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Production and characterisation of novel injectable chitosan/methylcellulose/salt blend hydrogels with potential application as tissue engineering scaffolds

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ABSTRACT

The properties of an injectable chitosan (CS)/methylcellulose (MC) blend hydrogel used as a three-dimensional synthetic matrix for tissue engineering were investigated. CS/MC hydrogels were prepared via blending of CS, MC and salts under mild conditions without organic solvent, high temperature or harsh pH. Such blends were liquid at low temperature (~4 °C), but gel under physiological conditions (37 °C). The effect of different salts including NaCl, Na_3PO_4, $NaHCO_3$ and glycerophosphate (GP) on the CS/MC gelation process was investigated by rheological analysis from which possible gelation mechanisms were inferred. Viscoelastic characteristics indicated that CS/MC gels formed using different salts had different gelation temperature, gelation rate, and gel strength. Gelation temperature followed the order NaCl > GP > Na_3PO_4 > $NaHCO_3$, gelation rate followed the order GP > $NaHCO_3$ > Na_3PO_4, and gel strength followed the order GP > $NaHCO_3$ > Na_3PO_4 (at 37 °C).

CS/MC hydrogels were also characterised by infrared spectroscopy (FT-IR), scanning electron microscopy (SEM), and X-ray diffraction (XRD). CS/MS gels formed with different salts had different gel structures, ranging from nonporous to microporous. When used as a scaffold for chondrocytes, CS/MC/Na_3PO_4 hydrogel resulted in good cell viability and proliferation.

1. Introduction

Hydrogels are hydrophilic polymer networks capable of absorbing large amounts of water. Injectable hydrogels that are able to undergo a temperature-triggered phase transition after injection have been intensively investigated. Such thermosensitive approaches that do not require the use of organic solvents, copolymerisation agents, or an externally applied gelation trigger, are very suitable for biomedical applications (Klouda & Mikos, 2008), e.g. drug delivery (Fang, Chen, Leu, & Hu, 2008) and tissue engineering (Yu & Ding, 2008).

Polysaccharides have been extensively studied for the development of thermosensitive hydrogel systems because they are biodegradable, a quality not possessed by most synthetic polymers (Coviello, Matricardi, Marianecci, & Alhaique, 2007; Prabaharan & Mano, 2006). Chitosan (CS), a polysaccharide derived from naturally abundant chitin, has received a great deal of interest (Tang & Du, 2008). A thermosensitive neutral hydrogel based on CS/polyol salt combinations that could undergo sol–gel transition at a temperature close to 37 °C has been developed (Chenite et al., 2000). Other researchers also evaluated the use of such a hydrogel in pharmaceutical applications (Ruel-Gariépy, Leclair, Hildgen, Gupta, & Leroux, 2002; Ruel-Gariépy & Leroux, 2004; Ta, Dass, & Dunstan, 2008), cartilage repair (Hoemann et al., 2001) and cell culture (Richardson, Hughes, Hunt, Freemont, & Hoyland, 2008). Recently, a CS-based thermosensitive hydrogel containing nanoparticles (Couto, Hong, & Mano, 2009) was reported for similar applications. Many modified CS copolymers also have thermosensitive characteristics, such as PEG-grafted CS (Bhattarai, Ramay, Gunn, Matsen, & Zhang, 2005), hydroxybutyl CS (Dang et al., 2006), N-isopropylacrylamide-grafted CS (Chung, Bae, Park, Lee, & Park, 2005), and quaternised CS (Wu, Su, & Ma, 2006), which are injectable liquids at low temperature and transform to semisolid hydrogels at body temperature.

Methylcellulose (MC) is a water-soluble cellulose polysaccharide derivative that is widely used as a binder or thickener in pharmaceuticals, foods, ceramics, etc. MC undergoes a two-stage thermoreversible gelation in aqueous solution with increasing

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temperature when the concentration is above a critical value. Investigations have found that different salts have an important effect on gelation temperature (Takahashi, Shimazaki, & Yamamoto, 2001; Xu & Li, 2005; Xu, Wang, Tam, & Li, 2004). Other additives, including alcoholic, glycolic, and polyester resins can also change MC gelation properties (Kuang, Cheng, Zhao, & Li, 2006; Kundu, Kundu, Sinha, Choe, & Chattopadhayay, 2003). However, for pure MC solution, gelation temperature is always in the region of ~50–70 °C, and therefore MC on its own cannot be used as an injectable product for gelation *in vivo* at 37 °C. Polymer blending is an important method for modifying and/or improving the physical properties of polymeric materials, and hence for increasing the range of their application. MC/alginate hydrogel blended with NaCl has potential as a suitable carrier for site-specific protein drug delivery in the intestine (Liang et al., 2004), and a fast reversibly thermosensitive copolymer based on poly(N-isopropylacrylamide)-g-MC has been developed (Liu et al., 2004). By controlling gel temperature, gelation rate, and mechanical strength, such copolymer hydrogels are promising as blood vessel barriers. A rapid gelling injectable blend of hyaluronan and MC was also found to be promising for localised delivery of therapeutic agents in spinal cord injury (Gupta, Tator, & Shoichet, 2006).

Detailed rheological investigations into the effect of different salts, including sodium chloride (NaCl), sodium phosphate (Na_3PO_4), sodium hydrogen carbonate ($NaHCO_3$) and glycerophosphate (GP), on the gelation process (gelation temperature, gelation rate, and gel strength) in CS and MC blends for potential application as an injectable product, which is a liquid at ambient temperature and undergoes gelation under physiological conditions, are presented, along with discussion of possible gelation mechanisms. Hydrogels are also characterised by spectroscopic, microscopic and X-ray techniques, and a preliminary assessment of their potential use as tissue scaffolds is presented.

