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Morphological evaluation of bioartificial hydrogels as potential tissue engineering scaffolds

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Poly(vinyl alcohol) hydrogels prepared by freeze-thawing procedure represent synthetic systems widely investigated as non-biodegradable scaffolds for tissue regeneration. In order to improve the biocompatibility properties of pure poly(vinyl alcohol) (PVA) hydrogels, blends of PVA with different biological macromolecules, such hyaluronic acid, dextran, and gelatin were prepared and used to produce "bioartificial hydrogels". The porosity characteristics of these hydrogels were investigated by scanning electron microscopy and mercury intrusion porosimetry. The morphology of bioartificial hydrogels was evaluated and compared with that of pure PVA hydrogels. In particular the effect exerted by each biological component on pore size and distribution was investigated. The obtained results indicate that when a natural macromolecule is added to PVA the internal structure of the material changes. A small amount of biopolymer induces the structural elements of PVA matrix to take on a well evident lamellar appearance and an apparent preferential orientation. Comparing the results of SEM and mercury intrusion porosimetry it was concluded that hydrogels containing 20% of biological component have the most regular structure and at the same time the lowest total porosity. On the contrary samples with the highest content of natural polymer (40%) show the less regular structure and the highest total porosity.

Introduction

Tissue reconstruction represents one of the most important uses of polymeric biomaterials. Although degradable polymers are those mostly employed, both degradable and non-degradable polymers are used as tissue engineering scaffolds. Non-degradable polymers such as poly(vinyl alcohol) (PVA), poly(ethylene), poly(N-isopropyl acryl amide) and polyacrylates [1] have found several applications especially in cartilage repair. Among these PVA is one of the first synthetic polymers tested to produce scaffolds.

When designing a non-degradable polymeric replacement for natural tissues, several parameters need to be addressed. Some of these considerations include providing adequate mechanical properties, having good biocompatibility, allowing the material to adhere well to surrounding tissue and resisting wear and fatigue. With the exception of mechanical properties, many synthetic hydrogels can meet these prerequisites [2]. PVA is able to form, by a freeze thawing procedure, hydrogels that have a water content similar to that of natural tissues. Like many hydrogels, those of PVA lack sufficient mechanical stability for use in their unmodified form [3]. However a PVA hydrogel offers several beneficial properties: it is relatively biocompatible, it can swell to accommodate a large water content, it can be sterilized and it can be molded into desired shapes. In addition growth factors or other bioactive agents could be incorporated, directly or by preliminary loading into nano- or microparticles [4, 5], into the PVA hydrogel matrix in order to promote favourable cellular responses.

In order to improve the biocompatibility properties of PVA hydrogels, blends of PVA with different biological macromolecules, such as collagen, hyaluronic acid (HA), dextran (Dx), chitosan, gellan etc. were used in our laboratories to prepare hydrogels (bioartificial hydrogels) by 8 freeze-thawing cycles [6–8]. The properties of these materials have been widely investigated [9] in order to establish their potential

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applications as biomaterials and in particular as drug release systems [10, 11].

In the present work dextran/PVA, gelatin/PVA, hyaluronic acid/PVA hydrogels, with various compositions, were prepared and their morphological properties were investigated by scanning electron microscopy (SEM) and Hg porosimetry.

It is well known that in order to promote tissue growth a material intended to be used as a scaffold must be highly porous. Pores must be large enough so that cells can penetrate and must be interconnected to facilitate nutrient and waste exchange by cells deep within the scaffold.

In the present work the porosity characteristics of PVA based hydrogels were studied. In particular the effect exerted by each biological component on pore size and distribution was investigated. The morphology of bioartificial hydrogels was compared with that of pure PVA hydrogels with the aim to establish the potential use of these materials as matrices for tissue regeneration.

Materials and methods

Poly(vinyl alcohol) 99% hydrolized (average molecular weight MW 85,000-146,000) Gelatin, type B from bovine skin (75 Bloom) and Dextran (MW 69,000, produced by *Leuconostoc Mesenteroides*) were supplied by Sigma Aldrich, Italy.

Hyaluronic acid (sodium salt, MW 200,000) was kindly supplied by Fidia Advanced Biopolymers S.p.A. Italy.

All chemicals were used as received.

Preparation of PVA hydrogels

A 10% PVA aqueous solution was prepared in an autoclave for 1 h at 120 °C.

Aqueous solutions of gelatin (Gel), dextran and HA respectively were prepared adding 3 g of each biological component to 100 ml of distilled water under stirring at about 50 °C.

