Poly(vinyl alcohol) as versatile biomaterial for potential biomedical applications

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In this paper, we present some new case examples where the chemical versatility of poly (vinyl alcohol) (PVA) can be used for potential biomedical applications.

PVA, the polymeric material used for designing new nanostructured devices, is water soluble, biocompatible and has excellent physical properties. We point out the possibility of obtaining wall-to-wall chemical hydrogels as well as microgels without diminishing the biocompatibility available in the starting PVA material. Injectability is another important factor to take into account in controlled drug delivery for gene therapy. In this respect, in this paper, established and more innovative methods are prospected in order to obtain particles with dimensions suitable for these applications.

Introduction

Multifunctional biocompatible materials can provide the basis for the obtainment of new devices in biomedical sciences. In this research domain, we are engaged in the development of polymeric materials with properties suitable for potential applications in the field of the controlled drug delivery devices with enhanced injectability and/or responsivity to external stimuli and as tissue substitutes.

Our starting material is poly(vinyl alcohol) (PVA) [1], a polymer already used in biomedical applications for its biocompatibility. The tendency of PVA to form physical gels through freeze-thawing thermal cycles due to the crystallinity of the polymer and studies on the properties of the resulting gel were reported in the literature [2]. However, the potential applications of this materials are somehow limited by the thermal reversibility, aging and opaqueness of the matrix. On the other hand, in chemical hydrogels based on PVA, other factors lower the potentiality of such systems. Glutaraldehyde and hexamethylene diisocynate are probably the most known crosslinking agent of PVA [3] based chemical hydrogels, but its toxicity can affect substantially the biocompatibility of the resulting material.

In recent years, we tackled the problem of preserving as much as possible the biocompatibility of PVA-based novel chemical hydrogels. Multifunctionality, internal architecture and diffusional properties of the solvent are other aspects to consider in the development of a versatile material for different biomedical applications.

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Experimental

Materials

PVA samples were Sigma product Lot #31K0008. A molecular weight of 67 000 g/mol was determined by static light scattering. Alginic acid, sodium salt, from *Macrocystis pilifera* (Kelp), medium viscosity, was Sigma Lot #50K0180. Calcium chloride dihydrate was purchased from Aldrich, sodium metaperiodate was a Sigma product. MilliQ water was used throughout this study. Glycidyl methacrylate was supplied by Fluka, DMSO was a Carlo Erba product.

Methods

Synthesis of wall-to-wall chemical hydrogels based on telechelic PVA is described in a previous paper by this group [4].

PVA coated microbubbles (air-filled) were prepared by interface crosslinking of telechelic oligomers stirring vigorously for 3 h 100 ml of the reaction medium with an Ultra-Turrax T-25 at 8000 rpm. The reaction was quenched bringing the solution to neutrality with the addition of the required amount of NaOH.

The methacrylate modified PVA was synthesized by esterification of pendant alcohol groups with glycidyl methacrylate in DMSO. PVA–methacrylate (PVA–MA) hydrogels were obtained by ultrasound-assisted crosslinking reaction. Ultrasound sonication was carried out with a Sonics VCX 400 equipped with a macro tip

immersed in 25 ml of 15% (w/w) PVA–MA in aqueous solution.

Atomic force microscopy (AFM) imaging was performed using a Digital Nanoscope™ IIIa controller and multimode scanning probe microscope operating in contact mode, equipped with an EV scanner, operating in contact mode. For imaging in liquid, a glass cell with silicon O-ring was used. Silicon nitride nanoprobe™ SPM NP-S20 (Digital) tips were used for both imaging in liquid and in air.

AFM sample preparation was carried out as follows: for imaging in air, 4 µl of the PVA beads suspension were deposited on freshly cleaved mica, dried under a gentle flow of nitrogen and immediately scanned. For imaging in liquid, the beads needed to be immobilized on the mica surface. For this purpose, a 3% (w/w) aqueous solution of alginate was prepared and 3 drops of it were deposited on a freshly cleaved mica surface. The suspension of PVA beads (2 µl) was added on top of the alginate film.

