JOURNAL OF MATERIALS SCIENCE: MATERIALS IN MEDICINE **8** (1997) 175–178

Non-destructive characterization of hydrogels

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Hydrogels have been prepared by a freezing–thawing procedure and investigations made of the effect of both number of freezing–thawing cycles and different content of hyaluronic acid (HA) on the mechanical properties of the PVA-HA hydrogels using non-destructive testing. The bulk elastic modulus K of hydrogels has been determined by pulse-echo measurements. It is noted that hydrogel elastic properties improve with the number of the cycles in PVA-HA 100/0; on the other hand samples with a high HA (1 000 000 molecular weight) content, beyond the third cycle, seem to be unaffected by the number of cycles. A bulk elastic modulus fall-off is then observed in samples submitted to an additional overnight freezing between two subsequent cycles. K increases in hydrogels with the highest HA content, when samples undergo pulse-echo measurements soon after their preparation. When hydrogels reach equilibrium, after having been kept in deionized water for 12 h, K values are lower, showing a nearly constant behaviour with different PVA-HA ratios and cycles. Furthermore, by means of scanning laser acoustic microscopy (SLAM) defects have been detected in the hydrogels. In samples which have reached equilibrium, SLAM images show that these defects disappear in PVA-HA hydrogels.

1. Introduction

From a physical and mechanical point of view, hydrogels are very similar to biological tissues due to their aqueous phase, the high permeability to small molecules and their good mechanical properties. The most recent research shows that improvements in the characteristics of synthetic hydrogels for biomedical applications could be achieved by the addition of biological macromolecules [1–3].

For this purpose the present study is focused on poly(vinyl alcohol) (PVA) hydrogels containing various percentages of hyaluronic acid (HA), a mucopolysaccharide existing in nature and which influences several cellular functions [4].

Hydrogels were prepared by a freezing-thawing procedure [5], and investigations made on the effect of the number of freezing–thawing cycles as well as on the different content of hyaluronic acid on the mechanical properties of the PVA-HA hydrogels by means of non-destructive testing. It is very important to characterize these particular materials avoiding any prior treatment of the sample, which can modify its properties.

2. Experimental procedures

2.1. Materials

The materials used are commercially available poly(vinyl alcohol) (Aldrich Chemie, Steinheim, Germany) in powder form with an average molecular weight 140 000 and a hydrolysis degree of 99%, and hyaluronic acid (FAB SpA, Abano Terme, Italy) in the form of sodium salt with an average molecular weight 1 000 000 determined by low angle laser light scattering.

2.2. Hydrogel preparation

An amount of PVA was dissolved in a flask of deionized water equipped with a reflux condenser for 2 h at 110 °C to obtain a final solution of PVA.

Hyaluronic acid solution was made at room temperature and the dissolution was completed in 12 h.

The two solutions were mixed in five different PVA–HA ratios: 100/0; 90/10; 80/20; 70/30; 60/40. The final concentration of PVA in all blends was 2.5% (w/v).

The hydrogels were obtained by submitting the samples to different cycles of freezing–thawing. The first cycle consisted of overnight at −20 °C and 1 h at room temperature, and the other cycles were 1 h at −20 °C and 1 h at room temperature.

In order to obtain hydrogels with a thickness of 2.5 mm, the same volume of mixed solution was used for the preparation of all blends.

2.3. Hydrogels characterization

The density of hydrogels was measured by a pycnometer immediately after their preparation and subsequently after water absorption.

This paper was accepted for publication after the 1995 Conference of the European Society of Biomaterials, Oporto, Portugal, 10–13 September.

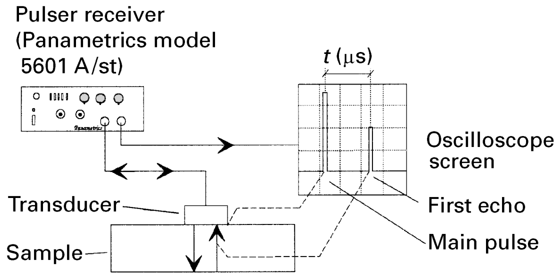

Figure 1 Pulse-echo operating system.

Pulse-echo measurements (Panametrics 560A1-ST equipment) were performed using the ultrasonic reflection method which utilizes the same transducer as pulser and receiver. The transducer, consisting of a piezoelectric crystal which converts electrical pulse into acoustic waves, transmits the waves into the sample [6]. Then the transducer receives the acoustic signal reflected by the opposite face of the sample. The received ultrasonic signal is amplified and digitalized by a PM 3323 Philips 500 MS/S oscilloscope and stored on hard disk (Fig. 1). The transit time of the ultrasonic wave is measured by PC and, knowing the thickness of the sample (fixed at 2.5 mm for all specimens) it is possible to calculate the velocity of the ultrasonic pulse in the material. To have comparable results, a water signal was taken as reference. All measurements were performed at 100 MHz.

