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A biodegradable *in situ* injectable hydrogel based on chitosan and oxidized hyaluronic acid for tissue engineering applications

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ABSTRACT

An "*in situ*" biodegradable gel consisting of chitosan, glycerol phosphate (GP) and oxidized hyaluronic acid (HDA) were synthesised and characterized .This is a two component hydrogel system where chitosan neutralized with GP resulted in instantaneous gelling when combined with HDA. The gels are cytocompatible and could be freeze dried to form porous scaffolds. The percentage porosity of the freeze-dried chitosan hyaluronic acid dialdehyde gels (CHDA) increased with increasing oxidation. Fibroblast cells seeded onto CHDA porous scaffolds adhered, proliferated and produced ECM components on the scaffold. Chondrocytes encapsulated in CHDA gels retained their viability and specific phenotypic characteristics. The gel material is hence proposed as a scaffold and encapsulating material for tissue engineering applications.

1. Introduction

Hydrogels derived from natural polymers are used for tissue engineering applications as they resemble the native extra cellular matrix of the tissue .(Tan & Marra, 2010). They also have an added advantage that they can support encapsulation of cells and growth factors, permit controlled release of chemicals/growth factors, support cell growth during tissue regeneration (Chenite et al., 2000; Kim et al., 2006) and also can be used for the repair of irregular defects with minimal invasive surgical procedures. Polysaccharide-based hydrogels have attracted much attention as matrices for application in tissue engineering due to their excellent biocompatibility and biodegradability(Kuang, Yuk & Huh, 2011).

Chitosan and hyaluronic acid are two polysaccharides that can form hydrogels and are widely exploited with various modifications for its use as scaffold for tissue engineering (Huaping Tan, Payne, & Marra, 2009). Chitosan has great potential as scaffold material in biomedical field (Muzzarelli, 2009) due to its minimal foreign body reaction, antibacterial nature, biocompatibility, biodegradability, adhesion to cells and the ability to be molded in various geometries (Di Martino & Risbud, 2005; Kim, Cho, & Chung, 2003). The amino group in chitosan has a pKa value of ~6.5, thus, chitosan is positively charged and soluble in acidic to neutral solution with charge density dependent on pH and the percentage deacetylation value. The ability of chitosan in promoting cellular proliferation and differentiation could be enhanced by incorporating growth factors indented for specific applications (Muzzarelli, 2011). Hyaluronate is a glycosaminoglycan component found in extracellular matrix and synovial fluid of joints and possess unparalleled properties for tissue engineering purpose compared to synthetic polymers. It provides soft connective tissues the counteracting force by absorbing water to resist compression and hence is the major component in the ECM of load-bearing joints. Hyaluronic acid (HA) acts as an environmental cue to regulate cell behavior during embryonic development, healing processes, inflammation.

Polysaccharide polymers have the disadvantage of low stability in water and faster degradation. This can be avoided by introducing cross linking in the system. However, most of the cross-linking agents in use are toxic to cells, and hence can create problems on implantation (Ferretti, Kobayashi, Defail, & Chu, 2006; Sung, Huang, Tsai, & Chiu, 1998). Hydrogel systems based on ionic interactions (Chenite et al., 2000; Dang et al., 2011) alone may pose the problem of faster degradation (leaching) and instability. This paper formulates a thermally independent, biodegradable, non-toxic neutral injectable hydrogel based on unmodified chitosan, and HDA which can be formed *in situ*, with no toxic cross linking chemicals involved.

2. Materials and methods

2.1. Materials

Chitosan, ≥75%deacetylated, glycerol phosphate, hyaluronic acid sodium, sodium periodate, ascorbic acid and proline were purchased from Sigma–Aldrich, USA. Dialysis tubing (Spectra/Por®,

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Table 1
Concentrations of reactants and their resultant gelling times.

Sample	Volume of 2% chitosan (ml)	Volume of 5% HDA (ml)	Gelling time (s)	Percent-chitosan:HDA
CHDA10	1	0.3	8 ± 2	57.1:42.9
CHDA25	1	0.2	8 ± 2	66.7:33.3
CHDA50	1	0.15	8 ± 2	72.7:27.3
CHDA75	1	0.1	8 ± 1.5	80:20

M.W.C.O 3500) was from Spectrum Laboratories Inc., CA, USA. Phosphate buffer saline (PBS), Dulbecco's Minimum Essential Medium (DMEM), penicillin, streptomycin, amphotericin, sodium pyruvate, glutamine, non essential amino acid (NEAA) and 0.25% trypsin–EDTA was obtained from GIBCO, Invitrogen Corporation. All the other reagents were of analytical or equivalent grade. Double distilled water was employed throughout.

