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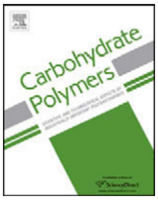

Dissipative convective structures and nanoparticles encapsulation in Cu/alginate/dextran composite hydrogels and sponges

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ABSTRACT

A novel approach is presented to produce ordered capillaries by dissipative convective process followed by freeze drying of anisotropically ordered alginate–dextran hydrogels produced by ionic diffusion of Cu^{2+} ions. The ionotropic gels consisted of capillaries, 10–100 μm in diameter which comprised small pores, 10–30 μm in size. Dextran-functionalized iron or gold nanoparticles can be readily incorporated into the gels with retention of its stability and patterned microstructures. The freeze-dried Cu/alginate/dextran gel was further cross-linked with epichlorohydrin in basic media to protect its internal microstructures. Mechanical strength of the anisotropically ordered Cu/alginate/dextran sponges also investigated.

1. Introduction

Alginates are well-known polysaccharides that can be cross-linked with di or trivalent cations (Eljaouhari, Muller, Kllermeier, Heckmann, & Kunj, 2006; Schexnaider & Schmidt, 2006). Encapsulation of nanoparticles in hydrogels significantly changes its properties and the resulting gels could be used in electronics, optics, catalysis, sensors, drug delivery, actuators and microfluidics (Peppas, Hilt, Khademhosseini, & Langer, 2006). The origin of generating dissipative structures in polysaccharide gels came from Rayleigh–Bennard heat convection (Cross & Hohenberg, 1993). Almost 60 years ago, hexagonally ordered capillaries were formed by non-equilibrium convective processes when sodium alginate was cross-linked with multivalent cations such as Ca^{2+} ions (Thiele & Anderson, 1955; Thiele & Hallick, 1958).

Recently, anisotropically structured hydrogels with considerable mechanical strength and extremely high extensibility have been prepared by a two-stage route in which acrylamide was polymerized within a self-organized poly(2,2′-disulfonyl-4.4′-benzidine terephthalamide; PBDT) gel produced by diffusion of a Ca^{2+} ion as cross-linking agent (Yang, Furukawa, & Gong, 2008). Sponge like structures of collagen-based composites can also be prepared by freeze drying where micropores are left behind by sublimation of ice crystals (Dittrich et al., 2007; Zhang, Cui, Liao, Zhu, & Han, 2003).

In this paper, we utilize the above approach to novel studies that consists of dissipative convective composite hydrogels with patterning microstructures prepared by alginate sol cross-linked with Cu^{2+} in the presence or absence of dextran. The gold, iron and titanium dioxide nanoparticles can also be encapsulated into the gels for studying its properties (Gutierrez, Garcia-carvajal, Jobbagy, Rubio, & Yuste, 2007; Zhang et al., 2005).

2. Experimental

2.1. Materials and methods

2 g of sodium alginate (1.96 wt.%) (Sigma–Aldrich, $M_w = 12\text{–}40 \times 10^4$) was dissolved in 100 g of water (98.04 wt.%) and sonicated for 3 h to obtain a homogeneous solution. The pH of the solution was 7.2. 5 g dextran (58.82 wt.%, 1.43×10^{-3} M, Fluka, $M_w = 2 \times 10^6$) was mixed with 3.5 g of water (41.18 wt.%) in a beaker and 30 mL of alginate solution was transfer into it and stirred well for 1 h to obtain the homogeneous viscous mixture. The alginate–dextran solution (pH = 7.4) was placed in a cylindrical glass mould, 10.5 cm × 3.4 cm in length and diameter, respectively, and 30 mL of an aqueous solution of $CuCl_2{\cdot}2H_2O$ (1.96 wt.%, 0.18 M, $M_w = 170.68$, Aldrich, 2 g copper chloride dissolved in 100 g of water) was carefully layered on the top of the alginate–dextran solution. The system was left under quiescent conditions for 40 h at room temperature to allow the Cu^{2+}-induced cross-linking to occur under diffusion–reaction conditions. The cross-linked wet gel body was cut at least 6 mm from the top and the remaining portion of the gel body was washed with deionised water several

