MSC-laden anti-inflammatory hydrogel improving wound healing of diabetic skin ulcer

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Statement of Purpose: Diabetic skin ulcer is a severe, persistent complication of diabetes, where sustained chronic low-level inflammation, decreased secretion of growth factors and difficulty in vascularization leads to the inefficiency of most existing treatments. Bone marrow mesenchymal stem cells (BMSCs) are reported to regulate wound healing through a series of paracrine growth factors (e.g., TGF-β, FGF), and can differentiate into wound healing related effector cells such as keratinocytes, fibroblasts and endothelial cells. Thus, BMSCs' active roles in wound healing set up the foundation for the use of BMSCs to treat diabetes caused ulcer. However, low-level inflammatory microenvironment of the ulcer, high concentration of inflammatory cytokines in the wound which lead to increased protease secretion, thereby resulting in the degradation or loss of growth factors secreted by BMSCs and the chronic inflammation microenvironment will impair the activity of BMSCs. In order to reach MSCs' full potential, we developed a biodegradable multifunctional crosslinker (PAA) based thermal sensitive hydrogel. The gel can suppress the inflammatory response and carry BMSCs to treat diabetic wound, meanwhile PAA contains an RGD-like motif that promotes cell attachment and differentiation of BMSCs.

Methods: The crosslinker PAA with guanidine as side chains and disulfide linkage in backbone was synthesized using Michael addition reaction (1). The morphology and mechanic properties of the PAA based thermal sensitive hydrogel were characterized. BMScs were isolated from healthy 8- to 12-week-old male C57BL/6 mice, and used with hydrogel for MTT assay and cytokine assay. In vivo diabetic skin ulcer model was employed to evaluate the wound healing of hydrogel and BMSCs.

Results: We employed the multi-functional crosslinker to fabricate a 3D hydrogel. The polymer presented sol status at the room temperature and it experienced sol-gel transition in minutes after immersed in a 33 °C water bath. The hydrogel was slowly degraded in DTT solution, the residual mass was (70 ± 3.99)% after 21 days. Co-culture of the hydrogel and BMSCs resulted in BMSCs in-growth and proliferation with enhanced growth factor secretion of BMSCs. In vivo model showed significantly greater wound contraction in the hydrogel+BMSCs group 5 days after treatment compared with the control or hydrogel groups. Furthermore, seven days after operation, the average unhealed area of the wound was only 24.6 ± 4.21% in the hydrogel+BMSCs group, whereas these areas were 79.54 ± 5.92% and 66.5 ± 6.67% for the control and hydrogel groups, respectively. In contrast to the control and hydrogel groups, histological observations revealed that the hydrogel+BMSCs group exhibited a significant formation of granulation tissue at the wound site 5 days after treatment. In addition, the joint hydrogel+BMSCs treatment has also been demonstrated to inhibit chronic inflammation, promoted the formation of granulation tissue, promoted keratinocyte proliferation and differentiation, and improve qualify of wound healing.

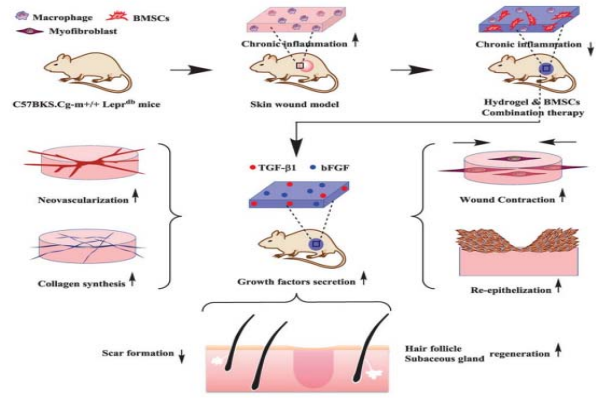

Fig.1. BMSCs laden hydrogel can inhibit the chronic inflammation, and promote growth factors secretion, resulting in accelerating wound contraction, EMC secretion, angiogenesis, re-epithelialization, hair follicle and sebaceous gland regeneration, and reduced scar formation.

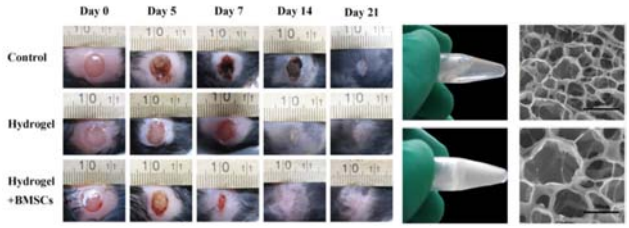

Fig.2. The hydrogel (left) promoting wound healing (right)

Conclusions: we designed the biocompatible hydrogel which can inhibit the chronic inflammation at the wound sites of diabetes caused ulcer, on this base, we used this hydrogel to package BMSCs for transplantation into these unhealing wound. This treatment resulted in the rapid healing of these unhealing wounds by promoting granulation tissue formation, angiogenesis, extracellular matrix secretion, wound contraction, re-epithelialization, regeneration of hair follicle and sebaceous gland, and reducing scar formation. Furthermore, we demonstrated that the hydrogel enhanced BMSCs' TGF-β1 and bFGF secretion, thereby producing a series of beneficial biological events.

References: Shi J. J. Mater. Chem. 2012; 22:23952-62.