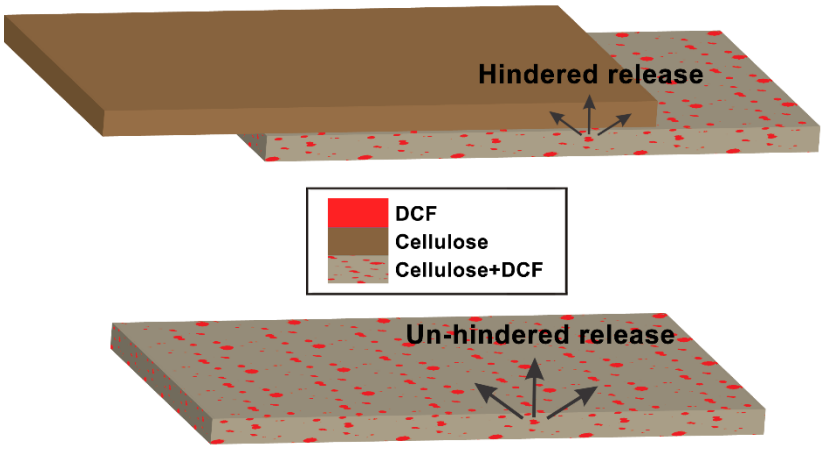

Figure 1: Proposed film stacking: BOTTOM – schematical depiction of the prepared drug loaded cellulose based model films; TOP – two layered model film with an additional cellulose layer on top, serving as a diffusion barrier enabling prolonged release.

For preparation of drug containing films, a TMSC/drug mixture was deposited on the chosen substrate (*i.e.* silicon wafers for most of the testing or flat gold QCM sensors for FTIR-ATR measurements) by spin coating followed by cleavage of the silyl groups by acidic vapors of HCl (Figure 2).

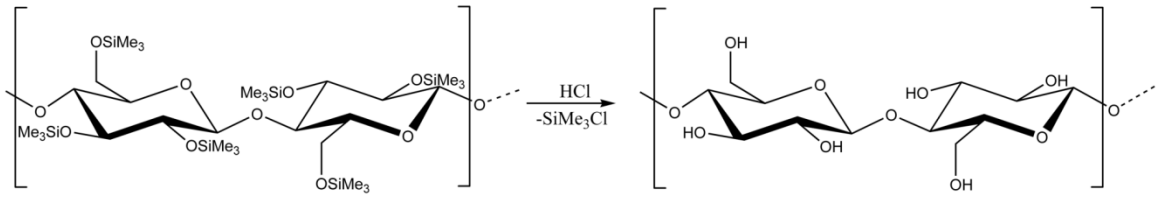

Figure 2: Conversion of TMSC to cellulose by HCl vapor treatment. Please note that the DS_{Si} of TMSC used in the study is 2.5.

In the first set of experiments, the model film preparation was optimized. In most literature reports, the preparation of TMSC films was realized by using 1 wt% solutions of TMSC (mainly from AVCEL) in the course of the spin coating process yielding films with a thickness of approximately 50 to 60 nm before regeneration. In contrast, the TMSC used in this study gives highly viscous solutions at 1 wt%, which do not yield ultrathin homogenous films after spin-coating (layer thickness 1 to 2 microns; results not included in this study). However, by diluting the films by a factor of three, homogenous TMSC films were obtained exhibiting a

thickness of 54.7 ± 0.9 nm (determined by AFM). DCF containing films were prepared by addition of DCF solution (1 mg/ml in THF) to a 1 wt% TMSC solution (ratio 1+2), resulting in a final TMSC concentration of 0.33 wt%. Using the same spin-coating parameters as for the preparation of pure TMSC films (mentioned in the *Experimental* section), a very similar film thickness could be achieved (54.9 ± 0.5 nm). In contrast to most polymers that show phase separation when mixed with TMSC (Kontturi et al. 2005; Hoeger et al. 2012), DCF is highly miscible with TMSC, while its incorporation does not lead to significant structural changes. Indeed, pure TMSC and DCF containing TMSC thin films exhibit very similar morphology and surface roughness as determined by AFM (Figure 4). The latter is rather high for both films indicating a porous film nature, suitable for loading of high amounts of drugs. Even more importantly, such porous structures having a high specific surface area can be compared to the macroscopic viscose porosity to some extent (and with some restraints), since viscose is composed of many intertwined and tangled fibers. Usually, cellulose films prepared from TMSC exhibit a smooth, flat surface (Mohan et al. 2011) with a rather low surface roughness (ca 0.7-2 nm), but obviously the change of the TMSC source (partially attributed to the different molecular weight and the use of a different solvents) resulted in formation of films exhibiting quite different morphologies, as indicated by a relatively high roughness (rms: ca. 10 nm over 3 micrometers).

