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Microwave assisted synthesis of bacterial cellulose-calcium carbonate composites

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ABSTRACT

The effect of microwave irradiation on the calcium carbonate ($CaCO_3$) deposition on bacterial cellulose (BC) membranes was investigated. The experiments were conducted at atmospheric pressure using calcium chloride and sodium carbonate aqueous solutions as starting reactants. The following parameters varied: the exposure time at microwave irradiation and the concentrations of calcium chloride starting solutions. The BC-$CaCO_3$ composites were characterized by means of scanning electron microscopy (SEM), X-ray diffraction (XRD) and Fourier transform infrared spectroscopy (FTIR). From the known polymorph of calcium carbonate, calcite and vaterite were deposed on bacterial cellulose membranes in the presence of microwave irradiation doses which were used in this work. Water vapor permeability and pencil scratch hardness were investigated for BC-$CaCO_3$ composites and compared with control samples obtained without microwave irradiation. Significant differences between morphology and even polymorphism of the calcium carbonate crystals were observed for the irradiated samples in comparison with those obtained in the absence of microwave irradiation.

1. Introduction

Organic–inorganic hybrid nanocomposites represent innovative materials which offer very interesting applications in different fields of activity from industry to medicine, due to their spectacular properties (John et al., 2012). Their interesting properties are the result of the synergy effects between the starting components. Many examples of complex organic–inorganic composite materials are also encountered in nature, bones, corals, pearls, mollusk shells, eggshells and exoskeleton of arthropods being the most known examples. Very good candidates, already used to obtain hybrid nanocomposites, especially for biomedical applications, are cellulose and calcium carbonate ($CaCO_3$).

Calcium carbonate is one of the most abundant biominerals, extensively studied for its industrial and also for its biomedical applications. From industrial applications it could be mentioned that $CaCO_3$ is a very common mineral filler in the paper, plastic and rubber making processes (Chen et al., 2011). Many additives and templates were used for mineralization in vitro of calcium carbonate (Matahwa et al., 2008; Yang et al., 2010; Isopescu et al., 2010; Ren et al., 2011; Yang and Xu, 2011).

Cellulose and its derivatives were already used as template to obtain hybrid composites with calcium carbonate (Dalas et al., 2000; Vilela et al., 2010; Shen et al., 2010; Ciobanu et al., 2010; John et al., 2012) and also with other inorganic materials (Xie et al., 2009; Jia et al., 2011; Li et al., 2013).

Bacterial cellulose (BC), a microbial polymer synthesized as nanofibrils by *Acetobacter xylinum*, Gram-negative acetic acid bacteria is also good template for the mineralization process and an interesting material for various medical and technical applications. The unique properties of BC as its higher purity, crystallinity, degree of polymerization and tensile strength have drawn the specialists' attention that it could be used as a new functional material to synthesize biocomposites. These ones would be suitable both as implants in the tissue engineering of artificial skin, artificial blood vessels, or cartilage and bones and also as membranes for wound dressing, to mention only the most important applications (Svensson et al., 2005; Czaja et al., 2006; Zaborowska et al., 2010; Bäckdahl et al., 2011; Shi et al., 2012; Torres et al., 2012). BC could be a good matrix for obtaining different types of BC-inorganic composites with improved biocompatibility or with antimicrobial properties. There has already been reported that BC composites with hydroxyapatite are suitable for bone healing applications (Zimmermann et al., 2011; Yin et al., 2011), while the ones with silver can be applied for wound dressing, especially due

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to their antimicrobial properties (Yang et al., 2012). Nanocomposites between BC and silica, titanium dioxide and magnetite have also been synthetized and characterized. All of them are promising materials for technical applications, having very interesting physical and chemical properties (Ashori et al., 2012; Gutierrez et al., 2012; Zhu et al., 2011).

Bacterial cellulose was not used as template for calcium carbonate deposition at its real potential, even if $CaCO_3$ hybrid materials using cellulose as template polymers had already been obtained and their biocompatibility had been successfully proved (Ma et al., 2012; Fu et al., 2013). A first attempt was done by our team, the result of which has been presented in a previous study where we have reported the BC-$CaCO_3$ composites that were obtained using ultrasound irradiation (Stoica-Guzun et al., 2012). A recent paper reports also the obtaining of lamellar calcium carbonate structures deposed on bacterial cellulose in the presence of egg white, the obtained materials being designated to tissue engineering applications (Liu et al., 2013).

There are many arguments to support the idea of obtaining hybrid composites from BC and calcium carbonate. The first of them refers to the biocompatability of bacterial cellulose with living cells (i.e. osteoblasts), which was already demonstrated (Shi et al., 2012). Also, by its fibrilar structure, BC is similar to the collagenous fibers of bone and its network could provide cell anchorage sites and also natural guidance for bone tissue regeneration. A guided bone regeneration membrane containing polycaprolactone and calcium carbonate nano-fibers was already obtained (Fujihara et al., 2005). We consider that the replacement of polycaprolactone could be done with bacterial cellulose. Having a reticulated nanofibrous structure, BC is a good matrix for mineral deposition, being also able to influence the polymorphism of the different inorganic compounds, as it was already demonstrated. For all these reasons, even if bacterial cellulose is an expensive biopolymer in comparison with cellulose, we consider that for biomedical applications BC provides important benefits over plant cellulose.

