

Translational Centre for Regenerative Medicine (TRM) Leipzig



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M.Sc. Marie Schneider

Regenerative Potential of Outer Root Sheath Melanocytes in Tissue Grafts

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About OMICS Group Conferences

OMICS Group International is a pioneer and leading science event organizer, which publishes around 400 open access journals and conducts over 300 Medical, Clinical, Engineering, Life Sciences, Pharma scientific conferences all over the globe annually with the support of more than 1000 scientific associations and 30,000 editorial board members and 3.5 million followers to its credit.

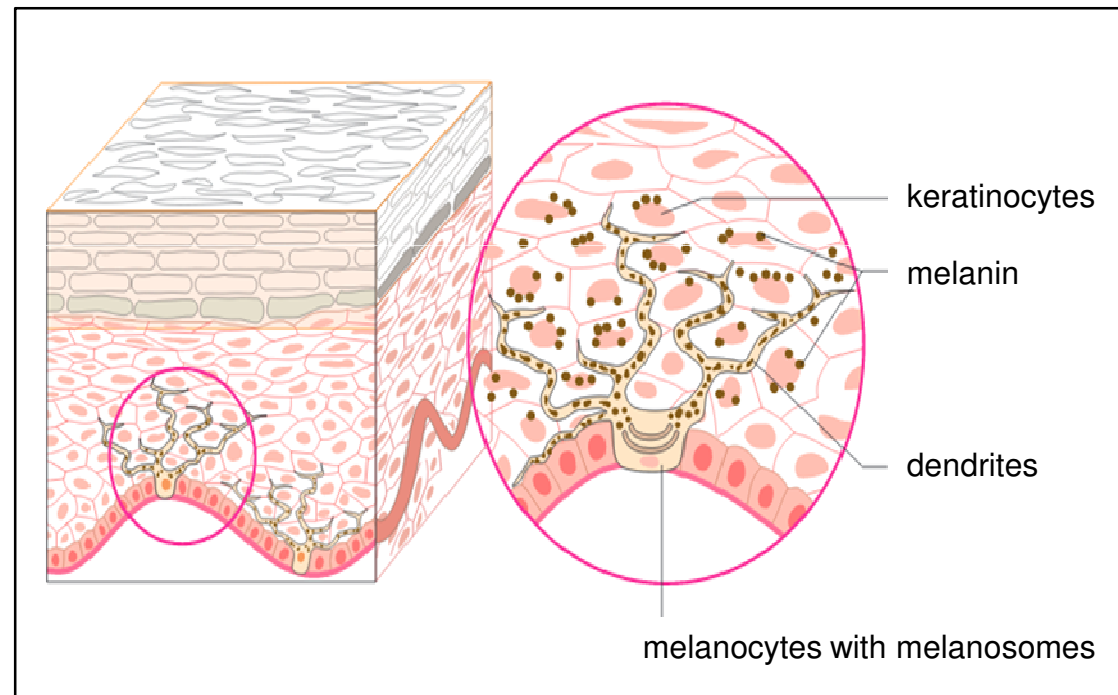
OMICS Group has organized 500 conferences, workshops and national symposiums across the major cities including San Francisco, Las Vegas, San Antonio, Omaha, Orlando, Raleigh, Santa Clara, Chicago, Philadelphia, Baltimore, United Kingdom, Valencia, Dubai, Beijing, Hyderabad, Bengaluru and Mumbai.

Depigmentation disorder Vitiligo

- prevalence: 1%
 - caused by a **lack of functional melanocytes in epidermis**
 - ➔ **no melanin**
- genetic defects
 - autoimmune reactions
 - reactive oxygen species



Indian J Dermatol. 2011; 56: 180-189.



Sterry, W. Kurzlehrbuch Dermatologie; Thieme: Stuttgart, 2011.

➤ phototherapy



Arch Dermatol. 2004;140:677-683

➤ topical or systemic immune modulation/suppression

- disadvantages: only temporary repigmentation/ can't be given for longer than 6 months
not causal