Regeneration by exposure to HCl vapors of the TMSC films with or without incorporated DCF led to formation of cellulose thin films (as depicted in Figure 2). The cleavage of the bulky silyl groups (present in TMSC) results in a decrease in film thickness to 19.0 ± 0.4 for cellulose and to 18.2 ± 0.6 nm for cellulose+DCF thin films, which is in the same range as reported previously (Djak et al. 2011; Kontturi and Lankinen 2010). Regeneration of TMSC to cellulose (with or without incorporated DCF) did not significantly alter the morphology and porosity of the films. The decrease in layer thickness is accompanied with a decrease of the static water contact angle from 98(1)° (TMSC) and 96(1)° (TMSC+DCF), to 37(1)° (Cellulose) and 54(1)° (Cellulose+DCF). The value for the cellulose film is in the range of reported contact angles for cellulose thin films (Mohan et al. 2011; Kontturi et al. 2003b; Nyfors et al. 2009). The cellulose+DCF films exhibit a higher water contact angle, most likely due to the unpolar moieties (phenyl rings) of the DCF molecule, decreasing the overall film wettability. Since this difference in the water contact angle is less pronounced before regeneration, the latter probably exposes a certain amount of the drug on the film surface. This is also in accordance with the results of the dissolution testing, where an initial burst effect is clearly visible. More details will be presented later in the article.

One of the commonly used methods to prove successful regeneration of TMSC films to cellulose, is FTIR-ATR spectroscopy. The DCF molecule exhibits some very specific bands that are clearly separated from vibrations in cellulose and TMSC. The obtained results are presented in three graphs. Figure 3a shows FTIR-ATR of TMSC before and after regeneration to confirm successful regeneration. Figure 3b and 3c present FTIR-ATR spectra of DCF and DCF loaded TMSC and regenerated cellulose films, respectively. Representative peaks indicating successful DCF incorporation are marked. DCF representing peaks in cellulose samples exhibit higher intensities in average, since the ratio between the host material and drug is changed during the regeneration process. Figures 3b and 3c serve for identification of peaks that can be attributed to DCF as well and can therefore be used for confirmation of its presence in the samples.

