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Wettable arrays onto superhydrophobic surfaces for bioactivity testing of inorganic nanoparticles

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ABSTRACT

Poly(L-lactic acid) superhydrophobic surfaces prepared by a phase-separation methodology were treated with 30 min exposition of UV/O_3 irradiation using hollowed masks in order to obtain patterned superhydrophilic squared-shaped areas. These wettable areas successfully confined bioactive glass nanoparticles (BG-NPs), by dispensing and drying individual droplets of BG-NPs suspensions. The obtained biomimetic chips were used to test the *in vitro* bioactivity of binary (SiO_2–CaO) and ternary (SiO_2–CaO–P_2O_5) nanoparticles produced using sol–gel chemistry by immersing such substrate in simulated body fluid (SBF). From SEM and EDX it was possible to conclude that the ternary system promoted an enhanced apatite deposition. This work shows the potential of using such flat disposable matrices in combinatory essays to easily evaluate the osteoconductive potential of biomaterials using small amounts of different samples.

1. Introduction

Superhydrophobic surfaces have attracted an increasing interest on worldwide research [1–4]. These surfaces with contact angles higher than 150°, exhibit extreme water repellency and have potential applications in a variety of scientific and industrial fields [5,6]. Some natural surfaces, like lotus leaves [7], show superhydrophobic characteristics due to the existence of a rough topography of the surface at both the micro and nano scales. Different methodologies have been proposed to produce artificial rough surfaces with similar features [8]. Rough surfaces made of poly(L-lactic acid), PLLA, exhibiting a superhydrophobic behavior were prepared using a phase-separation method [6]. The aim of this work is to demonstrate that such kind of biodegradable superhydrophobic substrates can be used to produce innovative chips that are able to act as a practical substrate to perform multiplexing tests of biomaterials. In this case we focus the bioactivity studies to address relationships between biomaterial characteristics and osteoconductive potential. The production of such chips is based on the fact that the wettability can be increased by exposing the surface to UV/O_3 radiation. By using adequate masks one can produce patterned superhydrophilic spots that can be used to confine different biomaterials.

Bioactive inorganic nanoparticles have a potential to be applied in a variety of biomedical applications, including bone tissue engineering and biomimetic nanocomposites [9–12]. The chemical composition of such nanoparticles and the processing conditions may influence their osteoconductive behavior. As many variables may be involved, combinatory methodologies should be developed to access biomaterial characteristics/property relationships. In this work bioactive glass nanoparticles based on the ternary and binary systems were prepared using protocols previously reported [13–15].

We demonstrate that biodegradable superhydrophobic substrates can be used to produce disposable chips that are able to easily evaluate important characterization aspects such as the *in vitro* bioactivity of materials. For the proof-of-concept binary and ternary formulations of bioactive nanoparticles will be tested to demonstrate the validity of the proposed methodology. We envisage that this kind of inexpensive chips has the potential to be applied to other kind of characterization tests needed in the biomaterial area where multiple effects are needed to be explored.

2. Materials and methods

2.1. Materials

Tetraethyl orthosilicate (TEOS, 99.90% pure), citric acid monohydrate (99–102%), ammonium phosphate dibasic, calcium nitrate tetrahydrate (99%), ethanol absolute, and ammonia water (ammonium hydrogen phosphate (98%), maximum of 33% NH_3) were purchased from Sigma-Aldrich. The used PLLA has $M_n = 69{,}000$ and $M_W/M_n = 1.734$.