2.2. Methods

2.2.1. Preparation of hyaluronic dialdehyde (HDA)

Into 1 g HA, dissolved in 100 mL distilled water, required amount of periodate for 10, 25 and 75% oxidized hyaluronic acid (hyaluronic dialdehyde or HDA) dissolved in 10 ml of distilled water was added and the content was stirred at 25 °C in the dark for 6 h. The degree of oxidation was found by determining the concentration of periodate left unconsumed by iodometry after 6 h (Lee, Bouhadir, & Mooney, 2007). Briefly, a 5 mL aliquot of the reaction mixture was neutralized with 10 mL of 10% sodium bicarbonate solution. Iodine was liberated by the addition of 20% potassium iodide solution (2 ml). This was kept under dark for 15 min and liberated iodine was then titrated with standardized sodium thiosulphate solution using starch as the indicator. After reaction, solutions were dialyzed against distilled water (2.5 l) for 3–4 days with several changes of water till the dialyzate was periodate-free. The absence of periodate was checked by adding a 0.25 mL aliquot of the dialyzate to 0.25 ml of a 1% solution of silver nitrate and ensuring the absence of any precipitate. HDA10, HDA25, HDA75 were prepared using this procedure. Oxidized hyaluronic acid thus formed was freeze-dried at −80 °C. Typical yield of the oxidized products ranged from 60 to 70%.

2.2.2. Preparation of neutral chitosan (CGP)

The procedure for preparing neutral chitosan is reported elsewhere (Chenite et al., 2000). Chitosan (≥75% deacetylated) solution (2%) was made by dissolving 200 mg in 9 ml 0.1 M hydrochloric acid. The chitosan solution was neutralized by adding 560 mg glycerol phosphate disodium salt dissolved in 1 ml distilled water by careful addition. The pH of the solution after addition is around 7.2.

2.2.3. Synthesis of chitosan-oxidized hyaluronic acid gel (CHDA)

The prepared 2% neutral chitosan solution is used for synthesis of gel. Hyaluronic dialdehyde (HDA) (10, 25, or 75%) oxidation is mixed with chitosan (2% solution in 0.1 M HCl) neutralized with GP (CGP) to form Schiff's base links between free amino groups of the chitosan and the aldehyde groups of the HDA. Different percentage solutions of both chitosan and HDA can be used to make the gel.

2.2.4. Gelling time determination of chitosan–hyaluronic dialdehyde gel

1 ml of 2% chitosan–GP was placed onto an 8 mm × 35 mm glass vial, under continuous stirring at 100 rpm. 200 μL of 5% solution of HDA 25 in phosphate buffered saline was then added to the vial. The mixture was stirred until it formed a solid globule; this was considered the gelation time. The results are reported as average of 2 experiments done in triplicates. The amount of hyaluronic acid dialdehyde required for gelation decreased with increasing percent of oxidation. 100 μl for 75% oxidized HDA, 150 μl for 50% oxidized HDA, and 200 μl for 25% oxidized HDA

This data was also verified by determining the viscosity change during gelation reaction between 5% solution of HDA and 2% chitosan–GP using a programmable viscometer (Brookfield, Model DV-11 +) at 37 °C with spindle SLV-64 and small sample adaptor (5 ml) at 100 rev/min. Gelling time was noted as the time at which the viscosity of mixture begins to increase sharply and reaches the maximum. 200 μl of 5% solution of HDA25 and 1 ml of 2% C-GP solution were mixed together by stirring and then the mixture was kept at 37 °C. The gel formation time was noted. The amount of HDA required for gelation decreased with increasing percent of oxidation, 100 μl for 75% oxidized HDA, 150 μl for 50% oxidized HDA, 200 μl for 25% oxidized HDA and 300 μl for 5% oxidized HDA. The C–GP solution without HDA did not gel at 37 °C, however, gel formation occurred at >75 °C. Gelling time for each oxidized sample of HDA is given in Table 1. The gelling time was found to be less than 2 min for the all HDA samples. The value given are average of two experiments each done in triplicates.

2.2.5. FTIR and thermogravimetric analysis

FTIR spectra of the freeze-dried scaffold samples were recorded using Nicolet 5700 FTIR Spectrophotometer with DTGS detector. The spectrum was used to confirm the formation of the Schiff's linkage between the amino and aldehyde groups. Thermogravimetric analysis (TGA) of CGP and CHDA10, 25 and 75 were done using TA instruments SDT 2960 in N_2 atmosphere. TGA was done to understand the extent of stability imparted to the hydrogel by cross linking of the amino and aldehyde groups. TGA data were obtained from the freeze-dried samples of the hydrogels

2.2.6. Biocompatibility studies: in vitro cytotoxicity test by direct contact method

In vitro cytotoxicity testing was done using the direct contact method with the test sample based on ISO 10993-5 standards. Briefly L929 cells were cultured in DMEM supplied with 10% FBS and incubated at 37 °C in a 5% CO_2 atmosphere. When the cells attained 70% confluency, hydrogels were kept in contact with the cells and incubated for 24 h at 37 °C in 5% CO_2 atmosphere. After incubation, cells surrounding the hydrogel were examined microscopically for cellular response.

