Hydrogels based on chitosan and dextran as potential drug delivery systems

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The release of human growth hormone (GH) from bioartificial polymeric materials in the form of hydrogels, was measured *in vitro* for up to 3 weeks. Poly(vinyl-alcohol) (PVA) was blended, in different ratios, with two biological polymers, dextran and chitosan respectively. These blends were used to prepare hydrogels, using a freeze–thawing method. The hydrogels were loaded with GH, and their potential use as delivery systems was investigated. The release with time of PVA, in aqueous medium, was also monitored and evaluated. Scanning electron microscopy was used to investigate the structure of the hydrogels.
The results obtained indicated that GH can be released from both dextran/PVA and chitosan/PVA hydrogels. The initial GH concentration used for sample loading affected the total quantity of GH released but not the pattern of release. The amount of GH released was affected by the content of the biological component. The percentage of PVA released was low but it was, however, related to the content of chitosan and dextran in the blends.
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1. Introduction

Synthetic and natural polymers are used for numerous applications in the biomedical field. However the properties of pure synthetic polymers and those of pure biological polymers alone are often inadequate to produce materials with a good combination of chemical, mechanical, thermal and biological performance characteristics. As a result of this, a set of new materials has been prepared by blending synthetic polymers with biological macromolecules [1–3].

These materials are defined as ''bioartificial polymeric materials'' and have been produced in various forms, for example films, sponges and hydrogels. These have been evaluated for different applications as biomaterials: dialysis membranes [4], wound dressing [5], drug delivery systems [6, 7] and so on. In particular, blends of poly(vinyl alcohol) with collagen (C) or hyaluronic acid (HA) have been used for the preparation of bioartificial polymeric materials in the form of hydrogels [8–10] by a freeze–thawing method [11]. As a result of their high water content, high permeability to small molecules and adequate mechanical properties, hydrogels are potentially useful systems as drug delivery devices.

The design of controlled drug delivery systems, whereby a drug is delivered in the correct dose to the target cells for the appropriate length of time, is particularly advantageous for GH and other growth factors [12, 13].

The development of a controllable, release system for the delivery of GH and other growth factors may help to improve wound healing and tissue repair in numerous biomedical applications. GH can be incorporated into different types of materials such as cements [14–16], ceramics [17] and polymers [18], to create a ''bioactive'' material. This can be released *in vitro* and has the ability to stimulate osteogenesis [19, 20].

The use of C/PVA and HA/PVA hydrogels as delivery systems for GH has been previously investigated [6, 7]. Both C/PVA and HA/PVA hydrogels proved to be useful for GH delivery.

The concentrations of GH released from these materials were within a physiological range and sufficient to have a local effect on cellular proliferation. In addition it was observed that increasing the content of the biological component, increased GH release and thus could be used as a control process [6, 7].

In this work hydrogels were prepared, starting from blends of PVA with chitosan and dextran respectively, and evaluated as potential delivery agents for GH.

Chitosan and dextran are both biological macromolecules belonging to the class of polysaccharides. As a result of the *N*-acetylglucosamine repeating units, chitosan has some similarity with glucosaminoglycans, and binds to growth factors normally present in the osseous trabecular tissue and endowed with mitogenic activity towards various types of mesenchymal cells, including osteoblasts [21].

Dextran is mainly used in the field of biomaterials as a plasma expander; however it is known that derivatized

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dextran is able to stimulate osteoblast phenotype expression and therefore may be useful to enhance bone metabolism and repair [22].

Chitosan and dextran were chosen for preparing bioartificial materials as due to the combined action of the biological component and of the hormone released could represent useful delivery systems.

The material's microstructure was investigated by scanning electron microscopy. The release with time of PVA, in aqueous medium was also monitored and evaluated.

The aim of this study was to investigate the effects exerted by both chitosan and dextran on the release properties of PVA hydrogels, and compare these effects with those exerted by other biological components such as collagen or hyaluronic acid used previously.

2. Materials and methods

2.1. Materials

Dextran (average molecular weight 78 000, produced by *Leuconostoc mesenteroides*), chitosan (from crab shells) and PVA, (average molecular weight 85 000–146 000), were supplied by Sigma-Aldrich, Milano, Italy. Growth hormone was supplied by Serono, Italy.