Treatments of Vitiligo

... only grafting of melanocytes is causal

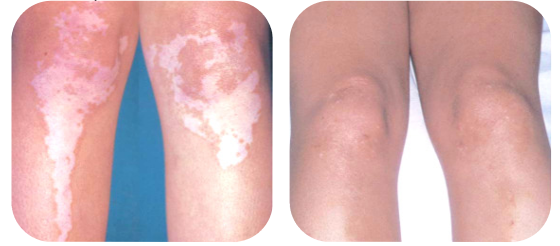
invasive epidermal biopsy



Van Geel et.al, Arch Dermatol. 2004;140:1203-1208

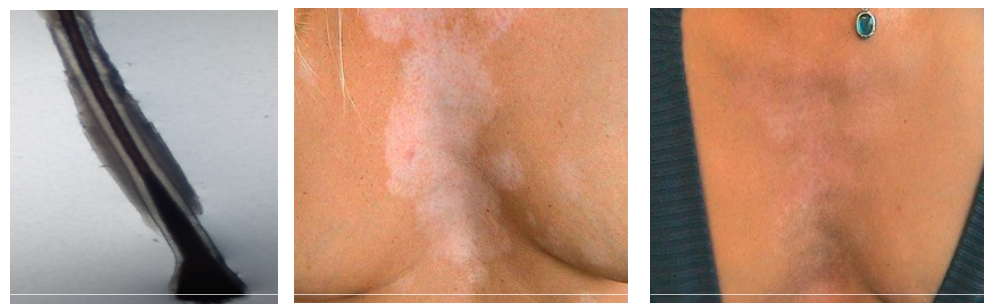


Mulekar et. al, Dermatol. Surg. 2009;35:66-71



Guerra et. al, Arch Dermatol. 2003;139:1303-1310

non-invasive hair root

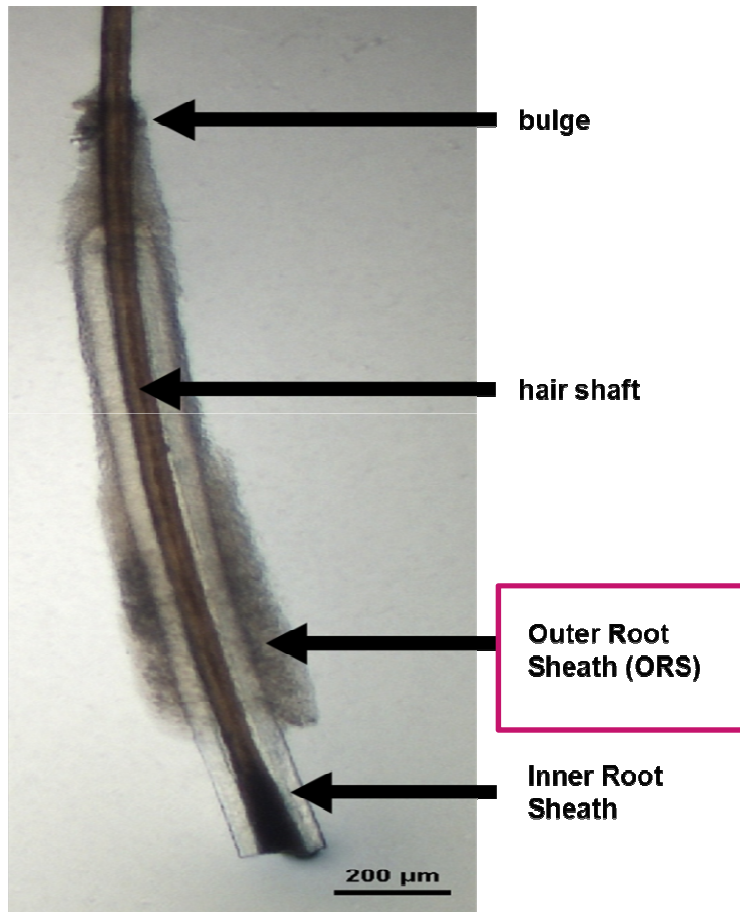


Vanscheidt & Hunziker, Dermatology 2009;218:342-343

disadvantages: 30% rejection due to mucosa
wound healing
need of post-operative care

➔ **non-invasive
causal
efficient**

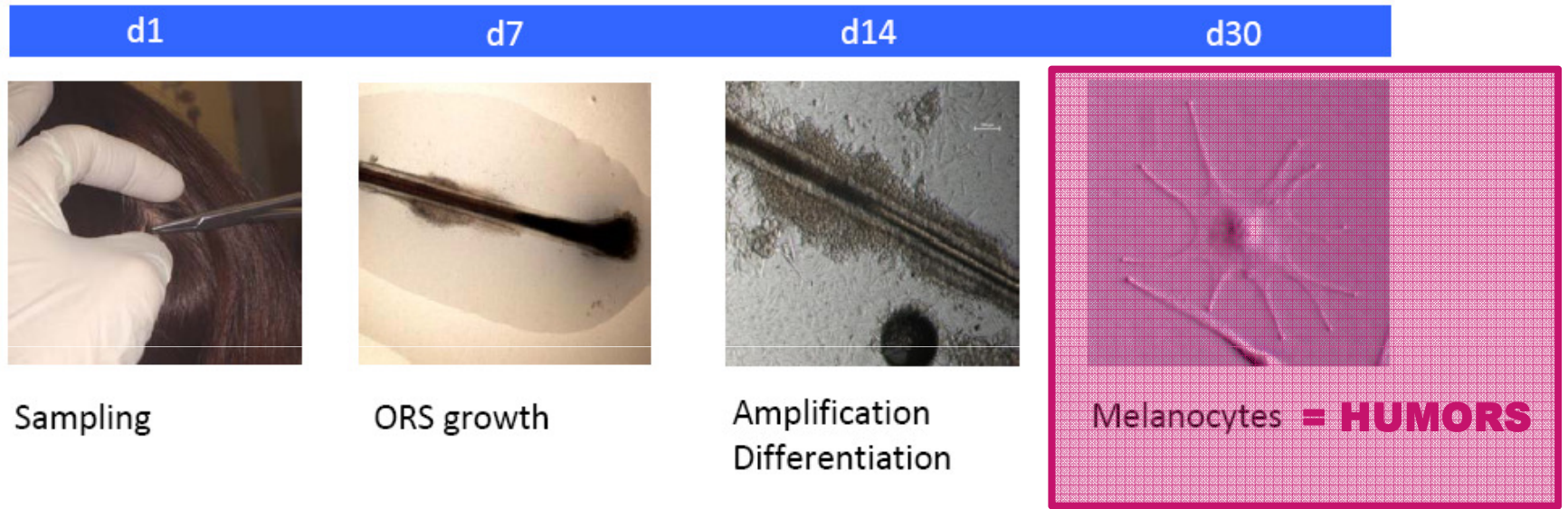
Hair follicle – Outer Root Sheath (ORS)



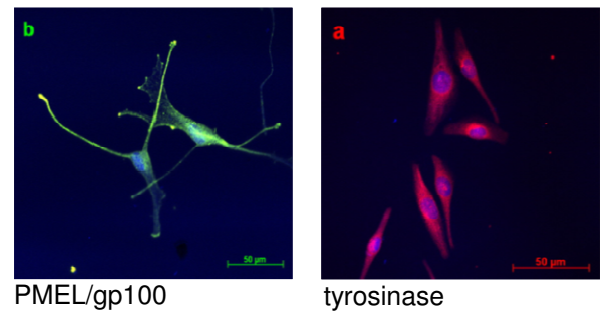
- Outer Root Sheath of hair follicle harbours pluripotent adult stem cells, transiently amplifying cells, precursors and differentiated cells
- more than ten cell types out of hair follicle
- **Outer Root Sheath (ORS)** as **regenerative reservoir**
- **non-invasive** source for regenerative therapies

