Pretreatment of Mesenchymal Stem Cells and Stromal-derived Factor-1α Delivery from Chitosan-based Injectable Hydrogels for Better Cell Guidance and Retention

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Abstract:
Clinical applications of mesenchymal stem cells (MSCs) rely on their capacity to home and engraft in the appropriate target tissues for a long time. Homing and engraftment capacity of these stem cells depend on the expression of Chemokines and their receptors. *Ex vivo* expanded MSCs exhibit homing potential when grafted to injury tissue but their homing efficiency has been observed very poor because of modifications in homing receptor expression and/or functions during culture and/or preparation steps. Hence, this study was designed to investigate the expression of surface CXCR4 by flow cytometric analysis (FACS) and *in vitro* modified Boyden chamber assay in adipose-derive MSCs (ASCs) stimulated with a hypoxia mimicking agents such as desferrioxamine mesilate (DFX), cobalt chloride ($CoCl_2$), lithium chloride (LiCl), valproic acid (VPA) and hypoxia. Intracellular CXCR4 were also evaluated by conventional and real-time PCR. Then we evaluated the homing ability of DFX-pretreated human DiI-labeled ASCs *in vivo*, 2 weeks after intravenous (IV), local infusion towards subcutaneously implanted chitosan-glycerophophate-hydroxyethyl cellulose (CH-GP-HEC) injectable hydrogels releasing SDF1 in dorsum of Wistar Rats. Presence of human ASCs in the CH-GP-HEC injectable, spleen, and lung were analyzed histologically by fluorescent microscope, and also quantified by PCR for human specific *CXCR4* gene, 2 weeks after transplantation in recipients' Rats. Results showed that short-term (24 hours) pretreatment to ASCs with the hypoxia mimicking agents up-regulate the CXCR4, increase in vitro migration capacity toward 100ng/ml SDF-1 (P<0.001) and *in vivo* homing capacity to the implanted CH-GP-HEC injectable hydrogel releasing SDF1. Fluorescence microscopic examination disclosed enhanced local accumulation of fluorescence-labeled ASCs in CH-GP-HEC in the DFX-pretreated group at 16th post-transplantation day. These results suggest that the SDF-1/CXCR4 axis plays an important role in the regulation of motility of ASCs, and increased expression of CXCR4 might be a potential strategy to improve homing and engraftment of ASCs towards SDF1 released by injectable hydrogels in different lesions.
Keywords: Chemotactic recruitment, Guided homing, Stem cells therapy.

Oral Presentation

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