The PVA solution was blended with each of the biological solutions to produce Gel/PVA, Dx/PVA, and HA/PVA blends with the following weight ratios: 10/90, 20/80, 30/70 and 40/60. The final PVA concentration was 2.5% in all the prepared mixtures.

The samples underwent eight freeze-thawing cycles to obtain hydrogels. Each cycle, with the exception of the first one, consisted of 1 h at −20 °C and 30 min at room temperature. The first cycle differed from the others due to a longer standing time (over night) at −20 °C.

Scanning electron microscopy

The internal structure of the hydrogels was analysed by a scanning electron microscope (SEM) Jeol JSM-5600 LV. The samples were freeze-dried and then mounted on aluminum stubs and coated with gold prior to examination.

Mercury intrusion porosimetry

Hg intrusion porosimetry was performed on freeze-dried hydrogels.

Pore size in the range 0.007–120 μm in diameter was evaluated by Hg intrusion using a Carlo Erba Pascal 140 Porosimeter, equipped with an automatic recording of intruded Hg volume. The pore volume distribution was obtained from the derivative curve of the cumulative intruded pore volume as function of pore diameter. This latter parameter is related to the measured pressure according to the Washburn model equation [12], developed for the intrusion of cylindrical shape pore:

$$d = (4\gamma \cos\theta / P) * 10$$

The cylindrical diameter d (μm) of the filled pores is inversely proportional to the intrusion pressure P(kg cm^{-2}), when Hg surface tension γ (0.48 Nm^{-1}) and the contact angle θ between Hg and the material are constant. Different values of the contact angle, around 140 °, had been measured for the different hydrogels.

Results and discussion

Macroporous hydrogels, based on blends of PVA with natural polymers, were prepared by freeze-thawing procedure.

The effects of blending PVA with a biological component on the morphology and pore distribution of the materials were investigated by SEM and mercury intrusion porosimetry.

SEM micrograph (Fig. 1) of a pure PVA hydrogel shows a porous morphology. The structural elements of PVA matrix do not have a preferential spatial orientation.

When natural macromolecules are added to PVA, independently on their content, no phase separation is observed at the considered magnifications, while the internal structure of the material changes.

A small amount of gelatin (10%) (Fig. 2) or dextran or hyaluronic acid (20%) (Fig. 3) induces the structural

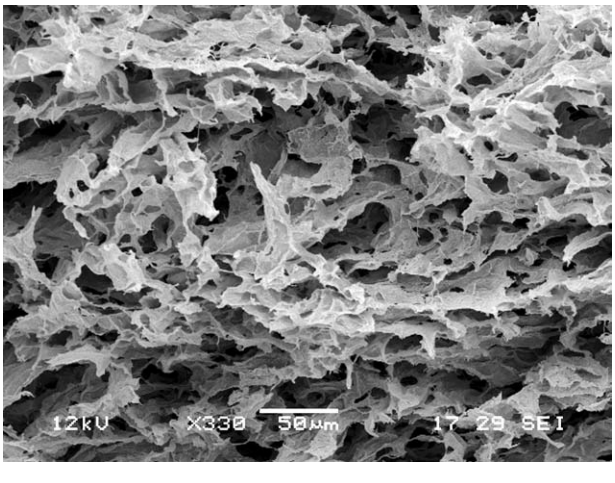

Figure 1 SEM micrograph of a pure PVA hydrogel.

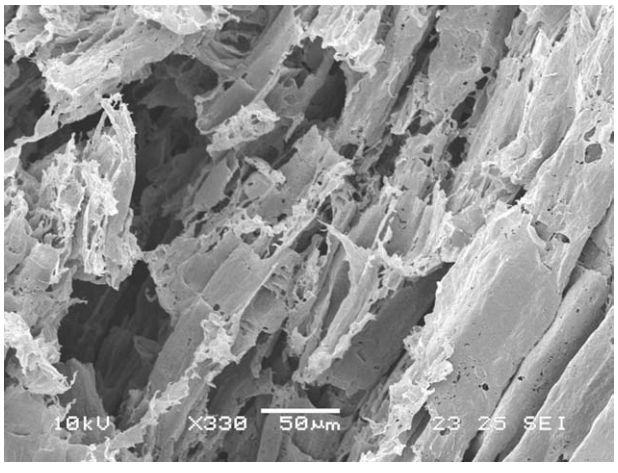

Figure 2 SEM micrograph of a gelatin/PVA = 10/90 hydrogel.

