Research Article

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Development of dual-triggered *in situ* gelling scaffolds for tissue engineering

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Abstract

Hydrogel-forming materials that mimic the three-dimensional architecture and properties of tissue are known to have a positive effect on cellular differentiation and growth. A subset of those are *in situ* gels, which utilise *in vivo* conditions like pH (e.g. acetate phthalate), temperature (e.g. poloxamer) and ionic concentration (e.g. Gelrite™), and can be used to facilitate the delivery of cells to an affected tissue. Hence, we have developed *in situ* hydrogels based on gellan and hydroxypropylmethylcellulose (HPMC), which are known to be triggered through ions and temperature, respectively, as matrices to deliver cells. Gellan/HPMC blends had a lower gelation temperature than gellan alone crosslinked with calcium, suggesting the role of the dual trigger. Average storage modulus at a frequency of 10 Hz for gellan crosslinked with 3 mmol L^{-1} calcium was 4.53×10^3 Pa; for 9:1 gellan/HPMC crosslinked with 3 mmol L^{-1} calcium was 5.59×10^3 Pa; and for 8:2 gellan/HPMC crosslinked with 3 mmol L^{-1} calcium was 2.13×10^3 Pa, suggesting tunable stiffness by changing the gellan-to-HPMC ratio. Hydrophilicity was confirmed using goniometry with a contact angle much less than 90°, facilitating the passage of cells and electrolytes when using the gels as scaffolds. The gels were also found to be porous and non-toxic to fibroblast cell line L929 and osteosarcoma cell line MG-63, which, when encapsulated within the gels, were able to grow and proliferate. These blended hydrogels are suitable as scaffolds to encapsulate cells, with tunable stiffness modulated by varying the concentration of gellan and HPMC.

Supporting information may be found in the online version of this article.

Keywords: gellan; HPMC; hydrogels; *in situ* gels; triggered systems; minimally invasive

INTRODUCTION

The phenomena that effect gelation of polymers have been studied in detail and have been characterised. This gelation occurs due to triggers which bring about a change in the polymer network enough for it to aggregate and form a hydrogel. The triggers range from chemical signals, such as pH, metabolites and ionic factors, to physical stimuli such as temperature or electrical potential. Furthermore, potential biomedical and pharmaceutical applications of such systems have been realised.[1–5] The main focus of much current research has been temperature-triggered and pH-triggered systems due to their physiological significance.[6–11] Additionally ion triggers and novel triggers like UV light have been considered for natural polymers after crosslinking with methacrylate.[12,13] Although a wide tunable range of stiffness is available, this lacks the ability to use physiological cues for gelation. For *in situ* applications it would be preferable to have materials that retain matrix stability by using physiological triggers such as ions, pH and temperature as against UV light.

Thermally responsive polymers can be defined as those polymers that have an upper critical solution temperature (UCST), that is, they shrink by cooling below the UCST, and as those with a lower critical solution temperature (LCST), that is, they contract by heating above the LCST. Hydroxypropylmethylcellulose (HPMC) is an example of such a polymer – chemically presented as $C_6H_7O_2(OH)_x(OCH_3)_y(OC_3H_7)_z$, with $x+y+z=3$ – which has the property of reversibility from sol to gel state, mediated by temperature.[14] This property of HPMC has been utilised in drug release systems.[15–17] This mechanism of gelation has been used to devise delivery systems and also in tissue engineering for non-invasive *in situ* systems.[18] Ion-triggered polymers like gellan gum, an exocellular microbial heteropolysaccharide that is secreted by the strain *Sphingomonas paucimobilis*, are also of interest.[19] Gellan is a linear anionic polysaccharide that consists of glucose, glucuronic acid and rhamnose in the molar ratio 2:1:116. Gelation in gellan is triggered by monovalent as well as divalent cations like sodium and calcium ions. A combination of HPMC and gellan as a blend scaffold was envisioned with use of the triggers for gelation of both polymers, in order to create a faster gelling matrix for tissue engineering applications.

There has been interest shown in the combination of thermally responsive polymers with other naturally occurring polymers to improve their gelation characteristics as well as to improve other properties like biocompatibility and

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biodegradability.[1] The polysaccharides that are under consideration are pH-sensitive polymers like chitosan and ion-sensitive polymers like alginate.[8,9,18,20] Blended polymer compositions like gellan and HPMC – ion- and temperature-responsive materials, respectively – have not been assessed in terms of their characteristics for tissue engineering applications. The study of such combinations that show dual characteristics, in terms of gelation and variable stiffness, can shed new light on the preparation of tissue-mimicking substrates.

