BIOACTIVITY AND ANTIMICROBIAL PROPERTIES OF CHITOSAN-TOBERMORITE MEMBRANES

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Introduction

Tobermorite ($Ca_5Si_6O_{16}(OH)_2.4H_2O$) is a layered calcium silicate hydrate phase whose bioactivity and biocompatibility with respect to bone and dental tissues are documented [1-3]. Chitosan is a biodegradable mucopolysaccharide derivative that has been evaluated as a tissue scaffold material for the *in situ* regeneration of bone and periodontal structures [2,3]. Recent studies have shown that tobermorite-chitosan composites are potential candidates for use as biodegradable guided tissue regeneration (GTR) membranes [2,3]. During the GTR process, a membrane is used to isolate the exposed root surface from invasive epithelial and gingival tissues in order to enable the slow-growing periodontal ligament and hard tissues to regenerate. Resistance to potentially pathogenic oral bacteria is a highly desirable property of GTR membranes which are prone to biomaterial-centred infection. Silver (Ag^+), copper (Cu^{2+}) and gallium (Ga^{3+}) ions are reported to confer antimicrobial activity when incorporated into bioactive materials [1,4,5]. In the present study, tobermorite was synthesised and ion-exchanged with Ag^+, Cu^{2+} or Ga^{3+} ions. The *in vitro* bioactivity and antibacterial properties of solvent-cast tobermorite-chitosan composite membranes were then evaluated with respect to their potential use as GTR membranes to repair damaged periodontal structures.

Materials and Methods

Tobermorite (TB) was prepared hydrothermally and characterised by X-ray diffraction analysis (XRD), Fourier transform infrared spectroscopy (FTIR) and scanning electron microscopy (SEM) [1]. Ion-exchanged tobermorites (TB-Ag, TB-Cu and TB-Ga) were, respectively, obtained by exposure to 5 mM Ag^+, Cu^{2+} or Ga^{3+} nitrate solutions at a mass:volume ratio of 1:400 g cm^{-3} for 1 week. Metal-ion uptake from solution was monitored by inductively coupled plasma spectroscopy (ICP) and the compositions of the ion-exchanged phases were determined by energy dispersive X-ray analysis (EDX). Tobermorite and chitosan were blended in 2% aqueous acetic acid solution, at a mass ratio of 35:50, cast onto a polycarbonate surface and dried in air at 60 °C. The *in vitro* bioactivity of the composite membranes was evaluated by monitoring hydroxyapatite (HA) formation on their surfaces in simulated body fluid (SBF) at 3, 7 and 14 days [6]. HA was confirmed by FTIR and SEM. Composite membrane discs (8 mm diameter) were placed on nutrient agar plates spread with *Escherichia coli*, *Staphylococcus aureus* or *Pseudomonas aeruginosa* (at ~10^6 CFU cm^{-3}). Zones of inhibition were measured following incubation at 37 °C for 24 h. All syntheses and analyses were carried out in triplicate.

Results and Discussion

Equilibrium metal ion-uptake by tobermorite was found to be 1.1, 2.0 and 2.2 mmol g^{-1} for TB-Ag, TB-Cu and TB-Ga, respectively. The pure chitosan control membrane did not demonstrate *in vitro* bioactivity; whereas, the characteristic HA doublet at 570 – 605 cm^{-1} was present in the FTIR spectra of all of the composite membranes following a residence time of 14 days in SBF. The formation of HA was also confirmed by SEM.

Zone of inhibition analysis verified that the composite blended with TB-Ag asserted antibacterial action against all three pathogens, as distinct clear zones were observed in all cases. Bacteria failed to populate the surfaces of the composite containing TB-Cu indicating that this material afforded some protection against direct biofilm formation. Conversely, the control membrane and those blended with TB and TB-Ga were observed to possess no antimicrobial activity, as their surfaces were readily colonised by the pathogens.

Conclusions

The bioactivities of composite membranes incorporating Ag^+-, Cu^{2+}- and Ga^{3+}-exchanged tobermorites were similar. Ag^+ exhibited significant antibacterial action, Cu^{2+} protected against biofilm formation and Ga^{3+} failed to exert any observable antimicrobial activity.

References

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