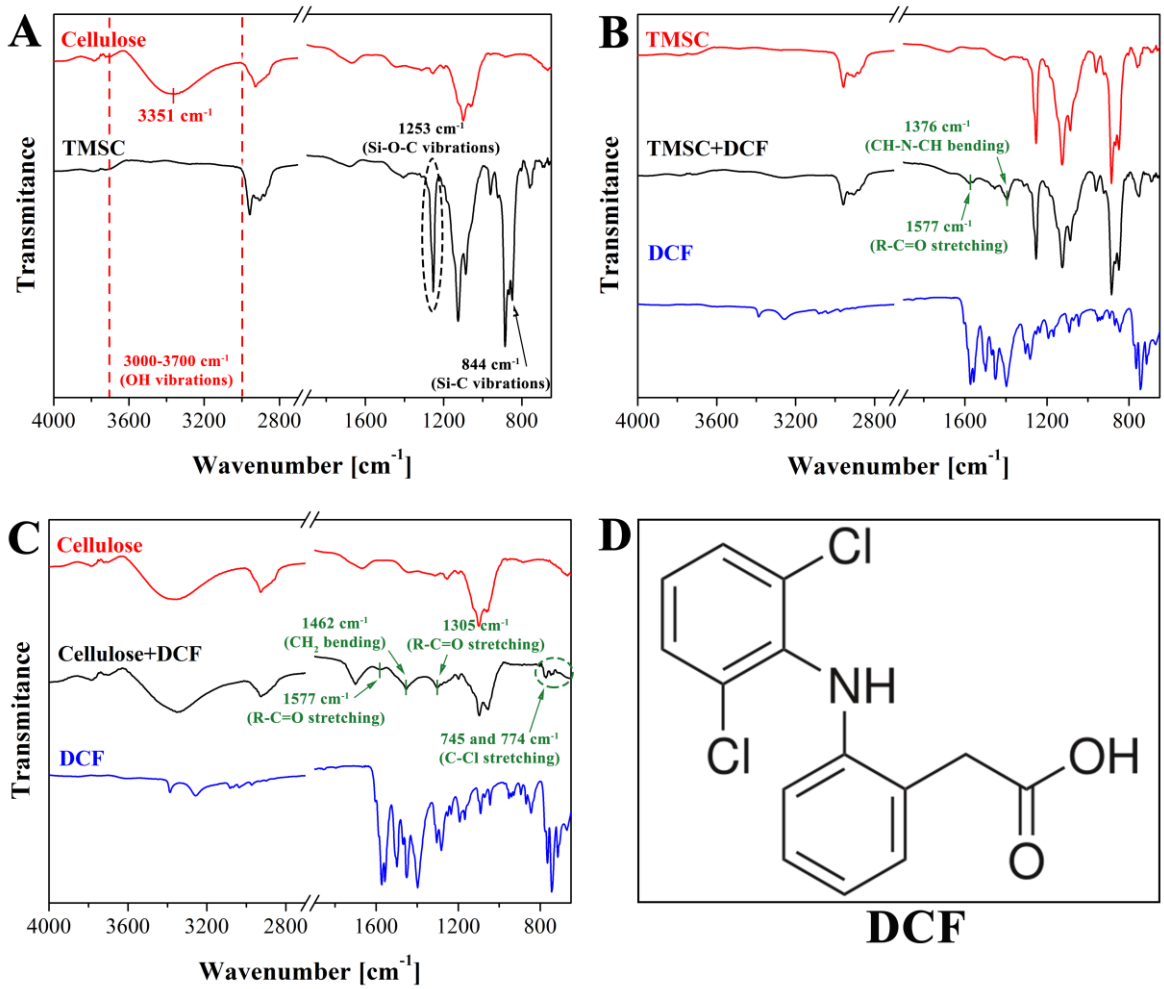

Figure 3: a) FTIR-ATR spectra of cellulose and TMSC, b) FTIR-ATR spectra of TMSC, TMSC+DCF and DCF, c) FTIR-ATR spectra of cellulose, Cellulose+DCF and DCF and d) molecular formula of DCF. Marked (green) regions and peaks correspond to DCF assigned peaks.

Similar to literature values, the observable bands in the TMSC spectra are C–Si rocking vibrations at 844, and 1253 cm^{-1} respectively (Kontturi et al. 2003a) while OH vibrations (3700– 3000 cm^{-1}) are negligible due to the high DS value of the used TMSC. Upon regeneration, bands corresponding to OH vibrations appear (centered at 3351 cm^{-1}) concomitant with the disappearance of the C–Si rocking vibrations. After DCF incorporation into the films, several new bands can be observed. Bands that can be assigned to C-Cl vibrations are visible in the region of 650 – 750 cm^{-1}, while a band corresponding to CH – N – CH vibration can be observed at 1376 cm^{-1}. At 1577 cm^{-1} R=C=O stretching can be observed as another indication of DCF presence.

Finally, FTIR-ATR measurement was performed also after the release to be able to evaluate possible DCF remainders in the samples. No peaks, assigned to DCF could be observed in the spectra of cellulose with incorporated DCF, indicating that no significant amount of the incorporated drug stayed inside the host cellulose film. On the contrary, the two layered cellulose sample with DCF in the first, even after 24h exhibits some peaks that can be assigned to DCF. This is in agreement with the results from release testing, more clearly described in the following section. After 48h no more peaks were observed that could be related to DCF, which again corresponds to the release testing results, where the two-layered sample release all of the incorporated amount after approximately two days. The additional FTIR-ATR spectra can be found in Supporting information – S1.