Hair follicle – Outer Root Sheath (ORS)

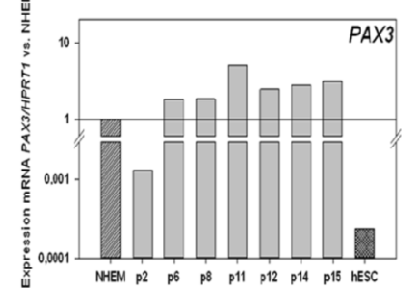
→ *patented Explant-method from Savkovic et al.*



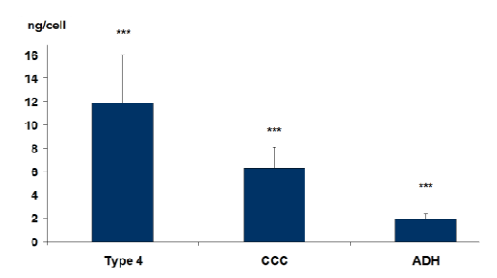
protein expression



gene expression



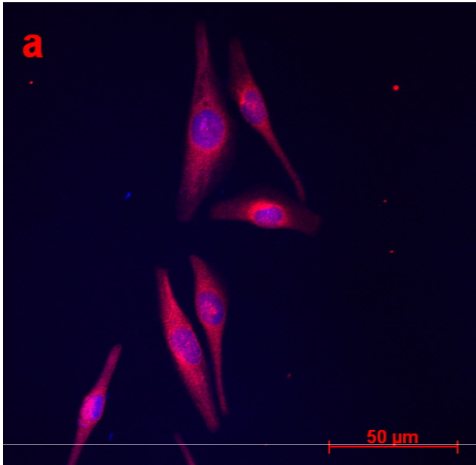
melanin content



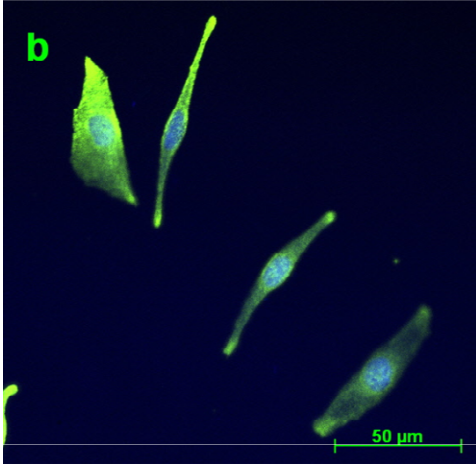
(Schneider et.al) Methods Mol Biol. 2014;1210:203-27
Patent WO 2013060899 A2.