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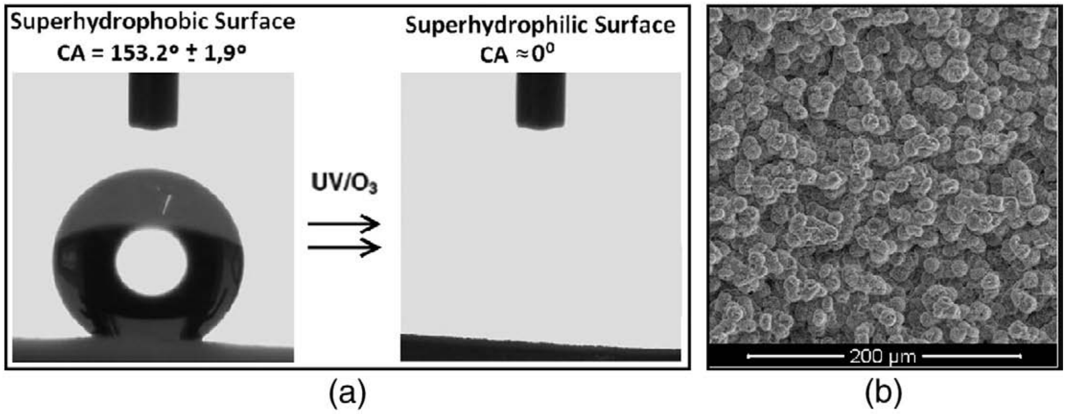

Fig. 1. (a) Change in wettability of the PLLA superhydrophobic surface after 30 min exposition with UV/O_3 irradiation; (b) SEM image of the superhydrophobic surface.

2.2. PLLA surface preparation

Flat smooth PLLA sheets were processed by compression molding. Superhydrophobic PLLA substrates were prepared by spreading a PLLA/dioxane 13% (wt/v) solution over pieces of smooth PLLA sheets (10×10 mm^2); After a few seconds the substrates were immersed in absolute ethanol during 1 h, to induce phase separation. The samples were dried in a vacuum oven for 24 h at 40 °C to eliminate all solvent residues. When the samples are completely dry, the upper part is removed. The surface of the original substrate exhibits in this way the desirable micro/nano-meter rough topography. A squared hollowed plastic mask with open regions with a 1×1 mm^2 size was used to improve the wettability in the desired areas by irradiating the surface for 30 min with UV/O_3 radiation using a BioForce UV/Ozone ProCleaner device.

2.3. BG-NP preparation

To prepare the bioactive glass nanoparticles (BG-NPs) with the composition SiO_2:CaO:P_2O_5 (mol.%) = 55:40:5, a protocol based on a previous work was followed [13–15]. The same procedure with the necessary adaptations was followed to obtain SiO_2:CaO (mol.%) = 70:30, where no phosphorous precursor was used [14].

2.4. In vitro bioactivity study

In vitro bioactivity tests were carried out by soaking the 10×10 mm^2 surfaces in 50 mL of SBF (simulated body fluid) solution during 0 (control, before SBF immersion), 3 and 7 days at 37 °C. The samples were then rinsed with distilled water and left to dry. The preparation of SBF followed the protocol described by Kokubo and Takadama using reagents from Sigma-Aldrich [16].

2.5. SEM and EDX

To study the composition and morphology of the surfaces, a NanoSEM-FEI Nova 200 (FEG/SEM) scanning electron microscope was used. A Pegasus X4M instrument was used to perform the EDX experiments.

3. Results and discussion

Superhydrophobic PLLA surfaces were successfully prepared with a CA higher than 150° (Fig. 1 (a)). Such behavior can be explained by the obtained roughness of the surface that exhibited a hierarchical structure at both the nano and micro-scales (Fig. 1(b)). Upon exposure with UV/O_3 radiation, the PLLA surface could acquire a

