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Encapsulation of adipose-derived stem cells and transforming growth factor-β1 in carrageenan-based hydrogels for cartilage tissue engineering

Pedro M Rocha, Vítor E Santo, Manuela E Gomes, Rui L Reis and João F Mano

Abstract

Tissue engineering (TE) is an emerging field for the regeneration of damaged tissues. The combination of hydrogels with stem cells and growth factors (GFs) has become a promising approach to promote cartilage regeneration. In this study, carrageenan-based hydrogels were used to encapsulate both cells and transforming growth factor-β1 (TGF-β1). The ATDC5 cell line was encapsulated to determine the cytotoxicity and the influence of polymer concentration on cell viability and proliferation. Human adipose-derived stem cells (hASCs) were encapsulated with TGF-β1 in the hydrogel networks to enhance the chondrogenic differentiation of hASCs. Specific cartilage extracellular matrix molecules expression by hASCs were observed after 14 days of cultures of the constructs under different conditions. The κ-carrageenan was found to be a suitable biomaterial for cell and GF encapsulation. The incorporation of TGF-β1 within the carrageenan-based hydrogel enhanced the cartilage differentiation of hASCs. These findings indicate that this new system for cartilage TE is very promising for injectable thermoresponsive formulation applications.

Keywords

Carrageenan, adipose derived stem cells, hydrogel, cartilage tissue engineering, Transforming Growth Factor, Controlled release

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3B's Research Group - Biomaterials, Biodegradables and Biomimetics, Department of Polymer Engineering, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, S. Cláudio do Barco, 4806-909 Caldas das Taipas, Guimarães, Portugal; ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal

Corresponding author:
João F. Mano, 3B's Research Group - Biomaterials, Biodegradables and Biomimetics, Department of Polymer Engineering, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, Zona Industrial da Gandra, S. Cláudio do Barco, 4806-909 Caldas das Taipas, Guimarães, Portugal
Email: jmano@dep.uminho.pt

Introduction

Tissue engineering (TE) is an emerging multidisciplinary field that may revolutionize the ways to improve the health and quality of life worldwide by restoring, maintaining, or enhancing tissue and organ functions.[1,2] Cartilage is an avascular, aneural, and alymphatic tissue composed of a unique type of cell, chondrocyte, which is embedded within a dense extracellular matrix (ECM).[3] The matrix is composed of collagens and proteoglycans, which provide the architectural structure and biochemical strength to the tissue.[4] Joint diseases, osteoarthritis, direct or indirect trauma from sports injuries, and premature or osteochondral cartilage degeneration are targets among cartilage TE repair.[5–7]

A typical cartilage TE approach combines cells, biomaterials, and signaling factors.[4,8–11] Cartilage regeneration using autologous chondrocytes has been indicated.[12,13] However, posterior *in vitro* cultures for cell expansion are required, which is typically associated with cell de-differentiation, leading to the downregulation of cartilage-specific genes.[14] Alternative cell sources, namely adult mesenchymal stem cells (MSCs), including adipose-derived stem cells (ASCs), have been reported.[15,16]

Adipose tissue is easy to access in individuals, and contains a large proportion of MSCs.[17] The easy access to ASCs by liposaspiration is one of the greatest advantages of this stem cell source. Furthermore, the ASCs can undergo differentiation into different lineages, including the chondrogenic cells.[18] Under specific conditions, which include culturing ASCs in an environment supplemented with transforming growth factor-β (TGF-β), dexamethasone, and ascorbate, stem cells undergo chondrogenic differentiation.[18–22]

TGF-β1 is known to be responsible for initial cell–cell interactions and for the stimulation of cells to grow and differentiate.[23–26] TGF-β is responsible for the control of chondrocyte proliferation and differentiation. Furthermore, it is able to induce chondrogenic differentiation of MSCs *in vitro*.[23–25,27] TGF-β1 is also important for the production of proteoglycans and other components of cartilage matrix.[27,28] Studies have been focused on the delivery of this growth factor (GF) in hydrogels and evaluated their influence in neocartilage tissue development.[28–30]

An important parameter in cartilage TE is the choice of the appropriate three-dimensional (3D) support. Biocompatibility, porosity, pore size, surface and mechanical properties, and biodegradability are essential parameters that should be specifically designed in order to achieve successful tissue regeneration.[31] Hydrogels are particularly suitable for cartilage TE as they resemble the 3D aqueous-rich environment of that tissue.[32,33] Hydrogels with appropriate mechanical properties, capable of entrapping cells and allowing the diffusion of nutrients through the mesh, can be used as a construct for cartilage TE. Moreover, stimuli-responsive (pH and temperature) hydrogels represent a promising approach as they can be injected by a minimal invasive procedure which is advantageous over other more complex treatments involving open body surgeries.[32,34]

Although the use of synthetic polymers to design hydrogels may seem promising, the choice of natural biomaterials assumes greater relevance in the objective of mimicking the nature due to their high biocompatibility.[35] Polysaccharides are the most versatile natural polymers due to the wide range of chemical, physical, and functional properties they convene in living organisms.[36–39]

Carrageenan, a sulfated polysaccharide that is extracted from red marine algae, is gaining interest for biomedical applications due to its facile gellation. It is comprised of repeating disaccharide units of 4-linked β-D-galactopyranose (G-unit) and 4-linked α-D-galactopyranose (D-unit) or 4-linked 3,6-anhydrogalactose (DA-unit), with a variable portion of sulfate groups.[39–41] According to the position and number of sulfate groups in the 1,3- and 1,4-linked galactose residues, carrageenans are divided into three families: κ, ι, and λ, corresponding to one, two, and three sulphate groups per disaccharide.[40] In aqueous solutions and in the presence of cations, both κ- and ι-carrageenans easily form, on cooling, thermoreversible gels.