2.2. Growth hormone solution

A solution of 0.4 ml^{-1} GH was made by adding 4 of GH powder to 10 ml 0.99% sodium chloride solution. This solution was used to incorporate GH into the hydrogels used in this study.

2.3. Hydrogel preparation

A 3% chitosan (Ch) solution was obtained by dissolving in 0.5 M acetic acid at 40 ℃. A 3% dextran (Dx) solution was prepared in distilled water. 10 g of PVA was added to 100 ml of distilled water and dissolved in an autoclave for 1 h at 120 ℃ to obtain a final concentration of 10% PVA.

The PVA solution was blended with the Ch and the Dx solution respectively in order to obtain blends with four different Ch/PVA and Dx/PVA ratios: 10/90, 20/80, 30/70 and 40/60. Each blend was dispensed in four wells of a 12-well plate (2.5 ml/well). One well was used as a control, and GH was added to the other three to produce final concentrations of 25, 75, and 100 mIU GH per ml of dextran or chitosan. Dx/PVA and Ch/PVA blends with the following biological/synthetic ratios: 10/90, 20/80 and 30/70 were also prepared and dispensed each in six wells of a 12-well plate (2.5 ml/well). GH was added to produce a final concentration in each well of 100 mIU independently of the content of the biological component. After GH addition, all samples underwent eight cycles of freeze–thawing to obtain hydrogels. Each cycle, with the exception of the first one, consisted of 1 h at –20 ℃ and 30 min at room temperature. The first cycle differed from the others due to a longer standing time (12 h) at –20 ℃.

2.4. GH elution studies

The elution of GH was monitored *in vitro*. The hydrogels were each placed in 3 ml of phosphate-buffered saline (PBS) in individual containers at 37 ℃. The elution fluid was removed at regular time intervals (every day for a week and then once a week for a further 2 weeks), and stored at –20 ℃. The eluate was replaced with PBS and the containers returned to 37 ℃.

2.5. GH assay

GH was measured using an enzyme-linked immunosorbent assay (ELISA) (Netria, London, UK). The microtitre plates were read using a 96-well plate reader (Biorad model 450). Optical density was measured at 450 nm with a reference wavelength of 655 nm.

2.6. PVA releasing test

The amount of PVA released from both Ch/PVA and Dx/PVA hydrogels, in aqueous solution, was measured *in vitro*. Each hydrogel was immersed in 30 ml of distilled water, for 144 h at 37 ℃.

At 1, 2, 3, 5, 8, 24, 48, 72, 96, 120 and 144 h the releasing solution was removed and replaced with 30 ml of fresh distilled water. The determination of released PVA was carried out spectrophotometrically according to the method of Bujanda and Rubin [23].

2.7. Scanning electron microscopy

Scanning electron microscopy (SEM) studies of the internal structure of the hydrogels were made. The materials were dehydrated by lyophilization, sputter coated with gold and observed using a Jeol T300 scanning electron microscope.

3. Results

3.1. GH release from Ch/PVA hydrogels

In the case of Ch/PVA hydrogels a two-stage release

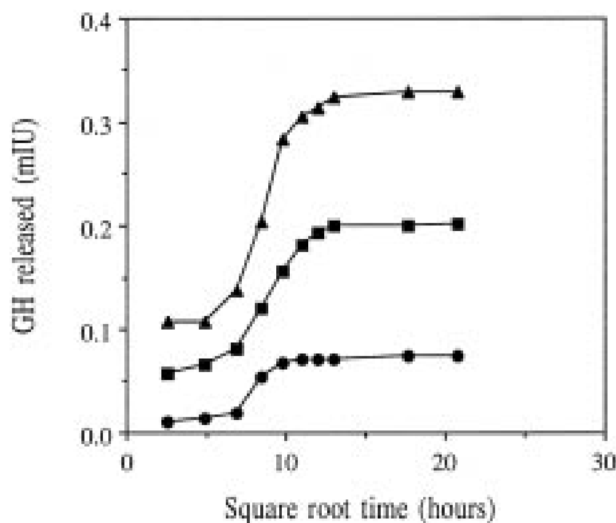

Figure 1 Release of GH from Ch/PVA hydrogels (20/80) with different initial GH concentrations: (●) 25 mIU/ ml Ch; (■) 75 mIU/ml Ch; (▲) 100 mIU/ml Ch.

