

## UNRAVELLING THE CROSSTALK BETWEEN CELL SHEETS OF HUMAN ADIPOSE STEM CELLS AND KERATINOCYTES

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We have previously shown that 3D constructs of human adipose stem cells (hASCs) cell sheets led to the formation of *de novo* hair follicles and rete-ridges like structures. An up-regulation of keratinocyte growth factor (KGF) was also observed in the experimental condition in relation to the control groups. We hypothesized that the natural adhesive character of the cell sheets promoted the direct interaction between the host and the transplanted cells<sup>1</sup>. In this sense, the present work aims at elucidating this communication between hASCs and human keratinocytes (hKC) and determining the extent of its mediation by KGF. In an *in vitro* scratch assays we showed that the secretome of hASCs in contact with hKC promotes cell migration and closure of the scrape. Moreover, when KGF-antibody was added diminished hKC migration was observed, suggesting that KGF might be one of the key cytokines involved in the interaction with hASCs. Furthermore, in order to assess the communication via gap junctions (GJ), a calcein-AM transfer assay was carried out in the presence/absence of a GJ inhibitor. The transference of the dye from hASCs to adjacent hKC, confirmed both by fluorescence microscopy and flow cytometry, showed that these cells also communicate via GJ. Moreover while hKCs expressed connexin (cx)43 and cx26, highly expressed at the wound margins, hASCs were only positive for cx43 as shown by immunocytochemistry and flow cytometry. Finally, the direct communication between transplanted hASCs cell sheets and hKC at the wound margins is being addressed in a human ex-vivo skin model with an artificial wound, to better mimic the previous *in vivo* conditions and confirm our hypothesis. So far we were able to demonstrate that hASCs and hKCs communicate directly through cx43 and indirectly via KGF secreted by hASCs, which promote KCs migration.

- (1) Cerqueira, M. T.; Pirraco, R. P.; Santos, T. C.; Rodrigues, D. B.; Frias, A. M.; Martins, A. R.; Reis, R. L.; Marques, A. P.; Cerqueira Pirraco, RP, Santos, TC, Frias, AM, Martins, AR, Reis, RL, Marques, AP, M. T. *Biomacromolecules* **2013**, *14*, 3997–4008.

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