The objective of this study was to design of a new carrageenan-based thermoreversible hydrogel for use in cartilage TE applications. Further, the effect of the TGF-β1 in the hydrogel network was studied with respect to the proliferation and chondrogenic differentiation of encapsulated human (hASCs).

Materials and methods

Hydrogel preparation

κ-Carrageenan hydrogels were formed by ionic crosslinking monovalent cations, such as K^+. κ-Carrageenan (Sigma–Aldrich) solutions with 2% and 2.5% (w/v) in distilled water. The dispersion was heated in a water bath at 60°C with stirring until a homogeneous solution formed. The solutions were sterilized in an autoclave (121°C for 30 min). The polymer solution was then poured into a Petri dish and allowed to gel. To croslinked the structure, 5% (w/v) potassium chloride (KCl) solution in phosphate-buffered saline (PBS) was added in a volume ratio of 4/1 (KCl solution/polymer solution), and allowed to react for 30 min. The hydrogel obtained was washed several times with PBS, and discs of ˜5 × 2 mm^2 were produced with a punch.

CryoSEM

The internal morphology of the hydrogels was evaluated by CryoSEM. Samples were cooled rapidly down to −210°C, followed by surface sublimation and coated with gold–palladium to avoid deposition of electron flux. Images were obtained in a microscope (SEM; JEOL JSM 6301F/Oxford INCA Energy 350/Gatan Alto 2500 from CEMUP laboratories (REEQ/1062/CTM/2005 e REDE/1512/RME/2005)).

Cell culturing and expansion

Two different types of cells, mouse embryonal carcinoma-derived clonal cell line ATDC5 (ECACC, UK) and human adipose-derived stem cells (hASCs), were used. Over the course of the cell culture period, samples were monitored with an inverted microscope (Axiovert 40 PG-HITEC, Zeiss).

Culture of ATDC5 cells

ATDC5 cells were defrosted at passage 8 and cultured for 2 weeks to obtain a sufficient cell number for the experiment. Cells were cultured in Dulbecco's modified eagle medium nutrient mixture F-12 (DMEM F-12) supplemented with 10% fetal bovine serum (FBS, Invitrogen Corporation, USA), sodium bicarbonate ($NaHCO_3$), and L-glutamine at 37°C, 5% CO_2 incubator. Medium was changed every 2 days.

Isolation and culture of hASCs

Human subcutaneous adipose tissue samples were obtained from lipoaspiration procedures performed on females, 35–50 years of age, under a protocol previously established with the Department of Plastic Surgery of Hospital da Prelada in Porto, Portugal. All the donations were provided with informed consent from the patients. All samples were processed within 24 h after the lipoaspiration procedure. Human ASCs were enzymatically isolated from subcutaneous adipose tissue as previously described.[19,20,42,43] The lipoaspirate samples were first washed with a solution of PBS and 10% Antibiotic/Antimycotic (Gibco, UK). Liposuction tissue was digested with 0.2% collagenase Type II solution (Sigma) for 11/2 h at 37°C with intermittent shaking. The digested tissue was filtered using a 100-μm filter mesh. The floating adipocytes were separated from the precipitation stromal fraction by centrifugation at 1250 rpm for 10 min. The cell pellet was suspended in lysis buffer for 10 min to disrupt the erythrocytes. After a centrifugation at 800 rpm for 10 min, cells were again suspended and placed in culture flasks with minimum essential α-medium (Invitrogen Corporation) supplemented with sodium bicarbonate, antibiotic/antimicotic (with 10,000 units of penicillin, 10 mg of streptomycin, and 25 μg of amphotericin B per mL (Sigma), and 10% of FBS. The cells were cultured until sufficient number of cells were obtained at 37°C in a 5%CO_2 incubator and changing the medium every 2 days.

Cell and GF encapsulation

To study the ability of the carrageenan hydrogels for cell encapsulation, ATDC5 cells were used as models to evaluate the efficiency of the system. Three different formulations were prepared: I – carrageenan-only hydrogels (2% and 2.5% (w/v)), II – carrageenan hydrogels (2% and 2.5% (w/v) encapsulating ATDC5 cells, and III – carrageenan hydrogels (2% and 2.5% (w/v), encapsulating ATDC5 cells and TGF-β1. The encapsulation of cells was performed by quick addition of the cell suspension in PBS to the polymer solution at a ratio of 1/20 (v/v) at 40°C. The solution was mixed every few seconds to disperse the cells and then 5 mL of the mixture poured into each Petri dish. GF (100 μg/mL) was dissolved in PBS and mixed with the carrageenan/cell suspension prior to the sol–gel transition to include TGF-β1 in the hydrogel network. ATDC5–carrageenan constructs were prepared with a cell density of 5×10^6/mL of final solution, and cultured for 1 week.