As microwave could offer interesting possibilities to obtain different composite materials, the present study was conducted using microwave irradiation in order to see if the polymorphism of calcium carbonate could be influenced in the presence of BC as template polymer.

To our best knowledge, a study on the microwave irradiation influence on calcium carbonate deposition on BC matrix has not been conducted yet. The primary focus of the present research is the structural characterization of the BC-calcium carbonate composites in order to prove that microwave assisted method could be used as a good strategy to obtain these new materials. Water vapor permeability and pencil scratch hardness were investigated for BC-$CaCO_3$ composites and compared with control samples obtained without microwave irradiation.

2. Experimental

2.1. Materials

Sodium hydroxide (min. 98%), sodium carbonate anhydrous (puriss), and calcium chloride purum powder (≥97%) were purchased from Sigma–Aldrich, being used without further purification.

2.2. Synthesis and purification of bacterial cellulose membranes

The BC membranes were obtained in static culture. *Acetobacter* sp. strain used was isolated from the traditionally fermented vinegar in Microbiology Laboratory of Chemical and Biochemical Engineering Department of University Politehnica of Bucharest. Bacterial cellulose (BC) membranes were obtained from a static culture on a modified HS medium containing 2% fructose (Hestrin and Schramm, 1954). The gel-like pellicles obtained were purified by boiling in an aqueous solution of NaOH of 0.5 M at 90 °C for 1 h, in order to eliminate the bacterial cells. The BC pellicles were washed several times with deionized water until the pH of water became neutral and then were stored in deionized water.

2.3. Synthesis of BC-calcium carbonate composites

Sodium carbonate (Na_2CO_3) (0.1 mol/L) and calcium chloride ($CaCl_2$) (0.1 and 0.25 mol/L) aqueous solutions were prepared using high quality deionized water. The pH of the working solutions was adjusted to 9. The bacterial cellulose gel-like membranes were cut in rectangular (9 cm × 6.5 cm) strip samples (25 g wet bacterial cellulose corresponding to 0.232 dry bacterial cellulose) and individually immersed for 10 min in a beaker containing 50 mL sodium carbonate of 0.1 M concentration. Afterwards, some of the samples were immersed in 50 mL calcium chloride solution and kept under microwave irradiation at 90 W for 3 or 5 min, the final measured temperatures in the solution being 50 ± 0.5 °C and 70 ± 0.5 °C, respectively. A household microwave oven (Panasonic, 2.45 GHz, maximum power 800 W) was used for MW irradiation. Other samples, with the same weight, but without microwave irradiation, were immersed for the same amount of time as the irradiated samples in 50 mL calcium chloride solutions, at 50 ± 0.5 °C or at 70 ± 0.5 °C. Finally, the membranes were rinsed with deionized water and dried at room temperature. The experimental conditions for all the samples are presented in Table 1.

2.4. Composites characterization

2.4.1. SEM, FTIR, XRD and TGA

The mineralized films were examined on a Jasco FT/IR6200 spectrometer (ABL& E-JASCO Romania S.R.L., Romania) with Intron μ Infrared Microscope with ATR-1000-VZ objective. The spectra were the average of 50 scans recorded at a resolution of 4 cm^{-1} in a range from 4000 to 500 cm^{-1} with a TGS detector.

For morphological observations a scanning electron microscope HITACHI S-2600N (Hitachi Romania, Japan) was used, with resolution in secondary electron Image 4.0 nm, electron gun with filament: tungsten hairpin type; accelerating voltage: 0.5–30 kV, emission current 10^{-12}–10^{-7} A. The work conditions were: accelerating voltage: 25 kV, WD (working distance) 13 mm and beam 30, in a good agreement with the physical characteristics of the sample. All samples were gold coated prior to SEM examination.

The X-ray diffraction (XRD) measurements were conducted using a Shimadzu XRD 6000 diffractometer (Ni filtered Cu-Kα radiation, 40 kV, 30 mA and 0.02° step scan).

The thermal behavior of the composites was tested using thermogravimetric analysis on a thermal analyzer (DTG-60-Shimadzu). The operating conditions were: temperature range of 20–1000 °C, with a heating rate of 10 °C/min, and air flow rate of 50 mL/min.

The digital image analysis was carried out using IMAGE J 1.47f software, a program developed at the National Institute of Health of the USA and available on Internet (http://image.nih.gov/ij).

2.4.2. Water vapor permeability test

Water vapor permeability was measured as described in literature (Limpan et al., 2010; Vargas et al., 2011). The samples, sealed on cups, were placed at 30 °C in a desiccator containing deionized water in order to control the relative humidity at 100% on one side of the film. Silica gel was used inside the testing cup to achieve 0% RH on the other side of the film. The chamber was equipped with a sensor which measures temperature and relative humidity. The moisture absorbed was determined by a periodical weighing of the