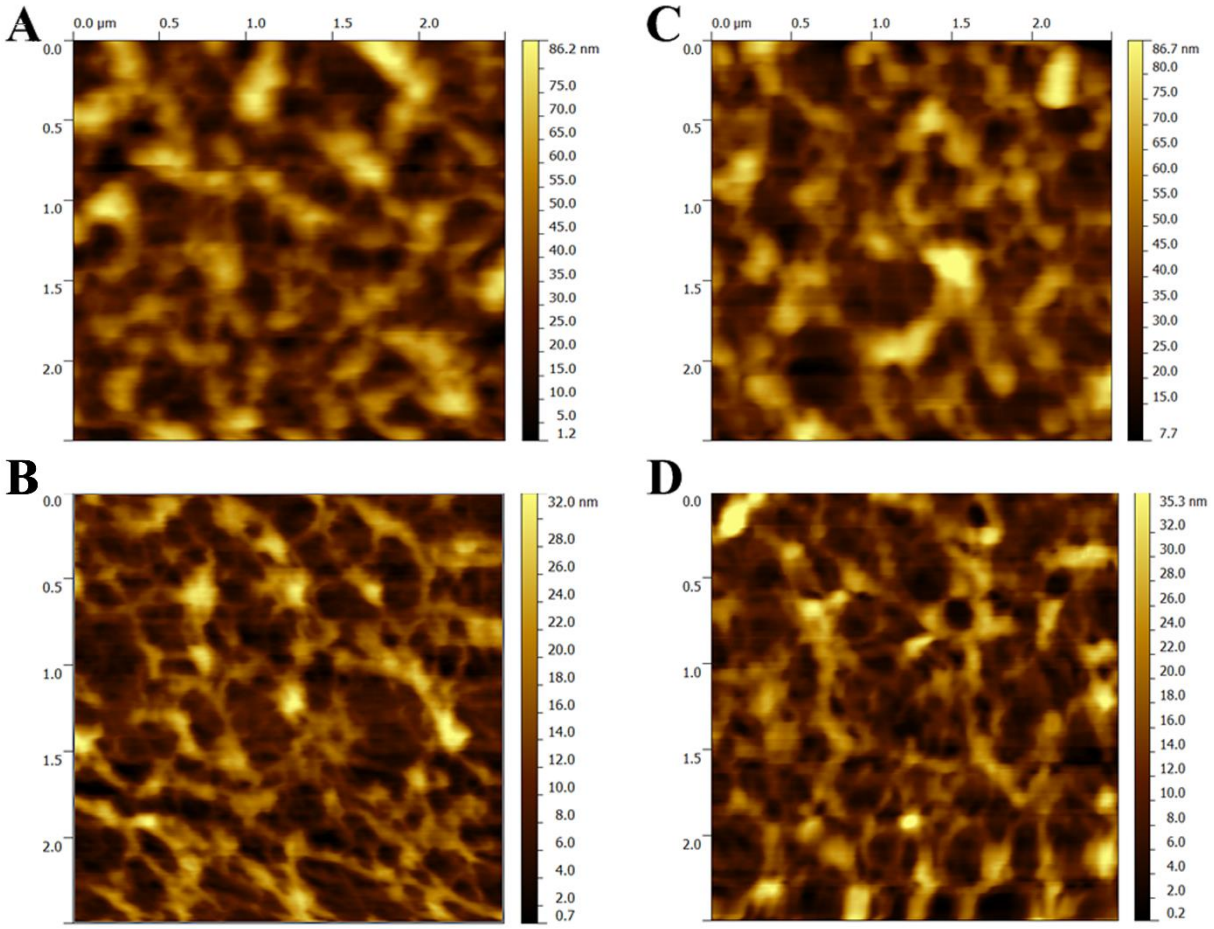

Figure 4: AFM topography images (2.5 µm x 2.5 µm) of the different films. A: TMSC (RMS: 15,99 nm), B: cellulose (RMS: 14,77 nm), C: TMSC+DCF (RMS: 5,65 nm), D: Cellulose+DCF (RMS: 6,69 nm).