The formulation chosen for the differentiation study was the 2% (w/v) κ-carrageenan hydrogels crosslinked in 5% (w/v) KCl for 30 min. The ASCs were incorporated into the hydrogels following the procedure described above for the ATDC5 cell line. Human ASCs were encapsulated in the carrageenan hydrogels with a final density of 2/mL of final solution and cultured for 14 days. Three different formulations A, B, and C were prepared as follows: (A) κ-carrageenan hydrogels encapsulating hASCs and TGF-β1 cultured in chondrogenic medium; (B) carrageenan hydrogels encapsulating hASCs cultured in chondrogenic medium; and (C) κ-carrageenan hydrogels encapsulating hASCs and TGF-β1 cultured in basal medium. The final TGF-β1 amount in each disc was 10 ng.

Culture conditions

ATDC5 constructs were cultured *in vitro* in 48-well plates with 500 μL of culture medium per sample of DMEM-F12 supplemented with 10% FBS, $NaHCO_3$, and L-glutamine. Medium was changed every 2 days, and specific discs were collected for analysis at days 1 and 7 for

3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay ($n = 3$), deoxyribonucleic acid (DNA) assay ($n = 3$), histology, and immunohistochemistry ($n = 3$).

Human ASCs–carrageenan constructs were cultured under two different conditions (all reagents were obtained from Sigma, USA). Formulations A and B were cultured in chondrogenic medium low-glucose DMEM supplemented with sodium bicarbonate, antibiotic/antimicotic, ITS(+) (insulin–transferrin–selenium), ascorbic acid, sodium pyruvate, L-proline, dexamethasone, and growth factor TGF-β1 (Sigma–Aldrich, Portugal). Discs from formulation C were cultured in DMEM supplemented with sodium bicarbonate, antibiotic/antimicotic (with 10,000 units of penicillin, 10 mg of streptomycin, and 25 µg of amphotericin B per mL), and 10% of FBS. The medium was changed every 2 days (500 µL), specific discs were collected for analysis at days 1, 7, and 14 for the MTS assay ($n = 3$), DNA assay ($n = 3$), and histology and immunohistochemistry ($n = 3$).

Live/dead assay. Viability live/dead analysis was performed with Calcein AM/Propidium iodide (PI) solution. Calcein AM (Invitrogen Corporation, USA) was diluted in serum-free basal medium, and PI working solution (Invitrogen Corporation, USA) prepared with RNAse A (USB corporation, USA) in PBS. Samples were collected after the encapsulation process, and 100 µL of each working solution was added to samples and incubated for 10 min at 37°C and protected from light. The cells were assayed with a fluorescence microscope (Stemi 1000 PG-HITEC Zeiss) with green and red filters.

Cell viability (MTS assay). The viability of the encapsulated cells within the hydrogel was evaluated using the MTS assay at specific times. At each time point, the culture medium from the samples was removed and replaced with 0.5 mL fresh serum-free medium supplemented with MTS stock solution in 5:1 (v/v) ratio. After incubating at 37°C, in a 5% CO_2 incubator, the medium was collected and analyzed with a Microplate Reader (Synergie HT Izasa) at 490 nm.

DNA quantification. DNA content in the scaffold was determined using the fluorescent picoGreen dsDNA (ds, double stranded) quantification assay (Invitrogen Corporation, USA). Samples collected at each time point were washed in PBS, 1 mL of ultra-pure water was added, and samples stored at −80°C until testing. Before testing, samples were sonicated for disrupting cell membranes in a water bath for 15 min. The samples were then analyzed with a microplate reader in a fluorescent mode at 485–528 nm (Synergie HT Izasa).

Histological analysis. The samples at each time point were washed in PBS and fixed with 10% formalin for about 1 h at 4°C. After fixation, the samples were rinsed in PBS and dehydrated by serial immersion in a series of increasing concentration of ethanol solutions (70%, 90%, 95%, and 100%) and xylene performed with a spin tissue processor (Microm ST120, INOPAT). Specimens were then embedded in paraffin and cut into 3-µm sections using a Microtome (Microm HM355S, INOPAT). Prior to staining, sections were dewaxed at 70°C for 15 min and rehydrated in a series of ethanol/water solutions. The toluidine blue and Alcian blue-stained samples were analyzed with a stereo microscope (Stemi 1000 PG-HITEC Zeiss) and the images collected in digital cameras.

Immunohistochemical analysis. Samples pre-fixed with 10% formalin for about 1 h at 4°C were cut into 3-μm section, and slides were deparaffinized and dehydrated in an automatic stainer (Microm HMS740, INOPAT). Slides were then rinsed in a PBS buffer for 5 min, and then immersed for 30 min into a hydrogen peroxide solution to inactivate endogenous peroxidase. The slides were washed again with PBS for 5 min and incubated with 5% powdered milk for 10 min at room temperature to avoid unspecific reactions. The first antibody used was Anti-type II collagen UNLB (goat) (SantaCruz, USA) for 1 h at room temperature. After the slides were rinsed in PBS for 5 min, the secondary antibody, polyclonal swine anti-goat, mouse, rabbit imunoglobu-biotinylated (Dako, Denmark), was incubated for 30 min at room temperature. After one more washing, the samples were incubated for 30 min with Vectastain R.T.U. Elite ABC Reagent. The final substrate reagent was added at room temperature for 10 min to develop staining, slides washed in tap water for 5 min and mounted. Positive control consisted of sections of HAC tissue, and negative controls consisted of sections incubated with PBS instead of the primary antibody.

Statistical analysis

All the experiments were performed with at least three replicates. Results are expressed as the mean ± SD. Differences between the experimental results were analyzed according to a Student's *t*-test, with the limit for statistical significance being defined as $p < 0.01$.