Expression of Tyrosinase and gp100 in HUMORS

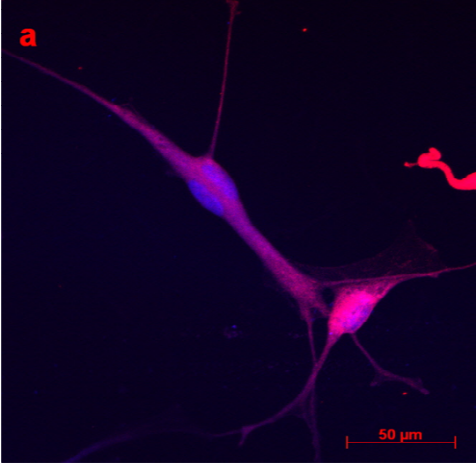
tyrosinase
HUMORS



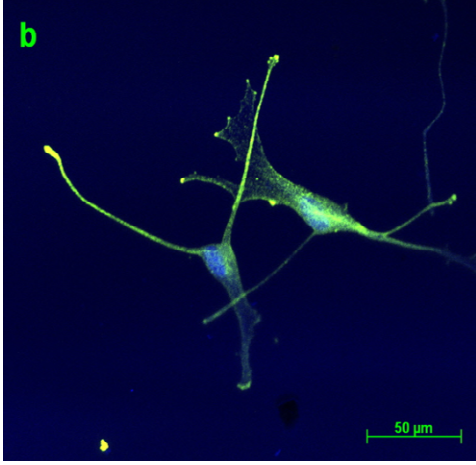
PMEL
gp100



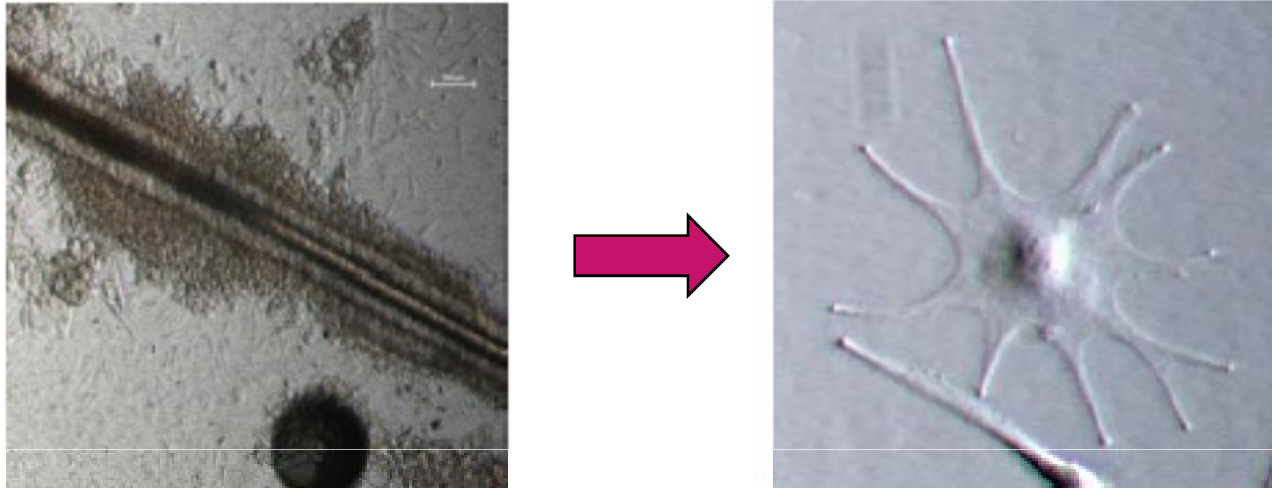
tyrosinase
NHEM



PMEL
gp100



Characterisation of heterogenous ORS mixture



heterogenous mixture in explant culture

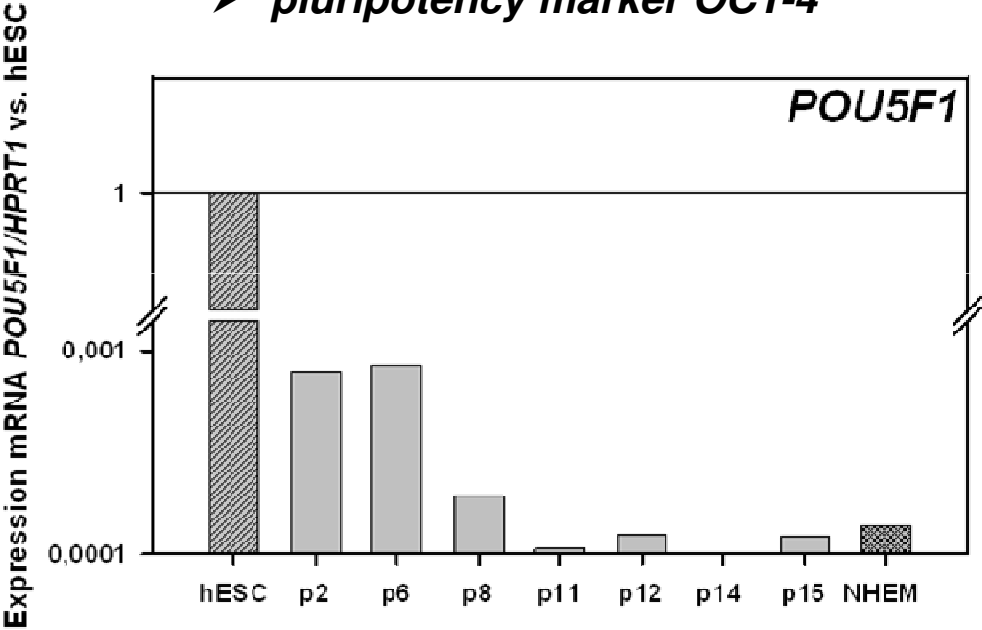
... stem cells, precursors or differentiated cells ...

... which give rise to HUMORS ?