2. Materials and methods

2.1. Materials

CS was obtained from Yuhuan Ocean Biochemistry Co. (Zhejiang, China). The deacetylation degree (DD) as determined by elemental analysis (Xu, McCarthy, Gross, & Kaplan, 1996) was 92%, and the molecular weight calculated by gel permeation chromatography (GPC) (Qin, Du, & Xiao, 2002) was $\sim 2.7 \times 10^5$ Da. Pullulan standards (for GPC calibration) were purchased from Showa Denko (Tokyo, Japan). MC was purchased from the Shanghai Medicine Company (Shanghai, China) and the specification indicated that the viscosity of a 2% (w/w) solution was 4.54 Pa s at 20 °C, and that the methoxyl content was 29.6%. All other reagents were of suitable analytical grade.

2.2. General CS/MC hydrogel preparation methodology

Clear solutions of CS (2%, w/w) were obtained by dissolving CS (200 mg) in aliquots of hydrochloric acid (HCl, 0.1 M, 10 mL) with cooling in an ice/water bath for 15 min. Stock MC solutions (2%, w/w) were prepared by gradual dispersion of MC into hot water (e.g. 5 g in 250 mL) to avoid coagulation. Full MC dissolution was achieved by storage in a refrigerator (~4 °C) for at least 1 day prior to use. Different salts were added to aliquots of 2% (w/w) MC solution (10 mL, at concentrations that did not result in any precipitation), mixed and similarly cooled for 15 min. MC/salt solution (10 mL) was slowly added to CS solution (10 mL) whilst cooling in an ice/water bath under magnetic stirring for 10 min. The resultant solution was degassed by centrifugation (3 min at 3500 G and 5 °C). The effects of the salts on pure CS and MC solutions (1%, w/w) were also investigated. Gelation was induced by incubation for 30 min at 37 °C, as required.

2.3. Rheological measurements

Rheological properties were investigated as detailed previously (Tang, Du, Hu, Shi, & Kennedy, 2007) using a strain-controlled ARES rheometer (TA Instruments, New Castle, DE, USA). Dynamic viscoelastic parameters such as dynamic shear storage modulus (G') and loss modulus (G'') were measured as a function of time, temperature, or angular frequency, for gelation of CS/MC/salt systems. Strain amplitude was checked to ensure that all measurements were carried out within the linear viscoelastic region, where G' and G'' are independent of strain amplitude. Accordingly, strain amplitude was 20% for all measurements.

2.4. FT-IR spectroscopic analysis

FT-IR spectra were recorded using a Nicolet FT-IR 5700 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at ambient conditions. CS, MC and dried hydrogel samples were triturated with KBr in the ratio of 1:100 and pressed to form pelleted samples for FT-IR spectroscopic analysis at 500–4000 cm^{-1}.

2.5. X-ray diffraction measurements

X-ray diffraction (XRD) measurements were performed on CS, MC and CS/MC/salt hydrogel samples using a D8 Advance diffractometer (Bruker AXS, Inc., Madison, WI, USA) with Cu target and Kα radiation ($\lambda = 0.154$ nm) at 40 kV and 50 mA. The scanning rate was 0.5°/min and the scanning scope of 2θ was 5° and 40° in a fixed time mode with a step interval of 0.02° at ambient conditions.

2.6. Microscopic investigations

Hydrogel samples were carefully lyophilised to maintain their tree-dimensional porous structure (without any collapse). Lyophilised hydrogel samples were immersed in liquid nitrogen, and the vitrified samples carefully cut with a cold knife. Cut samples were mounted, sputter coated with gold, and their morphology investigated by scanning electron microscopy (SEM) using a Hitachi S-570 SEM microscope (Hitachi, Tokyo, Japan).

2.7. Cell culture and morphology

Derived chondrocytes of mesenchymal stem cells (MSC) were obtained through isolation, expansion and chondrogenetic differentiation according to previously reported methods (Lei, Liu, & Liu, 2008). Hydrogel samples were lyophilised and sterilised with ethylene oxide at a density of 1×10^6/construct. Derived chondrocytes were cultured on the lyophilised/sterilised hydrogel scaffolds for 3 weeks. After this time, hydrogel scaffold samples were stained in the dark for 10 min using acridine orange (AO) solution (0.01%), rinsed with phosphate buffered saline (PBS) solution to remove AO adsorbed onto the scaffolds themselves, and cell morphology investigated by confocal laser scanning microscopy (CLSM) using a Leica TCS-SP CLSM microscope (Leica Microsystems GmbH, Wetzlar, Germany). The emission wavelength of AO was 488 nm.

3. Results and discussion

3.1. Gel formation mechanisms

Pure MC solutions can form thermoreversible gels on their own. At low temperatures, water molecules are presumed to interact