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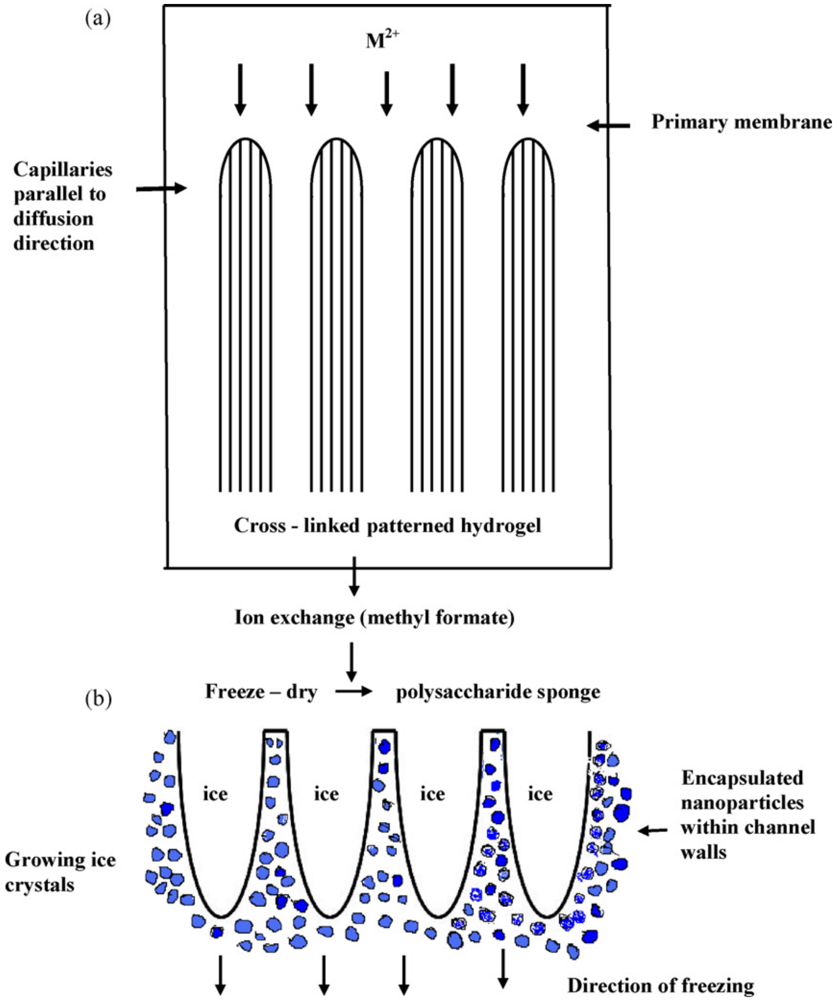

Fig. 1. Schemes of the different phases of alginate-based capillaries formation and freeze drying: (a) a unidirectional flow of cross-linking Cu^{2+} ions produces the alginate-based hydrogels with ordered capillaries by dissipative patterning processes. The hydrogels are subsequently freeze-dried to prepare polysaccharide sponges with complex microstructure, and (b) ice crystals formed are used to imprint higher-order porosity within the ordered hydrogels to produce sponge-like structures.

times to remove unbounded Cu^{2+} ions from the gel and then the gel body was soaked in 1 M methylformate (formic acid + methanol) for 3 h. Water and excess acid were removed from the gel body by bathing it in acetone–water mixture (1:5, v/v) for 2 h. To protect the internal organization of the copper cross-linked alginate/dextran hydrogels, samples were frozen in liquid nitrogen for 25 min; thereafter the gel bodies were put in freeze dryer (LABCONCO) to remove water by sublimation process. The freeze-dried gel when put in water overnight resulted in the loss of internal microstructures and weight due to the high solubility of dextran in aqueous media. This was alleviated to some extent by further cross-linking the freeze-dried gels with epichlorohydrin in basic media (0.1055 g of gel body was treated with 10 mL of 0.5 M epichlorohydrin and 5 mL of 0.5 M NaOH.

The copper cross-linked alginate gel was also prepared using above protocol in the absence of dextran.

2.2. Encapsulation of nanoparticles in gels

Nanoparticles like gold (Storhoff, Elghanian, Mucic, Mirkin, & Letsinger, 1998) (0.1 wt.%; size 11 ± 1.5 nm; 1.90×10^{16} particles/L), dextran-functionalized iron oxide (Kawaguchi & Hasegawa, 2000) (Fe_2O_3; 0.1352 wt.%; dextran:iron oxide = 80:20, w/w; size 2.5 ± 0.5 nm) or TiO_2 (Degussa; 20 wt.%; average size = 21 nm) were encapsulated in alginate/dextran sol before treatment of the hydrogels with copper chloride.