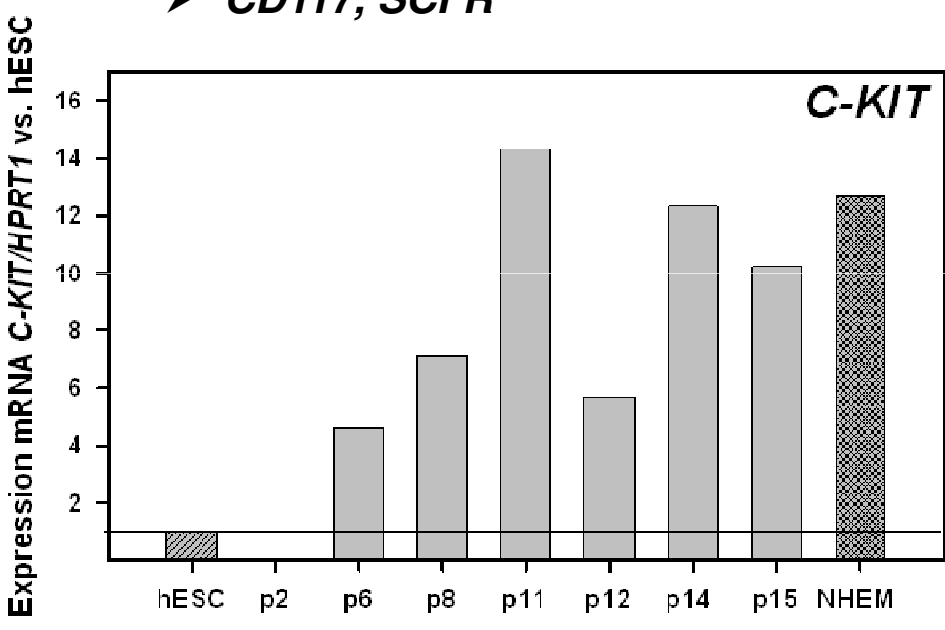
→ quantitative real-time PCR

Characterisation of heterogenous ORS mixture

➤ pluripotency marker OCT-4

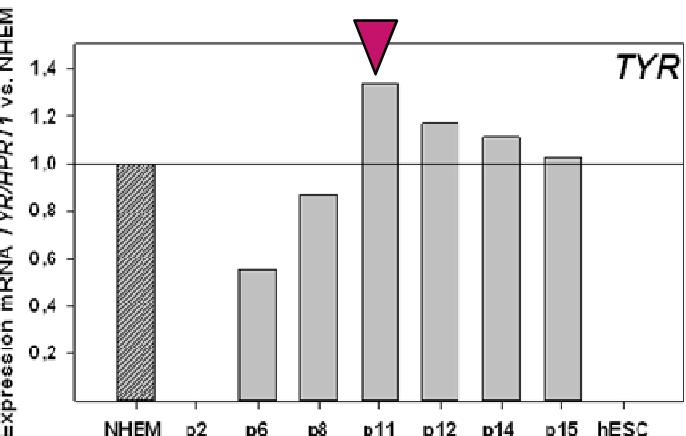
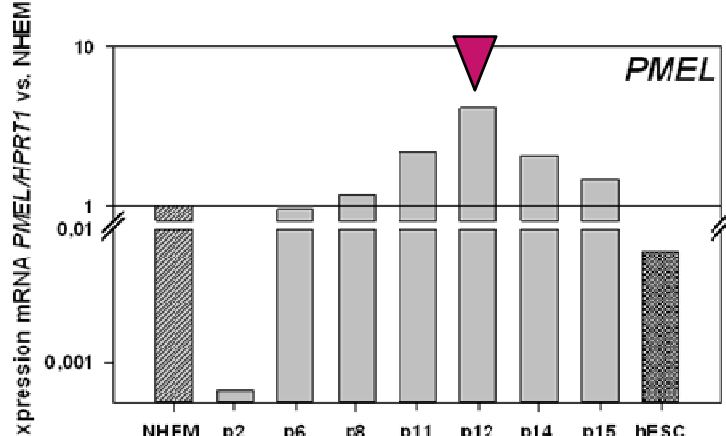
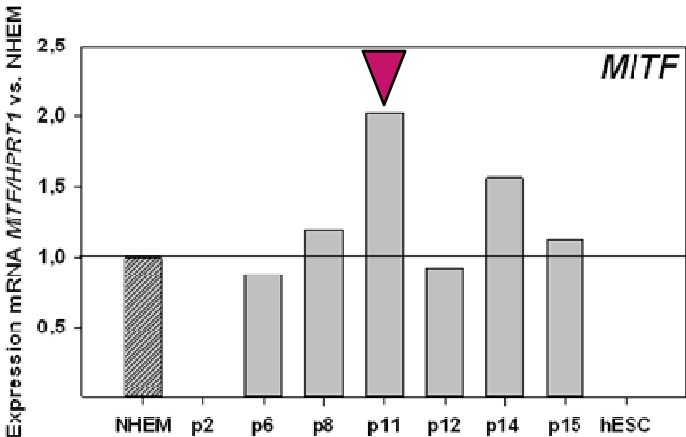
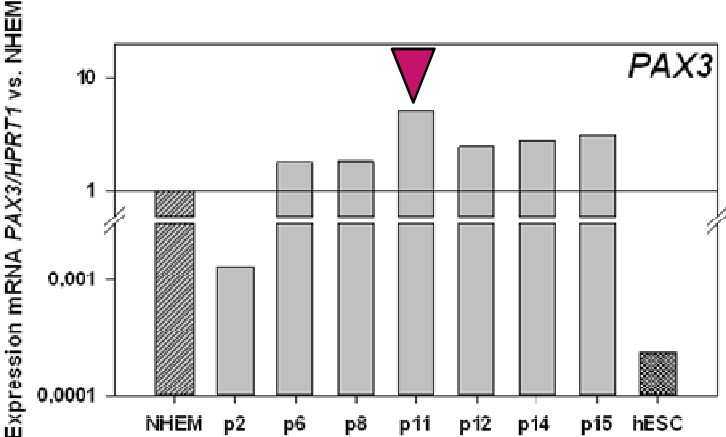


