Water dynamics in silanized hydroxypropyl methylcellulose based hydrogels designed for tissue engineering

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ABSTRACT

Silanized hydroxypropyl methylcellulose based hydrogels were developed for cartilage and intervertebral disc tissue engineering. Herein, study of dynamics of confined water showed two different populations, identified as hydration and bulk-like water. The diffusion coefficient showed that bulk-like water diffuses over distances ~10 µm without being affected by the hydrogel matrix. Addition of silica nanofibers leads to improved mechanical properties and enhanced diffusion coefficient. Good diffusion within hydrogels is essential for the application.

1. Introduction

Hydrogels are becoming increasingly important biomaterials because of their excellent biocompatibility and stimuli responsive characteristics. They are made of cross-linked molecules forming a three-dimensional network highly swollen by an aqueous medium. From a macroscopic point of view, hydrogels behave like solids: they can be shaped like solids and do not flow like liquids. Simultaneously, they present diffusive properties of real solutions with diffusion coefficients depending on the network (Adam et al., 1996). Therefore, hydrogels can be used for many applications, mostly in the biomedical field, such as contact lenses, drug delivery, superabsorbent etc. (Calvert, 2009; Deligkaris, Tadele, Olthuis, & van den Berg, 2010; Drury & Mooney, 2003; Hendrickson & Andrew Lyon, 2009; Satarkar, Biswal, & Hilt, 2010; Slaughter, Khurshid, Fisher, Khademhosseini, & Peppas, 2009; Van Vlierberghe, Dubruel, & Schacht, 2011; Wichterle & Lim, 1960). Among this, composite hydrogels are developed for tissue engineering. Indeed, their hydrated nature is close to that of extracellular matrix, their porosity has a significant role in oxygen and nutrients diffusion and they respond to ambient stimuli, making them effective to replace defective tissue (Asadi et al., 2018; Balagangadharan, Dhivya, & Selvamurugan, 2017; Bourges, Weiss, Daculsi, & Legeay, 2002; Buchtová et al., 2013; Jaikumar et al., 2015; Yu, Bao, Shi, Yang, & Yang, 2017).

Nevertheless, although a high water content is desired, it is also at the origin of the hydrogels' poor mechanical properties, limiting their applications (Anseth, Bowman, & Brannon-Peppas, 1996). The reinforcement of these mechanical properties was thus required. It is a fundamental challenge, motivated by the numerous future applications of toughened hydrogels (Chau et al., 2016; Gaharwar, Peppas, & Khademhosseini, 2014; Shapiro & Oyen, 2013; Yang, Wang, Yang, Shen, & Wu, 2016). New composites have been developed, using for instance chitosan nanofibers or graphene, leading to more compact microstructures and high mechanical strength (Balagangadharan et al., 2017; Yu et al., 2017). We have also proposed to reach such a mechanical reinforcement with colloidal silica nanofibers, thanks to their diameter and form factor close to 50 nm and 20 respectively, allowing their percolation with low charge level (Buchtová et al., 2013; Nojoomi, Tamjid, Simchi, Bonakdar, & Stroeve, 2017). Such colloidal silica were showed to be essentially nontoxic (Zhang et al., 2012). Since these nanocomposites hydrogels were validated for tissue engineering and are gaining consideration (Buchtová et al., 2013; Henry, Clouet, Le Bideau, Le Visage, & Guicheux, 2018), it remains mandatory to decipher the dynamics of the confined water. Since in the native tissues the nutriments are delivered to cells by simple diffusive processes, further application in cartilage and intervertebral disc tissue engineering needs understanding of the morphology of the hydrogels, and of the dynamics of confined water. The water confined within hydrogels exhibits physico-chemical characteristics which are different from classical bulk water. The dynamical behaviour of confined water is closely associated

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with the hydrogel's macromolecular network structure, and it depends on each given structure features (Caccavo, Cascone, Lamberti, & Barba, 2018). Numerous researchers have already used experimental techniques such as Nuclear Magnetic Resonance (NMR) and Differential Scanning Calorimetry (DSC) on several kinds of hydrogels to obtain insights on the confined water (Lang, Jiang, Li, & Zheng, 2008; Numata, Katashima, & Sakai, 2011; Sakai, Kuroki, & Satoh, 2008; Yoshida, Hatakeyama, & Hatakeyama, 1993). According to these results, the water in hydrogels can be generally classified according to three types of physical behaviour: (*i*) free (or bulk-like water) which can undergo liquid-to-solid transition at temperatures within usual ranges, and interfacial (bound) water that can be divided into (*ii*) non-freezing water and (*iii*) water which can freeze below or above regular temperature. The observability of these three water types is dependent on the hydrogel's matrix and its hydration level. Conversely, the physical state of confined water should provide useful information on the morphology and physical behaviour of hydrogels. We focus herein on a detailed description of water behaviour inside silanized hydroxypropyl methylcellulose (Si-HPMC) based hydrogels reinforced with mesoporous silica nanofibers (NFs).

2. Material and methods

2.1. Materials

Silanized hydroxypropyl methylcellulose (Si-HPMC) was prepared using 3-glycidoxypropyl trimethoxysilane (GPTMS, ≥98%, Sigma Aldrich, Germany) and hydroxypropyl methylcellulose (HPMC) with hydroxypropoxyl content 9.5% and methoxyl content 28.3% (E4M, Methocel®, Colorcon, The Dow Chemical Company™, UK) according to the procedure described elsewhere (Bourges et al., 2002). The final silicon content in Si-HPMC was 0.7 wt%. Silica nanofibers (NFs) were obtained by one-pot synthesis as reported previously (Rambaud, Vallé, Thibaud, Julián-López, & Sanchez, 2009). All the other reagents were of analytical grade.

