Applied Surface Science xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Full length article

# Cellulose acetate membranes functionalized with resveratrol by covalent immobilization for improved osseointegration

A.M. Pandele[a,b], P. Neacsu[c], A. Cimpean[c], A.I. Staras[c], F. Miculescu[d,*], A. Iordache[a], S.I. Voicu[a,*], V.K. Thakur[e,*], O.D. Toader[f]

[a] University Politehnica from Bucharest, Department of Analytical Chemistry and Environmental Engineering, Str. Gheorghe Polizu 1 - 7, 011061, Bucharest, Romania
[b] University Politehnica from Bucharest, Advanced Polymers Materials Group, Str. Gheorghe Polizu 1-7, 011061 Bucharest, Romania
[c] University of Bucharest, Department of Biochemistry and Molecular Biology, Splaiul Independentei 91-95, 050095 Bucharest, Romania
[d] University Politehnica from Bucharest, Metallic Materials Science, Physical Metallurgy Department, Splaiul Independentei 313, 060042 Bucharest, Romania
[e] University of Cranfield,School of Aerospace, Transport and Manufacturing, Cranfield, Bedfordshire MK43 0AL, UK
[f] Carol Davila University of Medicine and Farmacy, Str. Dionisie Lupu 37, 030167 Bucharest, Romania

ARTICLE INFO

*Article history:*
Received 11 May 2017
Received in revised form 24 October 2017
Accepted 6 November 2017
Available online xxx

*Keywords:*
Cellulose acetate membranes
Derivatization
Osseointegration
Resveratrol

ABSTRACT

Covalent immobilization of resveratrol onto cellulose acetate polymeric membranes used as coating on a Mg-1Ca-0.2Mn-0.6Zr alloy is presented for potential application in the improvement of osseointegration processes. For this purpose, cellulose acetate membrane is hydrolysed in the presence of potassium hydroxide, followed by covalent immobilization of aminopropyl triethoxy silane. Resveratrol was immobilized onto membranes using glutaraldehyde as linker. The newly synthesised functional membranes were thoroughly characterized for their structural characteristics determination employing X-ray photoelectron spectroscopy (XPS), infrared spectroscopy (FT-IR), Raman spectroscopy, thermogravimetric analysis (TGA/DTG) and scanning electron microscopy (SEM) techniques. Subsequently, in vitro cellular tests were performed for evaluating the cytotoxicity biocompatibility of synthesized materials and also the osseointegration potential of obtained derivatised membrane material. It was demonstrated that both polymeric membranes support viability and proliferation of the pre-osteoblastic MC3T3-E1 cells, thus providing a good protection against the potential harmful effects of the compounds released from coated alloys. Furthermore, cellulose acetate membrane functionalized with resveratrol exhibits a significant increase in alkaline phosphatase activity and extracellular matrix mineralization, suggesting its suitability to function as an implant surface coating for guided bone regeneration.



## 1. Introduction

Polymeric membranes possess an unique own place in the current materials usage because of their core property – selectivity [1], which allows their use in a large field of applications, such as water purification [2,3], proteins separation [4], fuel cells [5], or sensors [6–8]. One of the most studied domain is the biomedical applications field, such as haemodialysis and drug controlled release [9] or antimicrobial membranes [10]. Among the different polymers used in membranes applications, cellulose derivatives are among the utmost used polymers because of their wide spread nature (the most abundant material- $5 \times 10^{11}$ tones generated in the biosphere in a year [11]), pronounced mechanical properties [12,13], thermal resistance [14,15] and the versatility of processing methods [16,17]. A new and diminutive studied field is that of membranes for osteointegration – polymeric membranes favouring the welding of metal or graft implants, defective bones, membranes used especially in dentistry [18]. Membranes for such applications favour the proliferation of osteoblasts from the bone to the metallic implant [19–21], and must also be obtained from biodegradable and bioresorbable polymers [22–24]. The possibility to obtain these materials from biocompatible and bioresorbable polymers such as chitosan [25], collagen [26,27] cellulose derivatives [28], or caprolactone [29] has been studied in detail. Other studies have been performed on biocompatible, non-biodegradable polymers, but with high capacity for osteointegration such as polytetrafluoroethylene (PTFE) [30]. Different synthetic methods such

* Corresponding authors.
*E-mail addresses:* f_miculescu@yahoo.com (F. Miculescu), svoicu@gmail.com, stefan.voicu@upb.ro (S.I. Voicu), Vijay.Kumar@cranfield.ac.uk (V.K. Thakur).

https://doi.org/10.1016/j.apsusc.2017.11.102
0169-4332/

as precipitation or sol-gel synthesis [31] have also been studied, as well as the possibility of combining membrane osteointegration with controlled release [32,33].

The present study comes as a continuation of previous research performed by the same team on the synthesis of functionalized membranes with potential for osteointegration, the molecule initially studied being sericin [28]. Thus, in this case, cellulose acetate (CA) membrane was functionalized with resveratrol (Res), a natural polyphenolic compound that has been shown to have a stimulatory effect on bone formation both in vitro [34,35] and in vivo [36].

In vitro studies are often performed in order to assess the bioactvity of a material used as potential candidate for bone tissue engineering [34,37]. Bone formation involves active and differentiated osteoblaststs which induce the synthesis of extracellular matrix that will support the mineralization process [35,38]. This natural function of osteoblasts might be influenced by the presence of a degradable implant [36,39]. Recent investigations have shown that magnesium (Mg) itself has the capacity to stimulate the osteoblasts and to induce osteoinductive properties [37–40] [40–43]. However, the rapid degradation rate of Mg remains a critical challenge. To prevent the fast dissolution of a Mg alloy, namely Mg-1Ca-0.2Mn-0.6Zr alloy, in the present work cellulose acetate (CA) coatings or CA coatings functionalized with resveratrol (CA-Res) are proposed. In vitro tests of cellular proliferation and differentiation are performed in order to evaluate these novel biomaterials for their bone forming potential using MC3T3-E1 cell line. The obtained results could provide additional information for the development of novel materials for application in bone tissue engineering that enhance bone repair and regeneration.