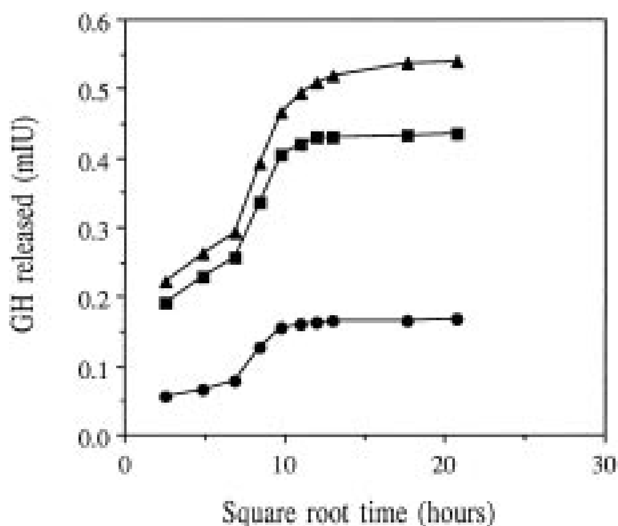

Figure 2 Release of GH from Ch/PVA hydrogels (30/70) with different initial GH concentrations: (•) 25 mIU/ml Ch; (■) 75 mIU/ml Ch; (▲) 100 mIU/ml Ch.

process was observed: a first phase, characterized by a slow release rate, was followed by a phase of rapid release until a plateau was reached. The trend was similar for all the compositions studied (Figs 1 and 2). There was a direct relationship between the amount of GH incorporated in the hydrogels and the total amount of GH released.

Fig. 3 shows the release curves for GH from hydrogels loaded with the same amount of GH (100 mIU GH/ hydrogel). It was observed that for Ch concentrations up to 30%, increasing the chitosan content of the hydrogels, decreased the total amount of GH released (Table I). Samples from the 10/90 blend released significantly more GH (paired *t*-test, $P < 0.05$) than samples from the 20/80 blend, which in turn, released significantly more GH (paired *t*-test $P < 0.05$) than samples from the 30/70 blend.

3.2. GH release from Dx/PVA hydrogels

Release profiles for Dx/PVA hydrogels all followed the same pattern, independently on the initial GH content. GH was released in a constant linear manner which subsequently reached a plateau.

The initial GH concentration did not affect the pattern of release but did, however, affect the total amount released. There was a direct relationship between the amount of GH incorporated in the hydrogels and the total amount of GH released. As an example the release profiles for Dx/PVA (20/80 and 30/70) hydrogels are reported in Figs 4 and 5.

Fig. 6 shows the release curves for GH from hydrogels loaded with the same initial amount of GH (100 mIU GH /hydrogel). It was observed that increasing the dextran content of the hydrogels, increased the total amount of GH released (Table I). Samples from the 30/70 blend released more GH than samples from the 20/80 blend, which in turn, released more GH than samples from the 10/90 blend. However this was not statistically significant ($P > 0.05$, paired *t*-test).

TABLE I Percentage of GH released from hydrogels loaded with the same initial amount of GH (100 mIU)

Blend	Composition	% of GH released
Ch/PVA	10/90	0.826
Ch/PVA	20/80	0.746
Ch/PVA	30/70	0.663
Dx/PVA	10/90	1.095
Dx/PVA	20/80	1.220
Dx/PVA	30/70	1.279

3.3. PVA releasing test

The percentage of PVA, released from Ch/PVA and Dx/ PVA hydrogels, normalized to the initial PVA content of the hydrogels, is reported as a function of time in Figs 7 and 8, respectively, where each point is the sum of the PVA amounts up to that sampling time.

