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EVALUATION OF THE POTENCIAL OF NOVEL HYDROGELS BASED ON CARRAGEENAN WITH ENCASPULATED ADIPOSE DERIVED STEM CELLS FOR CARTILAGE TISSUE ENGINEERING

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ABSTRACT
Cartilage tissue engineering approaches present a high potential of application in the repair of articular defects, where currently available therapies are not very successful. The use of injectable hydrogels for cell delivery in cartilage regeneration therapies have attracted more and more attention because of their promising advantages over pre-formed scaffolds. Temperature-dependent natural biopolymer k-carrageenan hydrogel has been investigated as a new cell delivery vehicle. Biological tests were performed using these hydrogels to encapsulate human adipose derived stem cells (hASCs) in order to evaluate their ability to maintain cells viability and proliferation. Furthermore, we examined the phenotype profile of hASCs in differentiated and undifferentiated state using RT-PCR to analyse the expression of specific cartilage markers (SOX-9, Col II, Col I, Col X, Aggrecan -) and immunohistochemical analysis (Col II and Col I immunolocalization). The results indicated that k-carrageenan hydrogels showed good cellular viability and proliferation after 21 days of culture and the constructs showed to be positive for specific cartilage markers. Based on this data, the feasibility of using k-carrageenan gel as a stem cell carrier vehicle and as potential injectable systems, due to its biocompatibility, gelling properties and ability to maintain viability and induce chondrogenic differentiation of encapsulated cells has been demonstrated.

INTRODUCTION

Adult cartilage tissue has extremely poor capability of self-repair, especially in case of severe cartilage damage due to trauma or age-related degeneration and remains a challenging problem for orthopedic surgeons (Wang, Blasioli et al. 2006). Numerous experimental and clinical attempts have been made to heal histological and macroscopically articular lesions aiming to reestablish their structurally and functionality (Hunziker 2002). To address the need for awareness and research into musculoskeletal injuries and disorders, cartilage tissue engineering strategies have been employed based on injectable *in situ* systems. The current cartilage regeneration strategy is to mimic as closely as possible the native environment and structure and to facilitate the replacement of damaged tissue with regenerated tissue that is designed and constructed to meet the needs of particular applications (Langer and Vacanti 1993). An injectable biomaterial must display a wide range of properties and characteristics in order to serve as an ideal scaffold for *de novo* cartilage formation. The advantages of injectable gelling systems over preformed matrices include introduction into the body in a minimally invasive manner, ability to provide a good fit, and delivery of bioactive molecules or cells to the defect site under mild conditions (Nair, Starnes et al. 2007). Carrageenan is a naturally occurring polysaccharide which forms a gel with potassium ions, but also shows gelation under salt-free conditions, helped by physical bonds, being a thermo sensitive hydrogel. The temperature-induced gelation permits easy formation of carrageenan based gels under mild conditions, enabling its application as an *in vitro* cell-carrier or as an *in vivo* injectable system. There are some challenges in the design of injectable scaffold-cell system, like optimization of kinetics in the gelation process, mechanical properties of the hydrogels maintaining an environment conducive to tissue growth; protection of cell damage and viability during preparation of scaffold and delivery; controlled release of growth factors; cell-material interaction and biocompatibility; rate of biodegradation and cytotoxicity; phenotype and tissue formation (Drury and Mooney 2003). Some of these aspects necessary in the development of injectable hydrogel were taken in consideration in the present work to evaluation k-carrageenan potential as cell carrier system. The present work focuses on evaluating the potential of this novel system, focusing on the viability and chondrogenic differentiation of encapsulated human adipose stem cells (hASCs) and

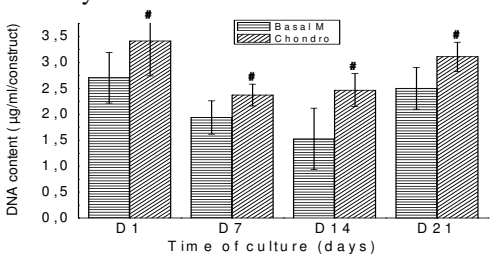
on the mechanical properties of carrageenan hydrogel with encapsulated hASCs along the time in culture.