2.3. Characterization

2.3.1. Scanning electron microscopic study

Scanning electron microscopic images were obtained using a JEOL 5600 microscope.

2.3.2. Mechanical strength study

Mechanical properties of the sponges were performed using rectangular section (typical dimensions, 8.6 cm × 8.5 cm × 8.4 cm) cut from the gel body with a razor blade. Stress/strain curves were obtained on samples oriented with the capillaries aligned parallel to the direction of loading using a stable Micro-system TA Xt plus texture analyzer.

2.3.3. Rheological study

Rheology studies were made using a Bohlin CVO rheometer with a 20 mm cone and plate geometry (Khan, Walsh, Patil, Perriman, & Mann, 2009).

2.3.4. FT-IR analysis

FT-IR spectra of Cu/alginate, Cu/alginate/dextran, dextran and the gel bodies cross-linked with epichlorohydrin were taken on a Perkin-Elmer Spectrometer using KBr pellets.

2.3.5. *BET surface area study*

Brunauer, Emmett and Teller (BET) surface area of Cu/alginate/dextran sponges treated with methylformate were determined using a Quantachrome Autosorb-1C instrument at −196 °C. Micropore size distribution were analyzed by the BJH model using desorption isotherms.

3. Results and discussion

Fig. 1a and b showed the formation of capillaries by dissipative convective process followed by freeze drying procedure. SEM images of hydrogels prepared from alginate cross-linked with Cu^{2+} were given in Fig. 2a and b. The sponges consist of capillaries with diameter varied from 25 to 100 μm with wall thickness 10–20 μm consisting of pores, sizes 10–30 μm. Addition of dextran to alginate hydrogels significantly affects the microstructures (Fig. 2c and d) that resulted in consisting of ordered capillaries of diameter 25 μm and wall thickness varied from 10 to 20 μm with interconnected channels of size 10 μm. In some cases, small pores of size 10 μm were also formed at the surface of the walls (Fig. 2d). In the presence of dextran, more ordered capillaries were obtained.

Fig. 3a and b are the SEM images of alginate/dextran hydrogels encapsulated with gold nanoparticles which have sponge like structures with thick walls of size 40–70 μm which have small pores of sizes, 5–10 μm. On careful observation, the sponge contains single and multinetwork gels (Yang et al., 2008). Highly sponge like structures were obtained when dextran-functionalized Fe_2O_3 nanoparticles were added to alginate/dextran gels (Fig. 3c). The sponges contain ordered capillaries of diameters varied from 10 to 20 μm with pores of sizes 5 μm at the surface and their own sides. This is probably to low viscosity of Fe_2O_3 containing gels. The colours of the Cu/alginate/dextran gels encapsulated Fe_2O_3 or Au nanoparticles were red or yellowish-blue, respectively (Supplementary Fig. S1). Fig. 3d is the SEM micrograph of dehydrated TiO_2 nanoparticles encapsulated alginate/dextran hydrogels. Disordered capillaries of sizes 50–100 μm were observed with no sponge like structures. EDAX analysis confirmed the presence of these nanoparticles (figure not shown). The nanoparticles were not readily observed in high magnification SEM images, suggesting that they were firmly encapsulated into the gel matrix. Typically, the dehydrated Cu/alginate/dextran/Au gel had surface area 27.35 $m^2\,g^{-1}$ with macropore size distribution (Fig. 4) in the range 5–10 μm.

FT-IR studies reveal that ECH or Ca cross-linked the OH– group in COOH group of alginate also OH– group in dextran with ECH due to which absorption frequency shift at 3400 cm^{-1} to lower side. Another shift of absorption frequency at 1700 cm^{-1} to lower side was observed due to the overlapping of C=O group in COOH in alginate (Fig. 5).

Mechanical strength studies reveal that dried gel under compression showed that anisotropically ordered Cu/alginate gels (Fig. 6a) were soft and highly viscoelastic and exhibited strain hardening at strain value greater than 22%. The elastic region was observed up to 10% with a corresponding modulus of 0.2 MPa. In case of Cu/alginate/dextran dried gel (Fig. 6d), it bears significant stress up to strain value of 24% with a modulus of 2.70 MPa after that it became viscoelastic. Similar behaviour is observed in case of Cu/alginate/dextran/Fe_2O_3 dried gel (Fig. 6c). In case of TiO_2 and Au containing Cu/alginate/dextran gels (Fig. 6b and e), both showed stiffer sponges with an initial elastic behaviours corresponding to modulus of 3 and 5.15 MPa, respectively, prior to crack formation at