➤ CD117, SCFR

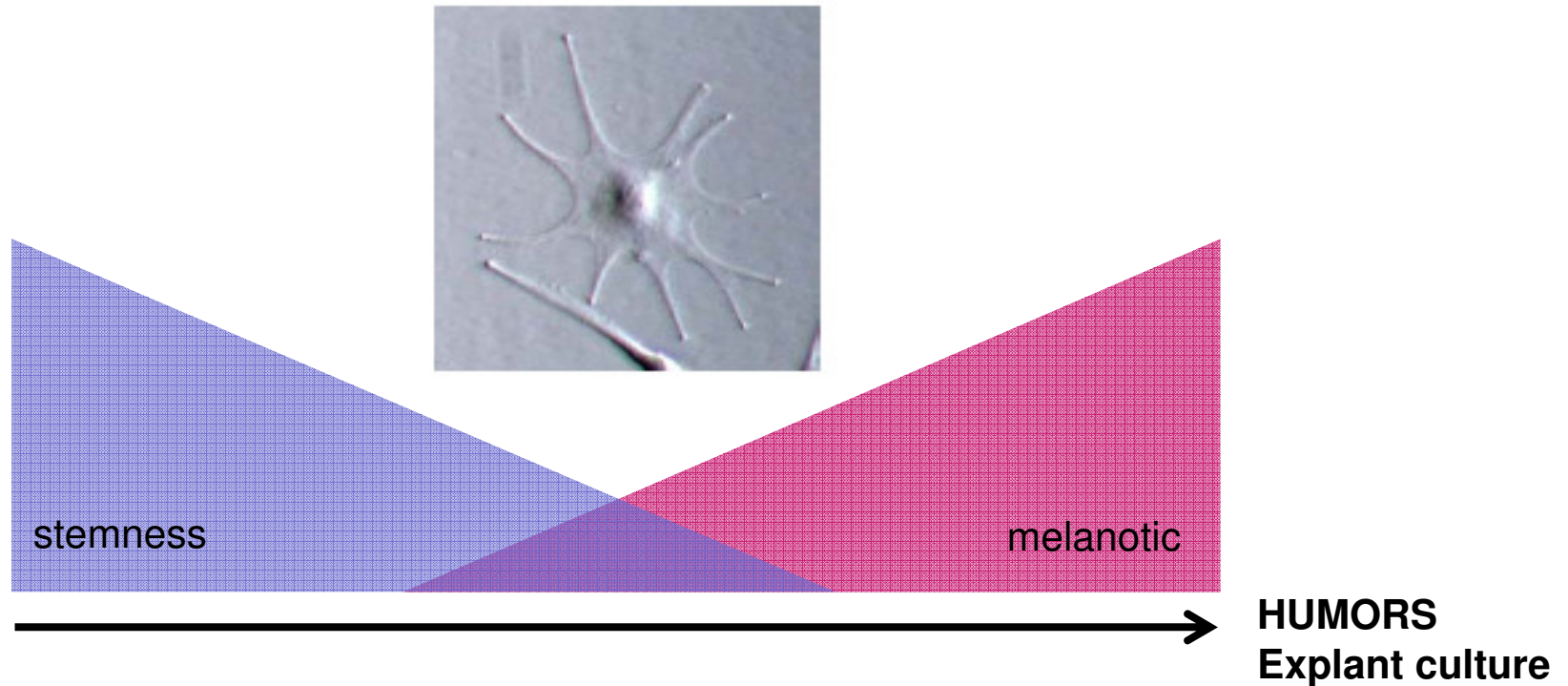


Characterisation of heterogenous ORS mixture

➤ *melanocyte marker*

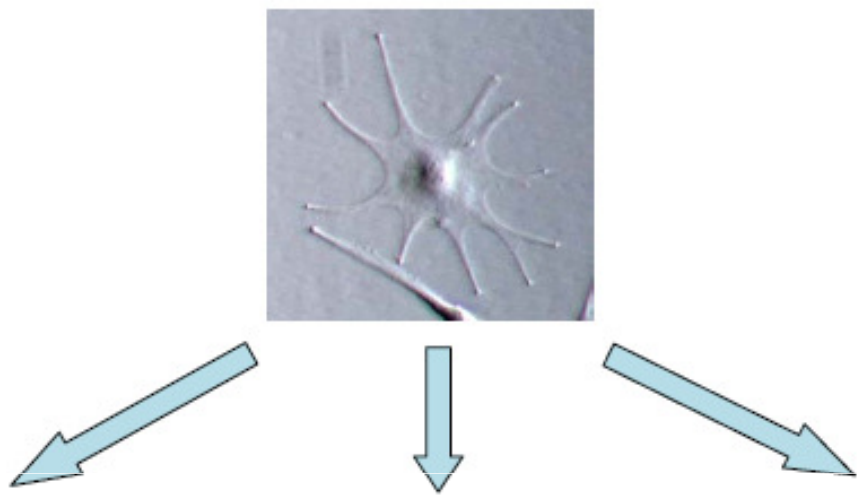


From stemness to melanotic identity



- highest expression of stemness marker in early passages in HUMORS culture
- highest expression of melanocyte marker in late passages (p10/11) in culture

Transplantation



spray, suspension

A diagram showing a blue spray nozzle on the left, emitting a fan-shaped orange spray towards a cross-section of skin tissue on the right. The spray is directed at the epidermal layer.

epidermal equivalent

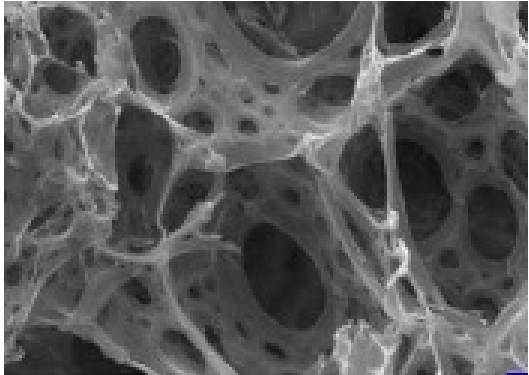
Two microscopic images are shown side-by-side. The left image is a brightfield micrograph of a skin cross-section, with a black arrow pointing to a specific layer. The right image is a fluorescence micrograph showing red and blue signals. Below the left image is the label "melanin" and below the right image is the label "tyrosinase".

scaffolds

A collage of various scaffold structures. It includes a blue and green fluorescent network, a grey fibrous mesh, a brown porous matrix, a circular pattern of cells, and a circular cross-section of a scaffold.

