Tissue and Cell 49 (2017) 35–44

Contents lists available at ScienceDirect

Tissue and Cell

journal homepage: www.elsevier.com/locate/tice

The direction of human mesenchymal stem cells into the chondrogenic lineage is influenced by the features of hydrogel carriers

A. Hansson[a,b,1], A. Wenger[a,b,1], H. Barreto Henriksson[a,c,*], S. Li[d], B.R. Johansson[e], H. Brisby[a,c]

[a] Department of Orthopaedics, Institute of Clinical Sciences, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[b] Department of Chemical and Biological Engineering, Chalmers University of Technology, Gothenburg, Sweden
[c] Department of Orthopaedics, Sahlgrenska University Hospital, Gothenburg, Sweden
[d] Department of Clinical Chemistry and Transfusion Medicine, Institute of Biomedicine, Sahlgrenska Academy, University of Gothenburg, Gothenburg, Sweden
[e] The Electron Microscopy Unit, Institute of Biomedicine, University of Gothenburg, Gothenburg, Sweden

ARTICLE INFO

Article history:
Received 5 July 2016
Received in revised form 1 November 2016
Accepted 12 December 2016
Available online 13 December 2016

Keywords:
Human mesenchymal stem cells
Intervertebral disc
Disc degeneration
Hydrogels
Cell carrier
Chondrogenesis

ABSTRACT

Low back pain is a major public health issue in the Western world, one main cause is believed to be intervertebral disc (IVD) degeneration. To halt/diminish IVD degeneration, cell therapy using different biomaterials *e.g.* hydrogels as cell carriers has been suggested. In this study, two different hydrogels were examined (*in vitro*) as potential cell carriers for human mesenchymal stem cells (hMSCs) intended for IVD transplantation. The aim was to investigate cell-survival and chondrogenic differentiation of hMSCs when cultured in hydrogels Puramatrix® or Hydromatrix® and potential effects of stimulation with growth hormone (GH). hMSCs/hydrogel cultures were investigated for cell-viability, attachment, gene expression of chondrogenic markers *SOX9, COL2A1*, *ACAN* and accumulation of extracellular matrix (ECM). In both hydrogel types, hMSCs were viable for 28 days, expressed integrin β1 which indicates adhesion of hMSCs. Differentiation was observed into chondrocyte-like cells, in a higher extent in hMSCs/Hydromatrix® cultures when compared to hMSCs/Puramatrix® hydrogel cultures. Gene expression analyses of chondrogenic markers verified results. hMSCs/hydrogel cultures stimulated with GH displayed no significant effects on chondrogenesis.

In conclusion, both hydrogels, especially Hydromatrix® was demonstrated as a promising cell carrier *in vitro* for hMSCs, when directed into chondrogenesis. This knowledge could be useful in biological approaches for regeneration of degenerated human IVDs.

1. Introduction

Low back pain is a major public health issue in the western world, which afflicts 70–85% of all people at some point in their life (Andersson, 1999; Freemont et al., 2002), and the prevalence of low back pain is reported to be as high as 84% (Airaksinen et al., 2006). In the Global Burden of Disease 2010 study it was concluded that low back pain causes the most disability compared with almost 300 other types of diseases or impairing medical conditions (Hoy et al., 2014). One of the main causes of low back pain has been proposed to be degeneration of the intervertebral discs (IVDs) (Freemont et al., 2002; Luoma et al., 2000). The IVD constitutes of two main types of tissues, the annulus fibrosus (AF) and the hydrogel-like nucleus pulposus (NP) (Humzah and Soames, 1988; Roughley, 2004). The chondrocyte-like cells produce the extracellular matrix (ECM) consisting mainly of proteoglycans and collagens. The expression of biomarkers in common for NP cells and chondrocytes in articular cartilage includes *e.g.* sex determining region box 9 (SOX9), collagen type II and aggrecan. SOX9 is a transcription factor necessary for the differentiation of human mesenchymal stem cells (hMSCs) into chondrocytes/chondrocyte-like cells (Chimal-Monroy et al., 2003; Goldring et al., 2006). IVD degeneration includes increased cell death, presence of ECM degrading enzymes, lack of nutrient supply and loss of gel-like structure in the NP, leading to tissue

* Corresponding author at: Dept. of Orthopaedics, Sahlgrenska University Hospital, SE 413 45 Gothenburg, Sweden.
E-mail address: helena.barreto.henriksson@gu.se (H.B. Henriksson).
[1] The first two authors contributed equally.