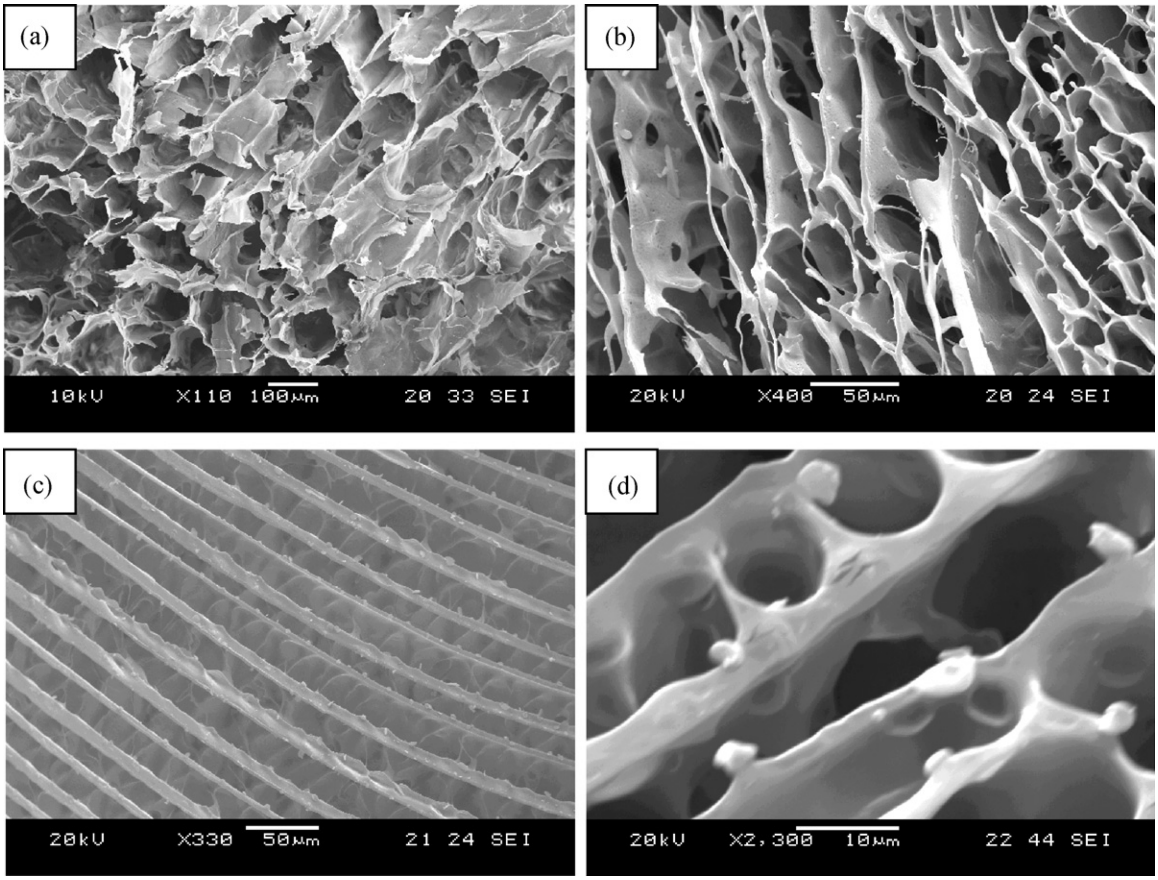

Fig. 2. SEM images of copper cross-linked alginate gels: (a and b) Cu/alginate gels and (c and d) Cu/alginate/dextran gels. Scale bars: (a) 100 μm, (b) 50 μm, (c and d) 50 and 10 μm.