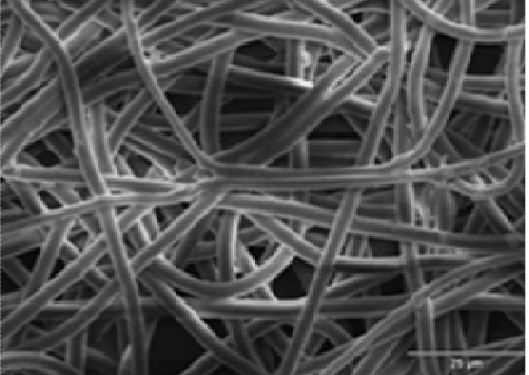
Scaffolds

Viscofan BioEngineering

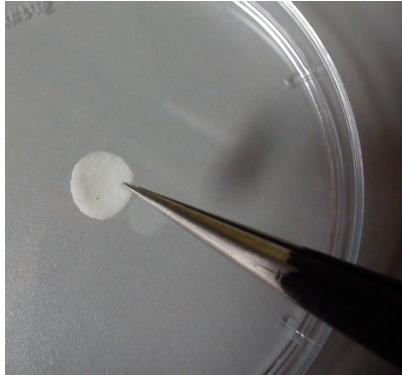


Collagen Cell Carrier – CCC

Pharmaceutical Technology University Leipzig

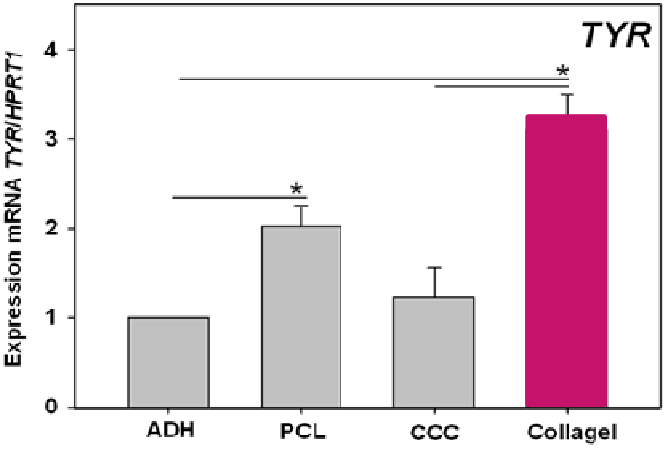
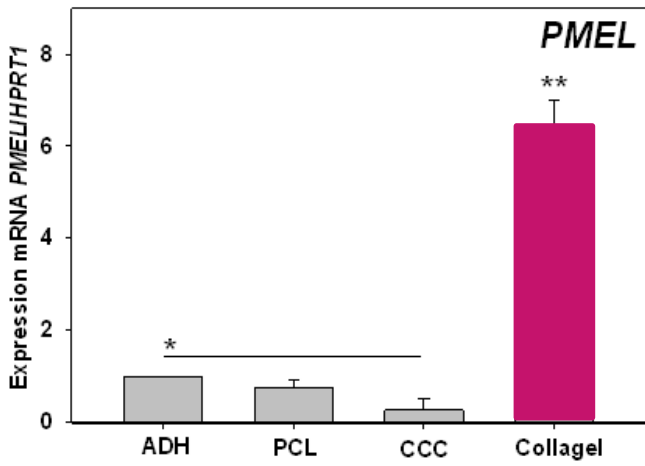
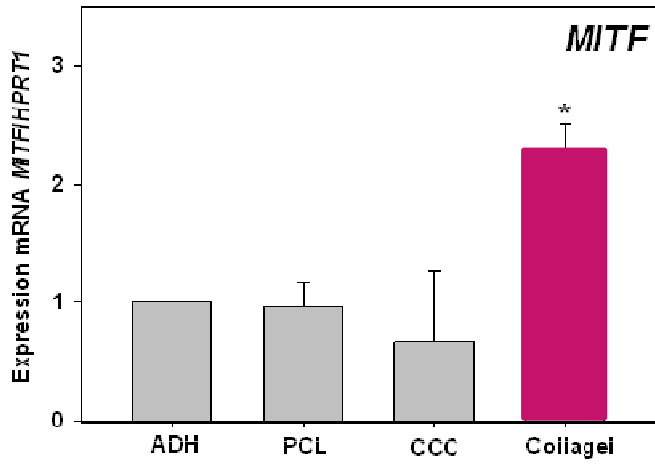
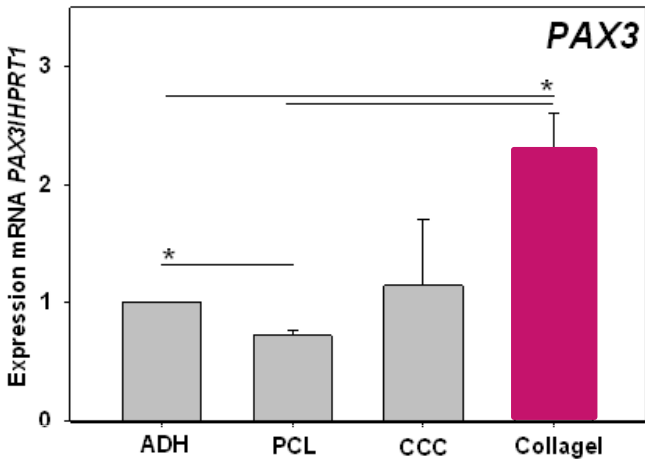