After 5 min, the alginate-PVA sample was completely covered with a 0.2 M solution of $CaCl_2$ and allowed to form the gel for 30 min. Finally, the sample was rinsed with 100 µl distilled H_2O and imaged in liquid (H_2O). Between sample preparation and imaging, particular care was taken not to allow the sample to dry. This procedure was adapted from an agarose gel preparation for AFM imaging [6]. AFM images were treated using a contrast enhancement processing.

Optical microscopy was carried out with a Zeiss Axioskop microscope at 400 × and 1000 ×.

Particle dimensions were measured also by dynamic light scattering using a Brookhaven BI-200SM photometer.

Ultrasound sonication was carried out with a Sonics VCX 400 equipped with a macro tip immersed in 25 ml of 15% (w/w) PVA–MA in aqueous solution. The network mechanical properties were determined by dynamic mechanical analysis with a DMA-7 dynamic mechanical analyzer (Perkin-Elmer DMA-7) equipped with a parallel plate accessory ($D = 15$ mm). Discs measuring 20 mm in diameter and 2–4 mm in thickness were cut from the slabs. The experiments were performed at room temperature by applying a different static stress between 30–300 mN, in order to assure a good contact between hydrogel and plates, and dynamic strain of 0.2% at frequencies of 1 Hz. For each formulation three replica samples were measured.

Results and discussion

As any vinyl polymer, poly(vinyl alcohol) (PVA) contains a small but detectable amount of head-to-head sequences. The occurrence of such arrangement is due to steric and electronic factors intervening in the radical polymerization and the fraction of these sequences is much smaller than the head-to-tail arrangement in atactic commercial samples of PVA. As already pointed out [7] a kinetic control of the amount of these sequences can be achieved increasing the reaction temperature. In the case of PVA, head-to-head sequences can be considered as 1, 2 glycolic units, being amenable to oxidative splitting to different extent by metaperiodate in aqueous solution, a well-known reaction in carbohydrate chemistry (see Chart 1).

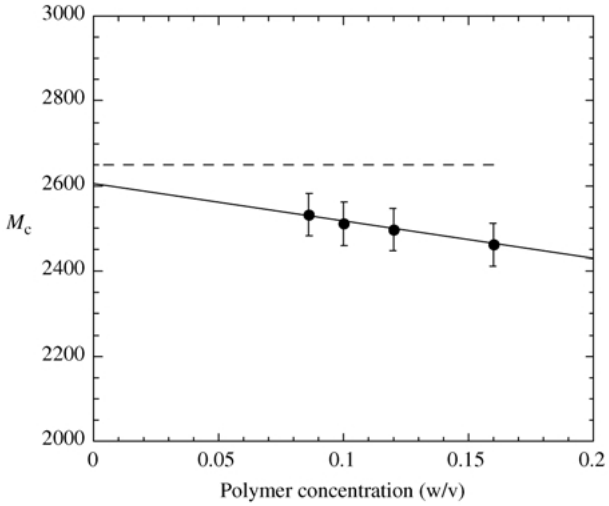

Figure 1 Molecular weight between crosslinks in hydrogels of telechelic PVA at a constant nominal degree of crosslinking of 0.8% as a function of the polymer concentration. Dashed line indicates the theoretical value of M_c.

By this procedure the obtainment of PVA macromers at different molecular weights carrying at each end a reactive aldehydic group is feasible. This opens the way to use the macromers of PVA, hereafter called telechelic PVA, as crosslinking agent by networking PVA chain by condensing the aldehydic end groups with the hydroxylic moiety of PVA. The result is a chemically homogeneous PVA network. The synthesis of the network is a one-pot reaction with a first step consisting in the production of the telechelic PVA followed by an acetalization crosslinking reaction in acidic aqueous medium. The structural characterization of this hydrogel has potentiality in biomedicine as the biocompatibility of the starting PVA is preserved.