The PVA amounts released from all the Ch-containing hydrogels ranged from 2 to 3% and were higher than that released from the pure PVA hydrogel. Among them, the

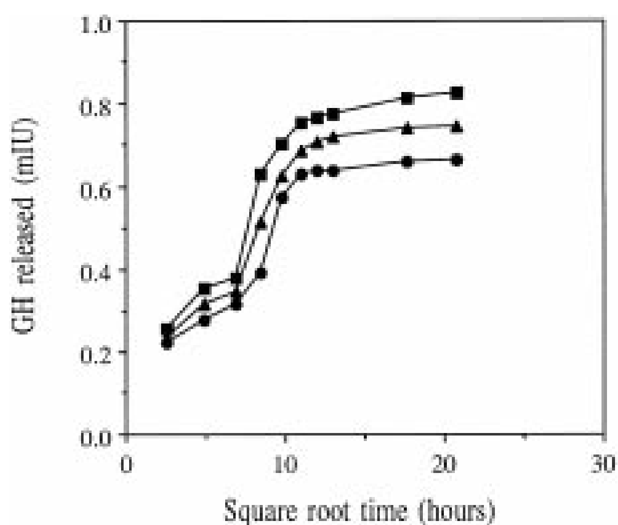

Figure 3 Release of GH from Ch/PVA hydrogels with the same GH concentration (100 mIU) but different Ch/PVA composition: (■) 10/90; (▲) 20/80; (•) 30/70.

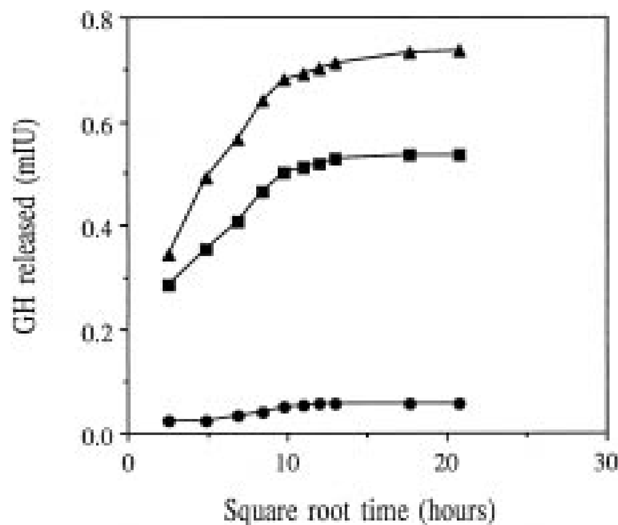

Figure 4 Release of GH from Dx/PVA hydrogels (20/80) with different initial GH concentrations: (•) 25 mIU/ml Dx; (■) 75 mIU/ml Dx; (▲) 100 mIU/ml Dx.

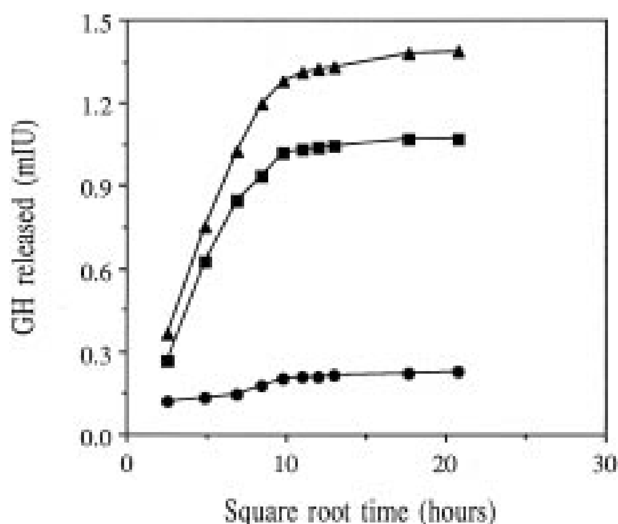

Figure 5 Release of GH from Dx/PVA hydrogels (30/70) with different initial GH concentrations: (•) 25 mIU/ml Dx; (■) 75 mIU/ml Dx; (▲) 100 mIU/ml Dx.

lowest values were observed for the 10/90 and 20/80 Ch/PVA samples.

The PVA amounts released from all the Dx-containing hydrogels ranged from 1 to 2% and were lower than that released from the pure PVA hydrogel.

3.4. Scanning electron microscopy

Fig. 9a–c show the internal structure of Ch/PVA hydrogels. The matrix is filamentous with interconnecting pores. It can be observed that by increasing chitosan content the structure becomes more macroporous.