Results

Different carrageenan hydrogels were successfully prepared based on two different polymer concentrations, 2% and 2.5% (w/v) in distilled water. CryoSEM of the internal morphology of the hydrogels is shown in Figure 1. The samples prepared with 2.5% (w/v) polymer had denser networks (Figure 1(b)), compared to 2% carrageenan hydrogels, which had a lesser

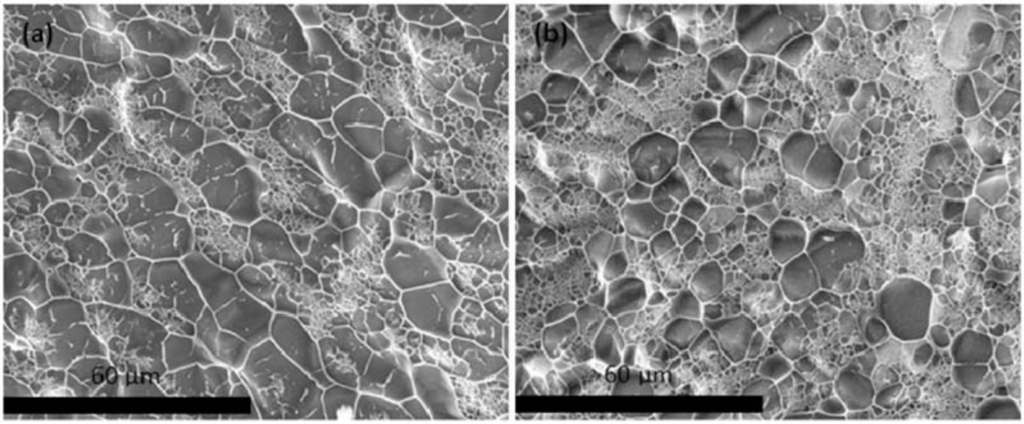

Figure 1. CryoSEM images of the surface fracture from carrageenan hydrogels prepared with different polymer concentrations: (a) 2% (w/v) κ-carrageenan hydrogel crosslinked with 5% (w/v) KCl for 30 min and (b) 2.5% (w/v) κ-carrageenan hydrogel crosslinked with 5% (w/v) KCl for 30 min. Formulation B presents a general denser crosslinked morphology.

crosslinked morphology (Figure 1(a)); thus, the water-uptake capability was greater as confirmed by water uptake measurements.

Viability and proliferation of ATDC5 cells

To assess the carrageenan hydrogels as a cell encapsulation system, the chondrocyte line ATDC5 was used. Cell viability and proliferation were evaluated by the polymer concentration (2% and 2.5% (w/v), crosslinking time (30 and 60 min), and TGF-β1 incorporation (0 and 100 ng/mL) within the hydrogel. The encapsulation process was achieved in all formulations and the hydrogels remained stable during the 7-day culture period.

Increase in polymer concentration and crosslinking degree did not show any negative effects on the cell viability immediately after the encapsulation step, proven by the live/dead assay (Figure 2). Moreover, the incorporation of TGF-β1 also did not affect the ratio of live/dead cells as all the hydrogels showed uniform distributions of viable cells after the encapsulation process (Figure 2), and similar results were obtained for the different formulations. At higher magnification (Figure 2(d)), the spherical cellular morphology typical from an encapsulation in a hydrogel network was clearly observed.

The cell viability and proliferation were followed for 1 week of culture. At days 1 and 7, constructs ($n = 3$) of each formulation were collected for MTS and DNA analyses. The encapsulated cells were viable for 1 week inside the gel. The cells maintained their viability

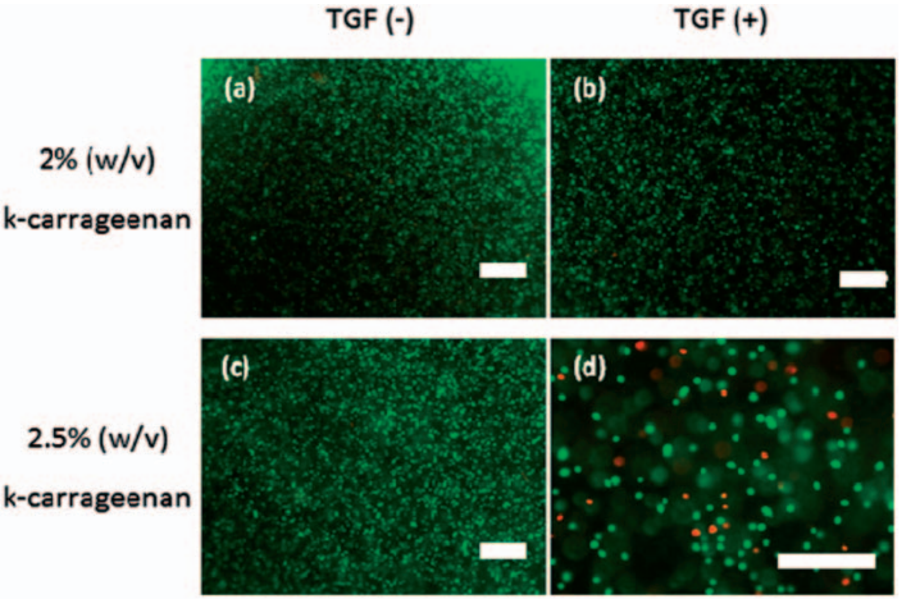

Figure 2. Live/dead assay, at day 0, for ATDC5 encapsulated in κ-carrageenan hydrogels with different polymer concentrations. Cells entrapped in the hydrogels display spherical morphology in all formulations. Figures (a) and (b) refer to 2% (w/v) κ-carrageenan, while (c) and (d) show cell viability for 2.5% (w/v) polymer. TGF-β1 was included in (b) and (d). Scale bar represents 100 μm.