The wall-to-wall telechelic PVA hydrogels prepared starting from different initial polymer concentrations have been characterized by dynamo-mechanic measurements (Fig. 1). Storage moduli measured for the equilibrium swollen samples were related to the crosslinking density and molecular weight between crosslinks, M_c, according to the rubber elasticity theory [7]. Measurements were carried out on different hydrogels increasing the initial telechelic PVA concentration from 8% to 16%. According to Equation 1, where ρ_p and

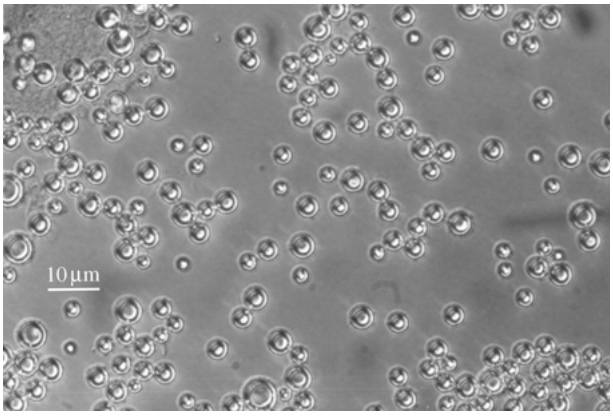

Figure 2 Optical micrograph at 400 × of an air-filled microspheres preparation.

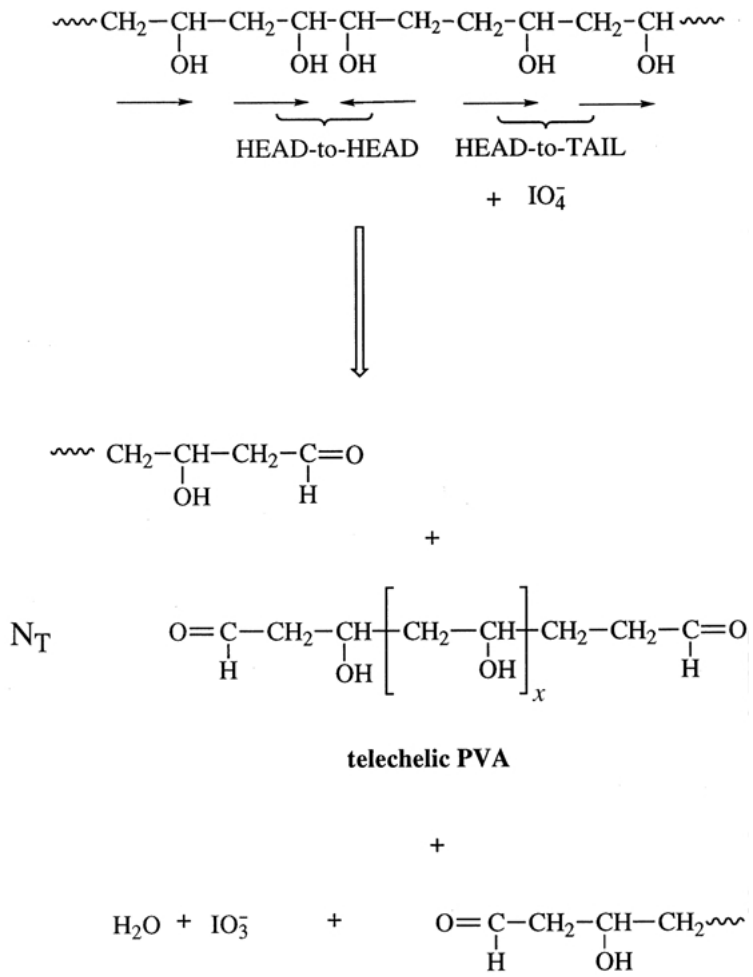

Chart 1 Descriptive scheme showing the method to obtain telechelic macromers of PVA: only head-to-head sequences are subjected to oxidation by metaperiodate (IO_4^-).

(ν_e/V_0) are the polymer density of atactic PVA and the density of crosslinks in the dried gel, respectively,

$$M_c = \frac{\rho_p}{(\nu_e/V_0) + (2\rho_p/M_n)} \quad (1)$$

an M_c decrease from 2530 ± 50 to 2460 ± 50 g/mol, respectively, was measured using a number average molecular weight, M_n, of the telechelic polymer estimated equal to 5280 on the basis of the molar ratio of metaperiodate and of the fraction of head-to-head sequences [4]. Extrapolated value of M_c at vanishing low polymer concentration is, within experimental errors, in agreement to with the theoretically predicted value ($M_{c,\text{theoretical}} = 2650$) based on the molar ratio between crosslinking agent (telechelic PVA and monoaldehydic chains) and repeating units.