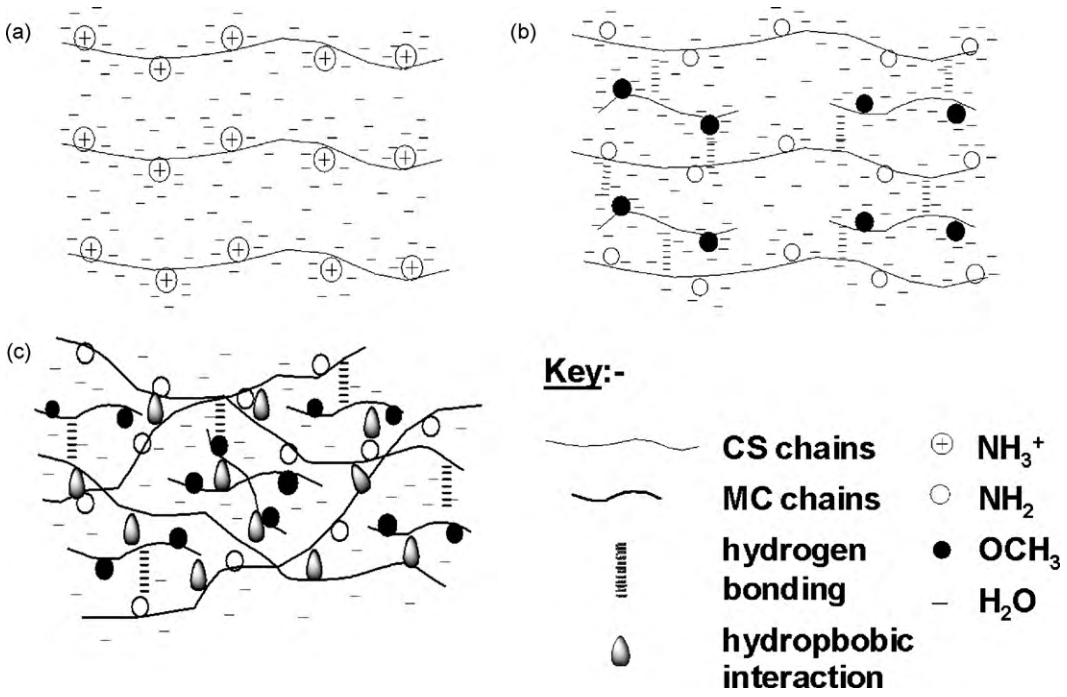

Fig. 1. Proposed interaction mechanisms for chitosan (CS) and methylcellulose (MC): (a) CS solution (low temperature); (b) CS/MC solution (low temperature); (c) CS/MC hydrogel (elevated temperature).

with the MC hydrophobic methoxyl groups via hydrogen bonding, forming 'cage-like' structures that surround the methoxyl groups, which effectively shields them from the hydrophilic environment, thereby causing MC to become water-soluble. Heating of such MC solutions causes destruction of the hydrogen bonds/cage structures, thereby exposing the hydrophobic regions of MC. This leads to the formation of intra- and inter-molecular chain hydrophobic associations, which ultimately result in gelation as the increasing number of interactions effectively produces a large 'hydrophobically cross-linked' network. CS is solubilised in dilute acid by protonation of the free-amino groups, which then undergo ionic/hydrophilic interaction with water molecules, resulting in solubilisation (Fig. 1(a)). Increasing the pH results in progressive deprotonation, gradually increasing the hydrophobic character of CS chains until gelation/insolubility occurs.

The mechanism of thermosensitive sol–gel transition in CS/poly(vinyl alcohol) blend systems has been previously discussed (Tang et al., 2007). For CS/MC gels, the gel mechanism is similar, since MC can be considered as a polyhydroxy material at low temperature (as discussed above). It is known that such polymers can stabilise certain compounds in solution and promote formation of a water 'shield' around macromolecules in aqueous solutions. When the MC solution is added at low temperature to CS solution, hydrogen bond formation between CS, MC and water can maintain/enhance CS chain dissolution (Fig. 1(b)). These interactions and the low temperature reduce macromolecular mobility, preventing CS chain association/precipitation. When temperature is increased, intermolecular hydrogen bonding interactions are reduced and the energised water molecules surrounding the polymers are dissipated, thereby allowing hydrophobic intermolecular chain association, resulting in gelation (Fig. 1(c)). Thus, hydrophobic interactions are presumed to be the main driving force in gelation of CS/MC systems at elevated temperatures.

The introduction of certain salts into CS/MC systems will obviously have a significant effect on gelation properties, since they are ionic species. For example they can disrupt intermacromolecular hydrogen bonding, and shield electrostatic/ionic repulsion/attraction effects, and thus alter polymer solution viscosity/rheology (Knill, Kennedy, Latif, & Ellwood, 2002). They can also alter system pH, which is particularly important with respect to CS (as discussed above). The effects of certain salts on CS/MC hydrogel gelation parameters are discussed below.

3.2. Effect of salts on gelation temperature

NaCl, Na_3PO_4, $NaHCO_3$ and GP were used to study the effect of salts on gelation properties, which were mainly characterised by rheological measurements. The storage modulus (G') reflects the solid-like component of the rheological behaviour, which is thus low at solution stage but increases dramatically as gelation occurs. Gelation temperature is usually defined as the sol/gel transition temperature at which G' is equal to G''.

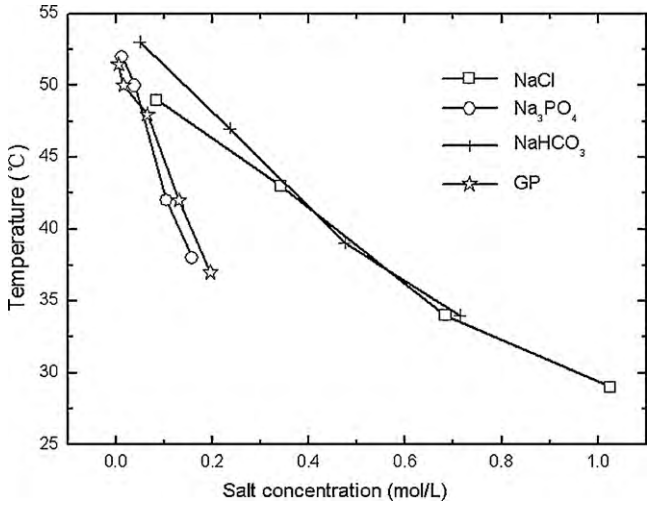

Fig. 2. Gelation temperature of MC/salt solutions as a function of salt concentration (MC concentration 1%, w/w).

