# Polysaccharide aerogel coatings in hip arthroplasty

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## ABSTRACT
Total hip replacement is one of the most widely performed procedures in orthopaedics and is therefore continuously evolving in terms of new materials, postoperative management, prosthetic design and surgical techniques. Materials that will have acceptable biocompatibility and better physical properties, hence better integration into bone, are the future of the hip design. Amongst biomaterial-based coatings, polysaccharides were found to be especially promising as regards enhancing the respective implant integration. In this research, we developed a composite pectin-xanthan coating on the medical-grade stainless steel. Aerogel coating was prepared by a novel route, developed by our group. Polysaccharide gel coatings were prepared in ethanol and then dried under supercritical conditions of $CO_2$. Prepared aerogel coatings were used as drug carriers for two non-steroidal anti-inflammatory drugs, diclofenac sodium and indomethacin. Both drugs were incorporated during the first step of the gel preparation due to their ethanol solubility. Electrochemical analyses were performed on the coated samples using electrochemical impedance spectroscopy and cyclic polarisation techniques. The results showed that all passivated samples were highly resistant to general corrosion. In-vitro dissolution tests showed the prolonged drug release of both model drugs. Both drug releases were completed after 24, additionally confirmed by IR spectroscopy after the final release point. The potential of samples for use in orthopedic applications was evaluated on a human bone-derived osteoblast cell and all samples were shown to be biocompatible. Moreover, the increased viability of some samples is a very promising start for the future research and possible clinical use of those materials.

## INTRODUCTION
Biomaterials are gaining high interest for their use in medical applications, especially in orthopaedics. Biomaterial-based coatings have been used as orthopaedic implants in order to modulate the surrounding biological environment [1-4]. Polysaccharides were found to be promising materials in such applications regards the enhancing the respective implant integration [5]. Polysaccharides are attractive for medical and pharmaceutical applications especially due to their ability to form a gel. Wet gels are produced by a sol-gel method. Later on, those gels should be dried in order to obtain stable structures. Among different drying procedures, the supercritical drying is the most attractive one. Since there is only one phase present above the critical point, no surface tension is present and the possible collapse of the structure is avoided [6]. Therefore, supercritical drying is considered as the best method for preserving the structure of a gel. The resulting materials, aerogels, possess a wet-gel-like structure and have very low apparent densities, large specific surface areas, are nanostructured, and are in most cases present in an amorphous form [7,8]. Aerogels could be used in various applications. Their pharmaceutical application is recently one of the most researched topics in the field. Biodegradable polysaccharide aerogels are suitable carriers for various active substances. They could be used to prolong the release of the model drug [9,10] or even to

enhance the bioavailability of water low-soluble drugs [11]. Due to those properties, aerogels are therefore promising materials for the use in total hip arthroplasty (THA). Materials for such applications need to be biocompatible with the body tissue and fluids, have similar mechanical properties as the bone etc. The most commonly used materials in THA are titanium alloys, different ceramics, and stainless steel [12]. Therefore, biomaterial-based coatings could be used in order to modulate the surrounding biological environment [1-4].

## MATERIALS AND METHODS

The preparation of medical-grade stainless steel samples (disc shape 15 mm in diameter) is reported in [4]. Samples, used in this study were additionally passivated for 1h in 30wt.% HNO3. We used high methoxyl pectin (hmP), provided by Herbstreith&Fox and xanthan (Xa), obtained from Sigma Aldrich for the preparation of aerogel coating in a ratio 1:1. First 0.5wt.% hmP aqueous solution was prepared. Xa (0.5wt.%) was added to a solution which was then mixed until homogenisation. The solution was transferred onto a medical-grade stainless steel and ethanol was slowly dropped onto the solution. Gel was set in the form of a coating on a medical grade stainless steel. Sample was aged in ethanol for 3 h before the supercritical drying. Aerogel coatings were additionally loaded with two model drugs, diclofenac sodium and indomethacin, respectively. Diclofenac sodium was added to the polysaccharide solution prior the gelation. Indomethacin was loaded into the gel during the aging process in ethanol. All samples were dried in the presence of supercritical CO2 at 314K and 12 MPa for 7h.
Prepared samples were characterized by the nitrogen adsorption (Micromeritics ASAP 2020) and by the field emission electron microscope (FE-SEM) Sirion 400 NC. Electrochemical impedance spectroscopy (EIS) and cyclic polarization (CP) techniques were employed for the electrochemical study. Drug loadings were determined after dissolving the drug loaded aerogel coatings in phosphate buffer saline (PBS). In-vitro drug release studies were performed as described in [13]. Drug release kinetics was evaluated by fitting the in-vitro release data to First-order model, Higuchi model and Korsmeyer-Peppas model. ATR-FTIR spectroscopy (Agilent Cary 630 FTIR) was used in order to prove the incorporation of drugs and to confirm the whole amount of the respective incorporated drugs has been released after 24 h. Biocompatibility testing was performed on a human bone-derived osteoblasts. Cell viability was determined after 24 h incubation via the reduction reaction of the tetrazolium salt. Cell cycle analysis was performed by a culture of human osteoblasts hFOB.

