Universidade do Minho
Escola de Engenharia

Semana da Escola de Engenharia
October 24 - 27, 2011

DIFFERENT TYPES OF CELLS COMBINED WITH K-CARRAGEENAN AIMED AT CARTILAGE REGENERATION

Elena G. Popa[1,2], Rui Reis[1,2] and Manuela E. Gomes[1,2]
[1]3B's Research Group - Biomaterials, Biodegradables and Biomimetics, University of Minho, Headquarters of the European Institute of Excellence on Tissue Engineering and Regenerative Medicine, AvePark, 4806-909 Taipas, Guimarães, Portugal [2]ICVS/3B's - PT Government Associate Laboratory, Braga/Guimarães, Portugal
E-mail: epopa@dep.uminho.pt; rgreis@dep.uminho.pt megomes@dep.uminho.pt

KEYWORDS
hydrogels, biomaterials, *in vitro* response, cartilage tissue engineering **.**

ABSTRACT

Articular cartilage lesions, with their inherent limited healing potential, remain a challenging problem for orthopedic surgeons. Engineering articular cartilage using hydrogels have attracted more and more attention. Thermoreversible natural polymer k-carrageenan hydrogel has been investigated as a new cell delivery vehicle for cartilage regeneration. Different cell types will secrete unique matrices that have different methabolic outcome on the surrounding enviroment. In this work we have analysed the in vitro cellular behaviour of k-carrageenan, with encapsulated cells of different types, namely a chondrocytic cell line (ATDC5), primary chondrocytes cells (human nazal chondrocytes, hNCs) and stem cells (human adipose stem cells, hASCs), often proposed for cartilage regeneration strategies. Biological tests were performed on these hydrogels with the encapsulated cells in order to evaluate their ability to maintain cell viability, proliferation and chondrogenic potential. The 3 types of cells encapsulated in k-carrageenan hydrogels showed good cellular viability and proliferation up to 21 days of culture and the constructs showed to be positive for specific cartilage markers. Based on this data, it is possible to conclude that k-carrageenan hydrogel demonstrate compatible biological properties to induce in vitro chondrogenic pathways for cartilage regeneration.

INTRODUCTION

Injuries of the articular cartilage are challenging to repair due to the poor ability of this tissue for regenerate (Lindahl et al. 2003; Redman et al. 2005). Cartilage tissue engineering strategies require the presence of cells and hydrogels which mimic closely the natural environment in the body and have tissue-like water content (Risbud and Sittinger 2002; Spiller et al. 2011). It is known that the extracellular matrix is not a static structure but a dynamic network of molecules secreted by cells (Buckwalter and Mankin 1998; Velleman 1999). K-carrageenan, is an ionic hydrogel recently proposed for TE approaches and forms a gel with potassium ions, but also shows gelation under salt-free conditions helped by physical bonds. Hydrogels such as carrageenan, melt at elevated temperature and by lowering the temperature results the gelation of the biopolymer. The temperature-induced gelation allows for the easy formation of gels of different shapes.

MATERIAL & METHODS

The k-carrageenan hydrogels were produced using an ionotropic gelation method and cells, namely ATDC5, hNCs and hASCs, were encapsulated at a density of $5*10^6$cell/ml and cultured for 21 days. The cell viability and proliferation was determined by fluorescence staining, MTS and DNA quantification. At a molecular level chondrogenic markers were evaluated by qRT-PCR analysis (Sox9, aggrecan collagen type I, type II and type X) and typical histological staining were performed for the detaction of proteoglicans production.

Semana da Escola de Engenharia
October 24 - 27, 2011

RESULTS & DISCUSSION

Measuring metabolic activity via the MTS assay is a rapid way to screen for toxicity caused by compounds of the hydrogel or by the crosslinking agents used for the formation of the gel and can allow the screening of different cell types as well. Our results demonstrated that the cells remain viable for up to 21 days of encapsulation/culture in the 3D hydrogels developed. Analyzing the importance of the cell type, we can observe that the highest metabolic activity was obtained for the hASCs encapsulated cells. The influence of cell type in the metabolic activity could be explained by the different cell proliferation rate of these cells. The data obtained from the DNA quantification assay demonstrated that the proliferation of all cell encapsulated in hydrogels tends to decrease with the culturing time, complementary to the MTS assay results Cells vary in their expression of antioxidant enzymes and greater expression of these enzymes can protect cells from oxidative damage. Each cell type was incubated in a different medium therefore we cannot rule out the effect of the cell culture medium and added antioxidants. The results from real time qRT-PCR confirm the expression of five important typical chondrogenic markers. In this system, hASCs were likely stimulated down the chondrogenic pathway compared to the ATDC5 and hNCs cell type. Chondrogenesis could be proved in the hydrogel system by the detection of glycosaminoglycans and proteoglycans proteins with the metachromatic staining Safranin O, Toluidine and Alcian blue. The biological evaluation of k-carrageenan hydrogel revealed that this polymer enables long term viability and proliferation of different cells. During 3 weeks of culture, cells encapsulated within the hydrogel developed a cartilage-like extracellular matrix rich in proteoglycans and type II collagen. Cartilage –like ECM deposition and production was found throughout all culturing periods indicating a stable chondrocyte phenotype in encapsulated cells. Nevertheless, encapsulated hASCs exhibit the highest proliferation rates and highest levels of chondrogenic markers expression.

CONCLUSIONS

K-carrageenan hydrogels enable the viability and proliferation of different cell types during long term cell culture. The results obtained indicated the feasibility of using these hydrogels in cartilage tissue engineering approaches due to its ability to support chondrogenic features of different cells types, particularly the hASCs.

AKNOWLEDGEMENTS

The author gratefully acknowledges Portuguese Foundation for Science and Technology (FCT) for the PhD grant (SFRH/BD/64070/2009).

REFERENCES

Atsumi, T., Y. Miwa. 1990. "A chondrogenic cell line derived from a differentiating culture of AT805 teratocarcinoma cells." *Cell Differ. Dev.*, 30 (2), 109-16.

Buckwalter, J. A. and H. J. Mankin. 1998. "Articular cartilage: tissue design and chondrocyte-matrix interactions." *Instr Course Lect*, 47 477-86.

Lindahl, A., M. Brittberg. 2003. "Cartilage repair with chondrocytes: clinical and cellular aspects." *Novartis Found Symp*, 249 175-86; discussion 186-9, 234-8, 239-41.

Redman, S. N., S. F. Oldfield. 2005. "Current strategies for articular cartilage repair." *Eur Cell Mater*, 9 23-32; discussion 23-32.

Risbud, M. V. and M. Sittinger. 2002. "Tissue engineering: advances in in vitro cartilage generation." *Trends Biotechnol*, 20 (8), 351-6.

Spiller, K. L., S. A. Maher. 2011. "Hydrogels for the Repair of Articular Cartilage Defects." *Tissue Eng. Part B*, 0 (0), null.

Velleman, S. G. 1999. "The role of the extracellular matrix in skeletal muscle development." *Poult Sci*, 78 (5), 778-84.

AUTHORS' BIOGRAPHIES (300)

ELENA G. POPA was born in Iasi, Romania and studied to Technical University, Faculty of Industrial Chemistry where she obtained her MSc in Natural products in 2007. For the BSc she went to University of Medicine and Pharmacy and obtained the degree in medical bioengineering in 2006. She is currently a PhD student at University of Minho in 3Bs Research Group. Her email address is epopa@dep.uminho.pt and her Web-page can be found at http://www.3bs.uminho.pt.