Poly(vinylalcohol)/Hydroxiapatite Hydrogels Obtained by Two Different Techniques: Characterization and Swelling Studies

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ABSTRACT

The applications of Poly(vinylalcohol)/hydroxiapatite hydrogels (PVA/HA) have been extended to the replacement of diseased or damaged articular cartilage due to its good physicochemical and especially tribological properties. The main objective of this work was to obtain and compare PVA/HA hydrogels with “in situ” generated HA by two different techniques: freezing/thawing (F/T), and ISISA (ice-segregation-induced self-assembly). For this purpose, morphological, chemical, thermal, mechanical and swelling analyses were carried out. The F/T method consists on: freezing (-18°C) a solution of PVA with Ca(OH)₂ and H₃PO₄ (stoichiometric ratio to precipitate HA) and thawing this solution (25°C), repeating the F/T cycle 3 times in order to crosslink the polymer. The ISISA process involves the submission of an aqueous gel, solution or suspension to liquid-nitrogen temperatures and subsequent sublimation. The controlled ice formation drives the segregation of every solute or colloid originally dispersed in the aqueous phase towards the zones in which the ice is absent, giving rise to areas where the ice resided before sublimation, resulting in an organized 3D scaffold. The results indicate that PVA/HA by F/T exhibits mechanical and swelling characteristics suitable for the use as articular cartilage replacement. In addition, the scaffold obtained by ISISA revealed the influence of the freezing rate on the final morphology, mechanical and swelling properties. Finally, similar studies will be carried out for scaffolds obtained by the combination of both techniques.

Keywords: Poly (vinyl alcohol), Hydroxiapatite, Hydrogel, Cartilage Replacement.

INTRODUCTION

In recent years it has been an increasing interest in using materials such as hydrogels for repairing damaged articular cartilage [1-2]. The hydrogels of poly (vinyl alcohol) (PVA) are similar to cartilaginous tissues and also have high biocompatibility and high elastic modulus even when the water content is very high [3]. The applications of these hydrogels have been extended to the replacement of diseased or damaged articular cartilage due to its good physicochemical properties and especially bio-tribological properties [4]. Moreover, the biggest problem of PVA in this kind of application is the fixation method. PVA hydrogels do not adhere inherently to human tissues for long-term and the fixation by suturing brings patient discomfort. This drawback could be overcome by mixing the PVA with a bioactive and biocompatible material such as hydroxyapatite (HA) [5]. The main objective of this work was to obtain and compare PVA/HA hydrogels with “in situ” generated HA by two different techniques: freezing/thawing (F/T), and ISISA (ice-segregation-induced self-assembly). For this purpose, morphological, chemical, thermal, mechanical and swelling analyses were carried out.

MATERIALS AND METHODS
Synthesis of PVA/HA hydrogels

PVA/HA hydrogels were obtained from aqueous solutions with 15 wt.% of PVA and 3 wt.% of HA. First, the PVA and Ca (OH)$_2$ were dissolved in distilled water and stirred at 90 °C. After 1 h of stirring, the H$_3$PO$_4$ was added under continuing agitation for 3 h. The content of Ca(OH)$_2$ and H$_3$PO$_4$ remained a stoichiometric ratio of Ca / P = 1.67 which satisfies the synthesis reaction of HA. Then the mixture was allowed to cool to room temperature and placed onto an ultrasonic bath for 30 minutes to remove all bubbles. The mixture was poured into plastic molds and three cycles of F/T (freezing at -18°C and thawing at 25°C) were performed for 12 hours per stage. To obtain the PVA hydrogel (without HA) was followed the same steps without the addition of Ca(OH)$_2$ and H$_3$PO$_4$.

Processing of ISISA: Once loaded into the moulds, typically insulin syringes (length 80 mm; diameter 4 mm), the homogeneous solutions were unidirectionally frozen by dipping the moulds at defined rate (3 mm/min) into a cold -196 °C (liquid-nitrogen) bath. The unidirectionally frozen samples were freeze-dried using an Alpha 1-2 LD Plus freeze-drier.

Swelling studies

Swelling determinations were carried out in saline solution and buffer solutions (pH 4 and 10) at 37 °C. All samples were dried before immersion until constant weight at 37 °C. The maximum swelling degree ($M_\infty\%$) percentage was determined by the following equation:

$$M_\infty\% = \frac{M_f - M_i}{M_i} \times 100$$

where $M_i$ and $M_f$ are the weights of the sample before and after immersion respectively.

