**Supplementary Information 1**

Complete list of keratinocyte differentiation protocols using human iPSCs or ESCs with functional outcomes reported in the experiment and variations in the ECM utilized, supplementing compounds, and the medium employed.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Cell type** | **Medium composition** | **Growth factors & compounds** | **ECM** | **Epidermal proteins analyzed** | **Functional analyses** | **Reference (s)** |
| 1. **Embryonic stem cells (ESCs)**
 |
| H9, I3, I6 | DMEM/Ham's F12 1:1 or FAD | BMP4 | PA6 cells fixed with 4% Formaldehyde  | p63, K18, K14 | N. D. | (Aberdam et al., 2008) |
| H9 | FAD  | BMP4 | MEF fixed with 4% Formaldehyde. Collagen I | K18, K12, Col IV | 3D Skin equivalents | (Hewitt et al., 2009) |
| H9, H13, H14; hiPSCs | DMEM/Ham's F12 1:1; D-KSFM | SU6656 (SFK: Src Family Kinase inhibitor); RA, BMP4 | MEF feeders; Matrigel | K8, K18, K14, K3; K10, IVL when Calcium was increased to 1mM |  | (Lian et al., 2013) |
| H1, H9 |  D-KSFM | RA, BMP4 | Gelatin, Matrigel | p63, K18, K14; IVL, FLG, K10  | 3D Skin equivalents | (Metallo et al., 2008; Metallo et al., 2010) |
| H1 | a) FAD, D-KSFM; b) D-KSFM | a) AA, RA; 20 days later activin A b) RA | a) H1-derived Fibroblasts; Collagen IV b) Decellularized H1-derived Fibroblasts + Ficoll as crowding agent; Collagen IV | p63, K14 | N.D. | (Movahednia et al., 2015) |
| ESC KCL034; hiPSCs  | mTESR1; FAD; D-KSFM; EpiLife + S7 supplement | RA, BMP4 | 3D decellularized Human dermal Fibroblasts; Collagen IV | p63, K14, Desmocollin 1 (DSC1), IVL, Filaggrin (FLG), Loricrin (LOR) | 3D skin equivalents | (Petrova et al., 2014) |
| hESCs; hiPSCs |  StemXVivo Ectoderm kit | BMP4, DAPT, SB431542 |   | K18, K8, K19, p63, AP-2a, AP-2g |  N.D. | (Qu et al., 2016) |
| H1 | DMEM/F12 (1:1) until D10; Thereafter D-KSFM for KC differentiation | BMP4, FCS +/-DAPT | Matrigel, Collagen I  | K18, p63, K14 | Transgenic mice | (Tadeu and Horsley, 2013; Tadeu et al., 2015) |
| H9 | KSFM | RA, BMP4, AA | Matrigel | K14, p63 | tissue-engineered skin | (Zhao et al., 2020) |
| H1 and H9; iPSCs (ND1-4, NL-1, NL-4) | DMEM/F12 | SB431542 (D0-6), CHIR99021 (D1-5), BMP4 (D1-8), DAPT (D4-8), BMP4/DAPT/EGF (D9-11) | Vitronectin; albumin-free conditions | p63, K1, K10, K14 | KC transplanted to mouse excisional wound model | (Zhong et al., 2020) |
| 1. **Induced pluripotent stem cells (iPSCs)**
 |
| iPSCs | NOD Cg-Prkdc-scid IL2rgtm1Wjl/SzJ mice (NSG) |   | Teratoma | p63, K5, K10, IVL, FLG, Col VII,  | Cells collected from teratoma. Skin equivalents grafted onto mice  | (García et al., 2016) |
| hiPSCs |  D-KSFM; KSFM | RA, BMP4 | Initially Matrigel. After passaging, Fibronectin and Collagen | p63, K14, DSG3, K1, COL7A1, Laminin 5, LOR | 3D Skin equivalents | (Itoh et al., 2011; Kajiwara et al., 2017; Matsumura et al., 2018) |
| hiPSCs | D-KSFM; CnT-07 after D4 | RA, BMP4 | Matrigel | H&E staining | 3D skin equivalent | (Itoh et al., 2013; Gledhill et al., 2015; Domingues et al., 2017; Matsumura et al., 2018) |
| hiPSCs | D-KSFM | RA+BMP4 until D4; hEGF, ROCKi | Vitronectin, Fibronectin + Collagen I | p63, K14, Laminin 5, K10, IVL,  | 3D skin equivalents | (Kajiwara et al., 2017) |
| hiPSCs | DMEM:F12; D-KSFM; D-KSFM:KSFM | RA, BMP4, EGF | Collagen IV | p63, K5, K14 | 3D skin equivalents; Xenograft onto NOD/SCID mice | (Kim et al., 2018) |
| hiPSCs | hEB (after D4); D-KSFM (after D6) ; CNT-07 after D30 | RA, BMP4, ROCKi (until D10) | Collagen IV | p63, K14, ITGA6, ITGB4, DSC3, DSG3 | iPSC-derived KC with characteristics of basal epidermal KCs. | (Koch et al., 2022) |
| hiPSCs | D-KSFM; CnT-07 | RA, BMP4 | Geltrex + Collagen I => Col I + Col IV | K14 |  - | (Kogut et al., 2014) |