http://dx.doi.org/10.1016/j.tice.2016.12.004
0040-8166/

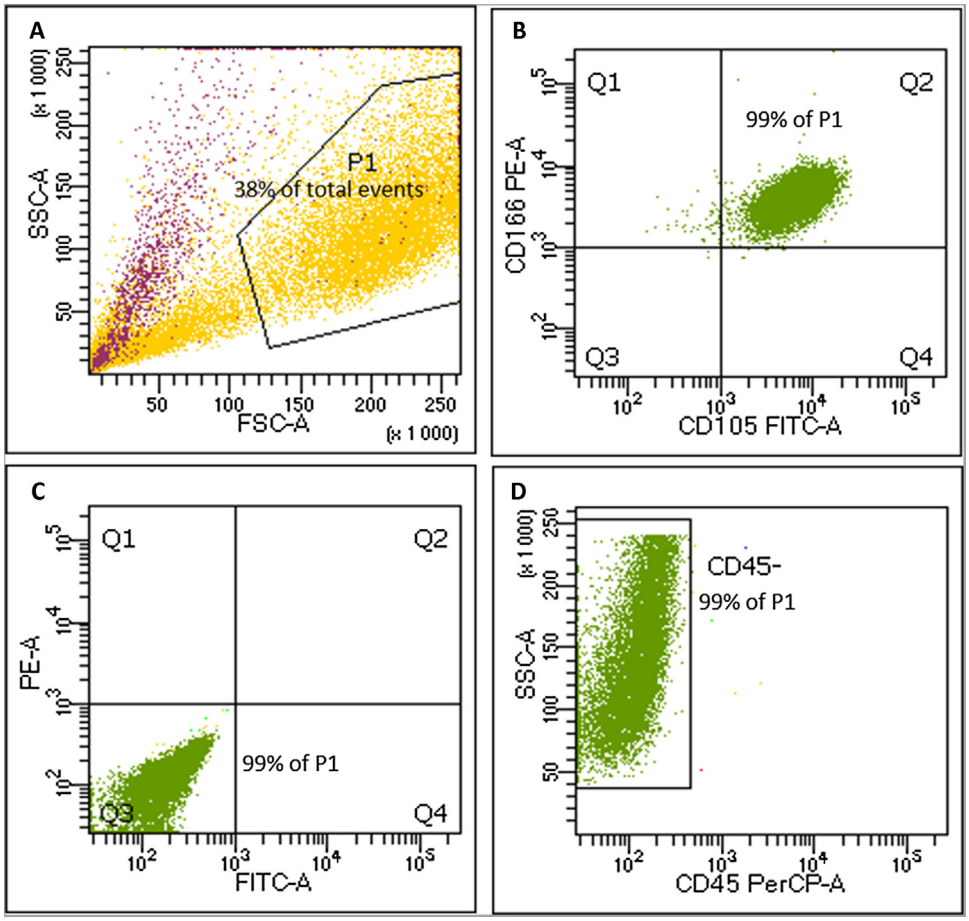

Fig. 1. The graphs display flow cytometry results of (donor 2), male, age 49 years: A) forward scatter plot, B) IgG isotype control, C) cells double-positive for the hMSCs markers CD105 and CD166 (P1) and D) cells negative for CD45, a hemapoetic stem cell marker.

dehydration, reduced IVD height and fissure formation (Urban and Roberts, 2003; Adams and Roughley, 2006). Current treatments for low back pain include physiotherapy and surgery, but these treatments are not fully dealing with the underlying factors and open surgery includes several risks *e.g.* postoperative infections (Fritzell et al., 2001; Vedicherla and Buckley, 2016). Minimal invasive treatments for halting or diminishing IVD degeneration are therefore desired and transplantation of cells, so called cell therapy, has been suggested (Gruber et al., 2002; Brisby et al., 2004; Henriksson et al., 2009; Hiyama et al., 2008; Feng et al., 2011). Stem cells have been considered for this purpose and hMSCs are of the most interest since these cells are easily obtained surgically, are able to differentiate into chondrocytes and to deposit ECM (Chamberlain et al., 2007; Pittenger et al., 1999; Svanvik et al., 2010). Different types of biomaterials such as collagen and hyaluronan hydrogels have been suggested as cell carriers to support cells transplanted into degenerated IVD (Hilborn, 2011; Sakai et al., 2003; Sawamura et al., 2009; Kisiday et al., 2002; Levett et al., 2014). Hydrogels, which are suitable for minimally invasive transplantations, would act as shock absorbers and may restore the IVD height (Schmidt et al., 2008; Silva-Correia et al., 2011). Hydrogels are further suggested to facilitate the distribution and differentiation of transplanted cells within the IVD (Chan and Gantenbein-Ritter, 2012; Henriksson et al., 2012; Fernandez-Muinos et al., 2015; Yamaoka et al., 2006). It is desired that the transplanted hMSCs attach to and migrate within the hydrogel. *In situ*, the hMSCs should further be able to differentiate into chondrocyte-like cells and produce ECM. Selected growth factors delivered by *e.g.* injections into the degenerated IVD are hypothesized to, either alone or in combination with cell therapy, increase cell proliferation, proteoglycan production and IVD height, thus regenerating the IVD by reversing the low grade IVD degeneration *in situ* (Revell et al., 2007; Masuda, 2008; Seelbach et al., 2015). Several *in vitro* approaches as well as *in vivo* studies including smaller and larger animal models have demonstrated the plausibility of this conjecture (Henriksson et al., 2009; Sakai et al., 2003; Henriksson et al., 2015; Masuda et al., 2004).

In this study, growth hormone (GH), Genotropin®, was applied, which is used clinically for patients with growth disorders including insufficient height and weight development (Albertsson-Wikland et al., 2008; Janssen et al., 1997).

The aim of the present study was to compare and examine cell survival and potential differentiation of hMSCs into chondrocyte-like cells, when cultured in two different types of hydrogel cell carriers. Further, the aim was to investigate if stimulation with growth hormone (Genotropin®) could improve chondrogenesis and ECM accumulation of hMSCs when encapsulated in these hydrogels.

2. Material and methods

2.1. Ethical permission

The study was approved by the local human ethics committee (ethical permission no: 532-04) and samples were collected from donors undergoing spine surgery with informed consent from all of the donors.