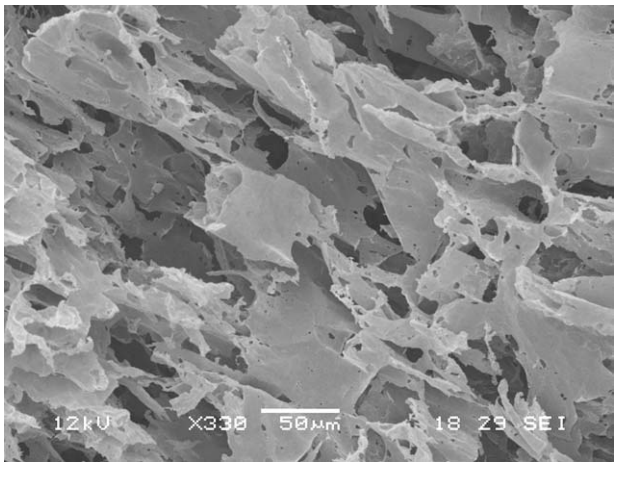

Figure 3 SEM micrograph of a dextran/PVA = 20/80 hydrogel.

elements of PVA matrix to take on a well evident lamellar appearance and an apparent preferential orientation.

In the case of HA/PVA hydrogels, the lamellar structure, well evident for a HA content equal to 20%, remains unchanged by increasing the HA content and a wider extension of the lamellas can be observed (Fig. 4).

Also in the case of Dx/PVA hydrogels, the lamellar structure results well evident for the 20/80 composition,

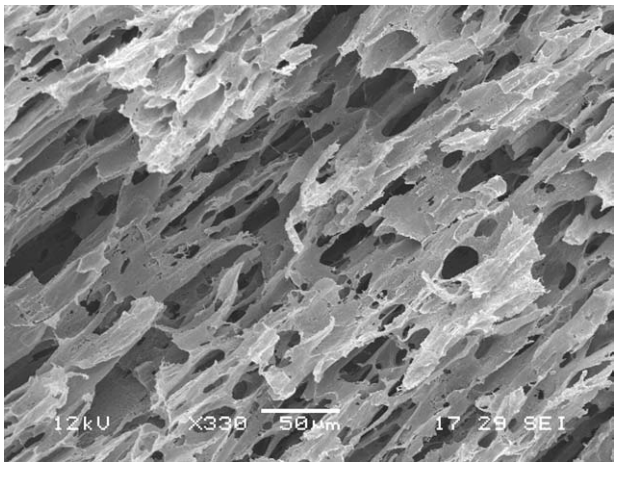

Figure 4 SEM micrograph of a HA/PVA = 40/60 hydrogel.

but it becomes gradually less ordered as dextran content increases.

SEM micrographs show that when the biological component of the hydrogels is a protein like gelatin, a lower amount of this (10%) is enough to produce the lamellar structure as shown in Fig. 2. This kind of structure remains unchanged for the 20/80 composition and then it becomes less ordered increasing the content of gelatin.

With regard to porosimetric analysis, it has to be underlined that the description of the pore volume distribution was limited to the 10–50 μm pore diameter range. In fact the contribute of the higher pore classes (50–120 μm) resulted negligible. In addition pores with a diameter lower that 10 μm were not investigated taking into consideration the potential application of the materials as tissue engineering scaffolds. It has been widely accepted that cell-scaffold interaction is greatly influenced by the scaffold porous structure and especially by its pore size.

It is known that there is an optimal pore size for cell infiltration and host tissue ingrowth; for instance 5–15 μm for fibroblasts [13], 20–125 μm for adult mammalian skin tissues [14, 15], 40–100 μm for osteoid tissues [13] and 100–350 μm for bone tissues [13, 16].

In Fig. 5 is reported the distribution of the total pore volume for a pure PVA hydrogel and a dextran/PVA = 40/60 hydrogel.

It is well evident that the 10–50 μm pore diameter range taken into consideration shows the highest volume.

Within the investigated range it was found that pure PVA hydrogels and bioartificial hydrogels containing gelatin have the most homogeneous pore volume distribution.

For all the investigated hydrogels the prevailing pore class is that in the range 10–20 μm (Figs. 6–8). In the case of bioartificial hydrogels, it was observed that increasing the content of the biological component, the pore volume progressively increases both in the 10–20 and in the 20–30 μm pore diameter range.