Release of DCF from the films

After successful preparation of DCF containing cellulose films, we became interested in the release kinetics of the DCF from the films and how it relates to viscose based wound dressings. Although both materials consist of cellulose, there are major differences that may influence the drug release from these materials. While films prepared from TMSC by spin-coating are highly amorphous (Kontturi et al. 2011; Mohan et al. 2012), viscose fibers have significant crystalline domains lowering the accessibility to water and functional reactants/molecules. In addition, the viscose fibers are already shaped into a fibrous form exhibiting rather high roughness while the cellulose thin films provide a discrete 2D confined space accompanied by usually low rms roughness. However, the films in these studies exhibit a higher roughness than those reported in literature. In order to tune the release kinetics and provide some insights about the release kinetics and hindrance induced by cellulose thin films, an additional cellulose layer was deposited onto the DCF containing initial cellulose film. In this study, the release from as-prepared films was studied using an adapted dissolution testing approach, based on the standard method for studying release from wound dressing materials. Quantification of the drug release was done by UV–VIS spectroscopy at 275 nm, which corresponds to the DCF highest UV absorption peak. The overall method was as follows. Each film was transferred to a 15 ml glass bottle which contained 7 ml of Milli-Q water. At defined time intervals (5, 10, 20, 30, 60, 120 and 180 min) an UV probe was immersed into the glass bottle to measure the absorbance. Quantification was performed by transforming the measured absorbance to concentration using a

calibration curve (obtained by measuring absorbance for DCF solutions with known concentrations). The results are presented either as the calculated concentration or as the released drug percentage, whereas the incorporated amount was defined as mentioned above. As is clearly visible in Figure 5a, the release is fast in the beginning for all samples and slows down after approximately 10 minutes. Obviously, the decrease in the release rate is more pronounced for the sample equipped with an additional cellulose layer. The almost identical release profile of the films within the first 5 minutes with and without the additional cellulose layer on top is most likely related to the intrinsic DCF solubility and the film morphology. Since the films are highly porous for both samples, a high "leaching" rate of the drug through the upper level is initially possible. However, after the first "burst release" (within the first 5 minutes), the release profiles of one and two layered films start to follow different paths. After the second measurement point at ten minutes, the release rate of the sample consisting of two cellulose layers is significantly slower, when compared to the single layer film. Apart from the sterical hindrance of the second layer, this decrease in the release rate can be attributed to possible hydrogen and Van-der-Waals interactions between cellulose and DCF. Since DCF was incorporated *in situ* into the first layer, the drug molecules are preferentially released from it via a diffusion controlled mechanism, while a portion (surface bound DCF exposed due to TMSC regeneration) is released almost instantly (burst effect). A different mechanism is more likely for the transport of DCF molecules through the second layer. Their most obvious path towards the solution is through the porous cellulose network (as seen from Figure 4 – AFM images of morphology), where a relatively large surface area is available for interaction with the host material. One may imagine this process to be very similar as in chromatography, where compounds interact with the stationary phase and consequently elute slower. This behavior can be exploited in order to generate materials whose release properties can be tuned over a wide range of release times, allowing for the simultaneous testing of wound dressing materials intended for treatments, where immediate pain reducing action is required as well as for patients with chronic diseases where a steady but slow release should be accomplished. It is clear that the shown approach is generic and that depending on the used top layer(s) the interaction capacity of the analgesic drug can be highly controlled leading to either fast or slow release. Anyway, the porous structure of the investigated cellulose thin films will always result in a burst like behavior in the initial stage of application ('burst'), while the top layer acts as a 'stationary phase' slowing or accelerating release depending on the envisaged application (acute vs chronic wounds). An immediate release of analgesic drugs is beneficiary for treatment of chronic wounds as well, but even more important is a more controlled (and slower) release in later stages of the wound healing process. Such combined effects significantly improve the patients' quality of life and lead to a lower frequency of wound dressing exchange and lower expenses for treatment.

Therefore, film stacking is very interesting in terms of final application, since different profiles can be achieved by adding layers with or without incorporated drug (immediate release, prolonged release and combined release). An important aspect is that such advanced drug releasing approaches are readily prepared and easily assessed using the proposed model films preparation platform.

The efficiency of the porous top layer for slowing down the release mainly depends on its interaction capacity with the drug of interest. Hence, the proposed model system is highly suitable for testing and evaluating different multilayered films for drug release control as well and it enables a versatile platform to compare a variety of host materials and/or incorporated drugs. One of the possible additional functionalities that can be included into such film-based systems, are layers based on cationic polysaccharides (i.e. chitosan and its derivatives), which are

capable to induce antimicrobial activities reducing the risk of infections in the course of the wound healing process. However, further investigation regarding these issues go beyond the scope of one paper.