Additionally, ultrasonic attenuation measurements were performed using a scanning laser acoustic microscope (SLAM) manufactured by Sonoscan. The SLAM system uses a piezoelectric transducer to produce plane continuous ultrasonic waves at frequencies of 10, 30 and 100 MHz, and a scanning laser beam to detect the amplitude of the ultrasonic waves after propagation inside the sample [7]. Absorption, scattering or reflection of ultrasonic waves crossing the sample are caused by changes of elastic properties and density inside the sample. The system performs the acoustic attenuation measurements by means of a comparison technique. As soon as the sample is placed in the stage, the CRT screen displays its acoustic image, allowing selection of the appropriate region of interest. The relative image brightness in this region, compared to that obtained when no material is present, is a measure of the attenuation in decibel (dB). The experiment is carried out at 20 °C in distilled water, used also as coupling fluid for ultrasonic wave transmission. At fixed times the attenuation of the hydrogel is measured during water absorption up to equilibrium.

3. Results

3.1. Pulse-echo measurements

Due to their high content of water, the elastic properties of hydrogels can be described, as for liquids, by the bulk elastic modulus K [8].

$$K = v^2\rho$$

where ρ is the density of the material and v the velocity of ultrasonic waves in the medium. In this work we refer to K for the evaluation of mechanical properties of hydrogels, calculated using the longitudinal velocity and the density measured for all samples just after preparation.

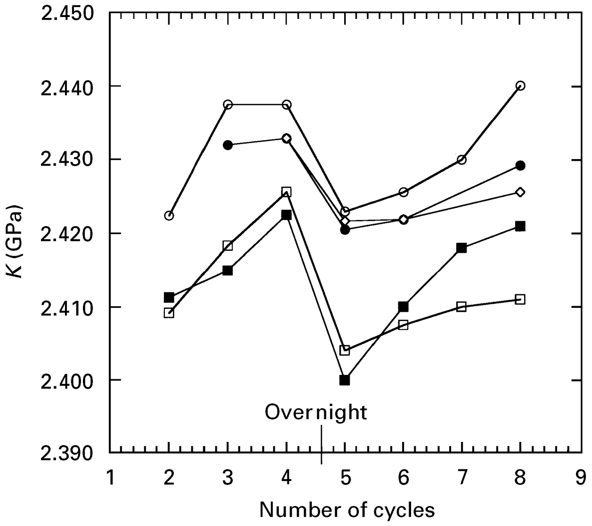

Figure 2 Bulk elastic modulus of PVA–HA hydrogels as a function of the number of cycles when a freezing overnight occurs between the 4th and 5th cycle: ■ 100/0; □ 90/10; ● 80/20; ◇ 70/30; ○ 60/40.

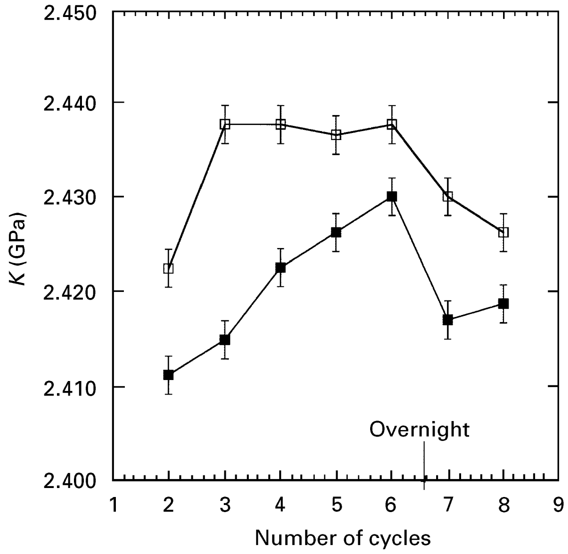

Figure 3 Bulk elastic modulus of PVA–HA hydrogels with 0% (■) and 40% (□) HA, as a function of the number of cycles when freezing overnight occurs between the 6th and 7th cycle.

We observe that the bulk elastic modulus increases with the cycle number if freezing–thawing routes are successive. On the other hand, if an overnight treatment at −20 °C is made between two cycles, the K value drops (Figs 2 and 3).

Fig. 3 shows bulk elastic modulus versus cycle number plots of the blends PVA–HA with ratios 100/0 and 60/40. Note that for increasing HA content, the K value does not change significantly after the third cycle.

Fig. 4 shows the behaviour of K as a function of HA content in hydrogels obtained after four freezing–thawing cycles. The bulk elastic modulus increases with the amount of HA in the hydrogels when the samples undergo pulse-echo measurements just after preparation. When the hydrogels reach the

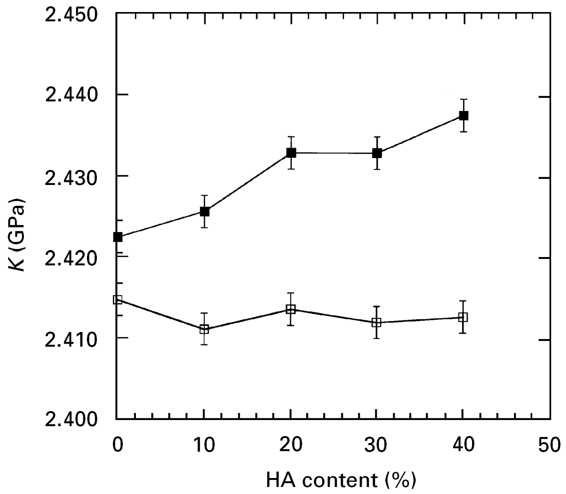

Figure 4 Bulk elastic modulus of PVA–HA hydrogels after four freezing–thawing cycles as a function of the percentage of HA: as prepared (■); at equilibrium (□).