2.2.7. Biodegradation studies

Degradation studies of the hydrogels of CGP and CHDA were studied in their wet form by incubating in phosphate buffered saline (PBS) at 37 °C. Each sample was kept in 10 ml PBS and incubated at 37 °C. After 24 h, the PBS was removed and the sample weight was taken. The wet samples were gently swabbed before weighing to remove any excess water. Fresh PBS was added after each weighing and again incubated at 37 °C. The procedure was continued till the end of the experiment. The experiments were done in triplicate and the values given are average of two separate experiments. Percent weight loss is the weight lost divided by the initial weight of the sample multiplied by 100

$$\text{Weight loss ratio} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100.$$

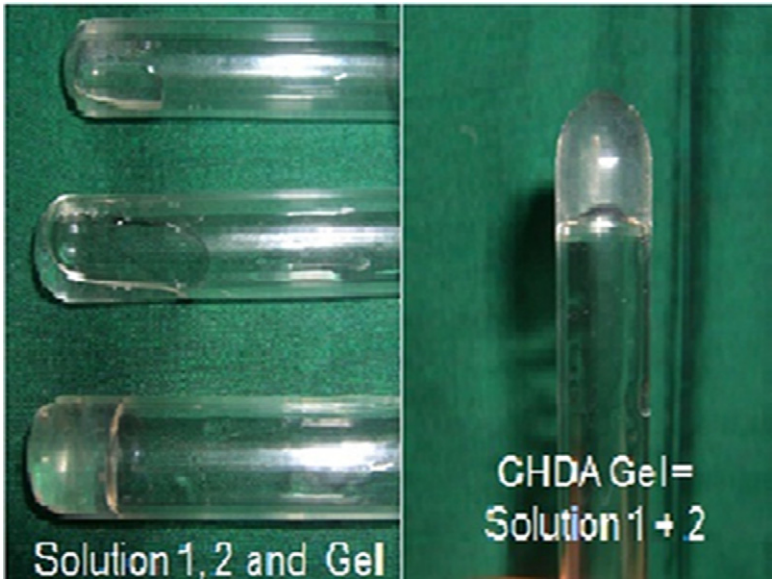

Fig. 1. Picture shows the gel formation when chitosan and HDA solutions are mixed in a test tube. Solutions 1 and 2 are chitosan and HDA solution, respectively.

2.2.8. Morphology and swelling studies

Morphology of the scaffold samples in the dried and wet form was studied by scanning electron microscopy (SEM), (Hitachi, Model S-2400, Japan) and Micro-computed tomography (μ-CT) (Scanco medical AG model 40). Morphology of the scaffold/gel after cell culture was studied in their wet form using ESEM (Quanta 200, FEI). Swelling studies were performed by immersing initially weighed freeze-dried hydrogels in PBS (pH 7.4) in preweighed containers for known intervals of time. The medium was carefully withdrawn at intervals and wet weight measured until equilibrium in weight was attained. Percentage swelling ratio was calculated using the formula

$$\text{Percentage swelling ratio} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Intial weight}} \times 100.$$

2.2.9. Cell seeding on freeze dried scaffold

In vitro cell culture studies were done using the freeze-dried samples of the gel. Mouse Fibroblast L929 cells were used for the culture studies. Briefly, about 1×10^5 cells/ml of cells were seeded onto the CHDA and CGP scaffold (5 mm diameter and 1.5 mm thickness) and cultured in DMEM supplemented with antibiotics at 37 °C, 5% CO_2 for specified period of time. Medium change was given every 3rd day.

2.2.10. Cell encapsulation studies using chondrocytes

Chondrocytes were isolated from the rabbit articular cartilage following enzymatic digestion with 0.2% Collagenase Type 2. The cells are cultured in chondrogenic media (DMEM supplemented with 10% FBS, penicillin (100 U/mL), streptomycin (100 mg/mL), sodium pyruvate (1 mM/mL),glutamine (1.4 mM/mL), NEAA (0.1 mM/mL), ascorbic acid (50 mg/L), proline (40 mg/L) and incubated at 37 °C, 5% CO_2 until confluent. The medium was changed every 2–3 days. Confluent monolayer was trypsinized using 0.25% trypsin–EDTA solution. Approximately 1×10^6 cells were suspended in 0.5 ml of 75% oxidized hyaluronic acid dialdehyde solution and was mixed with 1 ml of chitosan–GP solution to form encapsulated hydrogel. The gel with the cells were then transferred to chondrogenic media and cultured for a period of 28 days.

2.2.11. Cell viability assay

The viability and membrane integrity of L929 cells in freeze dried scaffolds as well as encapsulated chondrocytes were determined using LIVE/DEAD® viability/cytotoxicity kit (Molecular probes, Eugene). Cell seeded constructs were incubated in DMEM containing 4 mM Calcein-AM and 2 mM Ethidium homodimer-1 for 30 min. Live cells are permeable to the non fluorescent Calcien AM which is converted into fluorescent dye Calcein by the cytoplasmic esterase present in living cells. On the other hand, Ethidium homodimer-1 enters the cell if there is damage to the membrane integrity and is fluorescent when bound to nucleic acid. The imaging was done using Zeiss 510 Meta Confocal Microscope. Calcein Fluorecsence was excited at 495 nm using Argon laser and EtBr was excited at 528 nm using HeNe Laser.