The basis of the work reported in this paper was to ascertain if ion-responsive gellan and thermo-responsive HPMC, when blended, give a suitable triggered system for tissue engineering. We prepared different blends of the polymers using different ratios. The thermal behaviour of the hydrogel systems was evaluated using rheology measurements and cloud point measurements on blends, without crosslinking at various temperatures and with crosslinked hydrogels. The hydrogels were also tested for hydrophilicity using contact angle measurements with fluids like water, phosphate-buffered saline (PBS) and foetal bovine serum (FBS). SEM was performed to visualise the internal architecture of the gels. Furthermore, cellular compatibility was tested with the fibroblast cell line L929 and the osteosarcoma cell line MG-63, using the sulforhodamine B (SRB) assay to test cell viability in the presence of a gel extract. Finally, fibroblast cell line L929 and osteosarcoma cell line MG-63 were encapsulated within the gels, and cell viability was tested after six days in culture.

EXPERIMENTAL

Materials

Gellan, in its deacetylated form (food grade, KELCOGEL), was kindly provided by CP Kelco US (Chicago, IL). Calcium chloride, D-glucose, disodium hydrogen phosphate, potassium chloride, potassium dihydrogen phosphate, glacial acetic acid and hydrochloric acid were purchased from Qualigen Fine Chemicals (Mumbai, India). Calcein AM, propidium iodide and SRB were procured from Sigma Aldrich (St Louis, MO). Dulbecco's minimum essential medium (DMEM) was procured from HiMedia (Mumbai, India) and penicillin streptomycin and FBS were procured from Invitrogen (Carlsbad, CA).

Hydrogel formation and gelation time

Gellan (G0.5) and gellan/HPMC at ratios of 8:2 (0.5GH8:2) and 9:1 (0.5GH9:1) were weighed to a final concentration of 0.5% (w/v) and heated to 90 °C until a clear solution was obtained. The final concentrations of gellan and HPMC in 0.5GH8:2 were 0.4 and 0.1%, respectively, while in 0.5GH9:1 they were 0.45 and 0.05%, respectively. After this, samples were placed at 37 °C until the required temperature was reached; 50 mmol L^{-1} calcium chloride was also maintained at the same temperature. When both reached the required temperature, the calcium chloride was added into the gellan and gellan/HPMC solutions, with continuous stirring at 650 rpm, to a final concentration of 3 mmol L^{-1}.

Viscoelasticity

Rheological measurements were performed using a modular compact rheometer (Physica MCR 310). Fully hydrated samples were placed onto the plate of the instrument, using parallel plate geometry, with a gap of *ca* 3 mm.[21] The viscoelastic properties were quantified in terms of G' and G'', the real and imaginary components of the complex shear modulus of the material. G', the storage modulus, represents the elastic nature, while G'', the loss modulus, represents the viscous nature. Both moduli were calculated using the instrument software (Rheoplus/32, version V3.21, Anton Paar, Graz, Austria) that tracked the magnitude and phase lag of the torque for a given oscillatory frequency of the Plate. G' and G'' were plotted against the frequency of oscillatory stress and the resulting spectra were termed as mechanical spectra and were used to demonstrate the gel character and to discriminate between different classes of gels. G' and G'' were measured at various frequencies from 0.01 to 10 Hz, at a constant strain of 0.5%. All measurements were performed at the physiological temperature of 37 °C. The relative contribution of the elastic and viscous natures can be quantified by the loss tangent, tan a, which is the ratio of the two moduli:

$$\tan a = \frac{G'}{G''}$$

The higher the value of tan a, the more liquid-like the sample, with a value of 1 considered to be a threshold between liquid and gel behaviour.

A temperature sweep was also performed from 25 to 80 °C at a constant strain of 0.5% and at 10 Hz. Time sweep measurements were also conducted at a constant strain of 0.5% and at 10 Hz, at a temperature of 37 °C. Time sweeps were carried out after the addition of calcium maintained at 37 °C in order to obtain the *in situ* gelation time after the addition of crosslinker.