The aim of this paper was to develop a facile method for immobilizing resveratrol (Res) on the surface of a cellulose acetate CA membrane using aminopropyl triethoxysilane (APTS) and glutaraldehyde as linker molecules. The obtained membraness were morphologically and structurally characterized, and cell culture-based tests were also performed to verify the character of osteointegration.

In order to cover the implants made by Mg alloy, the membrane for coating can be synthesized by dipping the alloy in any shape in polymer solution, followed by solvent evaporation. By solvent evaporation, very compact polymeric films are synthesized with small-diameter pores, conferring a smooth character to the surface. The strength of the coating and also the adhesion is indicated by its block uniformity [44].

## 2. Materials and methods

### 2.1. Membranes synthesis

For the immobilization of resveratrol, the cellulose derivative −cellulose acetate was used. The membranes were prepared using a 12% cellulose acetate solution (CA, Sigma Aldrich, 67% acetylation degree) in N, N'-dimethylformamide (DMF, Sigma Aldrich analytical purity 99.96%). Membranes synthesis was done using inversion of the phase by precipitation in water. For this, a polymer film of 300 μm thickness is deposited on the glass and immersed in the clotting bath until the formed membranes detach from the surface of the support. After the membranes are formed, they are washed successively with ultrapure water and ethyl alcohol to remove any solvent.

To modify the membrane surface with APTS and glutaraldehyde, our previously established method was used [28]. For partial membrane hydrolysis, treatment with a 0.1N sodium hydroxide solution (Merck) was carried out for 2 h at 37° C. The immobilization reaction of APTS was performed in a weak basic catalysis using a sodium hydroxide (0.1 N) solution (2 mL per membrane) and 20 mL of 20% APTS in ultra-pure water for 24 h at 37 °C. After the completion of the reaction, the membranes were thoroughly washed with deionized water in order to remove any traces of unreacted APTS. For resveratrol immobilization (Sigma Aldrich), glutaraldehyde was used as the linker molecule to cross link the APTS and protein. To this end, 50% glutaraldehyde (20 mL) (GA, Fluka) in deionized water together with sodium hydroxide (2 mL; 0.1 N) solution was added over the membrane with APTS maintaining the reaction temperature for 2 h at 40° C. After two hours, membrane was washed and treated with 20 mL of 1% resveratrol solution at 37 °C for 4 h in deionized water in the presence of sodium hydroxide (0.1N; 2 mL) solution. After the completion of the reaction, the membranes were washed and stored in cold ultra-pure water to, so as to avoid the proliferation/formation of microorganisms on the membrane surface. Fig. 1 shows the schemes of derivatization reactions. For in vitro biological tests, Mg-based alloy discs with thickness of 2 mm and diameter of 16 mm were dipped for three times in CA solution. After solvent evaporation at 45 °C for 72 h, the polymer film, which coated the alloy, was functionalized by using the same procedures previously described.

### 2.2. Membranes characterization

The synthesized membranes were analysed by Fourier FT-IR using a Bruker Tensor 27 apparatus having a diamond ATR device (range 600–4000 $cm^{-1}$). Different spectra were recorded as an average of 32 successive measurements, eliminating noise bands, atmospheric carbon dioxide and atmospheric water vapor [28]. Scanning electron microscopy (SEM) (FEI XL-30-ESEM TMP instrument) was used to study the membrane morphology. The X-ray photoelectron spectroscopy (XPS) analysis was performed on a Thermo Scientific K-Alpha instrument and the TGA analysis was carried out in nitrogen atmosphere (room temperature to 800 °C) at a heating rate of 10 °C/min on a Q500 TA Instruments[28]. Deconvolutions of the C 1s, O 1 s peaks were performed after Shirley's inelastic background subtraction and were normalized for the measured transmission function of the spectrometer. A Sartorius installation system was used to study the water flows for the synthesized membranes using 4.5 cm diameter membrane discs and 500 mL deionized water (continuous recirculation). The vacuum in the system was always maintained below the being 0.1 bar. All the tests were performed by continuously recirculating the volume of water. For protein retention, analytical grade Bovine serum albumin (BSA, Merck) and Hemoglobin (Merk) were used. They were dissolved in ultrapure water at a concentration of $10^{-5}$M. A 10-point calibration curve was used to determine the concentration by measuring UV–vis protein absorbance. "For this, a Camspec spectrophotometer both in permeate and retentate from 10 to 10 min was used and the retention was calculated with the following formula" [28]:

$$C(\%) = \frac{Ci - Cf}{Ci} * 100$$

where Ci represents the concentration in the permeate, and Cf represents the concentration of the protein in the retentate.

### 2.3. In vitro biological testing

#### 2.3.1. Extract preparation and cell culture model

To perform in vitro tests, cellulose acetate CA membranes and resveratrol Res-functionalized membranes were immobilized on Mg-1Ca-0.2Mn-0.6Zr alloy discs with implantable potential, synthesized by the same procedure described above. Due to the fact that Mg-based materials proved to strongly affect osteoblasts viability over time in direct contact experiments (data not shown), in the present study the cellular response was tested using Mg extrac-

Please cite this article in press as: A.M. Pandele, et al., Cellulose acetate membranes functionalized with resveratrol by covalent immobilization for improved osseointegration, Appl. Surf. Sci. (2017), https://doi.org/10.1016/j.apsusc.2017.11.102