3. Results and discussion

A biodegradable, non-toxic, fast gelling hydrogel system based on chitosan, GP and HDA was formulated and characterized using FTIR, TGA, DSC, SEM, Micro CT, biodegradation and biocompatibility studies. Fig. 1a shows the picture of gel formation when the two solutions are mixed together in a test tube and a schematic representation of the crosslinked network of gel with GP are shown in Fig. 1b. GP is trapped inside the cross linked network and is bound by ionic interactions. Cross linking between chitosan and HDA was confirmed using IR spectra. The IR spectra of chitosan, HDA and the CHDA gels are given in Fig. 1c. The peaks of CHDA spectra at 1639 cm^{-1} and 1642 cm^{-1} corresponds to the C=N group, which confirms the formation of Schiff's linkage. The $-NH_2$ peak found at 1560 cm^{-1} for chitosan (bending vibrations) are absent in CHDA spectra. The aldehyde peak at 1600–1700 cm^{-1} found in HDA has diminished in CHDA. The 2800 cm^{-1} peak corresponding to –CH stretching of aldehyde is also absent in CHDA. These results from IR spectra confirm the formation of crosslinking between chitosan and HDA.

There have been previous reports (Chenite et al., 2000) of chitosan–GP (CGP) gel at 37 °C, however, the chitosan with GP (without HDA) used for our experiments did not gel at 37 °C, though the deacetylation percent was ≥75–80%. We tried two different chitosan with ≥75–80% deacetylation, gel formation occurred only at temperatures >55 °C and >75 °C, respectively. The gelling time and percent composition of the hydrogels are shown in Table 1.

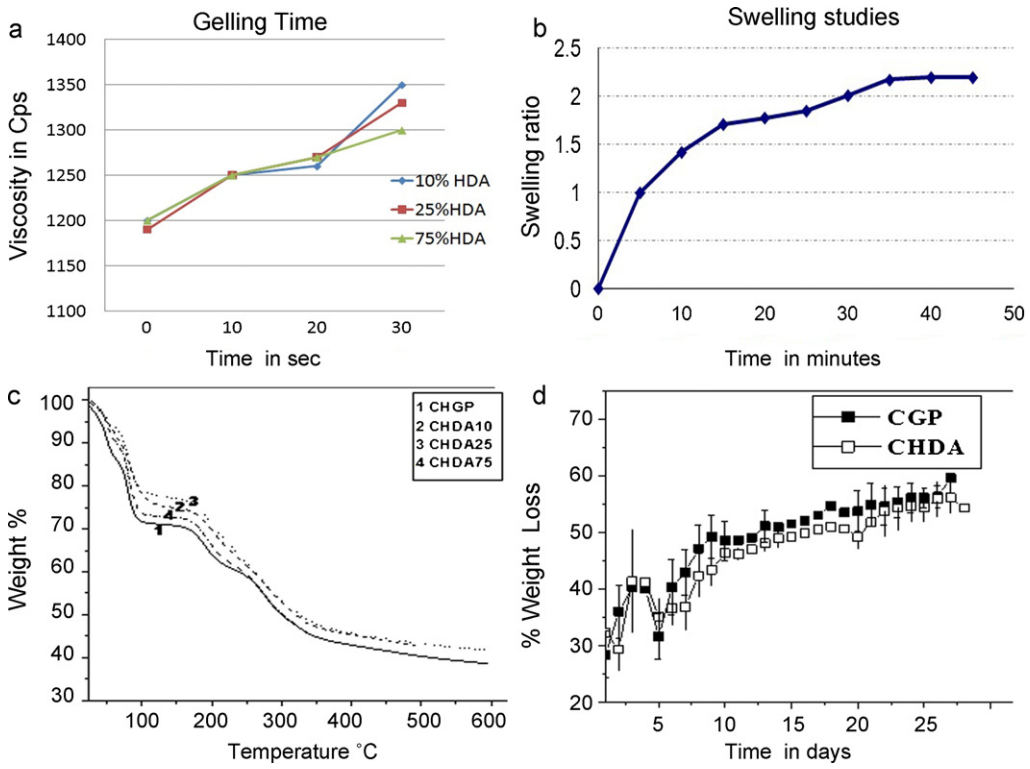

Fig. 2. (a) Gelling time measurement by Brookefield viscometer. (b) Swelling ratio of freeze dried CHDA75 gel is given. (c) TGA curves of the freeze dried form of gels are given (1) CGP (2) CHDA10 (3)CHDA25 (4)CHDA75. (d) Biodegradation curve for CGP and CHDA 75 gel. Plot of percent weight loss versus time (in days) of the gels.