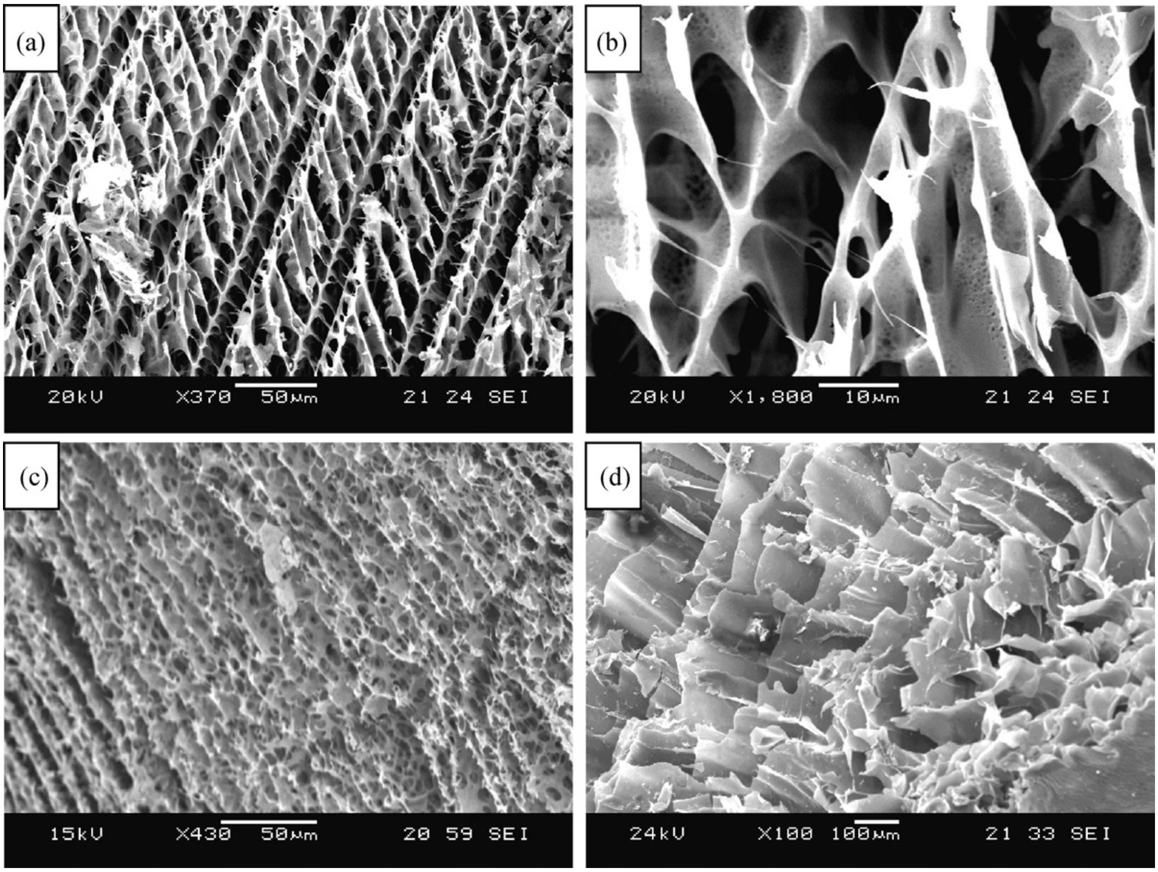

Fig. 3. SEM micrographs of polysaccharide sponges prepared with encapsulated nanoparticles: (a and b) alginate/dextran Au sponges, (c) alginate/dextran/Fe_2O_3 sponges, and (d) alginate/dextran/TiO_2 sponges. Scale bars: (a) 50 μm, (b) 10 μm, (c) 50 μm, and (d) 100 μm.

strain values of 8.5 and 13%. After cracks, both dried gels exhibited viscoelastic behaviours.

The rheological study confirmed the order of viscosity of sols sodium alginate ~ sodium alginate/dextran/TiO_2 < sodium alginate/dextran/Fe_2O_3 < sodium alginate/dextran < sodium alginate/dextran/Au which increased from 0.05 to 1.0 Pa s. The alginate/dextran mixture showed shear thinning at increased values of shear stress while alginate alone showed Newtonian fluid behaviour, showing strong interaction between the alginate and dextran molecules.

To confirm the effect of pre-structuring the hydrogel on the freeze drying process, unstructured Cu/alginate or Cu/alginate/dextran gels were prepared by mixing the compo-