Cloud point measurements

Thermal responsiveness of HPMC in an aqueous solution is connected to its insolubility in aqueous solution when the temperature is raised, which is referred to as its cloud point.[22] UV-visible spectrophotometry (PerkinElmer Lambda 35) was used to determine the cloud point of the gellan and HPMC and gellan/HPMC blends at ratios of 8:2 and 9:1, dispersed in MilliQ water to a final concentration of 0.5% (w/v). To obtain the spectra, the temperature was increased at a rate of 2 °C min^{-1} and the optical density was measured over the temperature range 25–90 °C. The LCST values were determined as given by Liu *et al.*[22]

Contact angle

To evaluate wettability, the contact angle of the gels was measured with MilliQ water and PBS and serum in accordance with the procedure of Zhang *et al.*[23] Contact angles of the gels were measured using the sessile drop technique with a CAM-100 optical contact angle meter (KSV Instruments, Finland), by depositing a 1 μL drop of water from a microsyringe on the surface of the gel which was dried onto the surface of a glass slide. The image of the drop was analysed with an automated curve-fitting program using in-built software. All measurements were made immediately following deposition of the drop. All reported data are mean values of three measurements.

Scanning electron microscopy

SEM (Hitachi 3400N, USA) was performed on lyophilised hydrogels. The imaging was performed at ×100 and ×200 magnification at 5 kV.

Cell cytocompatibility

Cellular cytocompatibility of the hydrogel composites was evaluated with the fibroblast cell line L929 and human osteosarcoma cell line MG63 using SRB assay according to the method of Skehan *et al.*[24] with slight modifications, in that gel extracts were incubated with cells to evaluate whether the gel extracts had a toxic effect on the cells. Cells were grown in DMEM supplemented with 10% (v/v) FBS (complete media) and incubated in a CO_2 incubator (IncuSafe, Sanyo, Osaka, Japan) at 37 °C under a 5% CO_2 and saturated humid atmosphere. Nearly confluent cells in 25 cm^2 tissue culture flasks were trypsinised with TRYple Select solution and re-suspended in fresh medium. Cell counts were determined using haemocytometry. Re-suspended cells were diluted accordingly and were plated at a concentration of 1×10^4 cells per well in a 96-well tissue culture plate and incubated in a CO_2 incubator. G0.5, GH8:2 and GH9:1 at different ratios were made up to a final concentration of 0.5% and the required amount of calcium chloride was added to make up the final concentration of 3 mmol L^{-1}. Samples were then autoclaved. Gels were allowed to set and were incubated with the complete media for 24 h at 37 °C for extraction. After 24 h the medium was replaced with a gel extract in quadruplicate and incubated. After another 24 h the SRB assay was conducted. In brief, the old medium with the gel extract was discarded and 100 µL of fresh medium was added. Cells were fixed by adding 50 µL of ice-cold 50% trichloroacetic acid slowly to the medium and incubating at 4 °C for 1 h. The plates were washed five to ten times with deionised water and dried in air. A 100 µL aliquot of 0.4% SRB dissolved in 1% acetic acid was added to the fixed cells, which was kept at room temperature for 20 min. The plates were washed with 1% acetic acid to remove unbound dye and dried at room temperature. An aliquot of 100 µL of 10 mmol L^{-1} Tris base (Sigma, USA) was added to each well and incubated at room temperature for 20 min to solubilise the dye. Plates were read at 560 nm with a plate reader (Thermo Electron Corp., USA). Cell viability was measured as

$$\text{Viability} = \frac{\text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

Cellular entrapment studies

Gellan/HPMC samples at 8:2 and 9:1 ratios were prepared at a final concentration of 0.5%. The gel was prepared under sterile conditions by adding sterile water and heating until a clear solution was obtained. Calcium chloride solution was maintained at 37 °C. Trypsinised cells were counted in a haemocytometer. The cells were plated in the gel at a final concentration of 5×10^4 cells per volume of gel plated. The cells were mixed with a calcium chloride solution and with a pre-warmed solution of gellan blends. Control included cells plated at a concentration similar to that loaded in the gel. Medium change was performed every alternate day. Cells were incubated on day 6 of incubation with calcein AM and propidium iodide solutions before confocal images were obtained at ×10 with an Olympus FluoView (FV500, Tokyo, Japan). A similar process was carried out with control for imaging. Z-series of approximately equidistant x–y scans at 7 µm were taken of a total thickness of 400 µm for the hydrogels, while for the TCP controls a total depth of 40 µm thickness was imaged at 3 µm. The cells were imaged and projected onto a single plane by the instrument's in-built software, after six days in culture.

