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Regenerative potential of outer root sheath melanocytes in tissue grafts

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Our group at the Translational Centre for Regenerative Medicine in Leipzig developed a method for melanocyte cultivation from the Outer Root Sheath(ORS) by expanding stem cells, precursors and dedicated cells and differentiating them into melanocytes. Based on this methodology, we are developing an autologous, transplantation-based, causal treatment of depigmentation disorders such as Vitiligo. In the scope of cultivation, we are tracking shifts in gene expression profiles, from pluripotency in undifferentiated cells to melanotic status in late differentiation stages of the ORS melanocyte culture.

For the purposes of melanocyte transplantation, a biocompatible, biodegradable graft carrier is needed. In order to identify candidates for biocompatible as well as mechanically stable niche, we are working on a palette of scaffolds, testing function and development of 3D cell cultures compared to 2D adherent culture.

Melanocytes were cultivated from plucked hair follicles by the means of an optimized explant method by Savkovic et al. Differentiated cells were characterized for expression of melanocyte markers and melanin production. ORS melanocytes were seeded and cultivated on different types of scaffolds– Polycaprolactone (PCL) microspunscaffold, decellularized bovine collagen backbone called Collagen Cell Carrier (CCC), as well as hydrogel produced of shortened, chemically cross-linked bovine collagen chains named Collagel – for a cultivation period of one week. Expression of melanocyte markers was assessed by immuno-fluorescence and gene expression was analyzed by the means of quantitative real-time PCR.

Gene expression of the ORS cells differentiating into melanocytes shifted from pluripotency-like profiles in early cultivation passages to melanocyte-like profiles in late passages. These results corroborate the hypothesis that the ORS melanocytes descend from the ORS stem cell and progenitor sub-pool and possess a high developmental potential within the follicle and in early passages of primary culture. ORS melanocytes showed correct melanocyte marker expression and displayed melanotic features in adherent cultures, which were potentiated on 3D scaffolds, especially on Collagel scaffolds. Their architecture and mechanical stability promote melanocyte features mimicking the native structure of the extracellular matrix, which makes them exceptionally good candidates for biomimetic graft carriers.

Biography

Marie Schneider has completed his Doctor in Translational Centre for Regenerative Medicine in 2013 and Master Thesis, in TRM Leipzig (Group Dr. Savkovic) on "Gene expression and melanotic properties of the hair follicle outer root sheath melanocytes in varying culture conditions". He is a Full Professor in the University of Leipzig, Gemany. The Bachelor Thesis was done in year 2011 Fraunhofer IZI Leipzig under the supervision of Dr. Lehmann. The High School Degree was done in Abitur during 2008.

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