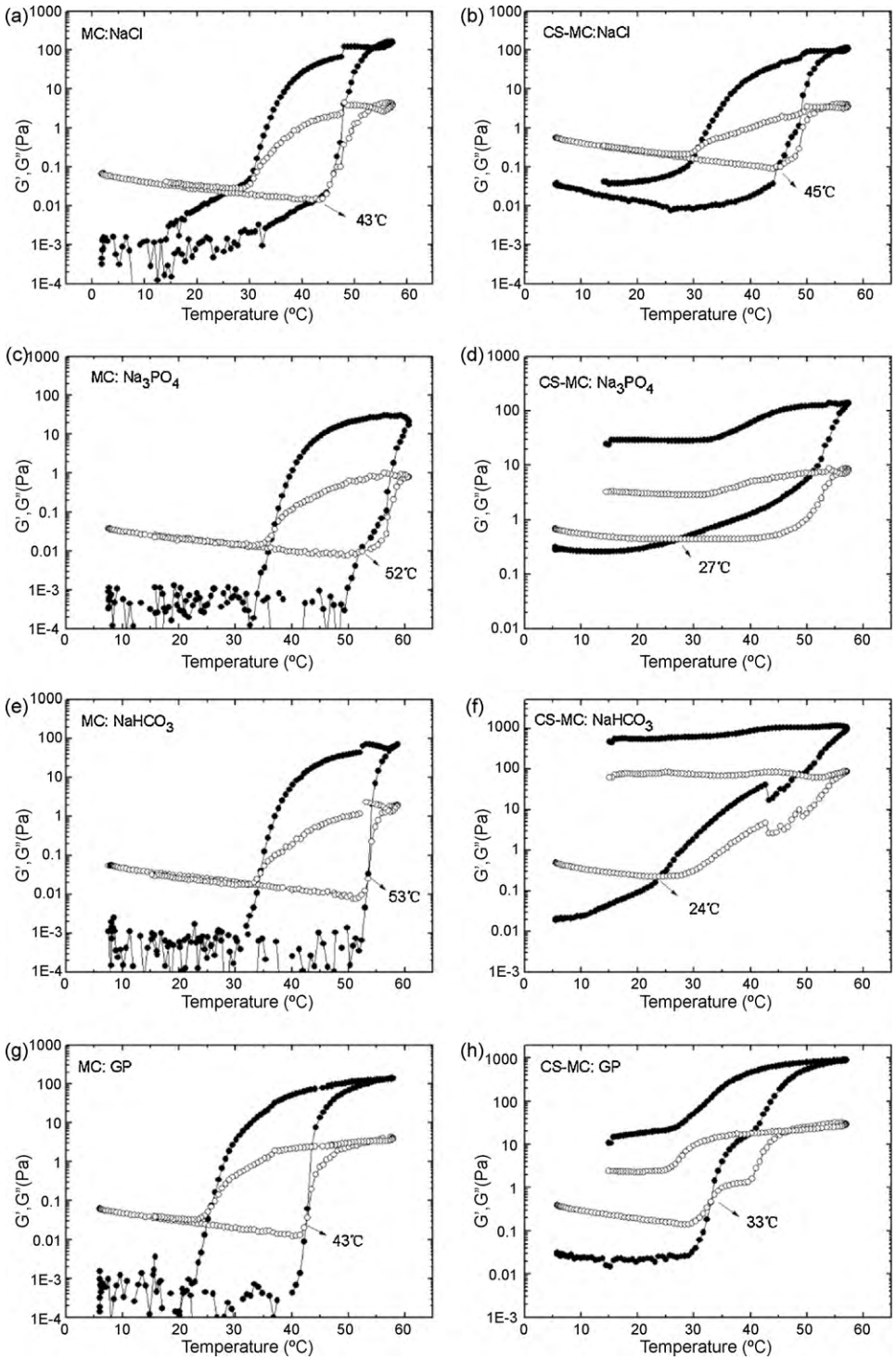

Fig. 3. Temperature dependence of storage modulus (G') and loss modulus (G'') of MC/salt and CS/MC/salt solutions: (a) MC/NaCl; (b) CS/MC/NaCl; (c) MC/Na_3PO_4; (d) CS/MC/Na_3PO_4; (e) MC/$NaHCO_3$; (f) CS/MC/$NaHCO_3$; (g) MC/GP; and (h) CS/MC/GP (heating rate 2 °C/min, frequency 1 rad/s, CS and MC concentrations 1%, w/w).

For pure MC solution, the gel temperature was 50 °C. The effects of the four salts investigated at different concentrations on MC gel temperature are presented in Fig. 2. As expected, ionic strength and anion charge had an important effect on gelation temperature (Xu & Li, 2005), Increasing ionic strength and higher anion charge density (i.e. 3− for phosphate compared with 1− for chloride) results in greater interaction with water molecules, thereby reducing intermolecular hydrogen bonding between water molecules and MC chains, which permits greater hydrophobic interaction between MC chains leading to more rapid onset of gelation (i.e. a decrease in observed gelation temperature). It is proposed that there exists a competitive attraction between anions and MC chains for water molecules. When salt concentrations are very low, they cannot attract water molecules away from MC chains. On the contrary, the salt ions are attracted by the water molecules and intensify the cage-like shielding effect around the MC chains (hence the initial increase in gelation temperature above the 50 °C observed for pure MC). However, as the salt ion concentration increases they become the dominant factor and attract the water molecules away from the MC chains, resulting in the effects discussed above. For Na_3PO_4