On the contrary, in the case of Dx/PVA hydrogels (Fig. 10a–c), increasing the content of the biological component, the internal structure becomes more compact and homogeneous.

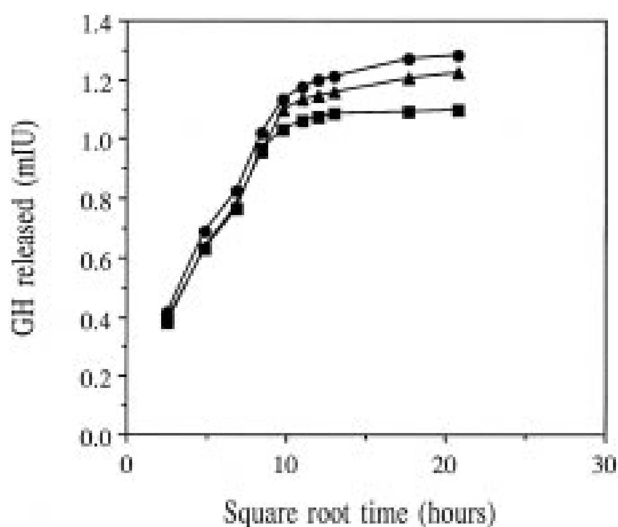

Figure 6 Release of GH from Dx/PVA with the same GH concentration (100 mIU) but different Dx/PVA composition: (■) 10/90; (▲) 20/80; (•) 30/70.

4. Discussion

In this work hydrogels were prepared starting from blends of PVA with chitosan and dextran respectively, and their potential use as delivery systems for GH was investigated. The results obtained in this study indicate that GH can be released from both Ch/PVA and Dx/PVA hydrogels.

The amounts of GH released were low with respect to the initial amount incorporated into the hydrogels. However, the GH quantities chosen for sample loading, released amounts of GH that are within a physiological range (3.125–100 ng ml^{-1}), (the conversion factor for GH is 100 ng = 0.3 mIU), and this is sufficient to have a local effect on osteoblast proliferation as demonstrated in a previous study [7].

In addition it was observed that for chitosan or dextran contents up to 30%, increasing chitosan content decreased the amount of GH released whilst increasing dextran content, increased the amount of GH released.

These differences can be explained by the fact that chitosan and dextran may have different effects on the crystallization process of PVA during hydrogel formation.

It was observed by differential scanning calorimetry (DSC) [24] that by increasing chitosan content, the degree of PVA crystallization decreases, whilst it increases with increasing dextran content. This was confirmed by the results of both SEM analysis and PVA release tests.

SEM micrographs of the internal structure of both Ch/PVA and Dx/PVA show a porous filamentous matrix which allows the transport of additives through the matrix. In the case of Ch/PVA hydrogels the structure becomes less ordered and more porous by increasing chitosan content while in the case of Dx/PVA hydrogels it becomes more homogeneous increasing dextran content.

From the results of PVA releasing test it is evident that Ch-containing hydrogels release a higher amount of PVA with respect to pure PVA hydrogels, whilst the presence of a little amount of dextran is sufficient to induce a decrease in PVA releasing.

It appears that chitosan has an adverse effect on the PVA crystallization process, whilst dextran favors PVA crystallization. This behavior could be attributed to the different chemical structure and steric hindrance of the two polysaccharides.

On the basis of these findings, a possible explanation of the results concerning GH release is that, as GH is excluded from the crystalline regions, during hydrogel formation it remains entrapped in the amorphous regions from which it is not easily released. This, therefore, increases the degree of crystallization of the material, also increases GH release.

In addition, in order to explain the two-stage release process showed by Ch/PVA and Dx/PVA hydrogels, it can be hypothesized that when the samples are immersed in the aqueous solution, the superimposition of two phenomena, that oppose each other, takes place: the uptake of water, that is an inward flux and the release of GH that is an outward flux.