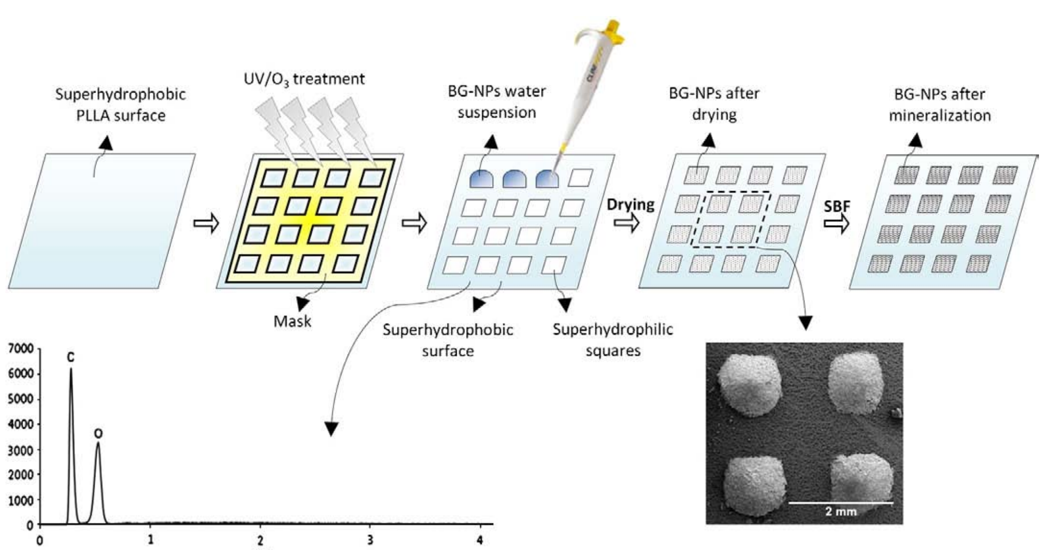

Fig. 2. Preparation of the chips used for the bioactivity testing showing the resulting EDX data for the superhydrophobic surface and low magnification SEM image for the areas containing the BG-NP.

superhydrophilic character (Fig. 1(a)). Fig. 2 shows how such superhydrophilic modification was controlled into approximately 1×1 mm^2 squared regions to produce an array where 1 μl droplets of suspensions of the nanoparticles were individually deposited. The droplets were kept confined and separated from each other due to the strong difference in the surface tension between the superhydrophilic and superhydrophobic regions. After drying the chip, the BG-NPs were kept in the superhydrophilic spots. Fig. 2 shows EDX spectra obtained in the PLLA region and a low magnification SEM image which demonstrate the formation of spots with BG-NPs on the array. *In vitro* biomineralization studies in SBF were performed to assess the osteoconductive potential of two different formulations of BG-NPs (binary and ternary). EDX spectra and SEM micrographs of the superhydrophilic arrays, with the two types of BG-NPs soaked in SBF for different incubation periods (0, 3 and 7 days), are present in Fig. 3. The carbon (C) peak corresponds to the substrate (PLLA surface); the oxygen (O) peak could be due to the substrate, to both BG-NPs, and to apatite; the phosphorous (P) peak could be attributed to ternary BG-NPs and to apatite, but only to apatite in the binary BG-NPs, as this formulation does not contain phosphorus; the calcium (Ca) peak could correspond to both BG-NPs and to apatite.

An indication of the development of an apatite precipitate in soaked samples, in comparison with non incubated samples, is that the concentrations of Ca and P gradually increase as the concentration of Si decreases due to the dissolution of the BG-NPs [13,15]. Moreover, the SEM images revealed the formation of mineral agglomerates — see