These findings can be easily converted in an average mesh size, ξ, of a network with a polymer volume fraction, ϕ_p, by means of Equation (2) assuming the Gaussian behavior for the chains with a characteristic ratio C_n and with a number of residues n between adjacent crosslinks having a length l:

$$\xi = \phi_p^{-1/3}[C_n n]^{1/2} l \quad (2)$$

In this way, a pore size value of about 10 nm is obtained, showing that the network is internally nanostructured.

Polymeric biomaterials for potential clinical applications as new contrast agent for ultrasound imaging [8] and for targeted delivery in gene therapy [9] can be envisaged for hollow microspheres. We carried out the telechelic PVA crosslinking process at the air–solution interface under vigorous stirring. The dispersion was separated in a precipitate and in a layer of finely dispersed polymeric material floating on top of the dispersion. Optical microscopy observations on this latter fraction indicated an ensemble of spherical particles with diameters in the range of few microns (Fig. 2), whereas the precipitate contained mainly PVA debris and collapsed or aborted microspheres. AFM analysis was made on dry samples as well as in wet environment by spreading the microspheres on a film of 3% (w/w) alginate hydrogel used as supporting medium. In Fig. 3 an AFM view of an air-filled microsphere with a diameter of 6.5 μm is reported. These dimensions were confirmed also by independent dynamic laser light scattering measurements in solution.

The chemical versatility of PVA is mainly due to the presence of the hydroxylic moiety in the backbone which is amenable of chemical modifications such as grafting and networking. The reaction with glicidylmethacrylate has been proven quite convenient on polysaccharides. Hennick *et al*. [10] have assayed this reaction with dextran samples showing the possibility to obtain a wall-to-wall hydrogel.

The possibility of obtaining injectable matrices can greatly enhance the bioavailability of the drug allowing lower dosage. We adapted the substitution method