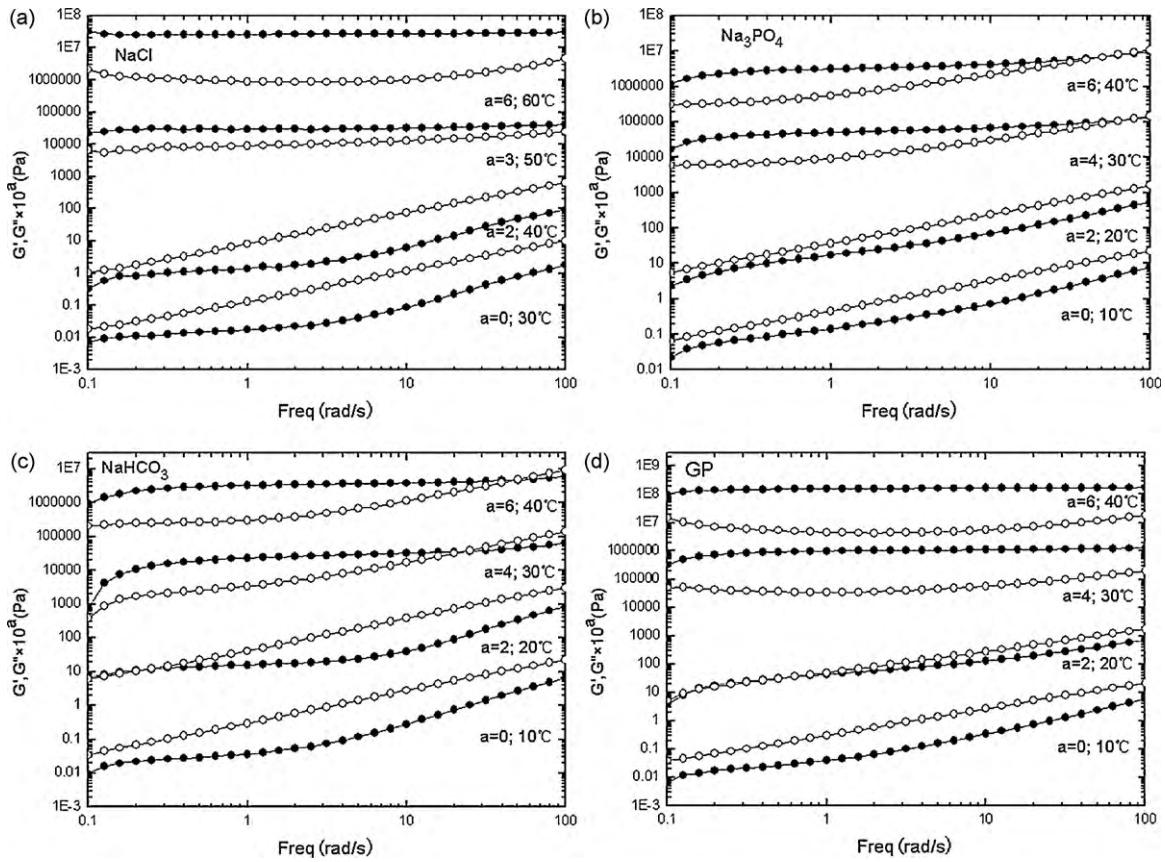

Fig. 4. Frequency dependence of storage modulus (G') and loss modulus (G'') of CS/MC/salt solutions at different temperatures: (a) NaCl; (b) Na_3PO_4; (c) $NaHCO_3$; and (d) GP (CS and MC concentrations 1%, w/w).

and $NaHCO_3$, the gel temperature was elevated to 53 °C and 52 °C, respectively, at low concentration.

In the case of pure CS solution, NaCl obviously had no effect on system pH and thermogelation could not be induced, even at high temperature. For Na_3PO_4 and $NaHCO_3$, the CS precipitated due to significant pH increase. However, due to its hydroxyl group content, GP could prevent CS chain association/precipitation (despite the pH increase) and resulted in a gelation temperature of 32 °C.

The temperature dependence of the storage modulus G' and the loss modulus G'' for MC/salt solutions/gels and CS/MC/salt blend solutions/gels (for different salt concentrations) at a frequency of 1 rad/s are presented in Fig. 3. For NaCl, the CS/MC/salt blend gelation temperature (45 °C) was similar to the pure MC/salt gelation temperature (43 °C), implying little interaction/gelation of the CS occurred. For Na_3PO_4, a significant difference in gelation temperature for the CS/MC/salt blend (27 °C) is observed compared to that for the pure MC/salt (53 °C), despite any pH increase. This may be due to either direct ionic interaction between phosphate ions and protonated CS amino groups and/or greater hydrophobic interaction between CS and MC. For $NaHCO_3$, some carbon dioxide was evolved as the salt reacted with the acid (HCl) used for CS solubilisation, keeping the pH low, resulting in a dramatic reduction in gelation temperature for CS/MC/salt blend (24 °C) compared with the pure MC/salt (53 °C), presumably due to greater hydrophobic interaction between CS and MC. For GP, a smaller reduction in gelation temperature for the CS/MC/salt blend (33 °C) is observed compared to that for the pure MC/salt (43 °C), despite any pH increase, due to the hydroxyl effect (discussed previously).

The effect of added salts on the CS in the CS/MC/salt system is of most importance with respect to having the greatest effect (reduction) in gelation temperature. In this study, the range of salt concentrations were somewhat limited, due to the adverse effects of pH increases on CS solubility (i.e. inducing precipitation).

3.3. Effect of salts on gel strength

The frequency dependence (0.1–100 Hz) of the viscoelastic properties of CS/MC/salt hydrogels at different temperatures is presented in Fig. 4, and observed differences in gel strength may be attributed to differences in gelation temperatures and interactions (ionic, hydrophobic, and hydrogen bonding, as discussed previously). For NaCl, a strong gel was mainly formed by the hydrophobic effects of MC chains above 50 °C, and it was thermoreversible. For Na_3PO_4 and $NaHCO_3$, the gel structure was relatively weak, even at 40 °C with the temperature exceeding their gelation temperatures of 27 °C and 24 °C, respectively. MC solution cannot form a gel on its own at 40 °C, therefore the gel structure is formed by the hydrophobic interaction of CS and MC, but the increased pH affects the ability of CS to do so. For GP, a strong gel was formed at 40 °C, i.e. close to its gelation temperature of 33 °C. Again the beneficial effects of the GP hydroxyl groups for enhancing the production of a complex network via multiple interactions (ionic, hydrophobic, and hydrogen bonding, as discussed previously) can be observed.

3.4. Effect of salts on gelation rate

The gelation time is defined as the time when the storage modulus becomes higher than the loss modulus, and reflects the changes of G' and G'' during the gelation process, indicating the gel rate and the gel strength. The influence of temperature (37 °C and 50 °C) on the gelation process for CS/MC/salt solutions at a fixed frequency of 1 rad/s was studied (Fig. 5). As expected, for all salts investigated,