MATERIALS AND METHODS

Newly developed carrageenan hydrogels discs were produced using an ionotropic gelation method and have been prepared by precipitation in KCl solution. Human ASCs, were obtained from liposuction procedures and isolated by enzymatic digestion; the resulting cells were resuspended in 1.5 % carrageenan solution (final concentration $1x10^6$ cells/mL) and casted into discs of 4mm diameter. The hydrogel discs with encapsulated cells were placed in 48 well culture plates with either DMEM-high glucose medium or in chondrogenic differentiation medium, containing 1X ITS, 100 mM dexamethasone, 0.1M sodium pyruvate, 35mM proline, 17mM ascorbic acid, 10 ng/ml transforming growth factor-β1 (TGF-β1). The viability (MTS assay, fluorescence staining) proliferation (DNA) and differentiate (RT-PCR) of encapsulated cells were evaluated after different culturing periods. We examinied the cell-loaded constructs for specific cartilage markers by immunohistochemical analysis (Col II and Col I immunolocalization. The mechanical properties of the hydrogels discs with encapsulated hASCs were assessed by dynamic mechanical analysis (DMA), performed after different culturing times, in order to analyse the effect of cells development and eventual formation of ECM on the properties of the constructs.

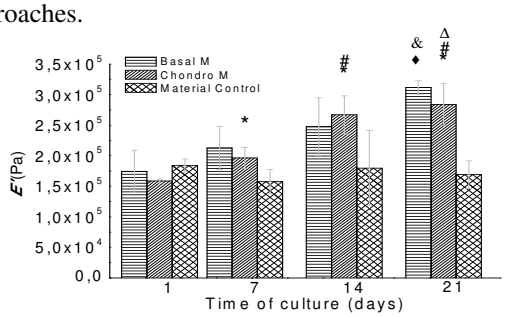
RESULTS

The *in vitro* proliferation of the encapsulated cells, cultured with either chondrogenic or basal medium is presented on Figure 1. Human ASCs show a higher proliferation rate when cultured with chondrogenic medium as compared to those cultured with basal medium. Nevertheless, after 21 days in culture, hASCs encapsulated in k-carrageenan hydrogels maintain a high viability.



Figures 1: Proliferation activity after 21 days in culture of hASCs encapsulated in carrageenan hydrogel cultured in differentiation medium. Statistical significance at $p < 0.05$.

At a frequency of 1 Hz, the compression modulus of the hydrogels was estimated to be 0.23MPa for hASCs in basal medium, 0.22MPa for cells culture in chondrogenic medium and 0.17MPa in k-carrageenan gels. After 21 days in culture, dynamically loaded k-carrageenan constructs indicated a significantly higher modulus than free cell discs, suggesting a positive effect of cells and of cartilage extracellular matrix components in the mechanical performance of the cell-carrier systems. These values (Figure 2) mimic the mechanical properties found in articular cartilage and they are higher or within the range of values found for other hydrogels used in similar cartilage regenerative approaches.



Figures 2: Dynamic Young's modulus data depicting the higher stiffness over time due to increase of the visco-elasticity. Statistical significance at $p < 0.05$.

CONCLUSIONS

Carrageenan appears to be promising material for cell encapsulation, due to its biocompatibility, gelling properties and ability to maintain the viability of encapsulated cells. Furthermore, the mechanical properties of carrageenan hydrogels are favoured by the presence of encapsulated cells and possibly by the formation of ECM.

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REFERENCES

Drury, J. L. and D. J. Mooney (2003). "Hydrogels for tissue engineering: scaffold design variables and applications." Biomaterials **24**(24): 4337-51.

Hunziker, E. B. (2002). "Articular cartilage repair: basic science and clinical progress. A review of the current status and prospects." Osteoarthritis Cartilage **10**(6): 432-63.

Langer, R. and J. P. Vacanti (1993). "Tissue engineering." Science **260**(5110): 920-6.

Nair, L. S., T. Starnes, et al. (2007). "Development of injectable thermogelling chitosan-inorganic phosphate solutions for biomedical applications." Biomacromolecules **8**(12): 3779-85.

Wang, Y., D. J. Blasioli, et al. (2006). "Cartilage tissue engineering with silk scaffolds and human articular chondrocytes." Biomaterials **27**(25): 4434-42.

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