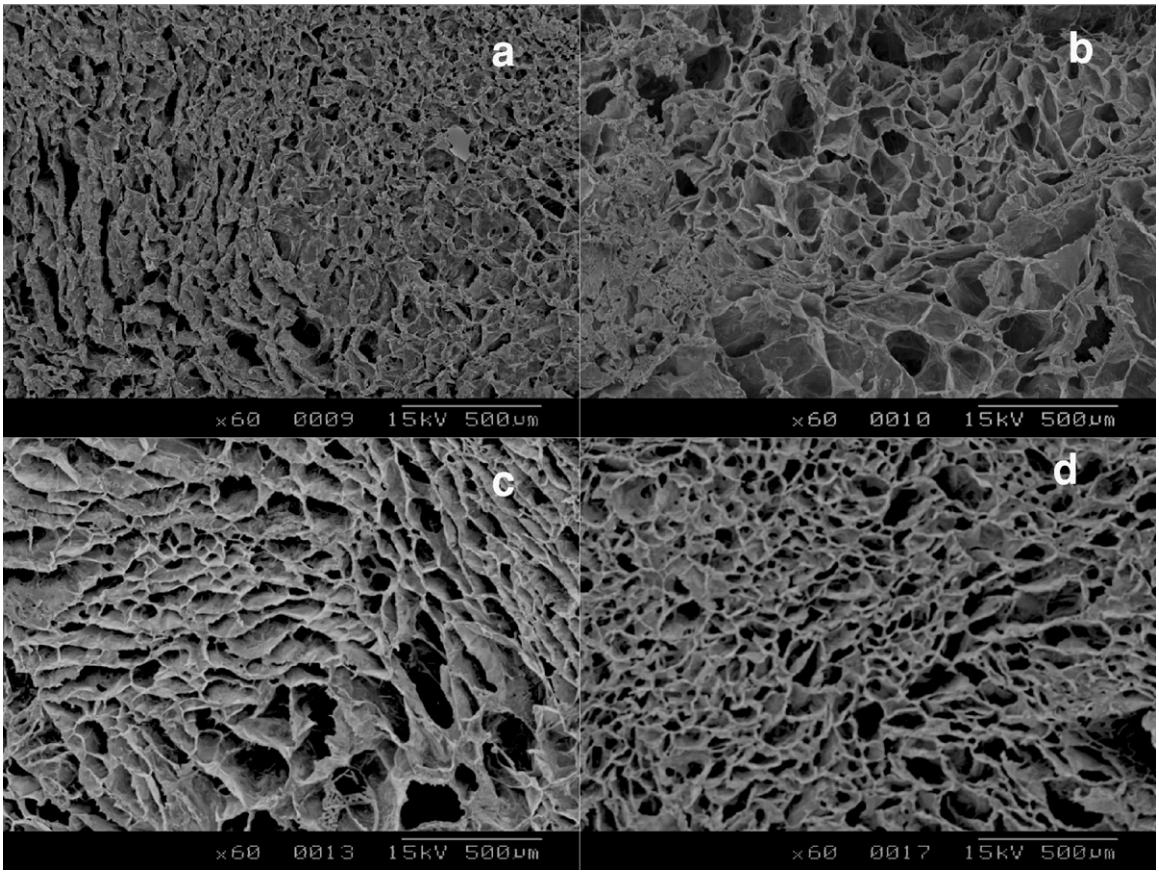

Fig. 3. SEM images of freeze-dried gels; clockwise from top left (a) CGP, (b) CHDA10, (c) CHDA25, and (d) CHDA75.