Table 1. Gelation time for gellan/HPMC hydrogels after the addition of calcium

Material	Average gelling time at 37 °C (min)
Gellan/HPMC 0.5% 9:1 with 3 mmol L^{-1} calcium	5
Gellan/HPMC 0.5% 8:2 with 3 mmol L^{-1} calcium	10
Gellan 0.5% with 3 mmol L^{-1} calcium	4

RESULTS AND DISCUSSION

Gelation time

The rationale for keeping gellan separated from the crosslinker at a concentration at which it cannot gel by itself is to facilitate *in situ* gelation via the crosslinker.[25] The gelation time becomes important when administering hydrogel polymers along with the crosslinker *in vivo* to obtain a solid gel *in situ*. Various researchers have mentioned standards for *in situ* gelation time, and a gelation time of *ca* 30 min has been found to be Suitable.[26,27] Furthermore, gelation time can be enhanced with the addition of HPMC, which is thermo-responsive.[28] The gelation time is taken as the point at which the $\tan\delta$ value begins to plateau (data given in the supporting information). In this respect, the average gelation for gellan/HPMC at a ratio of 8:2 is found to be 10 min at 37 °C with 3 mmol L^{-1} calcium, while at 9:1 it is found to be 5 min (Table 1). This suggests a difference of 6 min with addition of HPMC. The gelation time of gellan alone with calcium is found to be 4 min, which is much faster than that of the blend after crosslinking. The difference in the gelation time can be attributed to the reduction in concentration of crosslinkable gellan. Although this is the case, the blends reach a similar final saturation of $\tan\delta$, suggesting that the gel strengths are not affected. This also suggests that the thermo-responsiveness of HPMC at 37 °C has a role to play in the final gel strength of the gels.

Viscoelasticity

The properties of the gel formed after crosslinking were assessed with the rheometer at a frequency from 0.01 to 10 Hz and plotted at a frequency of 10 Hz as shown in Fig. 1. It is observed that, in the absence of calcium, the loss modulus of G0.5 is higher than the storage modulus, resulting in a loss tangent value greater than 1. This suggests a blend with a more viscous nature as compared to the blends of gellan and HPMC. The average $\tan\delta$ for 0.5GH8:2 is 0.3 and that for 0.5GH9:1 without calcium is 0.5, suggesting that HPMC increases the storage modulus, resulting in an elastic liquid as compared to G0.5. A comparison of the gellan, HPMC and gellan/HPMC blends crosslinked with calcium to form hydrogels shows that there is a significant difference between the loss moduli of 0.5GH9:1 (with and without calcium) and 0.5GH8:2 (with and without calcium) at $p < 0.05$. There is a significant difference between storage moduli of G0.5 and G0.5 with calcium ($p < 0.005$). There is also a significant difference in the storage and loss moduli of 0.5GH8:2 with and without calcium, suggesting, in both cases, that the increase in concentration of HPMC and addition of calcium affect both storage and loss moduli. In contrast, for the 0.5GH9:1 blend, only the loss modulus is significantly affected suggesting that the basal HPMC concentration has to be high enough to have a significant difference when calcium is added to the blend. This indicates that the gelation is triggered by calcium and the gelation