Scanning electron microscopy (SEM)

Micrographs were obtained by scanning electron microscopy JEOL JSM-6460 equipment LV.

Compression tests

Compression tests were conducted for the hydrogels of PVA and PVA/HA in a universal testing machine Instron 4467 at room temperature and at crosshead speed of 5 mm/min. The samples were swollen in saline solution, at 37 °C for 72 h approximately (until equilibrium water content) before tests.

Tensile tests

The tests were performed on hydrated films in a universal testing machine Instron 4467 at room temperature and traverse speed of 50 mm/min.

Friction tests

Friction tests were conducted, based on ASTM D1984-01 on PVA hydrogels and PVA/HA in a universal testing machine Instron 4467 at room temperature and at crosshead speed of 18 mm/min.

RESULTS AND DISCUSSIONS

Thermal and morphological characterization

It was found that the percentage of crystallinity decreased with the addition of HA (from 44.1% to 38.3%). This fact should be explained by the fact that the hydrogen bonds in PVA could not be created because PVA forms hydrogen bonds with HA [6].

Figure 1 shows the electronic micrograph of PVA samples with 3 wt.% HA, particles of uniform size dispersed in the PVA matrix and with no apparent signs of agglomeration can be observed.
Figure 1: SEM image of PVA/3 wt.% HA hydrogel.

Figure 2 presents a series of monoliths prepared under typical conditions inside cylindrical moulds. The resulting monoliths kept both the shape and size of the parent container in which the parent solutions were confined prior to ISISA. Because the ice-front progress state reached a stationary value (e.g., nominal) at the upper half of the monolith, the characterization and experiments were always conducted on that portion [7]. SEM inspection of their transversal section revealed a homogeneous texture and the radial ice front pattern in both cases (FT/ISISA and ISISA monoliths, (A) and (B) respectively). However, the FT monolith (C) reveals a random pattern similar to stacking layers. These differences could be assigned to the different interaction between water and the solute (PVA); in ISISA process the ice crystal formed covert the solute and grows while in F/T the interaction occurs between water and the solute.

Mechanical Properties

Table 1 summarizes the mechanical and swelling properties of PVA/HA hydrogels obtained from different tests.

<table>
<thead>
<tr>
<th>HA (%)</th>
<th>Compression tests</th>
<th>Tensile tests</th>
<th>swelling results of PVA hydrogels.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$E$ (MPa)</td>
<td>$\sigma_m$ (MPa)</td>
<td>$\varepsilon_r$ (%)</td>
</tr>
<tr>
<td>0</td>
<td>23.3±4.6</td>
<td>3.3±0.8</td>
<td>82.0±1.4</td>
</tr>
<tr>
<td>3</td>
<td>148.6±13.1</td>
<td>&gt;29.2</td>
<td>&gt;43.0</td>
</tr>
</tbody>
</table>

A notable increase in the modulus by incorporating the HA in the PVA matrix was observed for all tests. In fact, in the case of PVA with 3 wt.% HA the module obtained in compression ($E$) was 6 times higher than the corresponding neat PVA. A significant increase in strength for the hydrogels of PVA / HA with respect to PVA can be also observed. The friction coefficients for PVA/HA hydrogels obtained were $0.078 \pm 0.011$ in the case of PVA without HA (with $85.7 \pm 0.8\%$ of water content), $0.067 \pm 0.049$ for 3 wt.% HA samples (with $82.5 \pm 2.1\%$ of water content). It is important for hydrogel implants, used as prosthetic joint replacement, to have a low coefficient of friction in order to minimize the wear of the articular joints [8].
It is noted that $M_\infty$ decreased with the addition of HA in the three tested media. This effect may be due to HA particles promote the physical crosslinking of PVA, which limits the amount of water that can absorb the hydrogel and, in turn, hinders their diffusion into the gel [9].

CONCLUSIONS
It can be concluded that PVA/HA by F/T exhibit mechanical and swelling characteristics suitable for the use as articular cartilage replacement. Moreover, the scaffold obtained by ISISA revealed the influence of the freezing rate on the final morphology, mechanical and swelling properties.

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REFERENCES