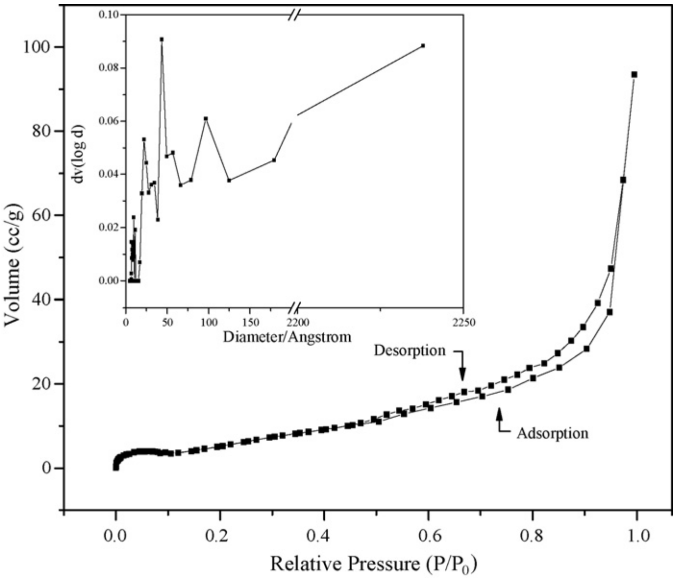

Fig. 4. N_2 adsorption isotherm for a Cu^{2+} cross-linked freeze-dried alginate/dextran sponge with encapsulated Au nanoparticles.

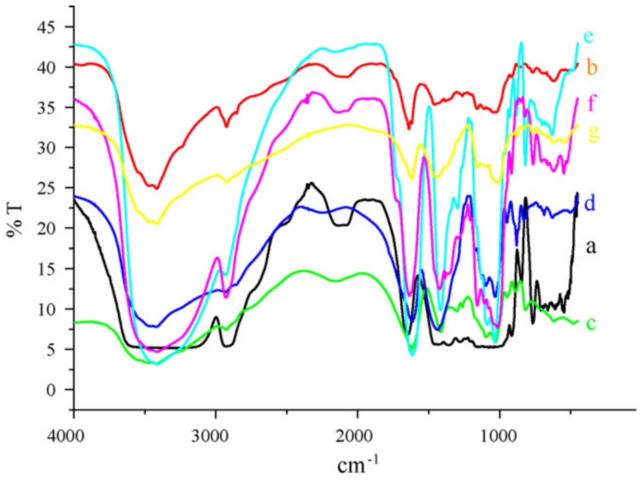

Fig. 5. FT-IR spectra of dried gels/sponges: (a) dextran, (b) dextran/ECH, (c) sodium alginate, (d) Cu/alginate/ECH, (e) Cu/alginate, (f) Cu/alginate/dextran, and (g) Cu/alginate/dextran/ECH.

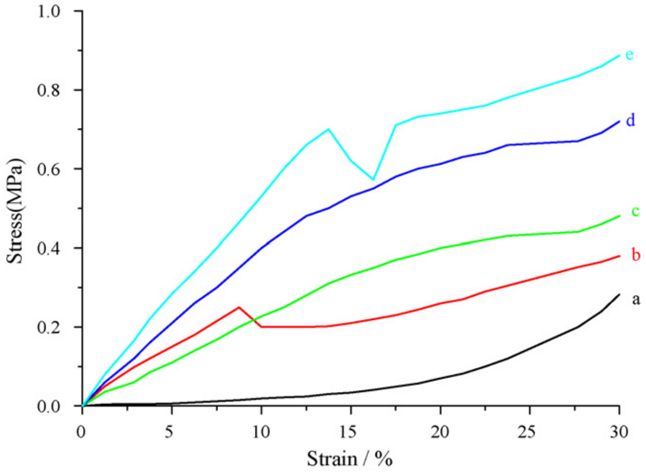

Fig. 6. Mechanical properties of copper cross-linked alginate gels/sponges: (a) Cu/alginate gels, (b) Cu/alginate/dextran/TiO_2 gels, (c) Cu/alginate/dextran/Fe_2O_3 sponges, (d) Cu/alginate/dextran gels, and (e) Cu/alginate/dextran/Au sponges.

nents under equilibrium (non-dissipative) conditions, followed by freezing in liquid nitrogen. In both cases, pattern microstructures were not observed, suggesting arrangement of the capillaries as well as the pores formed after freeze drying. Our results confirmed that the combination of pre-structuring and freeze drying was essential to get structural complexity in the Cu/alginate–dextran composite sponges.

4. Conclusion

We find that the origin of the capillaries and small pores in the patterned hydrogels is a result of dissipative convective phenomenon and freeze drying procedure. The structural complexity of patterned polysaccharide hydrogels can be achieved by the addition of dextran to alginate gels. The nanoparticles can easily be incorporated into the gels without perturbing the nonequilibrium nature of the process. Changes in the composition of the hydrogels result in modifications in the architecture of the sponge replicas. Our results show that the combination of dissipative convective patterning and ice templating technique could be exploited as a general procedure for fabricating ordered porous sponges. The fabrication of patterned hydrogels and sponges from polysaccharides/nanoparticles with environmentally benign process might be useful in soft matter technology.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carbpol.2010.08.021.

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