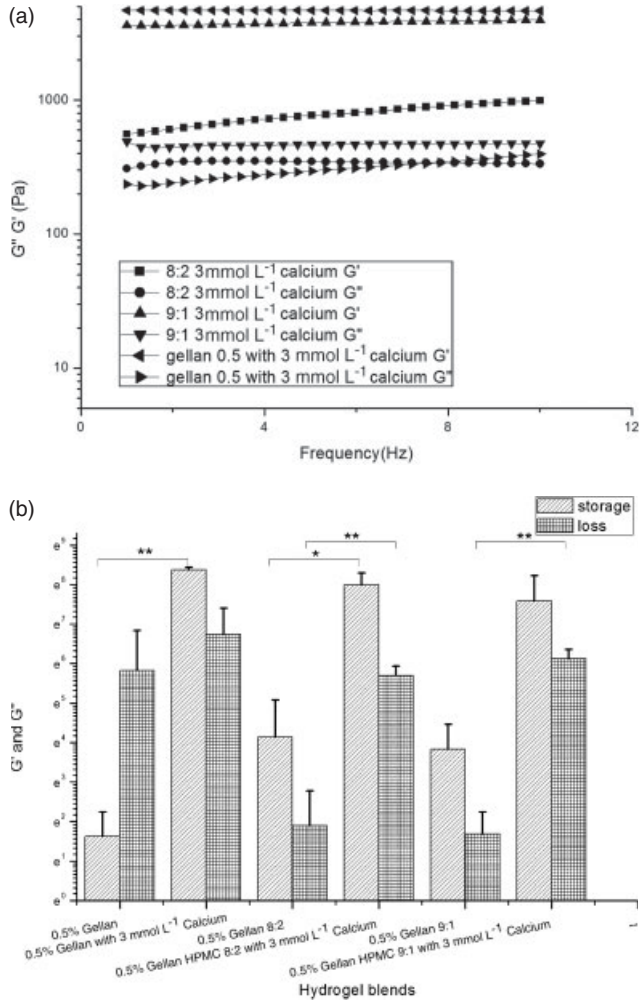


Figure 1. Storage G' and loss G'' moduli of gellan and gellan/HPMC of various ratios. (a) Frequency sweep of gellan and gelan/HPMC blends from 1 to 10 Hz. (b) Comparison of storage and loss moduli obtained at 10 Hz (*significant difference at $p < 0.05$, $n = 3$; **significant difference at $p < 0.005$, $n = 3$).

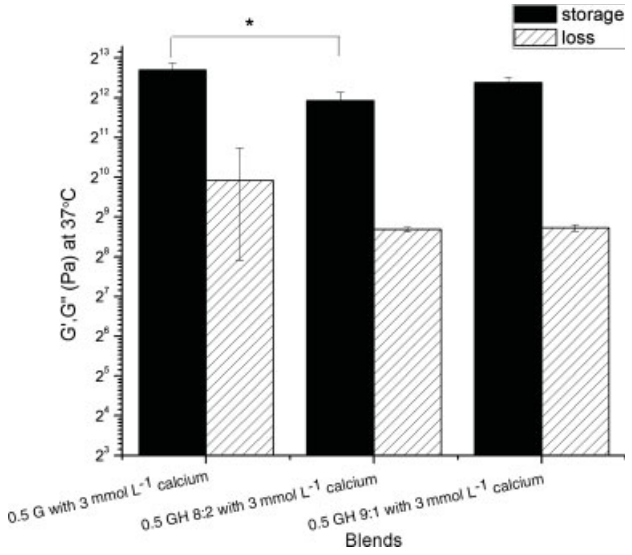


Figure 2. Storage G' and loss G'' moduli of gellan and gellan/HPMC of various ratios at 37 °C. Comparison of storage and loss moduli obtained at 10 Hz (*significant difference at $p < 0.05$, $n = 3$).

time improves after the addition of HPMC (Table 1). Although there is a significant difference between the storage modulus of G0.5 with calcium and that of 0.5GH8:2, tan δ is not significantly affected at $p < 0.05$. The tan δ value is also not affected for the 9:1 ratio, suggesting that the strength is similar to that of G0.5 crosslinked with calcium.

Temperature sweep measurements were performed from 25 to 80 °C at a constant frequency of 10 Hz, and data were plotted for 37 °C (Fig. 2). The results suggest a significant decrease in storage modulus of 0.5GH8:2 with calcium, as compared to G0.5 with calcium, while there is no significant difference for 0.5GH9:1. The reduction in storage modulus of 0.5GH8:2 shows no significant difference in the tan δ value, suggesting that the gel strength is comparable to that of G0.5, even with the relative concentration of HPMC increasing and that of gellan decreasing. Tunable viscoelastic properties are known to be an asset to hydrogel materials for tissue engineering applications, and gellan/HPMC gels can be used in this respect to be delivered as a support matrix and for cellular delivery.[29] Another property of relevance is the *in situ* injectability of these materials, which makes them ideal candidates for delivery of cells as well as of small molecules. It is evident for all the matrices prepared that this can be induced by calcium-mediated gelation.

Cloud point measurements

One assessment of whether the polymer chains precipitate out of solution when blended is done by measuring the cloud point of the blend. The change in transmittance on sweeping the temperature from 25 to 80 °C was measured for all blends, and for G0.5. It is observed that the transmittance decreases, attributed to the increase in opacity of the solutions, as the temperature increases – also known as the cloud point (Fig. 3). The results for pure gellan dispersed and solubilised in water show that there is no dramatic loss of transmittance due to an increase in temperature. As compared to gellan, there is a dramatic loss of transmittance as the cloud point is approached for the gellan/HPMC blends and HPMC alone dispersed in water. This can be attributed to the contraction of HPMC polymer strands in the solution after reaching their LCST. The difference in the cloud point between the gellan/HPMC blends and between the HPMC concentration can be explained by a concentration-dependent difference at the onset and progression in the plots in Fig. 3.[22] 0.5GH8:2 shows a sharper decline in LCST value as compared to the blends with just HPMC and that of 0.5GH9:1 and HPMC 1 mg mL^{-1} as well as 0.5 mg mL^{-1}. This suggests that HPMC in the presence of gellan has a higher LCST value than HPMC alone. This property of the blend can be of use when being applied *in vivo*. This phenomenon of concentration dependence of LCST can explain the increased gelling time of 12 min of the gellan/HPMC 8:2 blend over the 16 min of the gellan/HPMC 9:1 blend. Furthermore, this phenomenon can enhance the temperature stability of the final hydrogels after crosslinking with calcium.