Ch-containing hydrogels take more time in reaching the swelling equilibrium, because of their lower degree of crystallization and therefore of cross-linking with

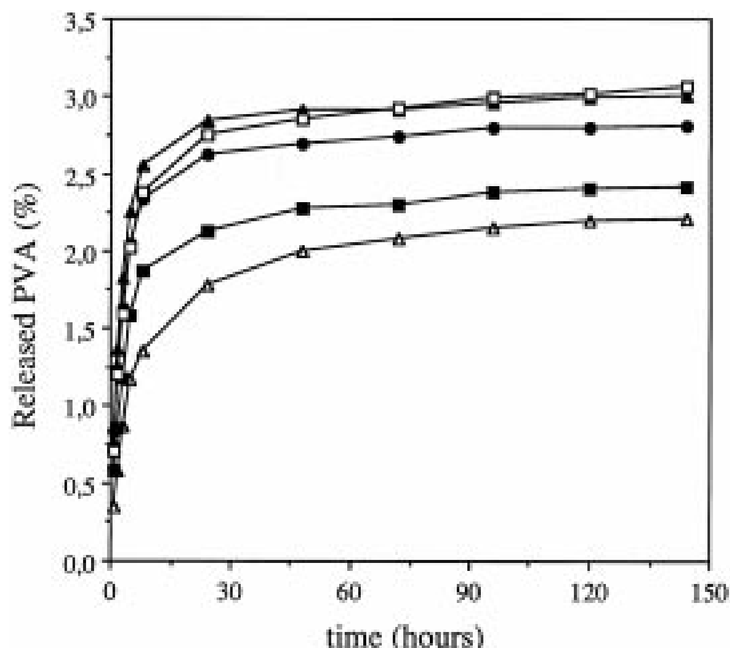

Figure 7 Percentage of PVA released from Ch/PVA, normalized to the initial PVA content of the hydrogels, reported as a function of time. Each point is the mean of two determinations (Ch/PVA: (Δ) 0/100; (■) 10/90; (•) 20/80; (▲) 30/70; (□) 40/60).

respect to those containing dextran, therefore a first phase is observed, in which water absorption opposes to GH release, followed by a second phase characterized only by GH release. On the contrary, Dx-containing hydrogels reach the swelling equilibrium very fast, because of their higher degree of cross-linking; thus the initial phase in which water absorption prevents GH release is less detectable and as a consequence GH release seems almost a single phase process.

It can be concluded that both Ch/PVA and Dx/PVA hydrogels proved to be useful for the delivery of physiological amounts of GH.

The amount of GH released was dependent on the content of chitosan or dextran; therefore, both the polysaccharides used in this study could be used as a tool in controlling drug release. In addition, their use could be advantageous because they are less expensive and more easily available in comparison with other biological macromolecules such as collagen or hyaluronic acid previously employed.

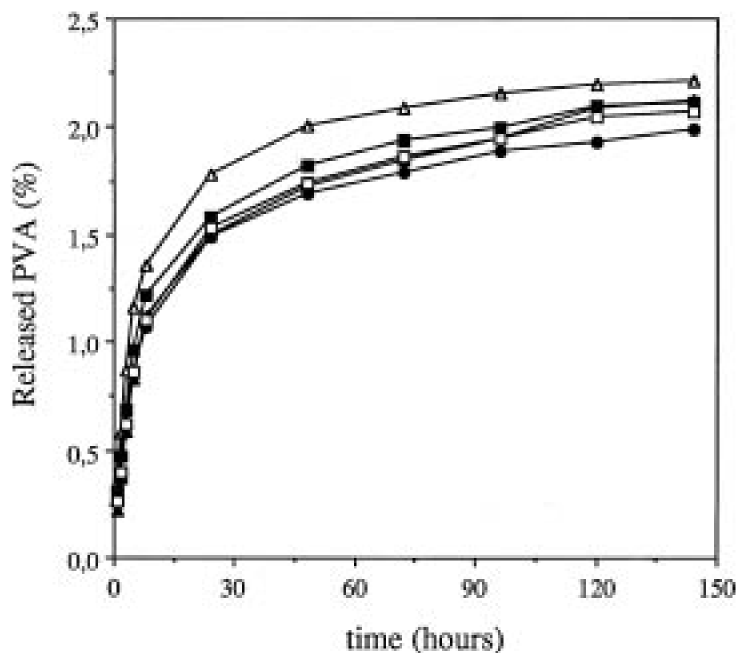

Figure 8 Percentage of PVA released from Dx/PVA, normalized to the initial PVA content of the hydrogels, reported as a function of time. Each point is the mean of two determinations (Dx/PVA: (Δ) 0/100; (■) 10/90; (•) 20/80; (▲) 30/70; (■) 40/60).