## RESULTS

Nitrogen adsorption analysis proved the porous structure of hmP:Xa aerogel coating on a medical grade stainless steel. The specific surface area was 289 $m^2g^{-1}$, the pore volume was 0.11 $cm^3g^{-1}$, and the average pore size was 6.8 nm, as determined by the BJH adsorption method. Figure 1 shows SEM micrographs of the prepared aerogel coatings. The highly porous structure of the hmP:Xa aerogel coating is clearly evident from Figure 1a. The microstructure of the hmP:Xa, hmP:Xa-DCF and hmP:Xa-IND samples is quite similar (Figure 1b, c, d) and morphological structure of a coating not significantly affected by the addition of DCF and IND. The FE-SEM micrographs show a highly compact, layered structure for all samples, confirming the low average pore size, obtained by nitrogen adsorption (reported above).

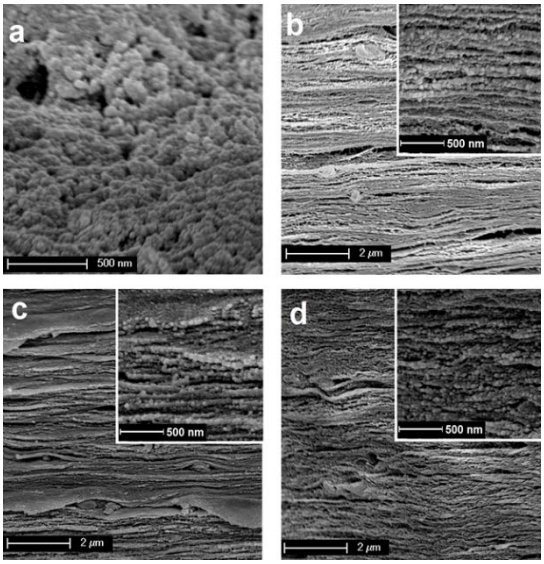


**Figure 1**. Scanning electron micrographs of (a) the surface of hmP:Xa coating, (b) cross-section of hmP:Xa coating, (c) cross-section of hmP:Xa-DCF coating, and (d) cross-section of hmP:Xa-IND coating.

The EIS measurements of both the coated and uncoated medical-grade stainless steel samples for different immersion times was determined in simulated physiological body fluid at 37 °C. The Bode modulus plots show distinctive behaviors of the samples at different frequency regions. The corrosion process of the medical-grade stainless steel in simulated physiological body fluid at 37 °C is under kinetic control as no diffusion was detected. The results of the CP measurements performed on the three studied passivated systems (uncoated, hmP:Xa-IND, and hmP:Xa-DCF) after 11 h of immersion are represented in the cyclic voltammograms, *E* vs. *i*, shown in Figure 2. The pit development is slower for the hmP:Xa-DCF sample compared to the non-coated passivated sample and hmP:Xa-IND. On the other hand, the non-passivated uncoated sample has the lowest $E_{bd} - E_{ocp}$ potential difference (a higher susceptibility to pitting corrosion) compared to the passivated samples. Based on this, we can conclude that all three passivated samples are more resistant to pitting corrosion compared to the uncoated non-passivated sample.

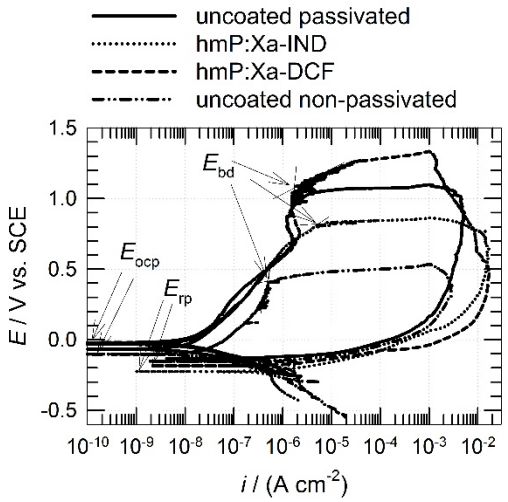


**Figure 2.** Cyclic polarization curves of medical-grade stainless steel, passivated uncoated, hmP:Xa-IND, and hmP:Xa-DCF, as well as non-passivated uncoated samples, after 11 h of immersion in simulated physiological body fluid at 37 °C.

Drug content was determined as the ratio between the amount of the NSAID and the total amount of coating. Similar results were obtained for both drugs, 4.5 ± 0.2% and 4.2 ± 0.3% for DCF and IND, respectively. Figure 3 shows the results of the respective NSAID release testing as the percentage of the release of DCF or IND as a function of time. In this research, hydrophilic DCF was incorporated in the initial step of the gelation process and lipophilic IND was incorporated by diffusion through ethanol. The same carrier (hmP:Xa aerogel coating) was used for both NSAIDs. Therefore, the drug release profiles were expected to be different for both NSAIDs [14]. DCF release is slower at the beginning compared to IND, while it surpasses the latter after 5 h. When hmP:Xa-DCF aerogel coating on medical-grade stainless steel comes into contact with PBS, PBS starts to diffuse into the pores of the aerogel, dissolving the DCF and also partially the aerogel. Dissolved DCF is released from the pores in the aerogel and slowly diffuses into the release media. If the intrusion of the PBS into the aerogel is faster than the DCF diffusion, the release profile is initially slower. On the contrary, IND release was faster in the initial 3 h of the *in vitro* drug release experiment. Since IND is a lipophilic drug incorporated into a hydrophilic carrier, an initial burst release may occur [14]. Upon contact with PBS, the IND is dissolved and released from the coating. However, since IND solubility in water is lower than that of DCF, the release of IND from the porous aerogel coating is delayed.

