

Functionalisation of PLLA nanofibers by collagen or collagen derived peptides - Effect on growth and differentiation of hMSCs -

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INTRODUCTION: In many native tissues, collagen represents a principal structural element of the extra cellular matrix. It can be obtained from a variety of sources, is conserved and relatively nonimmunogenic. Therefore, it has been used in different tissue engineering applications. Collagen I (COLI) can be electrospun to a non-woven nanofiber network, although the thickness of the COLI mesh obtained by electrospinning is limited, due to the fact that COLI nanofibers react with air moisture. Therefore, an immediate fixation is necessary. Although COLI nanofibers support the growth as well as differentiation of hMSC these fibres show a lack of stability during cell culture compared to PLLA [1].

Within this study, we examined the possibility of combining the stability of PLLA with the osteoinductive potential of collagen.

METHODS: The preparation of PLLA nanofibers has been reported in detail earlier [2]. In order to incorporate COLI into the nanofibers, PLLA and COLI were dissolved in hexafluoroisopropanol (HFIP) in the desired ratio resulting in a 4.5 to 6.0% w/v polymer solution. Cyclic RGD was incorporated into the fibers either as solid powder or pre-dissolved in water. Human mesenchymal stem cells were obtained according to [1]. Cells were seeded at a density of 3×10^4 cells/cm² on cover slips, cover slips coated with PLLA, collagen blended PLLA and RGD containing PLLA, in growth or differentiation medium. Gene expression analysis was according to [2] at desired time points. Immunofluorescence was performed after methanol-acetone fixation using primary antibodies for Ki67, osteocalcin (OC), COLI and corresponding cy2 and cy3 labelled secondary antibodies.

RESULTS: Depending on the blend ratio, the use of PLLA-collagen nanofibers increased cell densities of hMSC, especially during early cultivation. In this period of cultivation, increased gene expression of ALP, COLI and OC was detectable (Fig. 1 left).

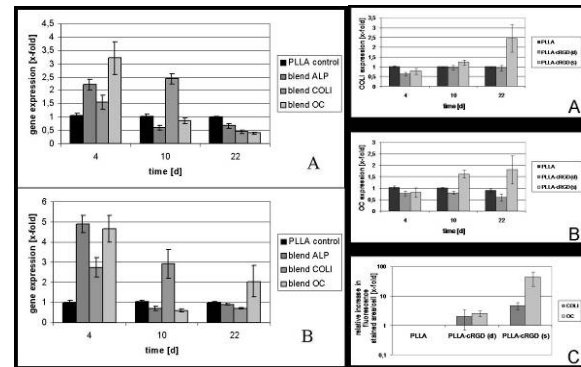


Fig. 1 left: Effect of PLLA blending with collagen on gene expression of hMSC cultured under growth (A) and osteoinductive conditions (B). Fig. 1 right: Effect of incorporation of cRGD into PLLA nanofibers on gene expression of COLI (A), OC (B) and deposition of protein (C).

The incorporation of cRGD peptides into the nanofibers had minor effects on hMSC growth. In addition, there were less osteoinductive effects using cRGD and these were restricted to the late phase of cultivation (Fig. 1 right).

DISCUSSION & CONCLUSION: The functionalisation of PLLA by blending improved the growth and differentiation of hMSC. However, the gene expression of osteoblast marker genes occurred in the early phase of cultivation, unlike in the case of cells cultured on pure collagen nanofibers [1]. One reason might be the alteration of COLI during electrospinning [3]. The direct incorporation of cRGD had effects similar to collagen nanofibers.

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