An important question is preparation of multi-layered films is how the preparation of additional layers can affect the composition of the bottom layer. The latter is an even bigger issue, if the bottom layer includes an incorporated drug, which can be possibly flushed away during the preparation of additional layers. Since the films in this study were prepared using THF, which dissolves the used drug DCF as well, an additional experiment was dedicated to preparation of the second layer using $CHCl_3$, which is equally good for preparation of films, but is a far worse solvent for DCF. The obtained results is available in Supporting information (S2-S4).

For all single-layered samples, the end point of release (dc/dt=0) was reached after a period approximately 240 minutes, while the same point was reached only after almost two days (2880 minutes – data not shown in Figure 5, but instead available as supporting info – S2) for the two-layered sample. In this context, a very important question is the amount of DCF that remains in the cellulose films. In order to evaluate this question, the dried films have been subjected to IR spectroscopy at different times after the release. In the case of the DCF/cellulose films, bands associated with DCF found prior to the release studies could not be detected beyond 240 minutes of release, while the films with an additional cellulose top-layer exhibited bands belonging to the DCF molecule until almost two days. Although the incorporated amount of the drug should be the same for the two layered sample (since the same procedure was used for the preparation of the first layer with DCF), the percentage of the released drug for this sample was quite smaller at 240 minutes, when the end point of release was reached for the sample with only one cellulose layer. Even after 24 hours, when the drug concentration almost stopped changing, the two layered sample still exhibited peaks previously associated with DCF, while the release studies showed that approximately 10% of the incorporated amount were still to be released. After 2 days of release, also the sample with the additional cellulose barrier layer, reached the end release point, concomitant with the disappearance of the DCF associated peaks in the IR spectra.

As mentioned above, the DCF release from the as-prepared model films is the sum of at least two different coupled release mechanisms, the initial burst, followed by a diffusion controlled release. To confirm the "multi-mechanism" release scenario, the first derivative was calculated from the obtained release data for the cellulose samples (Figure 5c). Since 1^{st} deviations from the obtained release data and taking into account the error bars revealed at least two different regions (judging by the always present break in the release curve at 20 minutes regardless of the sample, it could well be three coupled mechanisms) in the release profile (the regions are separated with a blue dashed line, showing possible three regions of different release mechanism), commonly used models to explain the drug release (Ukmar et al. 2012; Ukmar et al. 2011) could not be applied for additional data evaluation.

Finally, to get an impression whether release of DCF from model cellulose films is comparable to real systems, a commonly used wound dressing material, viscose, was impregnated with the same amount of DCF as was incorporated in the films. Cellulose model films and viscose can be compared since their chemical structure is the same, as well they are similar in terms of accessible sites for interaction of the media with the embedded drug molecules through their porous structure. The release testing of this sample was performed in an analogous way as for the thin films (as described in the *Experimental* section). Since the main purpose of this study was the

preparation of applicable models for development of novel advanced wound dressing materials, this was the most important validation point of our approach. Unexpectedly, the model film and the viscose fiber samples both show a similar release rate (Figure 5b). There are some minor differences in the early burst like release phase (in the region from 5 to 20 minutes); probably these differences originate from inhomogeneous DCF distribution in viscose. The final amount of released DCF is very similar for both samples with both reaching a release rates close to 100% after 24 h (final points are not shown).