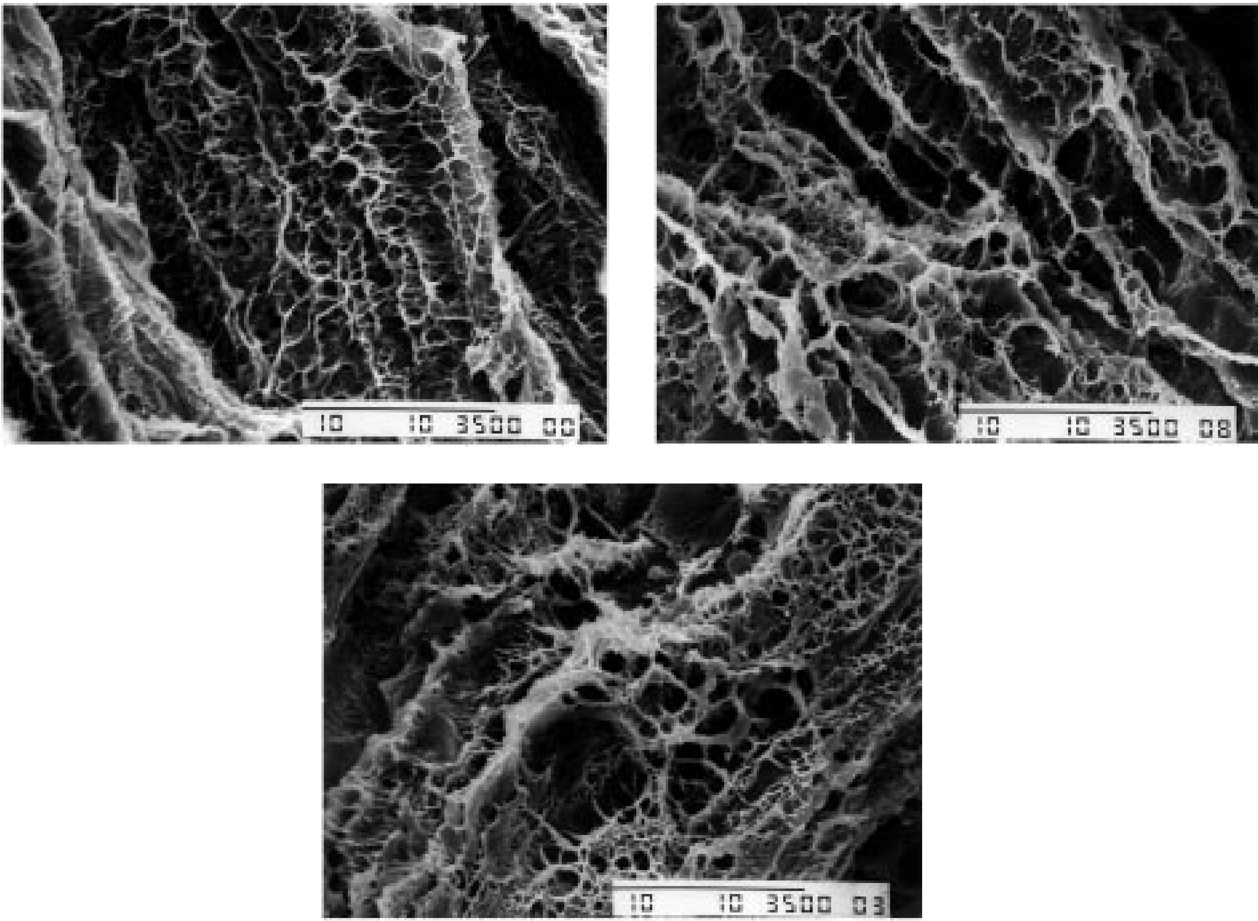

Figure 9 SEM image of the internal structure of Ch/PVA hydrogels at different chitosan concentrations: (a) 10/90; (b) 20/80; (c) 30/70. Bar = 10 μm