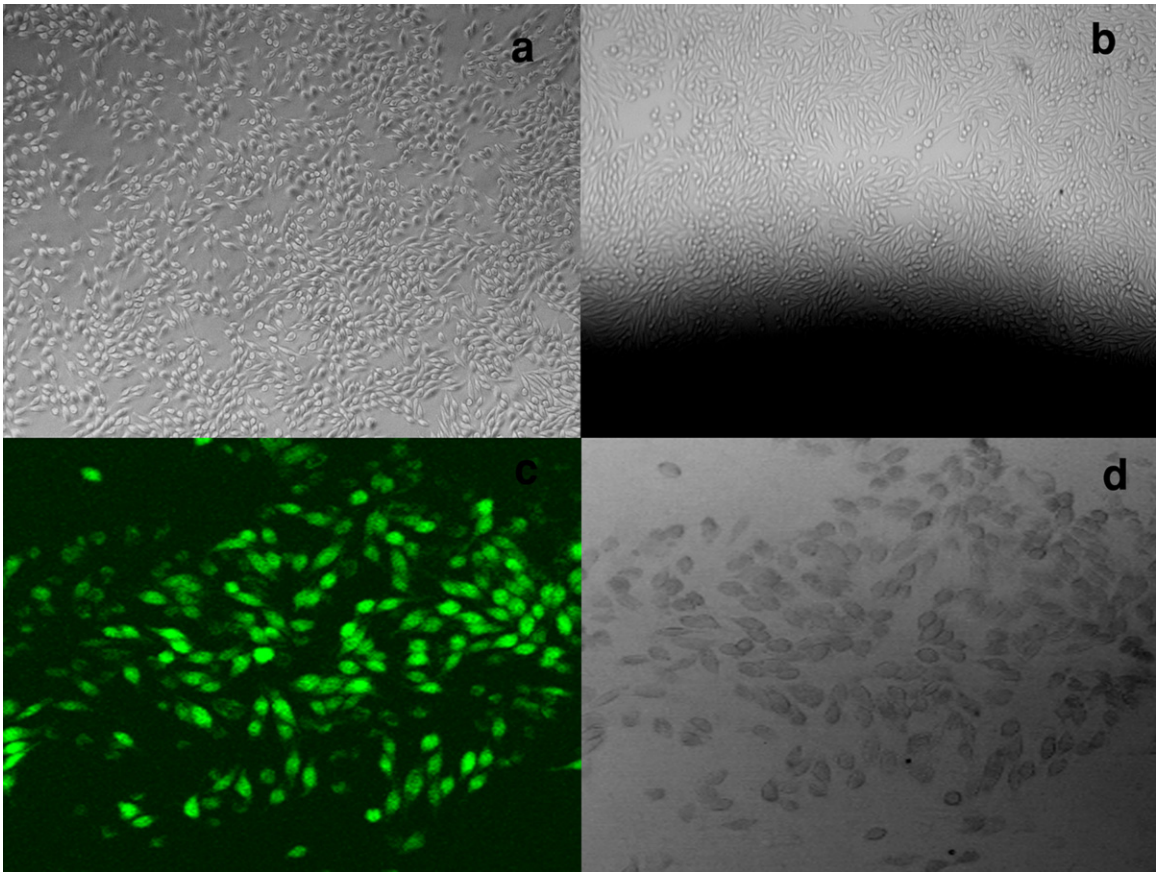

Fig. 4. Microscopic images of direct contact assay at 48 h (a) L929 cell monolayer (b) L929cells in contact with CHDA 75 hydrogel. Live dead assay of L929 cells in the freeze dried CHDA gel on 3rd day of culture. (c) Confocal image, live cells appearing as green (d) DIC image. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

The values given were standardized by trial and error method. All HDAs with different percent oxidation formed gel within 2 min. The results were also comparable to the viscosity changes in Gelling time measurement using Brookfield Viscometer (Fig. 2a). The result indicates that the percent oxidation did not have much effect on the gelling time. We however, observed that 2% chitosan solution and 5% HDA solution, when mixed, gel instantaneously and the change in the concentration of the solutions may vary the strength of the gel and the gelling time.

Swelling studies were done with freeze dried hydrogel samples prepared from chitosan–GP and HDA 75. The scaffolds attained equilibrium swelling rapidly (Fig. 2b) and it was evident that the scaffold can absorb and hold large amount of water. The high swelling behavior is a requirement for the 3D scaffolds to serve as a matrix for tissue regeneration. This is due to the wicking action of solution through the pores of the scaffold as well as hydration of free (–OH) groups in the system. This free diffusion does not seem to extensively change the dimensions of the scaffold. The high medium uptake ability of the freeze dried scaffold even in the first 2 min suggests the potential ability of the scaffold to absorb and supply nutrients to all the cells that are seeded within the porous structure of the scaffold. This may also facilitate diffusion of cells into the interior of the scaffold while seeding. This inherent property of the scaffold material could be taken advantage of, by considering them for application like engineering of hydrated tissues for, e.g. Cartilage.

The thermograms of CGP and CHDA hydrogel in their freeze-dried form are given in Fig. 2c. It is observed that, in general CHDA samples have higher thermal stability than the CGP. The TGA curves showed that the degradation for CGP gel started at lower temperatures compared to that of CHDA gels. The thermograms show that the thermal stability of the gels increased with the addition of HDA, and are as follows: CHDA25 > CHDA10 > CHDA75 > CGP. The result suggest that the presence of cross linking in the CHDA samples. It can be assumed that the cross linking between amino groups of chitosan and aldehyde group of HDA, in general, increases the thermal stability of the CHDA. However it was observed that CHDA75 has the least thermal stability among the CHDA gels and CHDA25 had the highest thermal stability out of the CHDA gels. Therefore, it can be concluded that there are other factors like the molecular weight of HDA which contributes to the thermal stability of HDA gels and the thermal stability could be a synergitic effect of all the factors.

The biodegradation test of CGP and representative CHDA75 hydrogel in their wet form was carried out in PBS at 37 °C in triplicates. The biodegradation was monitored by the weight loss of the material for 30 days. The biodegradation study results were given as weight loss versus time in days for both the samples and the plot of percent weight loss versus time (in days) for CGP and CHDA75 gels are given in (Fig. 2d). The biodegradation result shows that CHDA75 hydrogel has a slower degradation profile compared to that of CGP. The biodegradation profile shows a steep increase and then a decrease in biodegradation in the first 5 days. This is assumed to be due to the leaching of components from the network. After 5 days, the biodegradation profile assumed a gradual rise till the 10th day and then attained a plateau which remained approximately the same for the remaining days of observation. In 30 days a ∼50% biodegradation was observed for CHDA whereas ∼55% biodegradation was observed for CGP. The weight loss in the early phase was attributed to the leaching process and in the latter