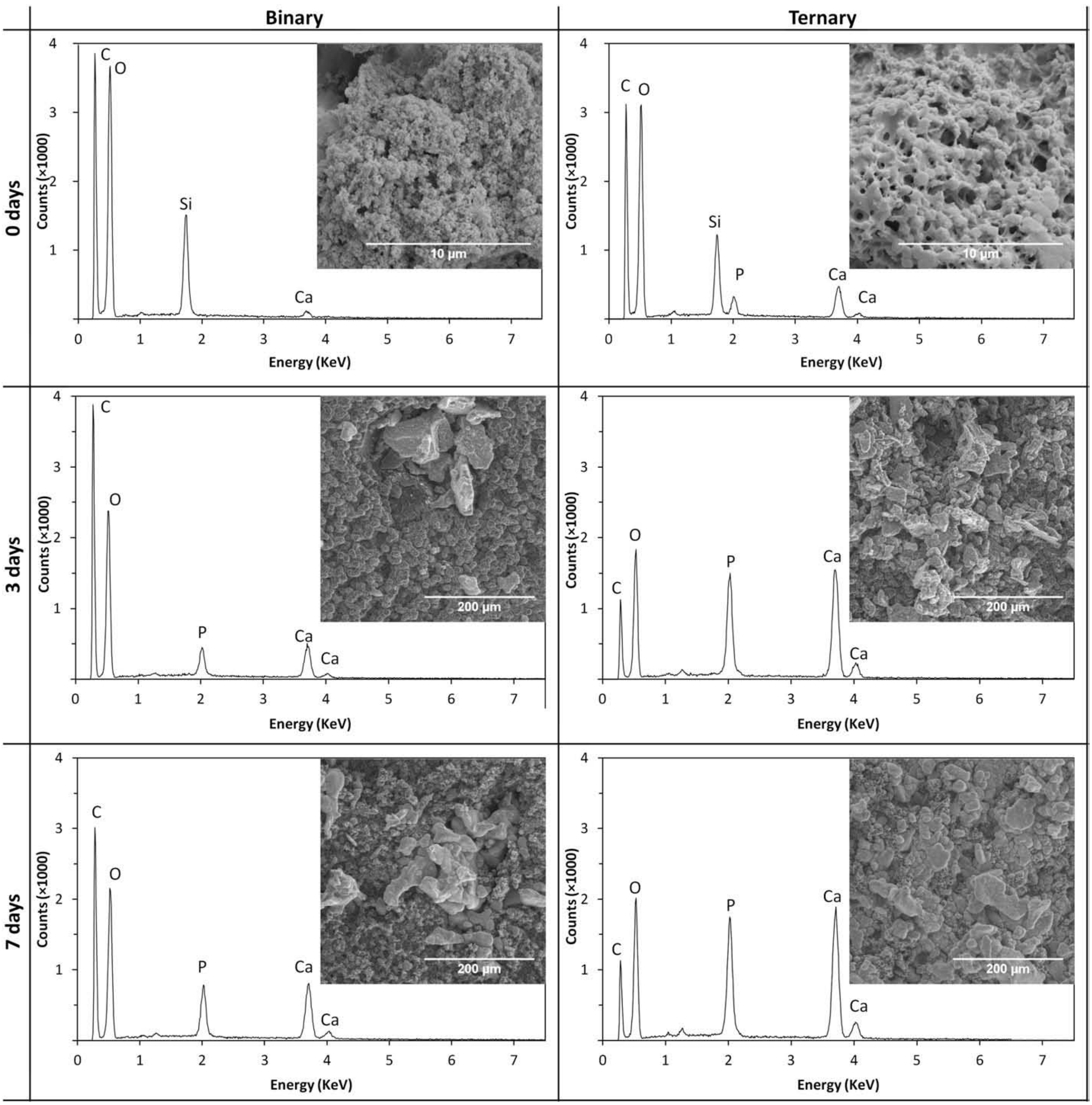

Fig. 3. Characterization of the chemical elements using EDX and the correspondent SEM micrographs of hydrophilic arrays which contained binary or ternary BG-NP soaked in the SBF solution during 0, 3 and 7 days.

Fig. 3. Furthermore EDX showed Ca/P ratios which are closed to the hydroxyapatite stoichiometric theoretical value (1.67): 1.72 for binary during 3 days; 1.61 for binary during 7 days; 1.56 for ternary during 3 days; and 1.60 for ternary during 7 days. These results confirm the bioactive nature of the BG-NPs. The ratio between the C and P (or Ca) peak intensity could provide a qualitative indication of the calcification extent in each spot. EDX of binary BG-NPs exhibits lower peaks of P and Ca than the ternary, which means that ternary BG-NPs are more bioactive than the binary composition. After 7 days of immersion in SBF the hydrophilic arrays presented a larger amount of apatite than for the 3 day case. This result is visible in the EDX graphs, where a slight relative increase in P and Ca peaks in both types of BG-NPs from 3 days to 7 days can be observed. In addition SEM images revealed a more uniform apatite layer after 7 days of immersion in SBF. The increase of mineral deposits with increased incubation time is related to the longer time available for apatite precipitation.

4. Conclusions

This work proposed a new straightforward methodology to test and compare the bioactivity of different BG-NP formulations, by confining reduced amounts of BG-NPs in wettable spots organized in an array onto superhydrophobic substrates. We envisage the use of such patterned substrates for other bioactivity combinatory tests.

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