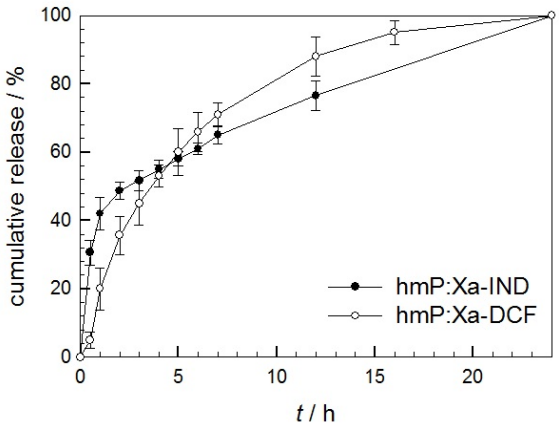


**Figure 3.** Cumulative release of DCF and IND from hmP:Xa aerogel coatings on medical-grade stainless steel. The results are shown with the corresponding 95% confidence intervals.

We applied first-order, Higuchi, and Korsmeyer-Peppas models to describe the respective release profiles. The best fitting to the experimental data was achieved by the first-order release kinetics and the Korsmeyer-Peppas [15] model for DCF and IND, respectively. The hmP:Xa, hmP:Xa-DCF, hmP:Xa-IND aerogel samples on stainless steel discs were studied using ATR-FTIR spectroscopy before and after *in vitro* drug release testing.

Figure 4 shows the combined results obtained from the biocompatibility testing using the dissolved sample solutions and their incubation with the human bone derived osteoblasts. Regardless of the time, all three aerogel samples (hmP:Xa, hmP:Xa-IND, and hmP:Xa-DCF) show a higher viability compared to the control sample. It is worth noting that also the hmP:Xa aerogel sample even outperforms the hmP:Xa-DCF sample, while the hmP:Xa-IND still exhibits a higher cell viability. The cell cycle was assessed with the MUSE Cell Analyzer. Although no significant differences are present among the samples, we can nevertheless observe that all samples again outperform the control samples in regard of the cell percentage in the $G_0/G_1$ phase, which is commonly related to a higher proliferation performance.

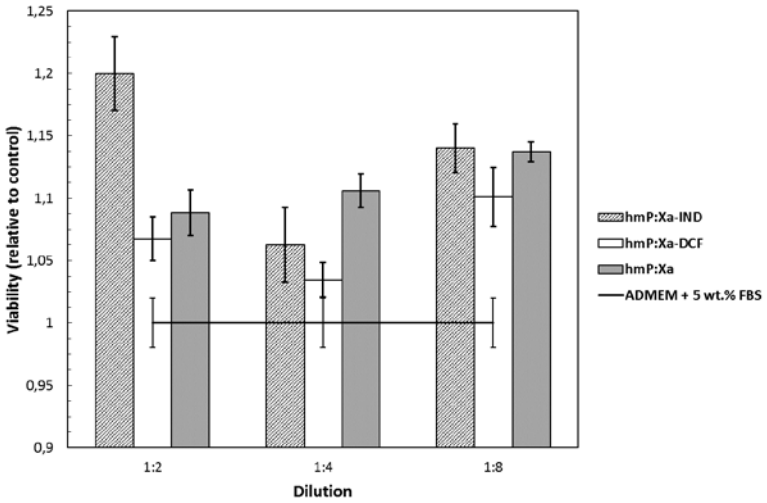


**Figure 4.** The cell viability of the aerogel samples. The results are shown with the corresponding 95% confidence intervals.

## CONCLUSION

The aim of this study was to develop novel biodegradable polysaccharide aerogel coatings on medical-grade stainless steel using pectin and xanthan. Two model drugs, diclofenac sodium and indomethacin, were incorporated into coatings in order to reduce pain and prevent local inflammation after hip arthroplasty. The results showed that all passivated samples are highly resistant to general corrosion. The resistance towards localized corrosion follows the trend hmP:Xa-DCF, passivated uncoated, hmP:Xa-IND, and non-passivated uncoated medical-grade stainless steel samples. The drug release profile of diclofenac sodium was different than that of indomethacin. Diclofenac sodium was released by the first order kinetics and indomethacin by Fickian diffusion. Both model drugs were completely released after 24 h, as confirmed by the plateau reached in the release profiles, as well as through post-release IR spectroscopy. All samples were shown to be biocompatible with human bone-derived osteoblasts. The increased viability of some samples indicates the high potential of the developed approach for future evaluation of possible clinical use.

## ACKNOWLEDGEMENTS

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