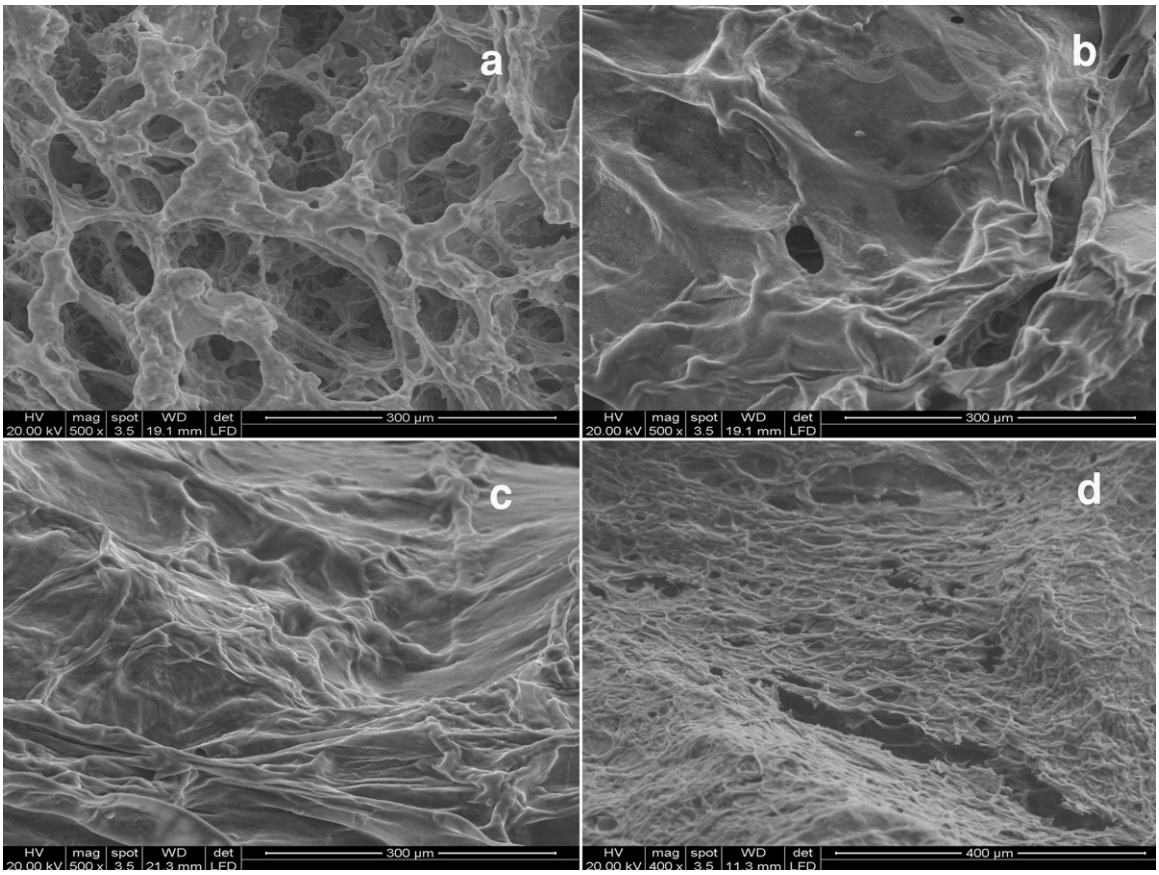

Fig. 5. ESEM images of freeze dried gels after 4 weeks of L929 cell culture. Clock wise from top left (a) CGP, (b) CHDA10, (c) CHDA25 and (d) CHDA75.

phase to degradation process. The result suggests that the hydrogel can support cell and tissue growth for more than 30 days frame.

Morphology of the hydrogels were studied and discussed using the μ-CT data and SEM results of the freeze-dried hydrogel. From μ-CT data volume of pores, total percentage porosity and pore size and its distribution of a sample were obtained. Table 2 gives the % porosity, pore volume, anisotropy and average pore diameter of all the gel samples in their freeze-dried form. Scaffolds with a high porosity and high pore sizes with interconnectivity are important for cell seeding and attachment, cell migration and angiogenesis. The CHDA gels of our study has pore size ranging from 80 to 140 μm in pore diameter and were likely beneficial in facilitating cell infiltration and tissue in growth. The CHDA gels showed higher pore volume compared to that of CGP. The average pore diameter of the CHDA gel is higher compared to that of CGP except for CHDA10. The result suggests a greater capability for the CHDA gels to encapsulate cells and to assist the proliferation and growth of cells. The pore volume and pore size data given in Table 2 clearly shows that CHDA gels are better for cell/tissue growth compared to that of CGP gel.

Micro CT data of the freeze dried samples of the gels showed that the CHDA gels had higher % porosity than that of CGP gel. The value shows that total % porosity increases with increase in cross linking density. Around 10% increase in % porosity was observed for CHDA75 compared to that of CGP. The increase in percentage porosity of the CHDA gels are also attributed to the cross linking of strands. It is well known that higher % porosity increases cell growth in a scaffold as high porosity supports diffusion of oxygen and nutrients towards the cells and drainage of waste products from the matrix. Therefore, our μ-CT data suggests that CHDA gels are better for cell growth than the CGP gels in terms of porosity.

The anisotropy of the CHDA gel samples are lower than CGP gel and nears the values of 'one' as the oxidation percent of HDA increases. This suggests that that CHDA gels have more uniformity in pore size towards all direction which in turn means better interconnectivity. Uniformity in all directions or interconnectivity is highly desirable for cell growth as it facilitates transport of nutrients, angiogenesis and thus tissue development.

Fig. 3 showed the SEM images of the gels in their freeze dried state. The images suggest that the freeze dried CHDA hydrogels have more oriented pores compared to that of CGP. From the images, it is evident that the cross-linking has altered the porous structure of the hydrogel. SEM pictures of the CGP hydrogel shows elongated pores separated by thick fibrous walls, while CHDA gels showcase a highly oriented pores separated by thin layers with patterned orientation. Combining the μ-CT data and SEM results we

Table 2
Porosity measurements of CGP and CHDA gels in their freeze-dried form.