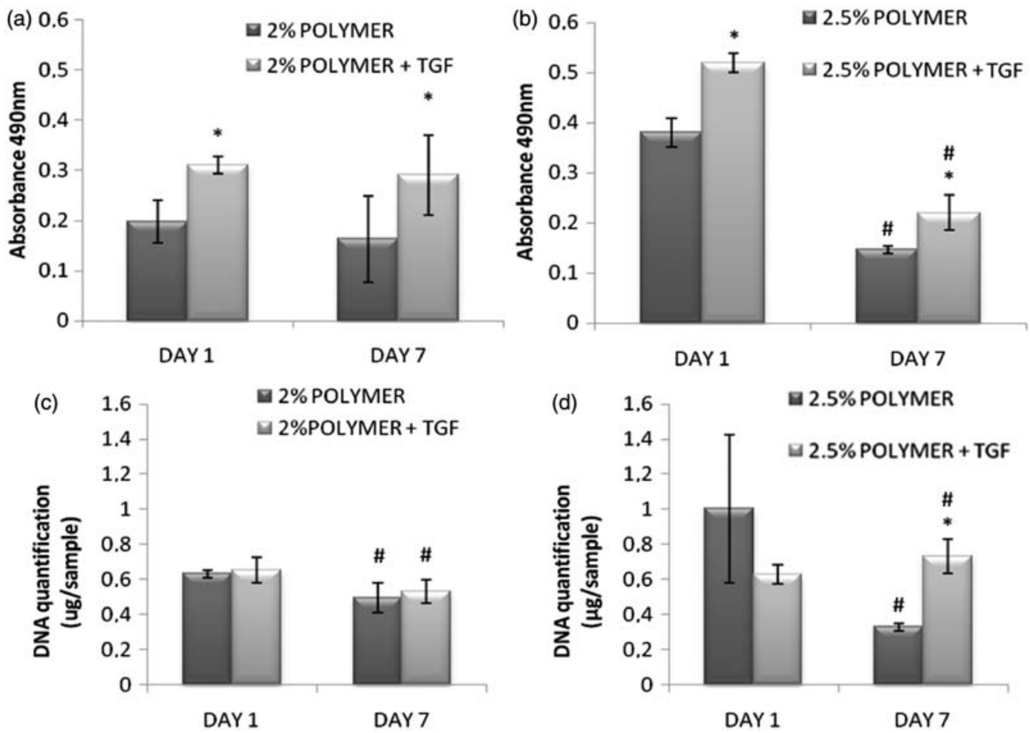

Figure 3. Evaluation of viability (a and b) and proliferation (c and d) of ATDC5 cells encapsulated in κ-carrageenan gels. The symbol * shows significant difference of samples where TGF-β1 is included comparing for the ones where the GF is absent; the symbol (#) shows difference of samples collected in day 7 from the ones collected in day 1 ($p < 0.01$).

for 1 week, without a significant decrease, in the hydrogels with 2% (w/v) κ-carrageenan. However, in the 2.5% (w/v) carrageenan hydrogels, the viability decreased significantly with time. It was noted that in most of the constructs with the presence of GF, the cell viability levels are significantly higher than in those without TGF-β1 ($p < 0.01$) (Figure 3). The presence of TGF-β1 in the hydrogel network seems to stimulate the proliferation of ATDC5 cells.

Viability and proliferation of hASCs

At day 0, when hASCs were encapsulated, the live/dead assay confirmed the entrapment of viable cells into the hydrogels (Figure 4), as the three tested formulations had higher ratio of green/red cells. The images are representative of the whole 3D cell distribution. In all three formulations, the cells were distributed homogeneously within the hydrogels with no dead cells and no significant differences between the three formulations.

The viability of the cells inside the hydrogel was assessed by the MTS assay from 1 to 14 days of culture (Figure 5). The human ASCs cultured in chondrogenic medium had significant higher viability at day 1 compared to constructs cultured in DMEM basal media. After the first day of culture, constructs entrapping TGF-β1 and cultured in chondrogenic medium had significant higher cell viability than the two other