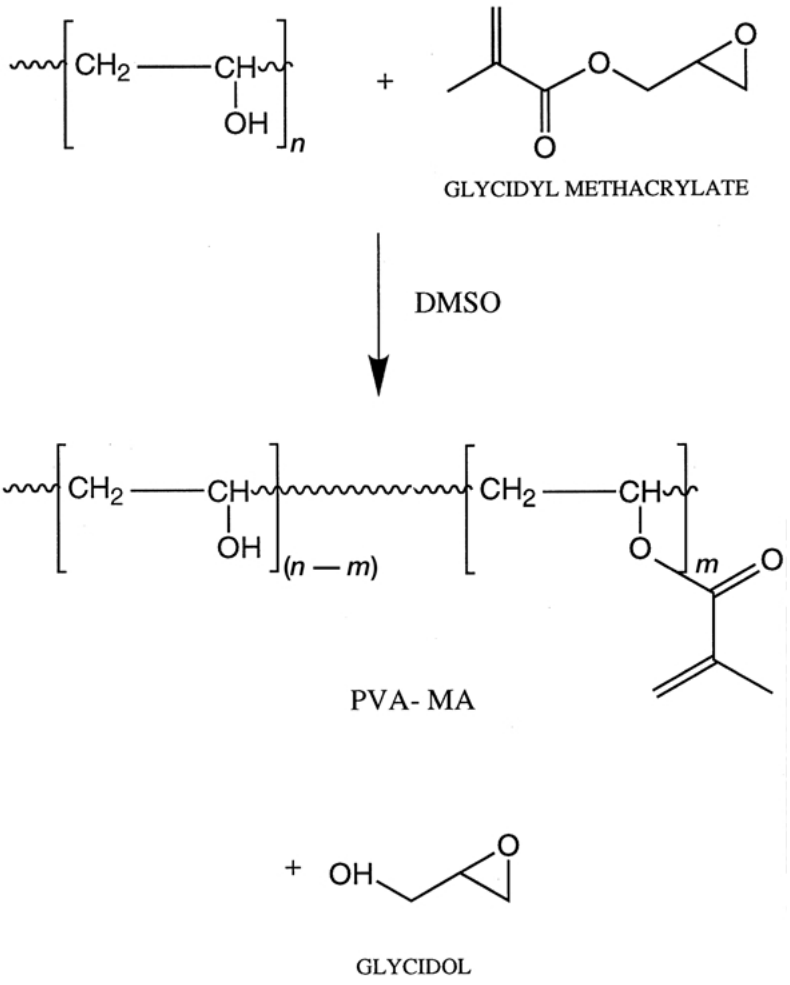

Chart 2 Reaction scheme for PVA–MA.

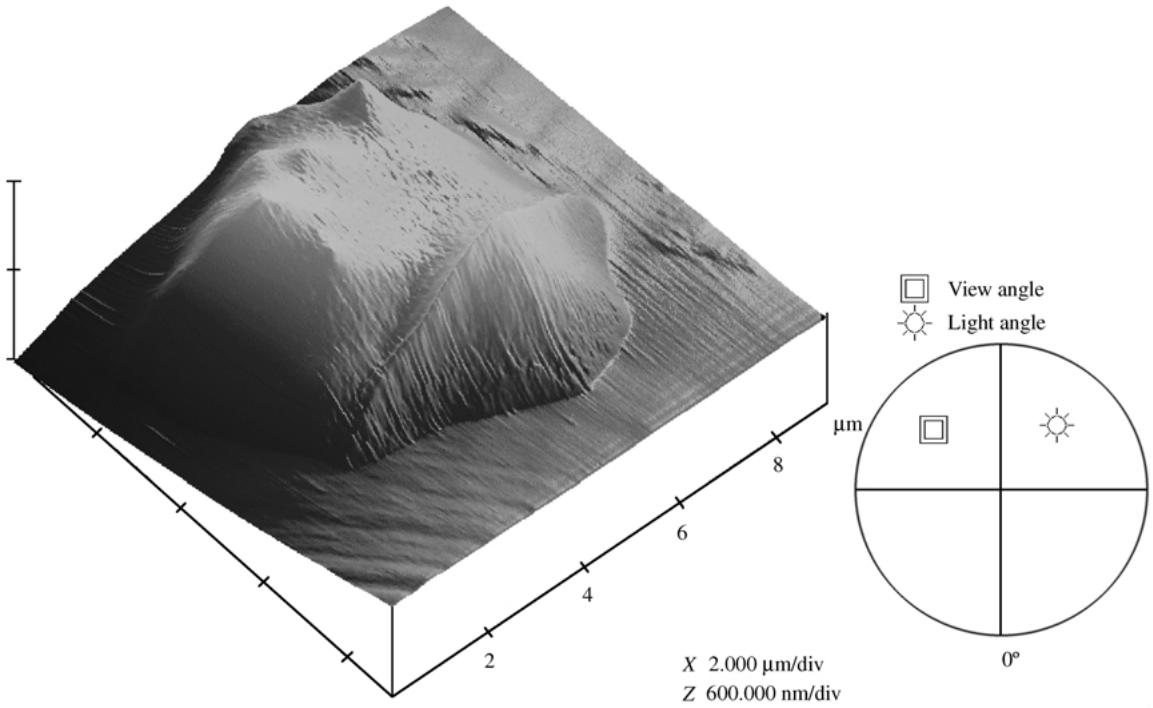

Figure 3 Contact mode AFM micrograph of an air-filled microsphere embedded in the alginate gel.

described in the literature [10] to PVA, see Chart 2. Transparent wall-to-wall hydrogels of PVA–MA can be obtained by photoinitiated reticulation. This suggests that this material can be developed as vitreous substitute, by injecting PVA–MA aqueous solutions in the ocular cavity promoting the successive formation of the gel in situ by light irradiation.

PVA–MA can also be used for obtaining PVA microspheres easily injectable but retaining the internal nanostructure proper of a wall-to-wall gel.