Polycaprolacton – PCL



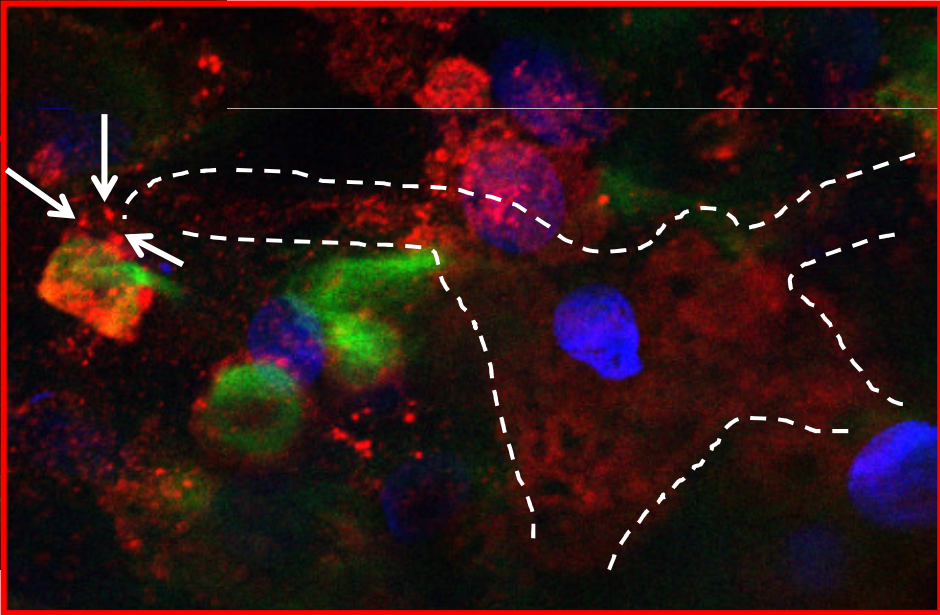
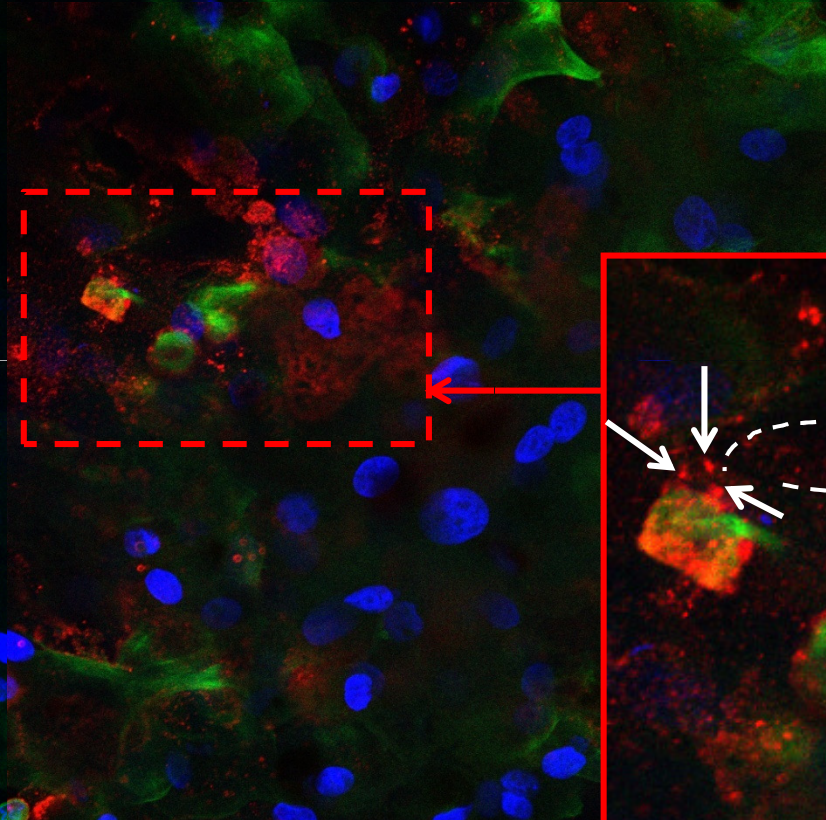
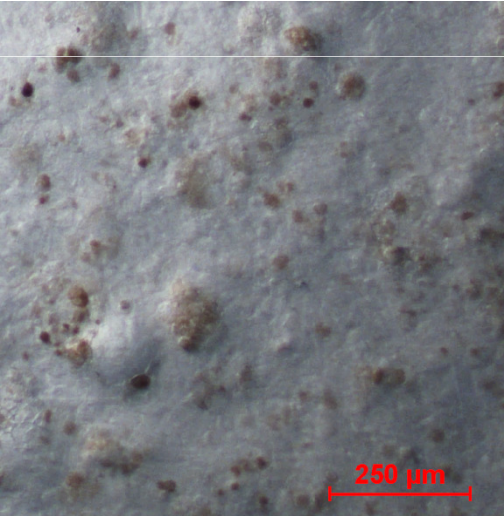
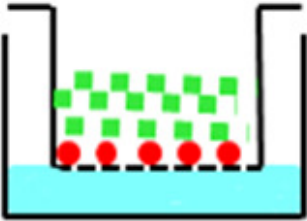
Collagel




Scaffolds

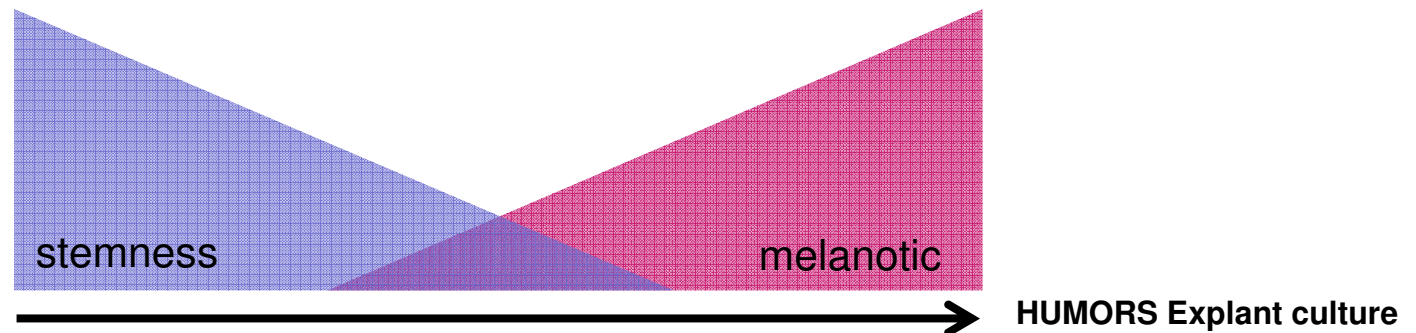


Epidermal equivalent

➤ HUMORS and keratinocytes (KORS) in co-culture (1:10)



-  pan-cytokeratin
-  PMEL
-  DAPI



- HUMORS mainly arise from stem cells and precursors due to the residual expression of stemness marker

→ lose their developmental potential and gain melanotic identity

- Collagel scaffolds provide a superior niche for melanocytes compared to the CCC, PCL-scaffold and to adherent polystyrene surface

→ good candidate for biocompatible scaffolds of melanocyte grafts

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Thank you for your attention



Prof. Dr. Thomas M. Magin



Prof. Dr. med. Jan-Christoph Simon



Dr. Mirjana Ziemer, Co-PI



Hanluo Li

SPONSORED BY THE



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