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Monocytes co-cultured with reconstructed keloid and normal skin models skew towards M2 macrophage phenotype

Limandjaja, G.C.; Waaijman, T.; Roffel, S.; Niessen, F.B.; Gibbs, S.

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Supplemental table 1: culture media

Medium	Components
KC-I	DMEM (Lonza, Verviers, Belgium) : F12-HAM nutrient mixture + L-glut. (HAMF12; Gibco, Grand Island, NY, USA) in a 3:1 ratio with 1% UltrosesG (Biosepra, Cergy-St-Christophe, France), 1% PenStrep (Gibco, Grand Island, USA), 2ng/ml human Keratinocyte Growth Factor, 0.09 µmol/L Insulin, 1µmol/L Hydrocortisone, 1µmol/L Isoproterenol
Fibroblast medium	DMEM (Lonza), 1% UltrosesG (Biosepra), 1% PenStrep (Gibco)
FSM-I	DMEM (Lonza), 2% UltrosesG (Biosepra), 1% PenStrep (Gibco), 5µg/ml Insulin 10-3M, 50µg/ml L-Ascorbic acid and 5ng/ml Epidermal Growth Factor
FSM-II	DMEM:HAMF12 (Lonza; Gibco) in a 3:1 ratio, 1% PenStrep (Gibco), 2% UltrosesG (Biosepra), 5 µg/ml Insulin 10-3M, 50µg/ml L-Ascorbic acid and 5ng/ml Epidermal Growth Factor
KC-II	DMEM:HAMF12 Lonza; Gibco) in a 3:1 ratio, 1% PenStrep (Gibco), 0.2% UltrosesG (Biosepra), 0.1 µmol/L Insulin, 1µmol/L Hydrocortisone, 1µmol/L Isoproterenol hydrochloride, 10 µmol/L L-Carnitine hydrochloride, 0.01 µmol/L L-Serine, 1µmol/L DL-α-Tocopherol, 0.4mmol/L L-Ascorbic acid, supplemented with a lipid mixture containing 7 µmol/L Arachidonic acid, 25 µmol/L Palmitic acid, 15 µmol/L Linoleic acid and 24 µmol/L Bovine Serum Albumin
Monocyte medium	DMEM (Lonza), 10% heat inactivated FBS (HyClone®, Northumberland, UK), 1% PenStrep (Gibco)
Medium for collection of 24-hr supernatant after 5 weeks	Skin equivalents with/without monocytes, monocytes cultured in KC-II: KC-II without Hydrocortisone For monocyte monocultures cultured with monocyte medium: monocyte medium

Supplemental Table 1: shows an overview of all the different culture media used for the construction of the skin models and monocytes co-cultures. All reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA) unless otherwise specified. Whilst in monoculture, keratinocytes and fibroblasts were cultured in KC-I and fibroblast medium respectively. The skin models with or without monocytes were cultured in FSM-I (for fibroblast seeding into MatriDerm®), FSM-II (t = 0-3 weeks after seeding), KC-I (for 3 days after seeding of keratinocytes onto the fibroblast-populated MatriDerm®) and KC-II (from air-exposure until end of culture) subsequently; see timeline in fig. 2A. Mono-cultured monocytes were also cultured in the same medium as the skin models: with the first batch of monocytes (cultured during week 1–3) cultured in FSMI-II and the second batch (cultured during week 3-5) cultured briefly (3 days) in KC-I, but mostly in KC-II medium. As an additional control group, monocytes were also cultured in their own monocyte medium.

Supplemental table 2: Flow cytometry antibodies

FL-1	FL-2	FL-3	FL-4
CD1a (Fitc) Mouse monoclonal IgG1 555806 BD Biosciences	Langerin (PE) Mouse monoclonal IgG1 PN IM3577 Beckman Coulter	CD14 (PerCp) Mouse monoclonal IgG2b 345786 BD Biosciences	CD11c (APC) Mouse monoclonal IgG2b 333144 BD Biosciences
CD40 (Fitc) Mouse monoclonal IgG1 555588 BD Biosciences	CD11c (PE) Mouse monoclonal IgG1 12-0116-41 eBioscience	CD206 (PerCp-Cy5.5) Mouse monoclonal IgG1 321122 BioLegend	25F9 (effluor660)* Mouse monoclonal IgG1 50-0115-41 eBioscience
CD34 (Fitc) Mouse monoclonal IgG1 555821 BD Biosciences	CD68 (PE) Mouse monoclonal IgG2b 556078 BD Biosciences	LSP-1 (PE-Cy7)** Rabbit polyclonal IgG bs-5154R-PE-Cy7 Bioss,	Collagen I (APC)** Mouse monoclonal IgG1 NBP1-05171 Novus Biologicals + Goat anti-Mouse IgG APC 731857 Beckman Coulter
CD45 (Fitc) Mouse monoclonal IgG1 555482 BD Biosciences	α -SMA (PE)** Mouse monoclonal IgG2a IC1420P R&D systems	CD45RO (PE-Cy5)* Mouse monoclonal IgG2a 555494 BD Biosciences	CD56 (APC) Mouse monoclonal IgG2a Ab28335 Abcam
MRP8/14 (Fitc)* Mouse monoclonal IgG1 BM40251 Acris antibodies	<i>PM-2K*</i> <i>Mouse monoclonal IgG1</i> <i>Ab58822</i> + <i>Goat anti-Mouse IgG1</i> <i>(PE)</i> <i>Ab99912</i> <i>Abcam</i>	CD90 (PerCp-Cy5.5) Mouse monoclonal IgG1 45-0909-41 eBioscience	
Fibronectin (AF488)** Mouse monoclonal IgG1 563100 BD Biosciences	CD19 (PE) Mouse monoclonal IgG1 561741 BD Biosciences	CD123 (PerCp-Cy5.5) Mouse monoclonal IgG1 306016 BioLegend	
CD3 (Fitc) M1466 Pelicuster Sanquin			

Supplemental table 2: Antibodies used for the immunophenotyping of the co-cultured monocytes by way of flow cytometry, followed by clonality, catalogue number and manufacturer. Italicized text: two-step staining procedure; * stained intracellularly and extracellularly, ** always stained intracellularly. Corresponding isotype antibodies were used as a control for each of the listed antibodies.