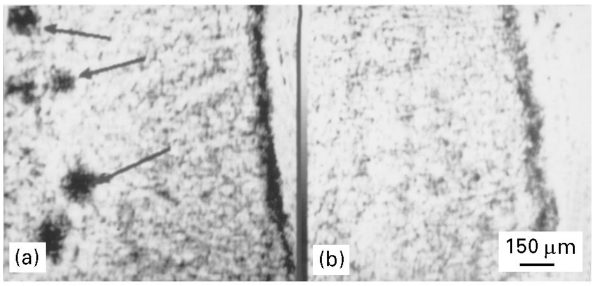

Figure 5 Defects detected by SLAM on PVA–HA 70/30 hydrogels: subjected to six freezing–thawing cycles: (a) as prepared; and (b) at equilibrium.

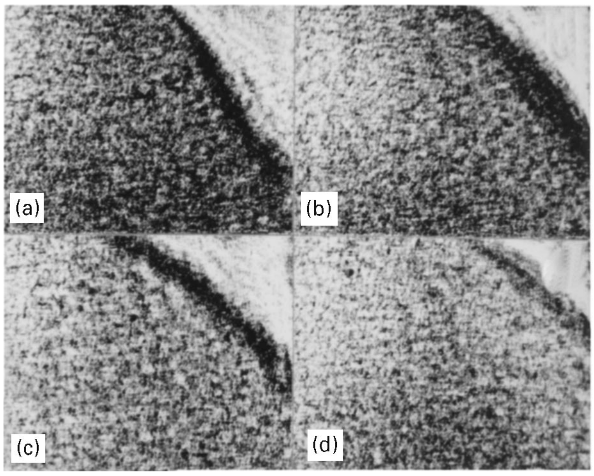

Figure 6 Acoustic attenuation of hydrogels subjected to six freezing–thawing cycles, and containing (a) 10; (b) 20; (c) 30; (d) 40% of HA at the equilibrium state.

equilibrium state, after having been kept in deionized water for 12 h, *K* values are reduced and show a nearly constant behaviour for different PVA–HA ratios and numbers of cycles.

3.2. SLAM measurements

In order to obtain information on the presence of defects and density change in the samples, SLAM characterization procedures were adopted. All the hydrogels show defects probably due to the formation of air bubbles during sample preparation. In particular for PVA–HA 100/0 samples the defects increase with the number of cycles. Moreover, in the case of hydrogels containing HA, defects are more evident with a higher percentage of acid (Fig. 5a). In the same samples these defects disappear after water swelling (Fig. 5b).

Ultrasonic attenuation measurements, providing information about density changes, were performed on samples with different HA content at a fixed number of cycles. As shown in Fig. 6, the attenuation decreases as the HA percentage increases.

4. Discussion

It is generally noted that the bulk elastic modulus of these hydrogels improves with the number of freezing–thawing cycles, but samples with a high HA content show a behaviour which, beyond the third cycle, seems to be independent of the number of cycles. Probably the high molecular weight of the hyaluronic acid permits rapid achievement of network consistency at low cycle numbers.

A bulk elastic modulus fall-off is observed in samples which undergo an additional overnight treatment at $-20\,^{\circ}\mathrm{C}$ between two cycles. This behaviour could be explained by a volume increase of the intrinsic water, due to the long freezing period, which probably leads to breaking of the bonds generated in the previous cycles.

The *K* value increase with hyaluronic acid content in hydrogels could be due to the presence of more HA functional groups which may be involved in crosslinking bonds for the network assembly.

It has also been shown that the elastic constant of all samples, after reaching equilibrium with the water, falls off to a similar value. This could be caused by a structure relaxation due to the high water content after swelling.

SLAM analysis shows that blends with HA exhibit a lower number of defects than PVA samples. Furthermore, after reaching equilibrium, the defects completely disappear in samples containing HA, while they remain trapped in the network in PVA hydrogels. Indeed the highest HA content produces the largest enrichment of water molecules, due to the ability of HA to bond with free water. This water absorption is confirmed by the weight measurements of hydrogels before and after reaching the equilibrium state and also by SLAM images that show a decrease in ultrasonic attenuation with increase of HA percentage.

5. Conclusions

By means of acoustic techniques an interesting mechanical behaviour of PVA–HA hydrogels during preparation has been observed.

An advantage of these techniques is the possibility of performing the analysis in a non-destructive way and without prior preparation of the sample. The information obtained in this way is related to the intrinsic properties of the materials.

Acknowledgements

The authors thank Dr C. Gerardi for helpful discussions.

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Received and accepted
7 September 1995