

Materials List for:

# Improving 2D and 3D Skin *In Vitro* Models Using Macromolecular Crowding

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## Materials

Name	Company	Catalog Number	Comments
Ascorbic acid	Wako	013-12061	Cell culture media additive
Cell culture inserts (6-well format)	Greiner Bio-One	657610	Organotypic cultures
Citrate solution	Dako	S2369	DakoCytomation Target Retrieval Solution Citrate, pH 6 (x10)
Collagen (rat tail)	Corning	354236	Organotypic cultures
CnT-57	CELLnTEC	CnT-57	Cell culture media
DAB Substrate + chromogen kit	Dako	K3468	Histology
Deep-well plate (6-well format)	Corning	355467	Organotypic cultures
ECL detection reagent	GE Healthcare Life Sciences	RPN2106	Amersham ECL Western Blotting Detection Reagent
Electron microscope (TEM)	JEOL	JEM-1010 (100kV)	Transmission electron microscopy
Fibroblast media (FM)			High Glucose-DMEM + 10% Fetal Bovine Serum + 1% Penicillin Streptomycin
Ficoll PM70	GE Healthcare Life Sciences	17-0310-05	Macromolecular crowder
Ficoll PM400	GE Healthcare Life Sciences	17-0300-05	Macromolecular crowder
Keratinocyte serum-free media (KSFM)	Life Technologies	17005-042	Cell culture media
Lysis buffer	ThermoFisher Scientific	89900	Lysis buffer for protein extraction. A protease inhibitor (Roche, #11836170001) was added to this lysis buffer.
Microscope	Zeiss	LSM510	Red, green and blue channels to visualize AF594, AF488 and DAPI fluorescent immunostainings (40X mag).
Mounting media (Hydromount)	National Diagnostics	HS-106	Fluorescent staining
Mounting media (Cytoseal)	ThermoFisher Scientific	8310-16	Histology (HRP)
OCT compound (Tissue Tek)	Sakura	4583	Embedding for cryotomy
Penicillin-streptomycin antibiotics	Sigma Aldrich	A5955	Cell culture media additive
<b>Primary antibodies</b>			
Anti-Collagen I antibody	Abcam	#ab6308	
Anti-Collagen Type IV	Novocastra	#NCL-COLL-IV	
Anti-collagen VII			LH7.2 (in house)
Anti-Fibronectin antibody	Abcam	#ab2413	
Reducing agent	ThermoFisher Scientific	NP0009	Western blot
Sample buffer	ThermoFisher Scientific	NP0008	Western blot

<b>Secondary antibodies (Immunostaining)</b>			
4',6-Diamidino-2-phenylindole dihydrochloride (DAPI)	Sigma Aldrich	#D9542	
AlexaFluor 594 goat-anti-rabbit	ThermoFisher Scientific	#A-11037	
AlexaFluor 594 goat-anti-mouse	ThermoFisher Scientific	#A-11005	
AlexaFluor 488 chicken-anti-rabbit	ThermoFisher Scientific	#A-21441	
AlexaFluor 488 goat-anti-mouse	ThermoFisher Scientific	#A-11001	
Secondary antibodies (HRP)	Dako	Envision + System - HRP Labelled Polymer Anti-Rabbit and Anti-Mouse	undiluted
Sodium deoxycholate	Prodotti Chimicie Alimentari		Decellularization
<b>Stratification media</b>			
Dulbecco's Modified Eagle's Medium			Used during the air-liquid interface of organotypic cultures. This media is added to the outside of the cell-culture insert.
Ham's F12			Used during the air-liquid interface of organotypic cultures. This media is added to the outside of the cell-culture insert.
10% fetal bovine serum			Used during the air-liquid interface of organotypic cultures. This media is added to the outside of the cell-culture insert.
100 U/ml penicillin and 100 U/ml streptomycin			Used during the air-liquid interface of organotypic cultures. This media is added to the outside of the cell-culture insert.
0.4 mg/ml hydrocortisone			Used during the air-liquid interface of organotypic cultures. This media is added to the outside of the cell-culture insert.
5 mg/ml insulin			Used during the air-liquid interface of organotypic cultures. This media is added to the outside of the cell-culture insert.
1.8·10 <sup>-4</sup> M adenine			Used during the air-liquid interface of organotypic cultures. This media is added to the outside of the cell-culture insert.
5 mg/ml transferrin			Used during the air-liquid interface of organotypic cultures. This media is added to the outside of the cell-culture insert.
2·10 <sup>-11</sup> M triiodothyronine			Used during the air-liquid interface of organotypic cultures. This media is added to the outside of the cell-culture insert.
Trypsin	Biopolis Shared Facilities	0.125% Trypsin/Versene pH 7.0 + 0.3	Used to trypsinize cells