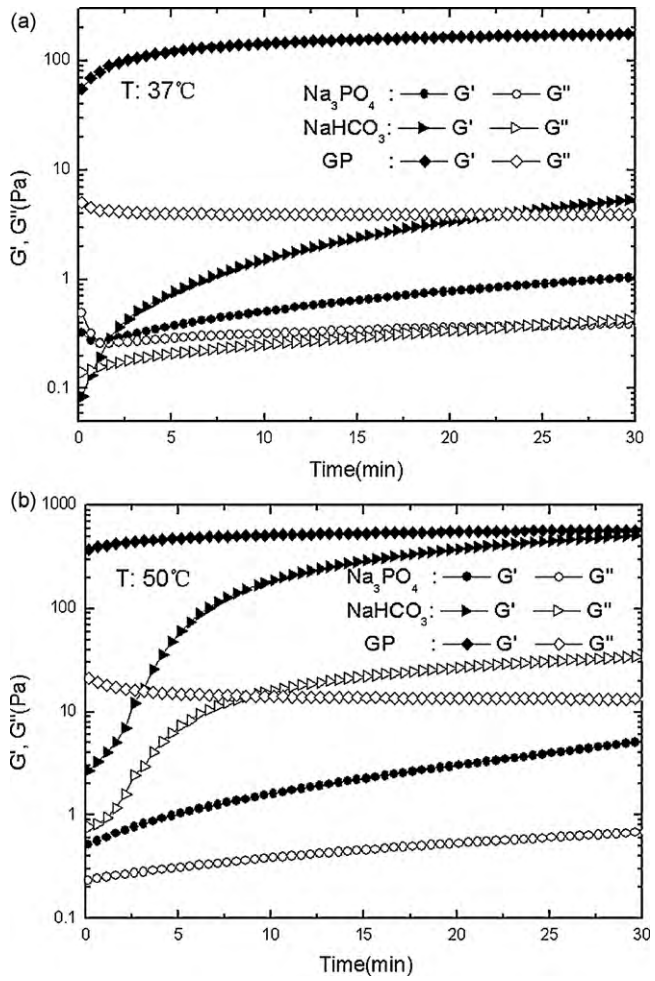

Fig. 5. Time dependence of storage modulus (G') and loss modulus (G'') of CS/MC/salt solutions at (a) 37 °C, and (b) 50 °C (frequency 1 rad/s, CS and MC concentrations 1%, w/w).

increasing the temperature increases the gelation rate. For the same temperature, the gelation rate for the different salts was in the order GP > $NaHCO_3$ > Na_3PO_4 and the gel strength was in the order GP > $NaHCO_3$ > Na_3PO_4. These results were in accordance with the gel frequency dependence with different salts (discussed previously). Since NaCl had no effect on CS gelation, the gelation rate depends on the effect of NaCl on MC (Fig. 3).

3.5. FT-IR spectroscopic analysis

FT-IR spectra of dried CS/MC/salt hydrogel samples (and pure CS and MC controls) are presented in Fig. 6. For pure chitosan, absorption peaks are observed at ~3400–3500 cm^{-1} (O–H stretching and HO–CH_2OH intra- and inter-molecular hydrogen bonding), ~2900 cm^{-1} (C–H stretching in CH_2), ~1660 cm^{-1} (C–O stretching in secondary amide, amide I), ~1550 cm^{-1} (C–O stretching in secondary amide, amide II), ~1420 cm^{-1} (C–H bending in, CH_2), ~1320 cm^{-1} (C–N stretching in secondary amide, amide III), ~1250 cm^{-1} (C–O stretching of ring ether) and ~1050 cm^{-1} (C–O symmetric stretching of primary alcohol) (Kasaai, 2008; Pinotti, García, Martino, & Zaritzky, 2007; Sionkowska, Wisniewski, Skopinska, Kennedy, & Wess, 2004; Van de Velde & Kiekens, 2004; Wu, Zivanovic, Draughon, Conway, & Sams, 2005). The amino group has a characteristic absorption peak in the ~3400–3500 cm^{-1} region (often masked by the broad OH absorption) (Sionkowska et al., 2004). For pure MC, absorption peaks are observed at

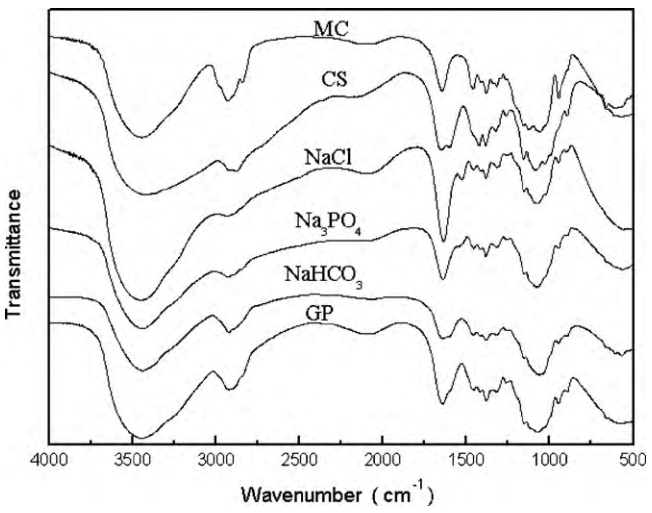

Fig. 6. FT-IR spectra of dried CS/MC/salt hydrogel samples (plus CS and MC controls).

~3400 cm^{-1} (O–H stretching), ~2900 cm^{-1} (C–H stretching in CH_2 and CH_3), ~1430 cm^{-1} (C–H bending in, CH_2), ~1250 cm^{-1} (C–O stretching of ether linkages) and ~1050 cm^{-1} (C–O symmetric stretching of primary alcohol) (Pinotti et al., 2007; Viera et al., 2007; Zaccaron, Oliveira, Guiotoku, Pires, & Soldi, 2005).

The FT-IR spectra of dried CS/MC/salt hydrogels contain no obvious additional absorbance peaks compared to those for CS and MC. This suggests that there is no direct chemical interaction between the components, i.e. no introduction of additional chemical functionality to account for gelation. Therefore, the gelation process is a direct result of physical interaction phenomena (i.e. ionic/hydrophobic interactions) between CS and MC chains, with the various salts contributing accordingly (as discussed previously).