This phenomenon, less evident in the case of Gel/PVA hydrogels (Fig. 6), becomes more evident for HA/PVA hydrogels (Fig. 7) and much more pronunced in the case of dextran-containing hydrogels (Fig. 8).

The analysis of the data, related to the overall porosity indicates that there is a contraction of the total porosity moving from pure PVA to the samples containing 10% of the biological component (20% in the case of HA).

This contraction results well evident also on the basis of the volume values regarding the individual pore classes reported in Figs. 6–8.

Comparing the results of SEM and mercury intrusion porosimetry it can be concluded that hydrogels containing a low amount (10–20%) of biological component have the more regular structure and at the same time the lowest total porosity. On the contrary samples with the highest content of natural polymer (40%) show the less regular structure and the highest total porosity.

The 20–30 μm pore class is that who better highlights this transformation.

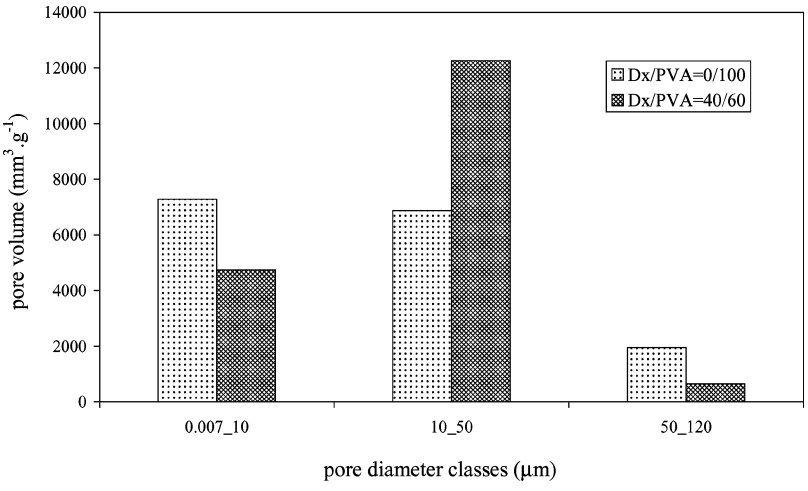

Figure 5 Distribution of the total pore volume for a pure PVA hydrogel and a dextran/PVA = 40/60 hydrogel.

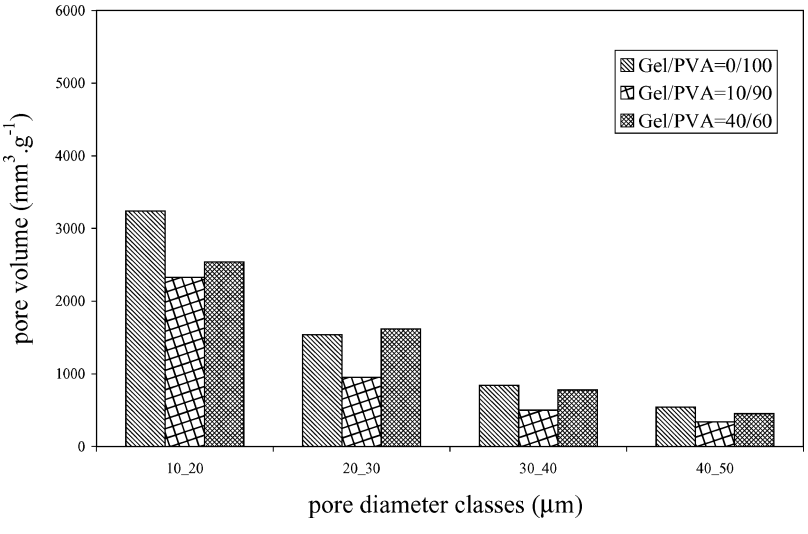

Figure 6 Pore volume as a function of pore diameter classes (μm) for gelatin/PVA hydrogels with the following compositions: 0/100, 10/90, 40/60.