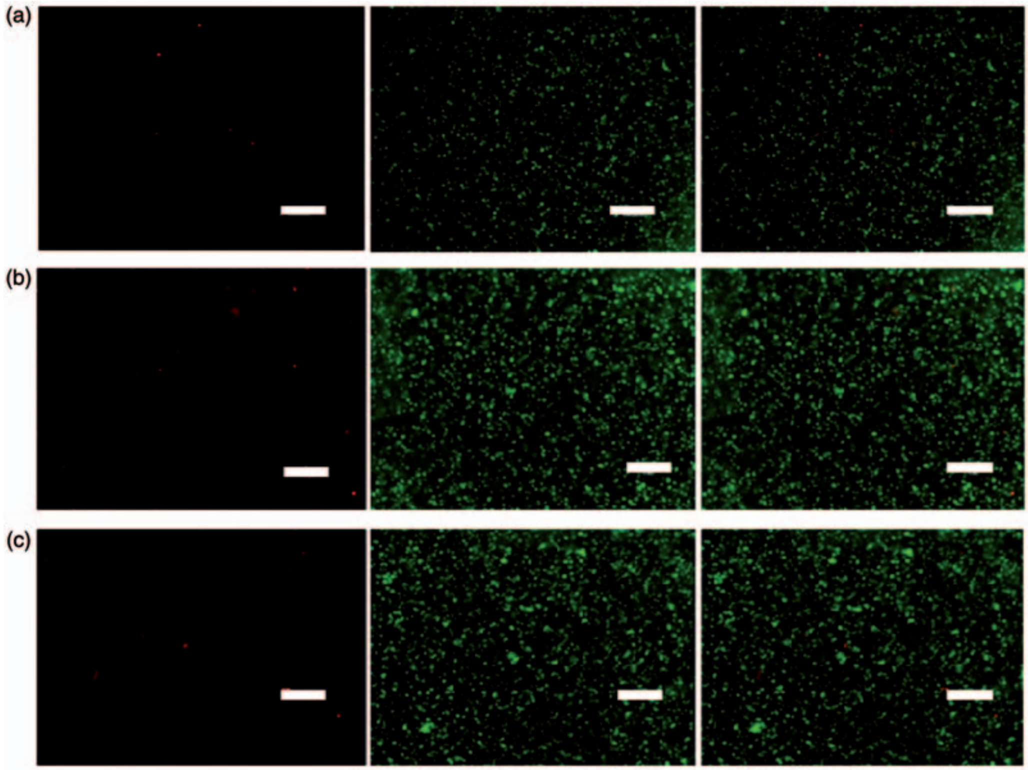

Figure 4. Viability of hASCs encapsulated in the κ-carrageenan hydrogels. Dead cells are presented with red fluorescence, while green one is indicative of live cells (first two columns, respectively). In third column, it is possible to observe the ratio between live and dead cells homogenously distributed in the hydrogel. There are no visible differences in hydrogels with TGF-β1 entrapping (formulations A and C) relating to its absence (formulation B). Scale bar represents 200 μm.

formulations. These culture conditions provided higher viability at day 7, but after 2 weeks, all three formulations were similar with no significant differences. The presence of TGF-β1 in the carrageenan hydrogel enhanced the hASCs viability throughout the culture period compared to cell-encapsulated hydrogels.

The constructs collected at the first day had higher numbers of cells compared to the other time points of culture. Both samples cultured in chondrogenic medium showed similar behavior, with a significant decrease in cells after 7 days followed by an increase past 14 days. With constructs cultured in DMEM, decrease was observed at each time point. The hydrogel entrapping both hASCs and TGF-β1 displayed a better proliferation results, while the formulation cultured in basal media was a more hostile environment for hASC culture.

Histology and immunohistochemistry

The organization and distribution of hASCs inside the hydrogel, as well as the accumulation of cartilage-specific macromolecules was examined by histological and

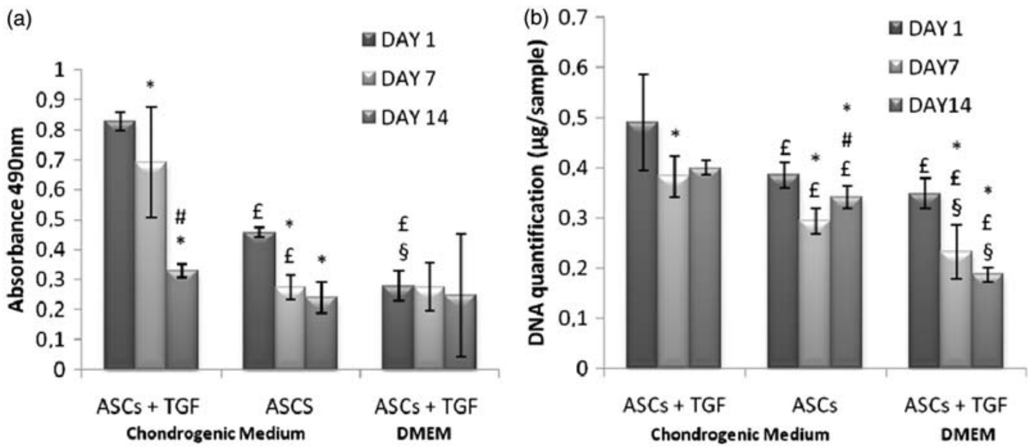

Figure 5. Evaluation of viability and proliferation of hASCs in κ-carrageenan hydrogels cultured for 2 weeks. The symbol (*) represents significant differences between the formulations related to the previous time point; (#) the significant differences related to the first day. The effects of TGF-β1 inclusion and the culture medium were also assessed and (£) represents significant differences related to formulation A, while (§) the significant differences to formulation B.

immunohistochemical methods. Histological stainings revealed that the cells adopted a rounded morphology and were well distributed inside the hydrogel. Samples collected after 14 days in culture showed a signs of sulfated glycosaminoglycans deposition on the pericellular regions and nearby (Figure 6).[44] Constructs cultured in chondrogenic medium showed a strong presence of cartilage-specific molecules and the cells that were encapsulated together with TGF-β1 appear inside a well-defined vacuole. Alcian staining proved the existence of proteoglycans in the constructs, more evidenced in formulation A. Cells appear surrounded by a blue ring correspondent to glycan molecules.

Immunohistochemical labeling against collagen type II showed the formation of a matrix composed of the two types of collagen studied. A strong evidence of collagen type II was seen in constructs from formulation A in the pericellular matrix of hASCs (Figure 7).