3.6. XRD analysis

X-ray diffraction patterns for CS/MC/salt hydrogel samples (and pure CS and MC controls) are presented in Fig. 7. Two typical peaks at $2\theta = 8.5°$ and 20° were observed for MC. The diffraction of CS showed typical peaks around 11° and 20°. These peaks are indicative of the crystallinity present in these polysaccharides. The MC diffraction intensity at 20° decreased dramatically and the peak at 8.5° disappeared completely in the CS/MC/salt hydrogels. Similarly,

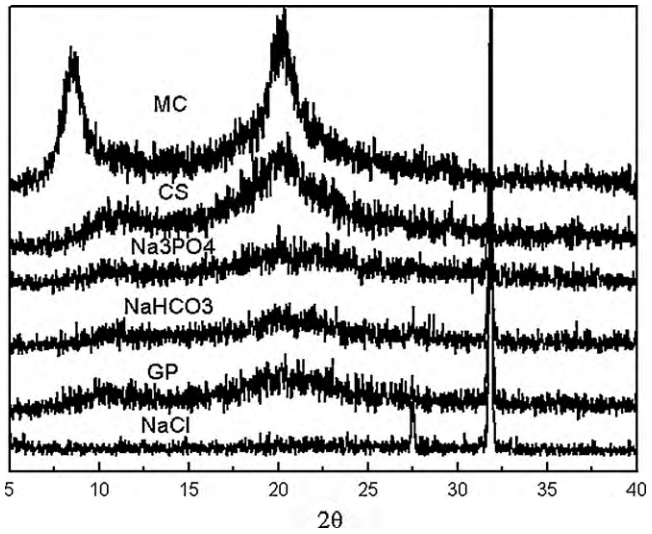

Fig. 7. XRD patterns for CS/MC/salt hydrogel samples (plus CS and MC controls).

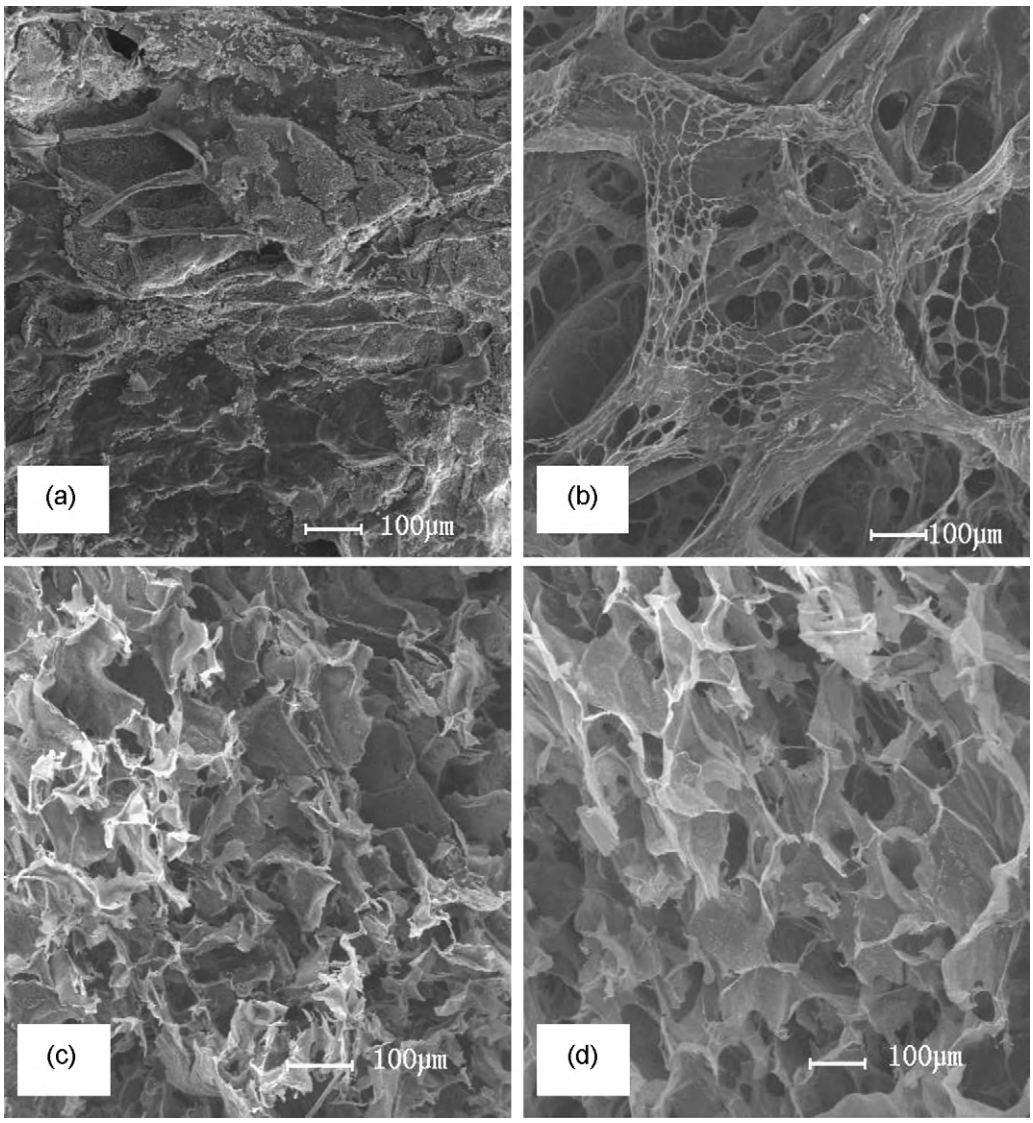

Fig. 8. SEM micrographs (~850 μm × 850 μm) of dried CS/MC/salt hydrogel samples, prepared using: (a) NaCl; (b) Na_3PO_4; (c) $NaHCO_3$; and (d) GP.

the diffraction peaks of CS at 11° and 20° decreased significantly in the CS/MC/salt hydrogels (and were not present at all in the case of NaCl). At the same time, a new very sharp peak at 34° was observed in the CS/MC/salt hydrogels. Overall, the CS/MC/salt hydrogels had greatly decreased crystallinity, compared with the polysaccharide substrates, indicating good initial component solubilisation and uniform distribution of components throughout the system during gelation, i.e. gel homogeneity.

3.7. SEM microscopic analysis

The morphology of the lyophilised CS/MC/salt hydrogels was examined by scanning electron microscopy (SEM), which showed that the hydrogel pore structure changes from nonporous to microporous with different salts (Fig. 8). For CS/MC/NaCl, a three-dimensional network did not exist because gelation could not occur at 37 °C (Fig. 8(a)). For Na_3PO_4, gel formation depended on the CS chains, and an interconnectivity among pores was observed, which is likely due to the ionic cross-linking effects between PO_4^{3-} and NH_3^+ (as discussed previously) and hydrophobic interactions between CS and MC chains (Fig. 8(b)). For $NaHCO_3$, the hydrogel gel did not have a uniform microporous structure and had poor pore interconnectivity (Fig. 8(c)). For GP, the hydrogel demonstrated a uniform microporous network, indicating good interaction between the components (as discussed previously).