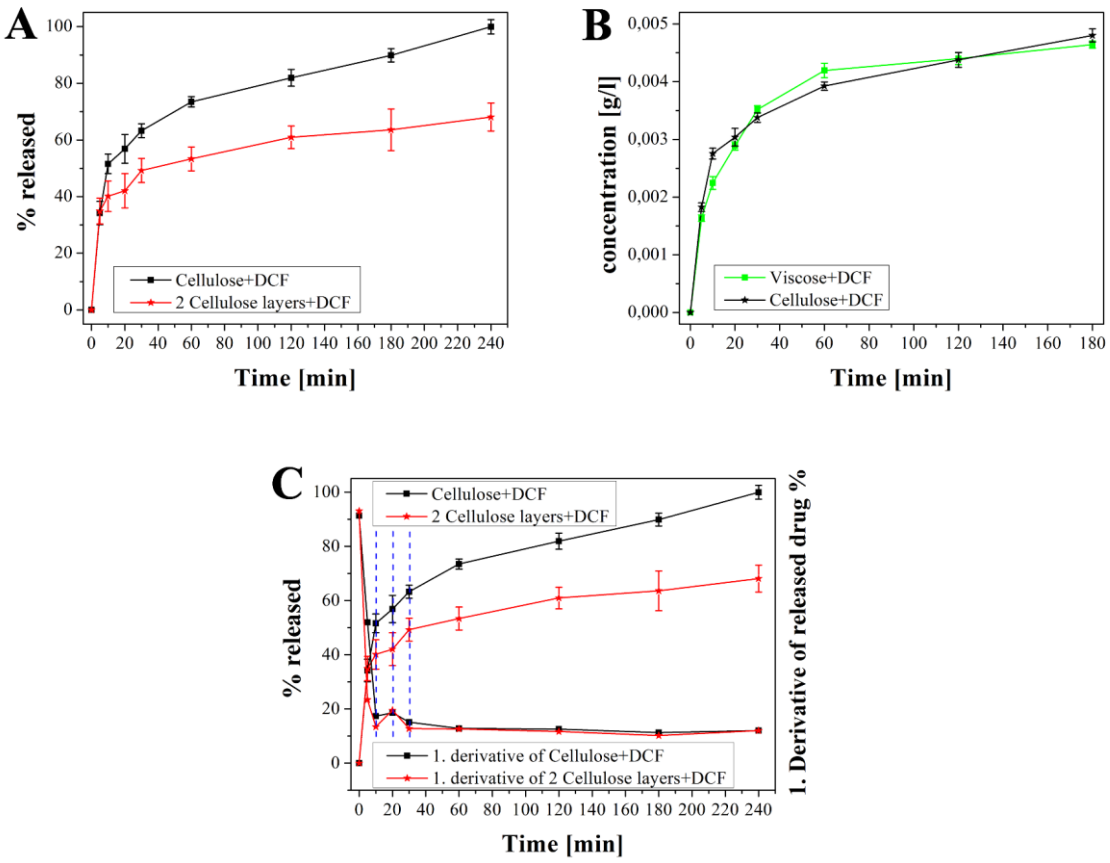

Figure 5: A) DCF release profile comparison between one layer and two layer cellulose films, B) comparison of DCF release from cellulose (model) and viscose (actual wound dressing) materials, and C) DCF release profile comparison between one layer and two layer cellulose films with additionally drawn 1. derivatives of the measured data to expose the multi-mechanism "complex" release from such materials. The release profiles present the average values from three parallel measurements, while the error bars present the standard deviations.

Conclusion

A model system for in vitro therapeutic wound dressing material testing is presented. Cellulose model films were chosen for this purpose, since viscose (build from cellulose) is one of the most frequently used materials in wound care. Additionally, films allow for thorough and relying characterization. The as-prepared samples were evaluated in regard of their physico-chemical and release properties. Cellulose based model films were shown suitable for tuning of drug release related characteristics and thorough characterization in order to better design possible future wound dressing products. Since cost reduction is nowadays the most limiting factor in development of new materials, optimization at the laboratory scale is certainly one of the possible solutions to decrease the development cost of advanced materials applicable in wound care. Although additional studies are necessary to fully exploit the possibilities and limitations of such model systems, the quite remarkable fit of the

release curves of the cellulose film and viscose sample for example, is already very promising. Using the proposed model system, we were able to show that layering can enable controlled tuning of the release properties. By adding an additional cellulose layer on top of the drug loaded one, a prolonged drug release could be achieved. Such layering seems a promising way for wound type-based wound dressing design. For example, our present system seems to be ideal for treatment of wounds, which require a reasonably fast start of activity, which is then maintained over a longer period (until the wound dressing exchange). The practical benefit from such system in light of the increasingly important cost reductions, could be the prolongation of the wound dressing exchange frequency, by which the overall wound treatment costs would be reduced. Further studies are already under way in order to fully understand the type of interaction between the cellulose host materials and the incorporated drug molecules, as well as to find exact correlation between the performance of this model system and actual wound dressings.

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Author contributions

Authors have contributed to the work equally.

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