Contact angle

The wettability of a material, and conversely its hydrophobicity, can be determined by the angle of contact made by water and other biological fluids, such as serum, with the material. The contact angle for the gel matrix when tested with water, PBS and serum is shown in Fig. 4. The contact angle is found to be much

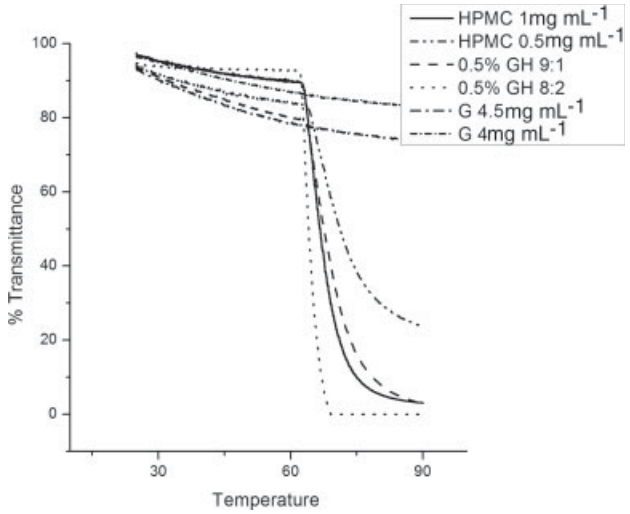


Figure 3. Cloud point plots for various ratios of gellan and HPMC (as indicated) without the addition of calcium, with a temperature sweep from 25 to 90 °C.

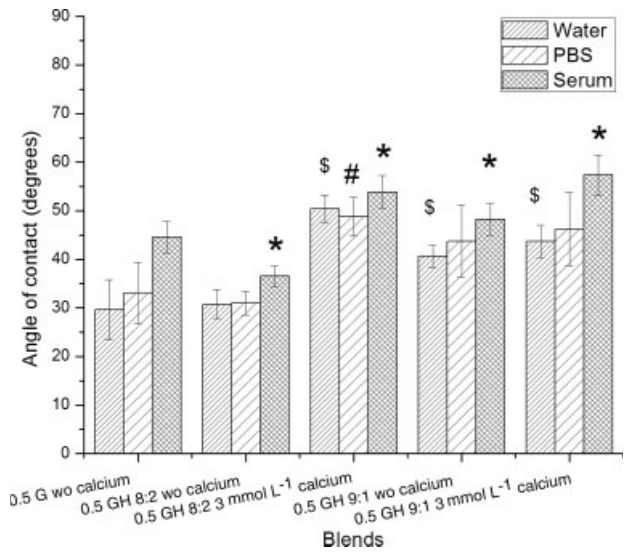
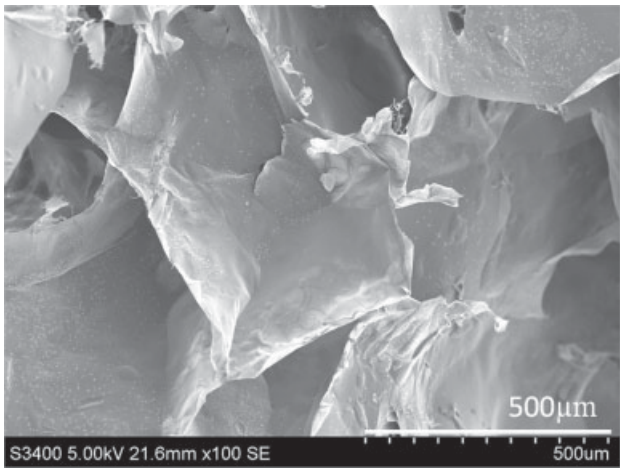


Figure 4. Contact angle measurements with water, PBS and serum of gellan/HPMC gels (with and without (wo) the addition of calcium). *, Significant difference of serum contact angle as compared to 0.5% gellan without calcium at $p < 0.05$; #, significant difference of PBS contact angle as compared to 0.5% gellan without calcium at $p < 0.05$; $, significant difference of water contact angle as compared to 0.5% gellan without calcium at $p < 0.05$.