3.8. Cell morphology

The effects of CS/MC/salt scaffolds (produced using Na_3PO_4 and $NaHCO_3$ and) on chondrocyte cell viability/proliferation were investigated. As indicated previously, the use of these different salts had a significant effect on the pore structure (size and interconnectivity) of their respective hydrogels (Fig. 8(b) and (c), respectively), which can have a considerable effect on cell growth and proliferation. Cell morphology was observed using CLSM after AO staining (as detailed previously). Cells on the CS/MC/$NaHCO_3$ scaffold were distributed in clusters after cultivation for 7 days (Fig. 9(a1)) and the quantities of cells significantly decreased after 14 and 21 days (Fig. 9(a2) and (a3), respectively). Initial cell growth on the CS/MC/Na_3PO_4 scaffold was slower than for $NaHCO_3$ (Fig. 9(b1)), however after 14 days the cells were clearly spreading (Fig. 9(b2)), and after 21 days had spanned the entire scaffold (Fig. 9(b3)). This clearly demonstrated the good viability of the CS/MC/Na_3PO_4 scaffold for MSC-derived chondrocytes.

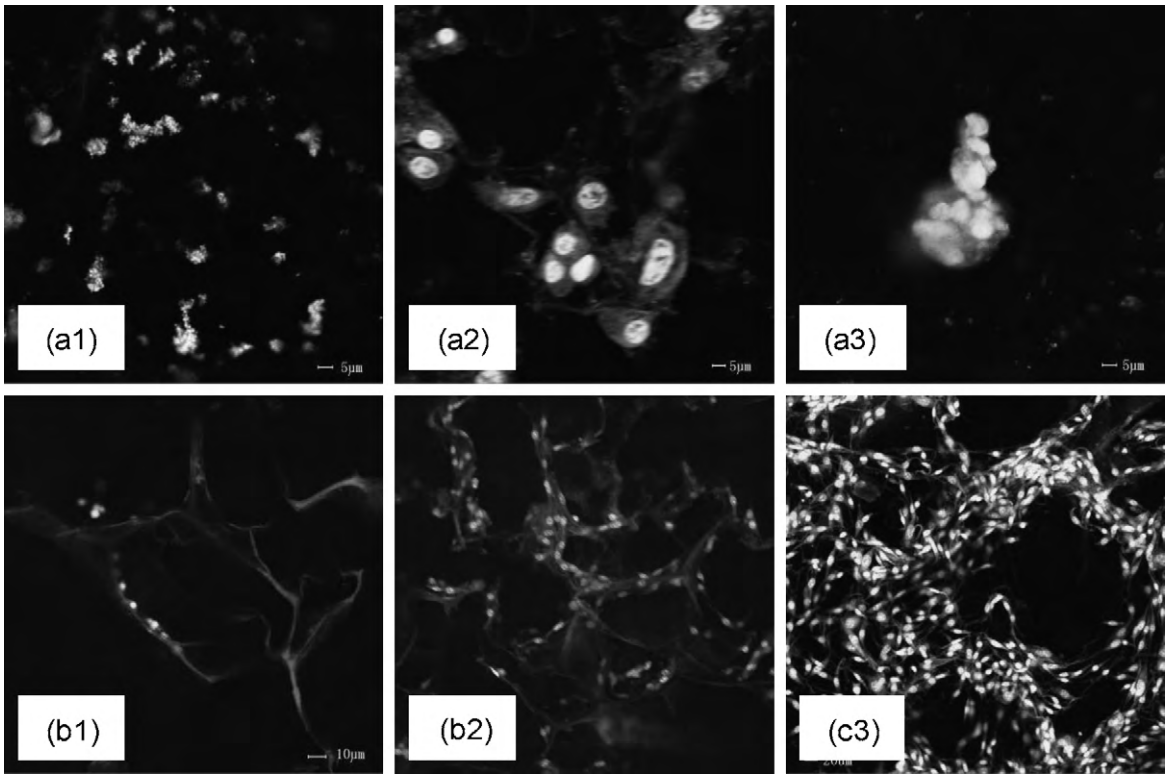

Fig. 9. CLSM micrographs (~85 μm × 85 μm) of dried, sterilised hydrogels: (a) CS/MC/$NaHCO_3$, and (b) CS/MC/Na_3PO_4, after incubation with MSC chondrocytes for (1) 7 days, (2) 14 days, and (3) 21 days (stained with AO, emission at 488 nm).

4. Conclusions

An injectable hydrogel was formed via ionic and hydrophobic interactions between chitosan (CS) and methylcellulose (MC) chains in the presence of various salts under mild conditions without organic solvent, high temperature or harsh pH. Such blends were liquid at low temperature, but gel under physiological conditions (37 °C). Rheological investigations demonstrated the different effects of NaCl, Na_3PO_4, $NaHCO_3$ and glycerophosphate (GP) on the CS/MC gelation process (gelation temperature, gel strength, and gelation rate), from which some understanding of possible gelation mechanisms could be inferred. Gelation temperature followed the order NaCl > GP > Na_3PO_4 > $NaHCO_3$, gelation rate followed the order GP > $NaHCO_3$ > Na_3PO_4, and gel strength followed the order GP > $NaHCO_3$ > Na_3PO_4 (at 37 °C). Characterisation by FT-IR spectroscopy, XRD, and SEM showed that the hydrogels were formed solely by physical interactions (ionic, hydrophobic, etc., i.e. not chemical cross-linking), had good miscibility/uniformity/homogeneity, with different pore structures (ranging from nonporous to microporous), respectively. When used as a scaffold for chondrocytes, CS/MC/Na_3PO_4 hydrogel resulted in good cell viability and proliferation, indicating potential use as a three-dimensional synthetic matrix for tissue engineering.

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