

Biocompatible xanthan/polypyrrole scaffolds for tissue engineering



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ABSTRACT

Polypyrrole (PPy) was electropolymerized in xanthan hydrogels (XCA), resulting in electroactive XCAPPy scaffolds with (15 ± 3) wt.% PPy and (40 ± 10) μm thick. The physicochemical characterization of hybrid XCAPPy scaffolds was performed by means of cyclic voltammetry, swelling tests, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), thermogravimetric analyses (TGA), scanning electron microscopy (SEM), atomic force microscopy (AFM) and tensile tests. XCAPPy swelled $\sim 80\%$ less than XCA. FTIR spectra and thermal analyses did not evidence strong interaction between PPy and XCA matrix. XCAPPy presented a porous stratified structure resulting from the arrangement of PPy chains parallel to XCA surface. Under stress XCAPPy presented larger strain than neat XCA probably due to the sliding of planar PPy chains. The adhesion and proliferation of fibroblasts onto XCA and XCAPPy were evaluated in the absence and in the presence of external magnetic field (EMF) of 0.4 T, after one day, 7 days, 14 days and 21 days. Fibroblast proliferation was more pronounced onto XCAPPy than onto XCA, due to its higher hydrophobicity and surface roughness. EMF stimulated cell proliferation onto both scaffolds.

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1. Introduction

Polysaccharides are important materials to create scaffolds for tissue engineering because they are biocompatible, abundant in nature and they can form hydrogels, which are similar to biological systems [1–4]. Xanthan gum is a high molecular weight polysaccharide with branched chains and acidic characteristics, produced by *Xanthomonas campestris* and with large industrial applications [5]. It is composed by D-glucosyl, D-mannosyl, and D-glucuronyl acid residues in a 2:2:1 molar ratio and variable proportions of O-acetyl and pyruvyl residues. Side-chains consist of a trisaccharide composed of mannose (β -1,4) glucuronic acid (β -1,2) mannose, attached to alternate glucose residues in the backbone by α -1,3 linkages. The deprotonation of O-acetyl and pyruvyl residues at $\text{pH} > 4.5$ creates negative charges along the xanthan chains, which in the presence of Ca^{2+} ions build physical networks [6–8]. Xanthan chemical networks easily produced by the reaction with citric acid, an efficient nontoxic crosslinker for polysaccharides [9,10], behave as hydrogels in the pH range between 4 and 10. Such xanthan hydrogels, here coded as XCA, have high negative charge density at $\text{pH} > 4.5$ and are bactericidal, if loaded with lysozymes [11]. In combination with xanthan-nanohydroxyapatite particles or their equivalent strontium substituted, XCA hydrogels were used successfully as scaffolds for osteoblast growth and induced high alkaline phosphatase activity [12]. Fibroblast proliferation onto nanocomposites of XCA hydrogels and

magnetite nanoparticles was favored, particularly under magnetic field of 0.4 T [13]. Hydrogels of binary mixtures of xanthan and chitosan (1:1) [14] or quaternary blends of xanthan, konjac gum, iota-carrageenan and kappa-carrageenan [15] have also been successfully used as scaffolds in the treatment of skin lesions.

Conductive polymers are also a promising class of materials for biomedical applications, because their electrically modulated properties could be engineered for biomedical devices [16]. Some examples include biosensor technologies [17], drug delivery systems [16,18,19] and substrates for neural implants [20,21]. Polypyrrole (PPy) is an interesting conducting polymer for biological and biomedical use due to its easy production, cytocompatibility, environmental stability and electrical conductivity, which can be controlled by the doping degree [22]. The literature shows reports about successful combinations of PPy with polysaccharides for tissue engineering. For instance, chitosan/PPy-alginate composite scaffolds can serve as substrates for bone tissue regeneration [23], membranes of PPy/heparin/poly(L-lactic acid) under electrical stimulation in the range of 100 mV mm^{-1} and 300 mV mm^{-1} favored the proliferation of osteoblasts [24], neuronal cells responded positively to PPy/bacterial cellulose scaffolds [21, 25]. In the view of these reports, in the present work PPy was electropolymerized in xanthan hydrogels (XCA) to produce hybrid functional materials. The resulting XCAPPy hybrid materials were characterized by means of cyclic voltammetry, swelling tests, Fourier transform infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), thermogravimetric analyses (TGA), scanning electron microscopy (SEM), atomic force microscopy (AFM) and tensile tests. The in vitro

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adhesion and proliferation of fibroblasts onto XCA and XCAPPy were evaluated in the absence and in the presence of external magnetic field (0.4 T).