2.2. Hydrogel synthesis

The synthesis of pristine Si-HPMC based hydrogel and corresponding Si-HPMC hydrogels reinforced with silica NFs have already been reported elsewhere (Buchtová et al., 2013). Briefly, to prepare such hydrogels the Si-HPMC polymer is dissolved in basic aqueous solution; then 1 vol of this solution is mixed with 0.5 vol of an acidic buffer solution (BS) by means of two interconnected luer-lock syringes. For silica NFs reinforced hydrogels, the desired amount of NFs is dispersed within BS via ultrasonication prior to its blending with the polymer solution. The final precursor solutions of hydrogels or reinforced hydrogels, which are injectable within the first ~10 min, are injected into plastic moulds and kept at room temperature in humid atmosphere (~80% RH) for 2 weeks to complete the polycondensation process.

2.3. Scanning Electron Microscopy in cryogenic mode (cryo-SEM)

Cryo-SEM was performed on Zeiss EVO LS10 microscope at −130 °C and at approx. 10^{-4} Pa with acceleration voltage of 7 kV. Prior any observation, each equilibrated swollen hydrogel was frozen in slush nitrogen and then quickly introduced into the microscope chamber. The sample's surface was cut off, slightly freeze-dried and gold-sputtered directly inside the microscope.

2.4. Differential Scanning Calorimetry (DSC)

DSC thermograms were obtained on Q200 DSC TA Instruments machine. Hydrogels and bulk aqueous solution of the same composition as inside the hydrogels were sealed in aluminum crucibles and rapidly

cooled to −50 °C at a heating rate of approx. 40 °C min^{-1}. Then, 10 cycles from −50 to +40 °C and back were realized with 5 °C min^{-1} speed. The melting temperatures and the corresponding enthalpies were determined for each sample as average values over 10 heating cycles.

2.5. Nuclear Magnetic Resonance (NMR) cryoporometry

NMR cryoporometry measurements were carried out on Bruker AVANCE 400 MHz NMR spectrometer using a 4 mm QXO probe. Hydrogels were prepared in deuterated water and cooled to 220 K within several hours. They were then gradually heated up to 315 K by 5 K steps and equilibration time of 20 min before starting NMR signal acquisition (temperature stability of ± 0.2 K). The NMR signal of both ^{1}H and ^{2}H was recorded at each temperature point across the process of heating. Temperature calibration was done before each set of measurements using a reference methanol sample.

2.6. Pulsed-Gradient Spin-Echo NMR (PGSE)

PGSE-NMR experiments were realized at 300 K on Bruker AVANCE 400 MHz NMR spectrometer equipped with a 5 mm BBOxyz probe. The diffusion coefficients were determined from the classical Stejskal-Tanner equation(Stejskal & Tanner, 1965):

$$\ln(I/I_0) = -DG^2\gamma^2\delta^2(\Delta - \delta/3) \tag{1}$$

where G is the magnitude of the two gradient pulses applied, δ is their duration and Δ is the time interval between them, γ is the gyromagnetic ratio of the nucleus under study and I and I_0 are the integrated intensities of the signals obtained, respectively, with and without gradient pulses. Here, the magnitude of the pulsed field gradient was varied between 0.674 and 32.030 G cm^{-1} in 16 steps. The pulse duration was fixed to 2.5 ms and two experiments were performed with Δ = 50 ms and Δ = 200 ms in order to check that no convection effects occur in the samples, neither restricted diffusion phenomenon. The gyromagnetic ration of hydrogen γ_H = 267.5 x 10^6 rad s^{-1}T^{-1}.

3. Results and discussion

3.1. Scanning electron microscopy analysis

Images of the 2 week-old Si-HPMC hydrogel monoliths containing 0 and 3 wt% of silica NFs are shown in the upper left inserts in Fig. 1a and b, resp. As can be seen, the pristine hydrogel, which contains 98 wt% of aqueous solution and only 2 wt% of chemically cross-linked Si-HPMC matrix, is self-standing, completely transparent and colorless. After the addition of 3 wt% of silica NFs, the Si-HPMC hydrogel monolith becomes opaque and whitish. Simultaneously, the compressive modulus of such hydrogels is enhanced as previously reported.(Buchtová et al., 2013) It is important to note that even if all the Si-HPMC based hydrogels contain 98 wt% of confined water, there is no water leaching out even upon compression.

To obtain information about the water-matrix arrangement inside Si-HPMC hydrogels, scanning electron microscopy in cryogenic mode (cryo-SEM) was performed. The fast sample freezing in slush nitrogen (Apkarian & Wright, 2005; Baker, Denton, & Herr, 2013; Sansinena, Santos, Zaritzky, & Chirife, 2012) used to prepare the sample should preserve the morphology of highly hydrated samples. However, it is known that even such fast freezing method leads to a certain deformation of hydrogel's morphology (Efthymiou, Williams, & McGrath, 2017; Aston, Sewell, Klein, Lawrie, & Grøndahl, 2016). Fig. 1a shows the morphology of the pristine Si-HPMC hydrogel. It is similar to morphologies observed typically on freeze-dried hydrogels. A highly porous structure with pore size distribution ranging from 1 μm to several tens of microns can be observed. The pore walls' thickness is about 100 nm, as shown in the upper right insert in Fig. 1a: they are formed