Discussion

The ability of κ-carrageenan hydrogels to support the chondrogenic differentiation of hASCs was assessed. Stem cells were efficiently isolated from adipose tissue by the previously mentioned procedure.[42,45] To emulate chondrogenesis *in vivo*, high cell density is required in a 3-D environment that favors cell condensation and cell–cell interactions, analogous to that which occurs in the formation of native cartilage.[22,46]

The encapsulation of hASCs inside the hydrogels was successful as only a insignificant number of dead cells were observed with live/dead assay. This fact indicated that the cells did not suffer a pronounced thermal shock in contact with the heated carrageenan solution. In both culture conditions (chondrogenic and basal media), the DNA content decreased with time. This finding is consistent with previous studies of hASCs grown within gel matrices, and it may be due to a combination of cell death or migration from the matrix.[19,27,47] Differences observed in cell viability (Figure 5), suggested a higher encapsulation success

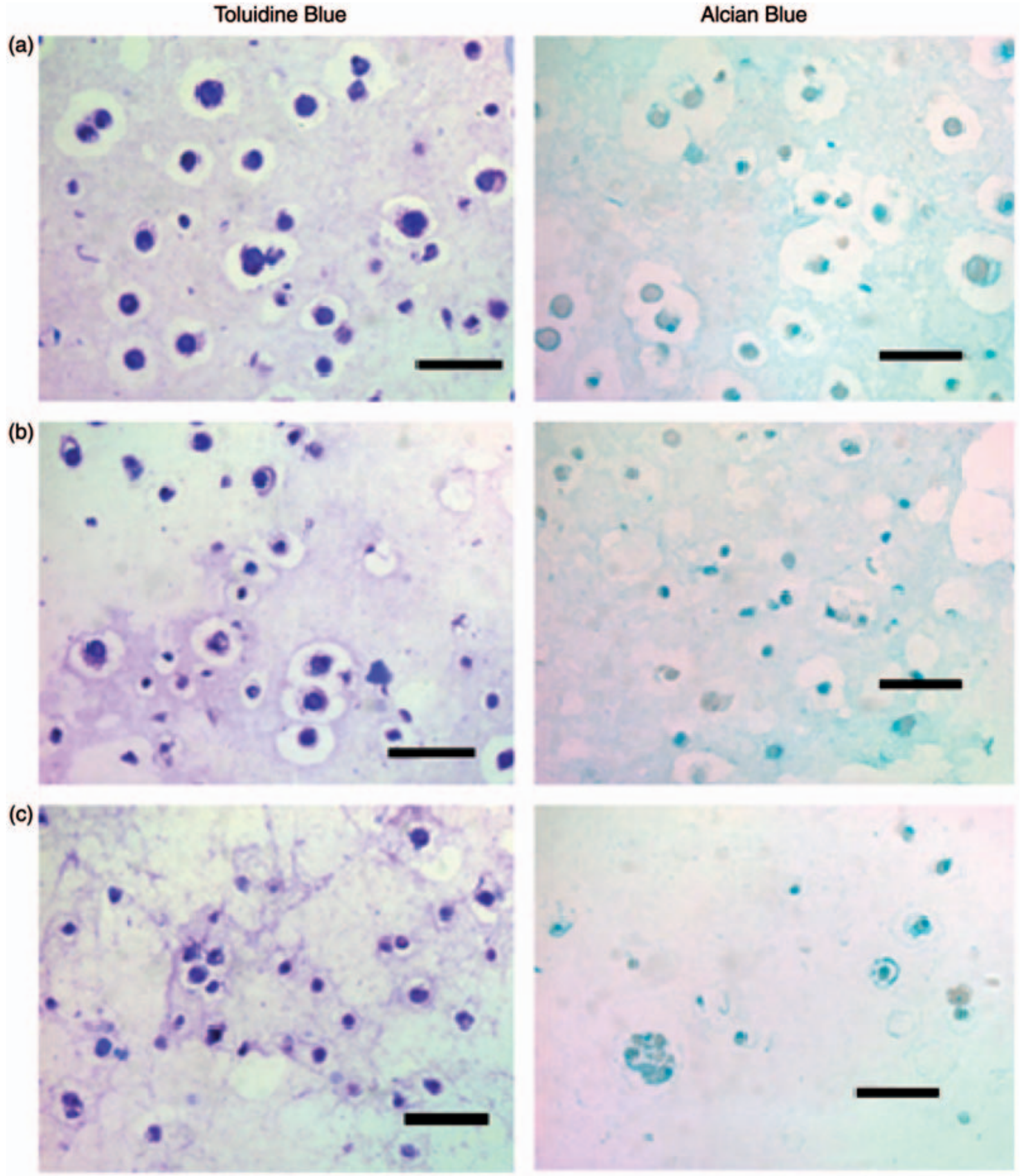

Figure 6. Histological images of hASCs cultured in κ-carrageenan hydrogels for 14 days. Toluidine staining showed the spherical shape of the cells, surrounded by a vacuole. Some extracellular matrix is clearly evidenced in formulation A, where cells were cultured in chondrogenic medium and TGF-β1 was included inside the hydrogel. Cartilage matrix-specific molecules were stained with Alcian blue and formulation A revealed higher accumulation, compared to B (chondrogenic medium without TGF-β1) and C (presence of TGF-β1 cultured in DMEM medium). Scale bar represents 60 μm.