2. Experimental

2.1. Materials

Commercial xanthan (Kelzan®, CP Kelco, USA, degree of pyruvate = 0.38, degree of acetyl = 0.41, $M_v \sim 1 \cdot 10^6 \text{ g mol}^{-1}$, degree of polymerization ~ 1072) was used as received. Citric acid (Synth, Brazil) was recrystallized twice before use. NaNO_3 (Synth, Brazil) was used as received. Pyrrole monomer (Sigma-Aldrich) was distilled using fractional distillation method prior to use. Deionized water was used in all experiments. The human fibroblast cells were obtained from the foreskins of University Hospital. The Ethics Committee of HU (HU CEP Case943/09) approved the process.

2.2. Xanthan (XCA) hydrogel preparation

Xanthan hydrogels were prepared as follows: xanthan films were produced as described elsewhere [10], by casting a 6 g L^{-1} xanthan aqueous solution in the presence of citric acid at 0.3 g L^{-1} . The solutions were homogenized with an Ika Turrax® stirrer at 18,000 rpm for 3 min and submitted to centrifugation for 5 min at 3600 rpm in order to remove air bubbles prior to casting. Crosslinking was achieved by heating the films at $165 \text{ }^\circ\text{C}$ for 7 min. The resulting xanthan hydrogels were swollen in water at $70 \text{ }^\circ\text{C}$, for 24 h to remove sol fraction and dried at $45 \text{ }^\circ\text{C}$, for 24 h.

2.3. XCAPPy preparation

The conducting polymer was electrochemically polymerized into XCA hydrogel matrices, according to scheme in Fig. 1. Swollen XCA samples presented high adhesion on tin-doped indium oxide (ITO) surfaces. Thus XCA swollen samples ($5 \text{ cm} \times 2 \text{ cm}$) were deposited on ITO and dried at room temperature during 24 h. After drying the XCA films remained firmly attached to ITO surfaces. XCA covered ITO was immersed into the polymerization medium (pyrrole at 0.4 mol L^{-1} in sodium nitrate solution 1.0 mol L^{-1}) for 15 min prior to reaction. Then, a constant potential of $+0.6 \text{ V}$ was applied until the charge achieved a value of 1.54 C cm^{-2} to form XCAPPy hybrid hydrogel. The reference and counter electrodes used were $\text{Ag/AgCl} (\text{KCl}_{(\text{sat})})$ and a platinum sheet, respectively. All the experiments were carried out with a potentiostat/galvanostat Autolab PSTAT 30. After polymerization XCAPPy/ITO system was immersed in deionized water for 24 h to remove unreacted substances. The XCAPPy samples were dried at room temperature and detached from ITO surfaces. The electropolymerization conditions were chosen based on previous reports about the optimization of

parameters for electropolymerization of PPy in hydrogels of poly(acrylic acid) [18] or polyacrylamide [19].

2.4. XCAPPy characterization

The electrical properties of XCAPPy films were analyzed by cyclic voltammetry in $\text{NaNO}_3 \text{ } 1 \text{ mol L}^{-1}$ electrolytic solution, sweeping the potential from -0.75 V to 0.75 V , at different scan rates ($5, 25, 50$ and 100 mV s^{-1}). All experiments were carried out with a potentiostat/galvanostat Autolab PSTAT 30; the working electrodes were the XCAPPy films and the counter and reference electrodes were those used for the polymerization process.

