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PROTEOMIC ANALYSIS TO IDENTIFY ROLE OF NASAL FIBROBLAST SECRETOME IN AIRWAY EPITHELIAL WOUND HEALING

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SUMMARY

The airway epithelium functions to moisten and protect the airways as well as being the primary absorption site for inhaled respiratory drugs. The nasal fibroblast secretome, which comprises various cytokines, chemokines, angiogenic factors, and growth factors, synergistically promote the respiratory epithelium restoration and regeneration after injury. However, critical role of nasal fibroblast secretome on respiratory epithelium wound healing have not been extensively studied. This study was undertaken to profile the proteins secreted by nasal fibroblasts, and subsequently, profile and quantitate the proteins derived from the fibroblast secretome that can enhance the wound healing of respiratory epithelium. Human respiratory epithelial cells and fibroblasts were isolated from consented patients undergoing turbinectomy and co-cultured. Fibroblasts from the co-culture were differentially trypsinized, leaving the colonies of AECs to reach confluency. For conditioned medium collection, the fibroblasts at passage 2 was cultured with F-12: Dulbecco's Modified Eagle's Medium (FD) + 10% FBS until it reach 90% confluency before the culture medium is changed to either serum free airway epithelial cell basal medium (AM) or serum free FD and maintained for three days for the collection of nasal fibroblast conditioned medium, denoted as NFCM_AM and NFCM_FD. Currently, NFCM_AM and NFCM_FD, each from 5 human samples have been collected. Optimization of seeding density showed that 7,500/cm² is the best cells seeding density to analyze the effect of the NFCM on the RECs proliferation. On the other hand, the migration assay protocol also was optimized, especially the time range taken by cells to migrate into the wound area of the airway epithelial cell layer. The conditioned medium that has been collected is currently processed to be send for mass spectrometry analysis and subsequently quantitative proteomic analysis. At the same time the pooled secretome will be used to perform the attachment, proliferation and migration assays.

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