Sample	Pore volume TV-BV	% Porosity (1-BV/TV) × 100	Average wall thickness (mm)	Average pore diameter (mm)	Anisotropy
CGP	8.86	60.88	0.0612	0.0867	2.28
CHDA10	14.11	62.08	0.0485	0.0796	2.69
CHDA25	9.48	69.72	0.0628	0.139	1.66
CHDA75	33.28	69.63	0.0491	0.0973	1.30

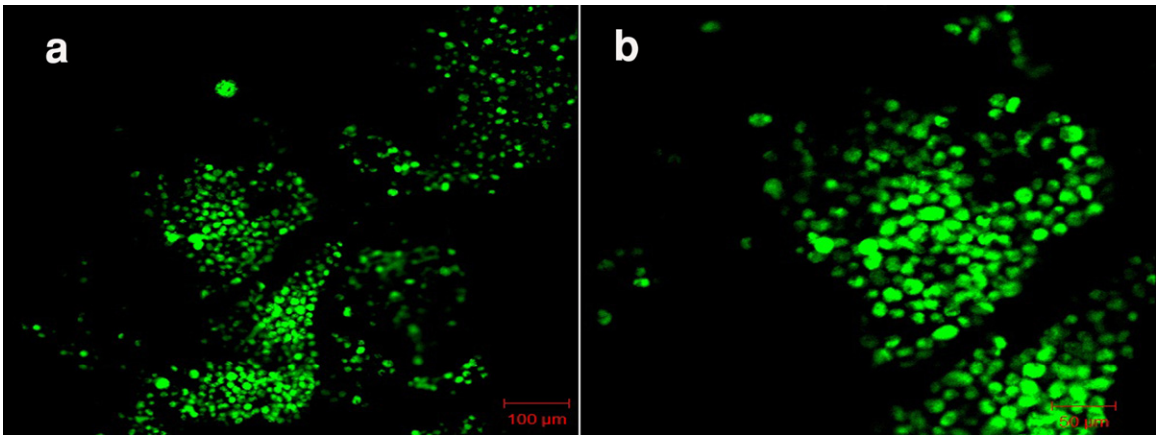

Fig. 6. Live dead assay of encapsulated chondrocyte on 28-day of culture in CHDA 75 gel Live chondrocytes appearing green (a) 10× magnification and (b) 20× magnification. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

conclude that the CHDA gels, possessed higher porosity, average pore diameter and pore volume than that of CGP gel and thus can act as a better injectable gel or scaffold for cell/tissue encapsulation and growth.

The preliminary cytotoxic evaluation of the hydrogels by direct contact test for 24 h showed that the material is non-toxic to a monolayer of L929 fibroblast cells. The cells showed their characteristic spindle shaped morphology (Fig. 4a and b). Having found that the material is non cytotoxic, it was used for further cell culture studies.

We examined the freeze dried gel for its suitability as a scaffold by culturing L929 cells in it; as described in the methods section. Live dead assay was performed on three day culture construct and results were shown in Fig. 4c and d. 100% viability was seen with cells showing its characteristic spindle shaped morphology. Macroscopically the physical appearance of the scaffold showed changes after 14 days of culture, wherein a white layer deposit started to form on or within the scaffold. The white substance was assumed as the ECM of fibroblasts and to confirm the fibrous nature, ESEM of the samples were done during the third week of culture. The ESEM images showed that the pores have been covered by a fibrous deposition/layer (Fig. 5). Therefore, the results of *in vitro* cell culture studies using fibroblast L929 cells showed that the freeze dried gel is able to promote ECM secretion/deposition just after 2 weeks of culture and also suggest that the hydrogel in its freeze dried form can be used as a porous scaffold for cell seeding applications.

Encapsulation studies were performed to prove its suitability as an injectable hydrogel system. Hydrogels were encapsulated with rabbit chondrocytes and were cultured for a period of 1 month. LIVE/DEAD assay of 1 month construct showed that >95% of the cells were viable. Chondrocytes are characterized by their round phenotype. Preserving this feature inside hydrogels is a prerequiste for efficient matrix production. As observed in Fig. 6, chondrocytes inside the gel still maintained their round morphology upto a period of 1 month.

4. Conclusion

We herein proposed a fast gelling, thermally independent hydrogel comprised of chitosan and HDA. This hydrogel formation, is independent of temperature and amount of GP added and have the advantage of higher stability than neutral chitosan gel due to covalent bonds formed by the cross linking of amino group of chitosan and aldehyde group of HDA and with a gelling time of less than 2 min. The gels are cytocompatible and could be freeze dried to form porous scaffolds. Moreover, cells could be encapsulated in the gel that retained more than 95% of the cells viable making it an ideal injectable system for tissue engineering. The degradation rate and encapsulation property of this hydrogel could be exploited in drug and gene delivery. We also suggest that the scaffolds of chitosan and hyaluronic acid with their inherent properties are good choice of materials for skin and cartilage tissue engineering applications.

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