less than 90°, suggesting that the hydrogels are wettable and hydrophilic in nature after gelation. The contact angle for water with 0.5GH8:2 and 0.5GH9:1 (with calcium) and 0.5GH9:1 (without calcium) is significantly higher than that with 0.5G without calcium. This can be attributed to the reduction in the number of charged carboxyl groups due to crosslinking with calcium. The contact angle with PBS is significantly higher only for 0.5GH8:2 with calcium. The contact angle with serum is significantly lower for 0.5GH8:2 without calcium, while significantly higher for all other samples tested, as compared to G0.5 without calcium.

Hydrophilicity is of great importance when cells are combined in the presence of hydrogels to be administered as scaffolds at tissue sites. The hydrophilicity allows cellular contact and adhesion

(a)



(b)

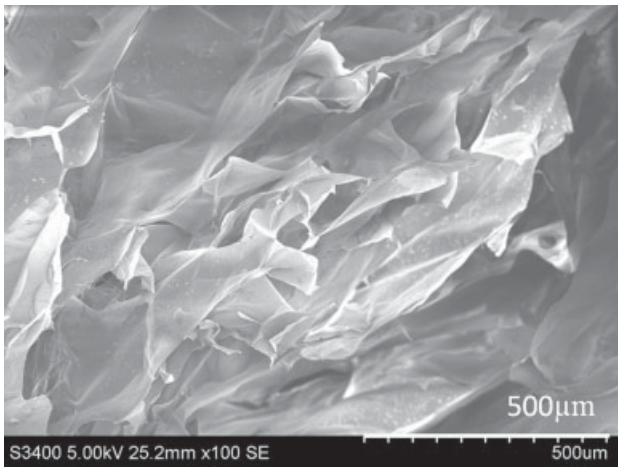


Figure 5. SEM micrographs of gellan/HPMC gels: (a) 0.5GH8:2 with 3 mmol L^{-1} calcium; (b) 0.5GH9:1 with 3 mmol L^{-1} calcium.

for support. This also facilitates the passage of metabolites across surfaces and into the cells, resulting in the growth and proliferation of cells. Although the contact angle increases for the hydrogel scaffolds as compared to those that are uncrosslinked, it is not greater than 90°. Also, the hydrogels form stiffer matrices, as determined from rheological analyses, after crosslinking, and are suited for entrapment of cells *in vivo* and *in vitro*, while uncrosslinked gels flow.

Scanning electron microscopy

The use of the scanning electron microscope to map the cross-section of the lyophilised gel shows us to investigate the internal porosity of the hydrogel matrix, as shown in Fig. 5. The porosity of the gel is substantial, considering that the gel is lyophilised. This allows a three-dimensionally relevant space for cells to grow and proliferate.

Cell cytocompatibility

Cytocompatibility was investigated using two cell lines: fibroblast L929 cell line and osteosarcoma MG-63 cell line. This was to demonstrate that normal cells as well as pluripotent stem cells are unaffected by the gel products that leach out into the surrounding

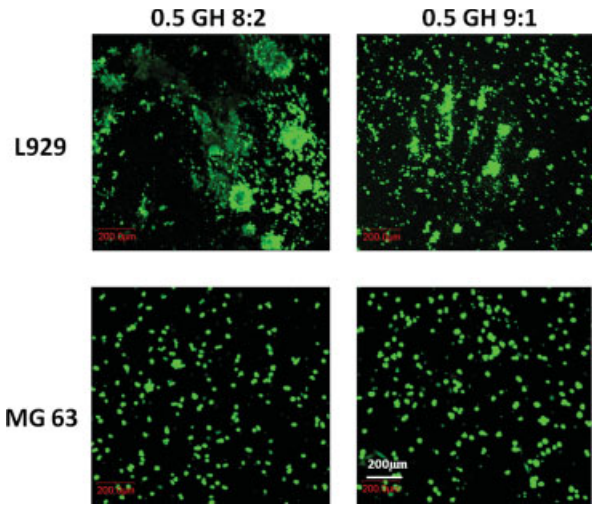


Figure 6. Morphology of fibroblast L929 cell line and osteosarcoma MG-63 cell line within gellan/HPMC blend scaffolds. It is observed that the fibroblast cells tend to aggregate while some tend to spread within the hydrogels and form aggregates. In contrast, for the osteosarcoma cell line, the cells retain a circular morphology.