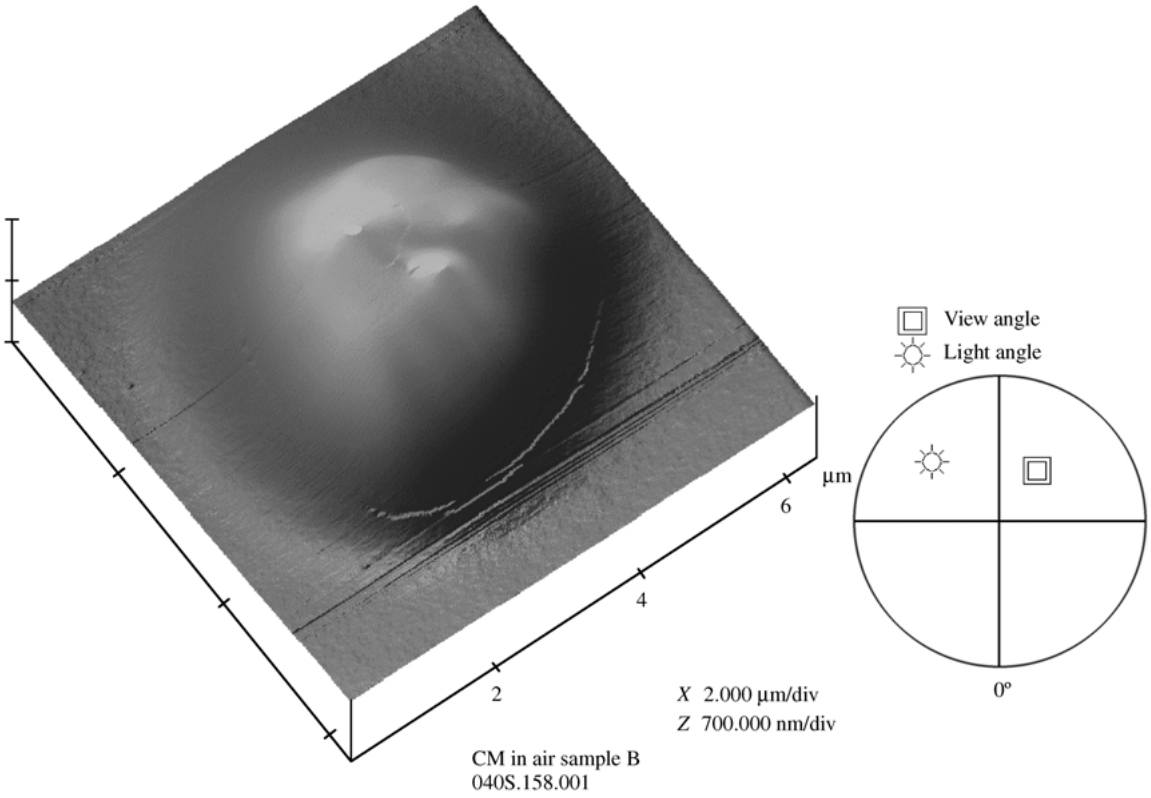

Figure 4 Contact mode AFM micrograph of microsphere of PVA–MA with uniform density.

We explored the possibility to activate the vinyl moiety of the side chains by ultrasonic irradiation. This method was already used to accelerate the radical polymerization process of mixtures of styrene sulphonate and vinylpyrrolidone [11].

In our case, the PVA chain networking process was started in the absence of any radical initiator. An increase of the viscosity of the polymer solution indicated the setting of the polymerization process. After the gel formation the amplitude of the ultrasounds is increased up to the disruption of the gel into a particle suspension. After dialysis a precipitate is collected. The AFM observation, reported in Fig. 4, shows the obtainment by sonication treatment of spherical particles of PVA with a diameter of about 4 µm.

In conclusion, the versatility of PVA is an important advantage for the formulation of new multifunctional materials and hydrogels with several potential applicative targets. In this paper, we showed the feasibility of synthetic and methodological approaches for the obtainment of microdevices suitable for regulated drug delivery and as contrast agent for ecographic.

Acknowledgments

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