Characterization of the cytokine profile of endothelial cells cultured onto dermal substitutes used for tissue bioengineering

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PURPOSE: Chronic wounds are defined as a break of the skin barrier lasting longer than 42 days. There are many risk factors for the delay of wound healing, including vascular insufficiency, ageing, diabetes, chronic diseases, nutritional deficits, neurological defects. Current treatments are not very successful, nonetheless, the use of acellular dermal matrices as skin substitutes is indicated in some patients, as these scaffolds could support the repairing process and help restoring the skin integrity. One of the key factors for the correct wound healing process is the revascularization, thus the use of a biocompatible scaffold pre-seeded with autologous endothelial cells (ECs) could support the healing [1,2]. Since leukocyte recruitment and formation of granulation tissue are essential processes for the *restitutio ad integrum* of skin [3], we evaluated whether the seeding onto different skin substitutes, already used in clinic practice, induced a modulation in cytokine and chemokine expression by dermal ECs.
We compared five commercial scaffolds for their interaction with Adult Dermal Microvascular Endothelial Cells (ADMECs) and evaluated if the 3D culture could modify the angiogenic and inflammatory behavior of cells.

METHODS: AMDECs were isolated from skin biopsies of patients undergoing reductive plastic surgery using a trypsin digestion; cells were positive selected for CD31 using magnetic beads and characterized by immunofluorescence for EC-markers. The adhesion capability was analyzed seeding fluorescent-labeled cells onto the dermal substitutes (Integra® Bilayer Matrix Wound Dressing, PELNAC®, PriMatrix® Dermal Repair Scaffold, Endoform® Natural Template, and Myriad® Matrix) at three time points and the proliferation drive labeling the cells for Ki-67 and quantifying the fluorescence by Odyssey fluorescence scanner.
To investigate the modulation of gene expression profile of pro-angiogenetic and inflammatory cytokines, we performed RT-qPCR of dermal substitute seeded ECs after 24h of culture. The protein production was evaluated by ELISA assays of the culture supernatant.

RESULTS: Adhesion assays demonstrated that Integra®, PELNAC® and Myriad® were the most pro-adhesive dermal substitute for ADMEC, and Integra® induced the greater proliferative activity. The seeding onto Integra® and PELNAC® was able to induce a pro-angiogenic drive, increasing the gene expression of the main pro-angiogenic factors (VAGF-A, PlGF, and ANGPT1) and their receptors (KDR, Flt-1, and TEK). Integra® was also able to increase the expression of TNF, IL-8/CXCL8, MCP1/CCL2 and IL-6, whereas PELNAC® up-regulated the expression of IL-8/CXCL8 and IL6 and down-regulated TNF.

DISCUSSION AND CONCLUSION: Tissue-engineered skin substitutes are essential for tissue repair, regeneration of wound and modulation of inflammatory phase. ADMECs onto Integra® demonstrated the greater adhesion and proliferation capability, and were able to produce MCP1/CCL2, a macrophage recruiting chemokine, that could stimulate the formation of the granulation tissue, a fundamental step towards the wound healing. The interaction between ADMECs and Integra® or PELNAC® induced an angiogenic drive and a controlled inflammatory response.
The combination between ECs and dermal substitutes represents a promising strategy for tissue revascularization.

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