Swelling degree at equilibrium (Q) was calculated according to Eq. (1):

$$Q = \frac{m_{\text{water}}}{m_{\text{driedgel}}} = \frac{m_{\text{swollengel}} - m_{\text{driedgel}}}{m_{\text{driedgel}}} \quad (1)$$

where m_{driedgel} is the mass of dried hydrogel, m_{water} is the amount of water absorbed by the gel and $m_{\text{swollengel}}$ is the mass of swollen hydrogel.

Fourier transform infrared (FTIR) spectra were obtained in a Bomem MB100 equipment with the resolution of 4 cm^{-1} and 32 scans per spectrum. Samples were prepared using KBr pellets. Thermal behavior was investigated by differential scanning calorimetry (DSC, TA-DSC Q10V9.0 equipment), according to the procedure described by Guru and coworkers [26] for xanthan. The samples underwent heating/cooling/heating cycle. In first cycle of measurements, samples were heated up to $90 \text{ }^\circ\text{C}$ and equilibrated to remove remaining water content in the hybrid material films, then cooled down to $-10 \text{ }^\circ\text{C}$ and reheated up to $250 \text{ }^\circ\text{C}$. The heating/cooling/heating rate was set at $10 \text{ }^\circ\text{C/min}$. Samples ($\sim 3 \text{ mg}$) were placed inside hermetically closed Al pans. Thermogravimetric analyses (TGA) were performed in a TGA-STA i1500 equipment. For TGA analyses the samples were dried in an oven at $50 \text{ }^\circ\text{C}$ until constant weight and kept in desiccator. They were removed from desiccator just prior to the measurements. The samples ($\sim 10 \text{ mg}$) were heated from $25 \text{ }^\circ\text{C}$ to $900 \text{ }^\circ\text{C}$, at heating rate of $10 \text{ }^\circ\text{C/min}$, under dynamic N_2 atmosphere (50 mL/min), using Pt crucibles. SEM analyses were performed in a Jeol microscope FEI7401F equipped with a Field-Emission Gun. Samples were prepared by cryo-fracturing freeze-dried hydrogels. Resultant surfaces were analyzed after gold coating (sputtering). Atomic force microscopy (AFM) analyses were performed with a PICO SPM-LE (Molecular Imaging) microscope in intermittent contact mode in air at room temperature, using silicon cantilevers with resonance frequency close to 300 kHz . Areas of $1 \text{ } \mu\text{m} \times 1 \text{ } \mu\text{m}$ were scanned with a resolution of 512×512 pixels. Image processing and the determination of the root mean square (rms) roughness were performed using the Pico Scan software. Mechanical properties were evaluated in a DMA Q800-TA Instruments for dried films 0.010 mm

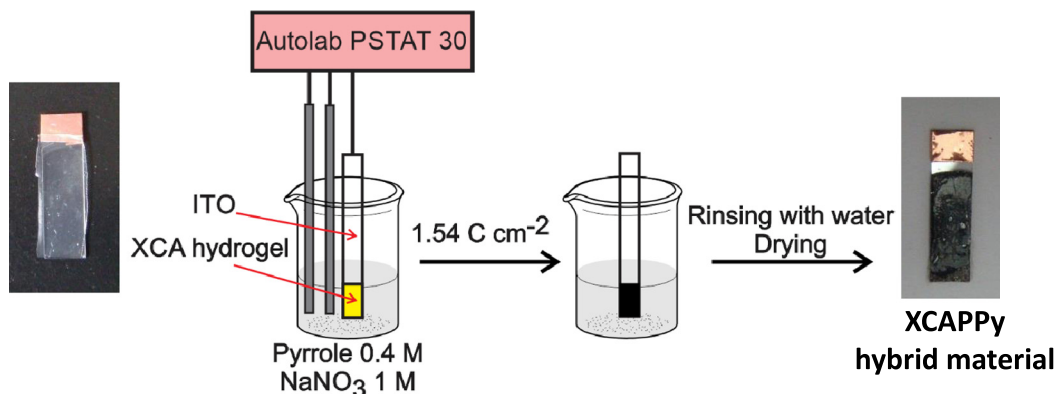


Fig. 1. Schematic representation of the experimental setup for the production of XCAPPy hybrid materials.

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