| hiPSCs | D-KSFM | RA, BMP4 |   | K8, K18, p63, K14,  | Skin equivalents | (Larribere et al., 2017) |
| hiPSCs | D-KSFM; CNT-07 (after D9) | RA and BMP4 (until D9); ROCKi [Y-27632] (after D28) | Matrigel; collagen (after D28) | p63, K14, K10, IVL, ITGB4, K5 | 3D skin equivalents | (Sah et al., 2021) |
| h 209.2 PSC | D-KSFM; CNT-07 after D4 | RA, BMP4 (until D4); ROCKi Y-27632 (after D10) | Fibronectin, Col IV | p63, ITGA6, INTβ4, COL17A1; also,　C1ORF68, LCE2B, TGM1, TGM2 | ESPCs exhibited basal cell phenotype | (Ruiz-Torres et al., 2021) |
| hiPSCs | FAD D4, N2 D7; D-KSFM | RA+ROCKi D2; RA+BMP4 D7;  | Gelatin => MEF=> CELLstar | K18, p63, K14 | 3D skin equivalents; Mouse skin xenografts | (Sebastiano et al., 2014) |
| hiPSCs | FAD | BMP4, Ascorbic acid, TGF-b inhibitor (SB431542) | 3T3 feeders | p63, K14, K18 |  - | (Shalom-Feuerstein et al., 2013) |
| hiPSCs | D-KSFM or CnT-07 until D22-28 =>CnT-57 | RA, BMP4 |   | DSG3, Col VII, Desmoplakin, K1, K5, K14, LOR | 3D skin equivalents In vivo reconstituted skin on SCID mice | (Umegaki-Arao et al., 2014) |
| hiPSCs | D-KSFM | RA, BMP4 | Collagen I | K18, TP63, K14 | HF morphogenesis in vivo in patch assay | (Veraitch et al., 2013) |
| hiPSCs | 1. FAD.2. D-KSFM; 3. KSFM | Initially low BMP4, followed by RA, then RA+ BMP4+EGF, then BMP4+EGF, finally EGF alone | Matrigel. Differentiation on 3T3 feeders | CD200, ITGA6, K15, K5, K14, ITGB1, K1, K10 | Folliculogenic potential: 3D-skin equivalents; HF patch assay; Reconstitution assay (Si chamber). Permeability assays (toluidine blue dye) | (Yang et al., 2014a; Yang et al., 2014b)  |
| 1. **Organoids**
 |
| hiPSCs | KCs: D-KSFM; Cnt-Prime Epithelial Medium | Spheroid: D-KSFM; D-KFSM+EGF, BPE; D-KSFM+supplements; EBM; EGM | RA, BMP4 (until D6); KGF stabilized mRNA (D0 and D2) | - | K1, K10, K14, VIM | Organoids, xenotransplantation of skin wounds in mice | (Ebner-Peking et al., 2021) |
| hiPSCs | KCs => DMEM:F12 (D0-8); D-KFSM (D9-12); D-KFSM:KSFM (D13-30) | Organoid => DMEM:F12 (3:1) (D5-7); DMEM:F12 (1:1) (D8-9); F12 (after D10) | RA, BMP4, EGF (until D12); BMP4, EGF (from D13-30) | Col IV (KCs); Col I (organoid) | p63, K5, K14, LOR | 3D skin organoid, engraftment in mice (cord blood mononuclear cells-derived iPSCs) | (Kim and Ju, 2019) |
| 1. **Patient-derived induced pluripotent stem cells**
 |
| hiPSCs (psoriasis patients)  | DMEM/F12; DMEM/12+N2 (after D5); N2 (after D7) | RA, BMP4 (until D5); EGF (after D5) | Geltrex | p63, K1, K14, K18, LOR, IVL, LAM | KCs derived from PsO-iPSCs with dysregulated transcripts associated with psoriasis and KC differentiation | (Ali et al., 2020) |
| hiPSCs (Gorlin patients) | D-KSFM | RA, BMP4 (until D4); EGF (until 1st passage); EGF, ROCKi [Y-27632] (after D4) | Matrigel (until D4); Col I, vitronectin, i-Matrix (after 1st passage) | K14, ITGB4, p63 | N. D. | (Morita et al., 2021) |
| hiPSCs (RDEB patients) | E6; D-KSFM (after D7) | RA, BMP4 | Vitronectin | K14 | 3D skin equivalents; engraftment in mice | (Rami et al., 2020) |
| hiPSCs (ARCI1, ARCI2, TTD1, WT2) | KCM-mod | BMP4, RA (until D7) | 3T3  | p63, K5, K14, K18, IVL | basal keratinocyte-like cells derived from non-syndromic autosomal recessive congenital ichthyosis | (Lima Cunha et al., 2021) |
| hiPSCs (trisomy 21 rescued disomy 21) | D-KSFM | RA, BMP4; EGF (after D4); EGF + ROCKi [Y-27632] (after D14) | Vitronectin; Col I, fibronectin (after D14) | K10, K14, p63, IVL, LOR, FLG, ITGB4 | 3D skin equivalent  | (Tanuma-Takahashi et al., 2021) |
| hiPSCs (RDEB patient-derived iPSCs) | E8; D-KSFM (after D7) | RA, BMP4 (until D7) | Vitronectin | K14, K10, LOR | 3D skin equivalents; engraftment in mice | (Jacków et al., 2019) |

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