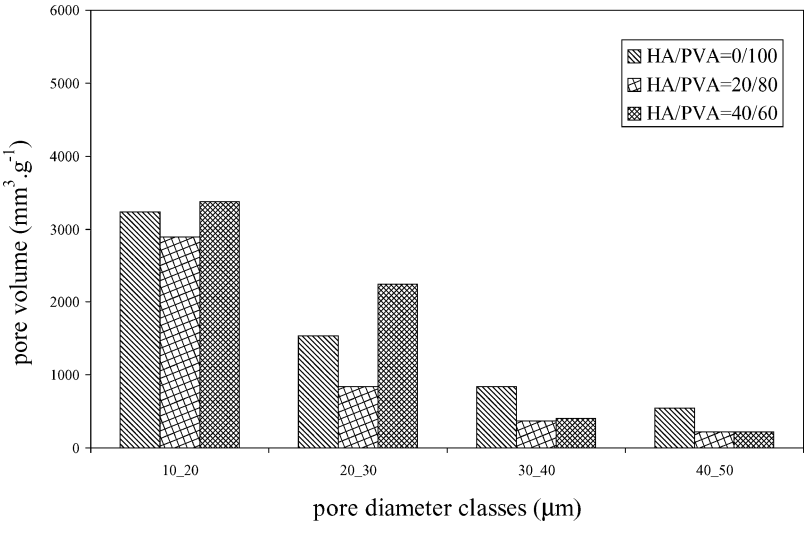

Figure 7 Pore volume as a function of pore diameter classes (μm) for HA/PVA hydrogels with the following compositions: 0/100, 20/80, 40/60.

On the basis of these data it can be hypothesized that the lamellar structural units take on a prevalent disposition face-to-face, when the content of the biological component is low (10–20%). This disposition allows the lamellas to get closer and thus the porosity is reduced.

Increasing the content of the biological component the lamellas change their disposition toward a face-to-edge configuration that hinders them to get closer and this induces an increase of the total porosity.

Worth underlining that Dextran/PVA = 40/60 hydrogels show not only a value of the overall porosity higher

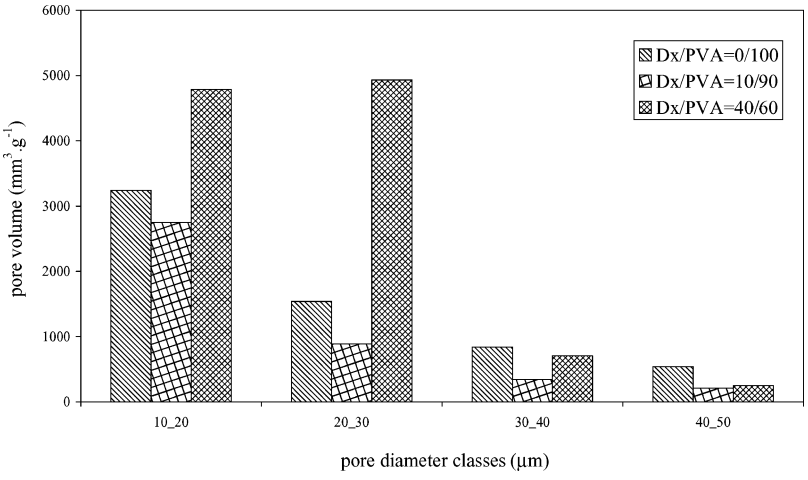

Figure 8 Pore volume as a function of pore diameter classes (μm) for dextran/PVA hydrogels with the following compositions: 0/100, 10/90, 40/60.

with respect to that of pure PVA hydrogels but also by far the highest value of overall porosity.

In order to explain this behaviour of dextran, different with respect to that of the other investigated biopolymers, the results of a previous study on the properties of dextran/PVA hydrogels can be considered. It was observed [7] that dextran affects the structure of PVA matrix at a molecular level exerting a stabilizing effect on PVA crystallization. This is probably dependent on its chemical structure and molecular weight but further investigation are necessary for a better understanding.

Conclusions

The morphology and porosity characteristics of bioartificial hydogels based on PVA and containing different amounts of biological polymers were investigated.

It was observed that when a natural macromolecule is added to PVA the structural elements of PVA matrix take on a well evident lamellar appearance and an apparent preferential orientation. A contraction of the total porosity is induced by the presence of a small amount (10–20%) of biopolymer while increasing the content of the natural component (40%) the total porosity increases.

It seems that the addition of gelatin or hyaluronic acid, independently on their content, does not improve the porosity characteristics of the PVA matrix since pure PVA hydrogels show a value of the overall porosity higher with respect to that of hydrogels containing gelatin or hyaluronic acid. On the contrary the presence of a high amount of dextran (40%) induces a significative increase of the overall porosity.

It can be concluded that, as a whole, the investigated materials show porosity characteristics (overall porosity and pore diameters) that allow them to be tested as substrates for fibroblasts growth.

In vitro tests based on the cell culture method are in progress. The results of these tests will be fundamental to verify the correspondence between the porosity properties, determined in the present study, and the ability of the materials to work as scaffolds supporting cell adhesion and proliferation.

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