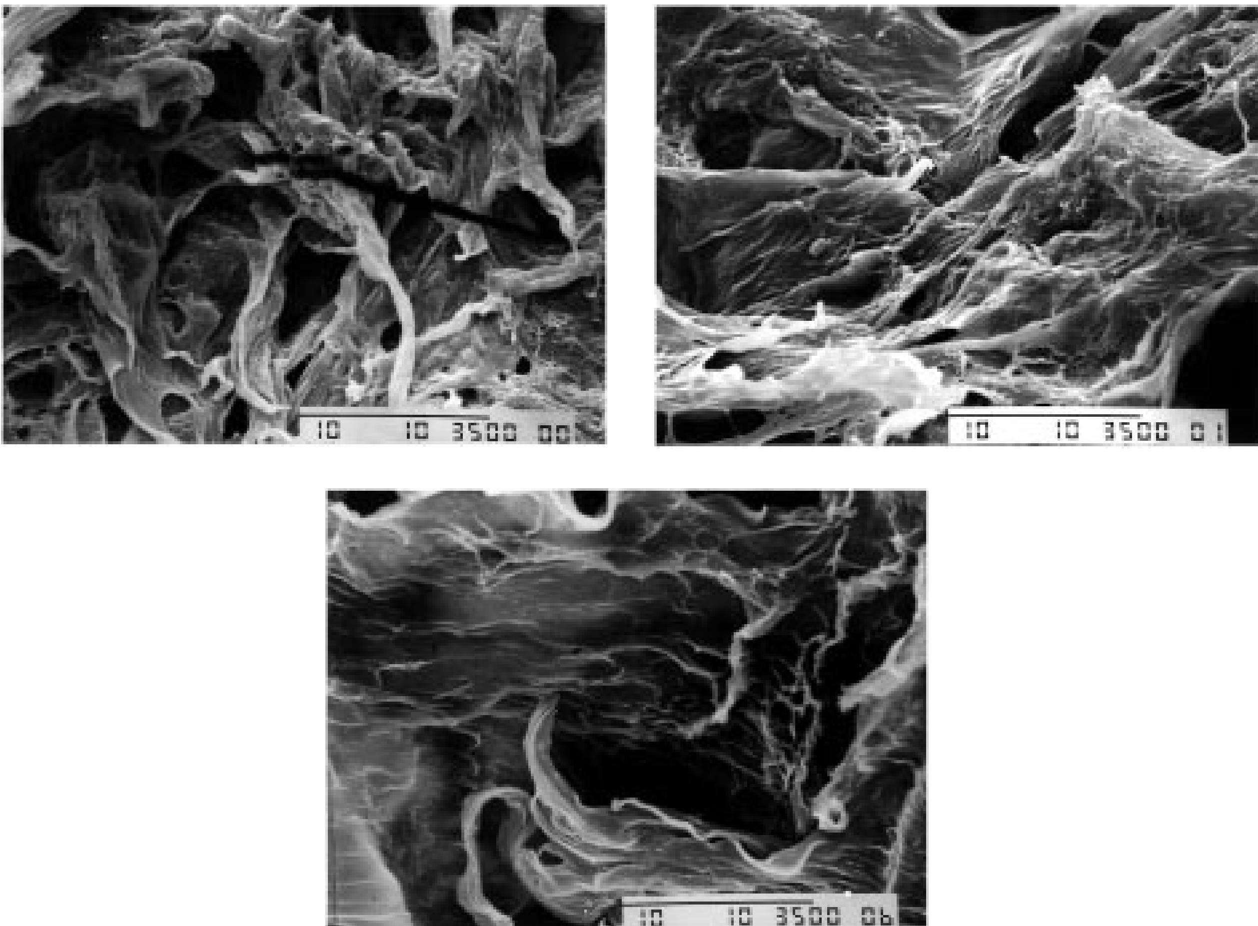

Figure 10 SEM image of the internal structure of Dx/PVA hydrogels at different dextran concentrations: (a) 10/90; (b) 20/80; (c) 30/70. Bar = 10 μm

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