Adipose-derived Mesenchymal Stem Cells treatments for fibroblasts of fibrotic scar via Down-regulating TGF- β1 and Notch-1 Expression enhanced by Low Level Laser Treatment

Wounds of skin often heal with scar formation. Generally, adverse scar (keloid or hypertrophic scar) formation is characterized with negative cosmetic and psychological effects. Various therapeutic approaches have been studied for keloid and hypertrophic scar, which include occlusive dressings, local injection, surgical excision, etc. Some identified genetic, systemic, and local factors make contributions to the pathogenesis of keloid and hypertrophic scar. And local mechanical forces also play a significant role in the formation of adverse scar. Previous studies demonstrate that epithelial-mesenchymal transition (EMT)and myofibroblast transition plays an important role in aberrant scar formation, which can be induced by a regulatory network formed by multiple signaling pathways, especially transforming growth factor beta 1 (TGF-β1) as a critical driver. Similarly, TGF-β1- induced EMT can also be meditated by other signaling pathways through direct interactionsor transcription. Notch signaling, an evolutionarily conserved pathway that regulates multiple cellular process, was proved to play a key role in regulating fibroblast activation and EMT. And interestingly, a great body of evidence supports that TGF-β and Notch signaling regulate EMT and fibrogenesis cooperatively. However, understanding into the keloid and hypertrophic scar pathogenesis is still lacking, which contributes to less proper treatment strategies. As lately studies show that formation of these abnormal scars share several pathological processes with other fibrotic diseases, and may occur as a result of fibroproliferative disorder. Mesenchymal stem cells (MSCs) has been a potential treatment forf ibroproliferative disorder by recruitment of macrophage and T cell, upregulation of vascular endothelial growth factors and initiation of angiogenesis, secretion of anti-fibrotic factors, promotion of dermal fibroblast functions, and differentiation into cutaneous cell types.Among the MSCs, adipose-derived mesenchymal stem cells (AMSCs) with advantages of abundant in source of tissue, easy to harvest, minimal donorsite injury, and fine histocompatibility has drawn attention from scientists. Recently, evidence support that use of AMSCs can remodel fibrotic matrix in a scar, as well as reduce the proliferation, extra cellular matrix (ECM) production, and con- traction of myo fibroblasts. Also, levels of profibrotic factor TGF-β1 decreased while pro-angiogenic factors vascular endothelial growth factor (VEGF) increased. Together, we surmise that growth factors and cytokines of AMSC have the potential to prevent or even to reverse fibrosis. Yet the mechanisms of how AMSCs work in treatment of fibrosis have not been thoroughly investigated.

Photobiomodulation therapy (PBMT), used to be known as low level laser treatment (LLLT), or low level light treatment (LLLT) is considered as a laser or light with low power and energy, when act on tissues, which can induce positive photobiological processes in cells compared to the same light delivered at high doses. At molecular and cellular level, photons emitted by PBMT devices are absorbed by mitochondria, and then the production of ATP is elevated, yet levels of ROS re- mains low, which paly a pivotal role in activation of transcription factors, to produce a number of gene transcript products responsible for the positive effects of PBMT . Given that laser biostimulation theory, PBMT was used in many kinds of cells to investigate its influence on cell proliferation and differentiation. Among those cells, AMSCs and other kinds of mesenchymal stem cells have been frequently investigated .Studies have shown that PBMT might generate stimulatory benefits of AMSCs, such as regulating cell secretion, promoting cells proliferation, differentiation and migration, enhancing immunological functions, and therefore accelerating tissue repairing .However, the parameters of lasers used in previous studies have varied, and various effects have been observed when differing type, wavelength, energy density, and power were applied. Recent evidence has suggested that AMSCs exposed to PBMT at 5 J/cm^2 caused pronounced cell proliferation and secretion . Therefore, we speculate that PBMT could enhance AMSCs' treatment potential for fibrotic scar.

Therefore, in our study, we analyzed the key proteins concerned TGF-β and Notch signaling in vitro, to test the effects of conditioned medium gathered from post-PBMT AMSCs (PBMT-AMSCs-CM) on the fibrotic phenotypes associated with hypertrophic scar fibroblasts (HSFs) and keloid fibroblasts (KFs).The outcomes demonstrated that PBMT-AMSCs-CM inhibited cell proliferation and down-regulation of the profibrotic growth factor and collagen synthesis associated with HSFs and KFs, especially KFs. Based on our current results, we presume that the inhibiting effect is related to downregulating TGF-β1 and Notch-1 expression.