A one-step combined therapy for cartilage repair: Development & performance assessment

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Introduction

With a progressively ageing and physically active population, joint ailments appear as responsible for activity limitation in adults 18-65 years of age, resulting in reduced quality of life and high socio-economic burden. Despite intensive efforts on the development of cartilage repair strategies worldwide, no current approach has proven full efficacy. In this work, it is proposed a novel one-step combined therapy where human adipose-derived stem/stromal cells (hASC)[1] develop cartilage-like tissue within a novel hydrogel based on methacrylated gellan gum[2] (mimsys® G). A complete xeno-free approach was developed and both *in vitro* and *in vivo* experiments were conducted to evaluate the performance of this system for focal cartilage repair.

Materials and Methods

Human ASC (hASC xeno-free, irisbiosciences, Portugal) were expanded in xeno-free media until sub-confluency. mimsys G hydrogel (mimsys® G, irisbiosciencies) was prepared to yield a 2% w/V solution. Cells were encapsulated at $10x10^6$ cells/mL, ionically crosslinked and cultured with or without chondrogenic induction for 21 days. Cell viability was determined by live/dead assay, and cell metabolic activity by MTS reduction. A *in vivo* study evaluated regeneration of focal cartilage lesions on a rabbit knee model for 8 weeks, using an autologous approach. Chondrogenic development was assessed on both *in vitro* and *in vivo* samples by histological staining of cartilage matrix using safranin O and immunolocalization of col-II. Expression of chondrogenic-related genes such as aggrecan, col-II and sox-9 (Applied Biosystems) were also determined at different time points.

Results

In vitro analysis of the combined system demonstrated high viability of the cells and progressive metabolic activity along culture (Fig.1A). Additionally, intense cartilage matrix deposition was detected by safranin O staining (Fig.1B). Correspondent up-regulation of chondrogenic genes were quantified. As for *in vivo* performance, analysis of rabbit cartilage lesion explants demonstrated filling of the defect and indication of focal regeneration.

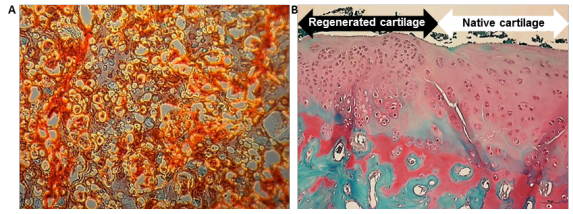

Fig.1 Safranin-O staining of combined system: A. After in vitro chondrogenic induction. B. After in vivo regeneration of cartilage lesion.

Discussion and Conclusions

The proposed one-step combined therapy system demonstrated both safety and efficacy *in vitro*, revealed respectively, by high cell viability and intense cartilage development, demonstrated by expression of specific hyaline cartilage markers. *The in vivo* study revealed promising repair performance in focal cartilage lesions, suggesting a valuable approach for further therapeutic exploitation.

References

1. Correia C. et al. Tissue Eng Part A 18, 1979-91, 2012; **2.** Silva-Correia J. et al. Adv Healthc Mater 2, 568-75, 2013

Acknowledgments:

ARTICULATE: QREN-13/SI/2011-23189