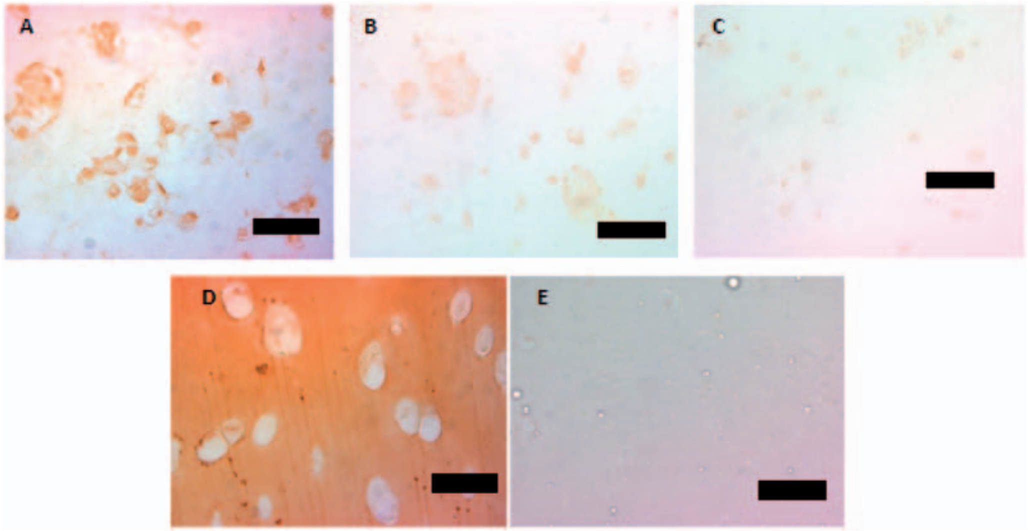

Figure 7. Immunolocalization of collagen type II in hASC–carrageenan constructs after 14 days of culture (A, B, and C). Positive and negative controls were performed with human articular cartilage (D and E). Higher formation of collagen type II was found in constructs incorporated with TGF-β1 and cultured in chondrogenic medium (A). Scale bar represents 60 μm.

in constructs from formulation A, in which the GF is dispersed within the matrix. Additionally, culture conditions and the presence of TGF-β1 also influenced the cell viability and proliferation, revealing better results when the amount of TGF-β1 is higher, i.e., when the GF was included in the gel and the constructs were cultured in chondrogenic medium. TGF-β1 is responsible for initial cell–cell interactions and has the ability to stimulate, grow, and differentiate the cells.[23–25] After days 1 and 7, the formulation A, with TGF-β1 inside the hydrogel, presented an enhanced viability and proliferation, compared to other formulations. However, this difference was not observed after 14 days of culture, which might be explained by the partial release of the GF from the structure, according to the GF release profile of κ-carrageenan hydrogels observed in a previous study.[40] The effect of the TGF-β1 became weaker with culture time, which might justify the similarities of the results after 2-week period. Based on viability and proliferation results, the GF inside the carrageenan cultured in basal media is less capable of sustaining chondrogenic differentiation of hASCs compared to constructs cultured in a chondrogenic medium. These suggest that higher GF concentration may be needed in such hydrogels to induce a better cellular response for hASCs without using culture medium containing the GF and other chondrogenic stimulators.

The histological and immunohistochemical analyses confirmed the presence of cartilage-specific matrix in the constructs. The Alcian staining was most intense in the pericellular matrix, characteristics associated with cells found in native cartilage.[19] When cultured in chondrogenic medium and with TGF-β1 encapsulated into the hydrogel, higher expression of collagen type II was obtained (Figure 6), confirming the influence of the GF in chondrogenic differentiation of hASCs, and that TGF-β1 was the ability to stimulate production of proteoglycans and other components in the cartilage matrix.[27,28]

The chondrogenesis of adult MSCs requires an environment containing strong chondrogenic inducer, such as, TGF-β1, dexamethasone, or ascorbic acid.[23–25,28] This is the reason why constructs cultured in basal DMEM medium displayed significantly lower expression of cartilage-specific molecules. Constructs A and C were designed with a final concentration of GF similar to the typically applied for the chondrogenic differentiation of progenitor cells (10 ng/mL).[27] Thus, the inclusion of TGF-β1 in the hydrogel is not enough to induce chondrogenic differentiation by itself but strongly enhances it when acting synergistically with chondrogenic medium.

The cell morphology inside the hydrogel was also affected by changes in culture environment (Figure 6). The majority of the cells had a spherical morphology, necessary for the expression of chondrocytic phenotype which is related to the synthesis of ECM components of cartilage.[48] Similar cell morphology was observed previously in 3-D carrageenan-based cultures of chondrocytes.[49] These findings confirm that carrageenan-based hydrogels are suitable for cell encapsulation[19] and κ-carrageenan hydrogel networks may mimic extracellular cartilage matrix.[50] Additionally, due to the gelling properties of the biomaterial and the transition temperature of the material close to the physiological one, carrageenan-based hydrogels might be used in the future as an injectable system for TE, envying a minimally invasive approach.

Conclusions

κ-Carrageenan can be a suitable material for cartilage TE as it is noncytotoxic and an efficient cell entrapment. The simultaneous entrapment of TGF-β1 and hASCs inside the hydrogel network enhanced the in-cell viability and proliferation during the first week of culture as well as, an increase in the expression of chondrogenic differentiation markers when cultured in chondrogenic medium. It is possible to observe the initial steps of chondrogenic differentiation of hASCs after just 14 days, showing that this cell source could be an adequate option for cartilage TE.

Acknowledgements

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