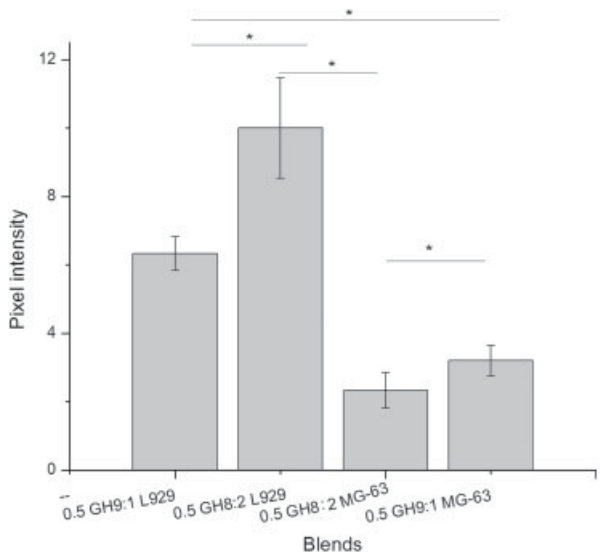


Figure 7. Fluorescence intensity of dye calcein AM for different gellan/HPMC blends (*significant difference at $p < 0.01$ with $n > 3$ images for assessment 0.5 GH 9:1 L929 and 0.5 GH 8:2 L929 are L929 fibroblast cells encapsulated in 0.5% Gellan HPMC at ratios of 9:1 and 8:2, while 0.5 GH 9:1 MG63 and 0.5 GH 8:2 MG63 indicates Osteosarcoma cell line MG63 encapsulated within 0.5% Gellan HPMC at ratios of 9:1 and 8:2. All gel blends were crosslinked with calcium at a final concentration of 3mM.).

medium. The viability is found to be in the range 90–100% when compared to the control. This property of the gels can further be used in cellular differentiation in combination with pluripotent and multipotent stem cells.

Cellular entrapment studies

Cellular entrapment was investigated with the fibroblast L929 cell line and human osteosarcoma MG63 cell line. Cells were incorporated into gellan/HPMC gels prepared at different ratios (8:2 and 9:1) at a final concentration of 0.5% (w/v). Cell survival was then analysed using a live–dead assay in which living cells fluoresce green and dead cells fluoresce red, as observed under a fluorescence microscope (Fig. 6). This shows that almost all the cells are stained green in the hydrogel, similar to those plated onto tissue culture plastic. The intensity plot of the images shows there is a significant difference between the 0.5GH8:2 and the 0.5GH9:1 blend for both cell lines at $p < 0.01$ (Fig. 7). There is also a significant difference between the MG-63 and L929 cell lines for the 8:2 blend as well as the 9:1 blend. This indicates that the 8:2 blend with a higher concentration of HPMC as compared to the 9:1 blend shows a higher growth of cells. The good biocompatibility of gellan/HPMC can be attributed to the use of calcium for crosslinking and relying on the thermo-responsiveness of HPMC. The residual calcium will be removed as a result of solubilising within the gel. The staining of the cells with calcein AM and counterstaining with propidium iodide suggest that cell encapsulation within the gel matrix has no detrimental effect on the survival of the cells. The cells tend to grow as aggregates occupying the interstitial spaces within the hydrogel, while, on other occasions, the cells tend to align themselves along the gel matrix.

CONCLUSIONS

Hydrogel blends with improved gelation time were prepared with the incorporation of HPMC. Based on SEM data it was clear that the porosity was high for the gel blends formed. Rheology proved without doubt that the addition of HPMC improved properties like gelation time and elastic properties of the blend before the addition of crosslinker, and HPMC was non-toxic. The gellan/HPMC blends additionally improved growth and proliferation of cells within the gel matrix. The calcium-crosslinked gels were porous and non-toxic suggesting that suitable networks are present within the gels to entrap cells with greater viability. Materials that can be administered under minimally invasive conditions *in vivo* are those that form the basis of delivery systems. As suggested, these require a mechanism by which they can be administered in the sol form and must gel at a required site in order to enhance their capabilities as localised, self-sustaining entities. These entities must further support the infiltration and growth of cells by mimicking the environment at the site. The blend of ion-responsive gellan and thermo-responsive HPMC[29] can be explored for tissue engineering applications. The tunable rheological properties of the gellan/HPMC blend can further be used for the differentiation of stem cells *in vitro*.

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SUPPORTING INFORMATION

Supporting information may be found in the online version of this article.

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