Development of 3D bioprinted reconstructed skin models using native and non-native bioinks

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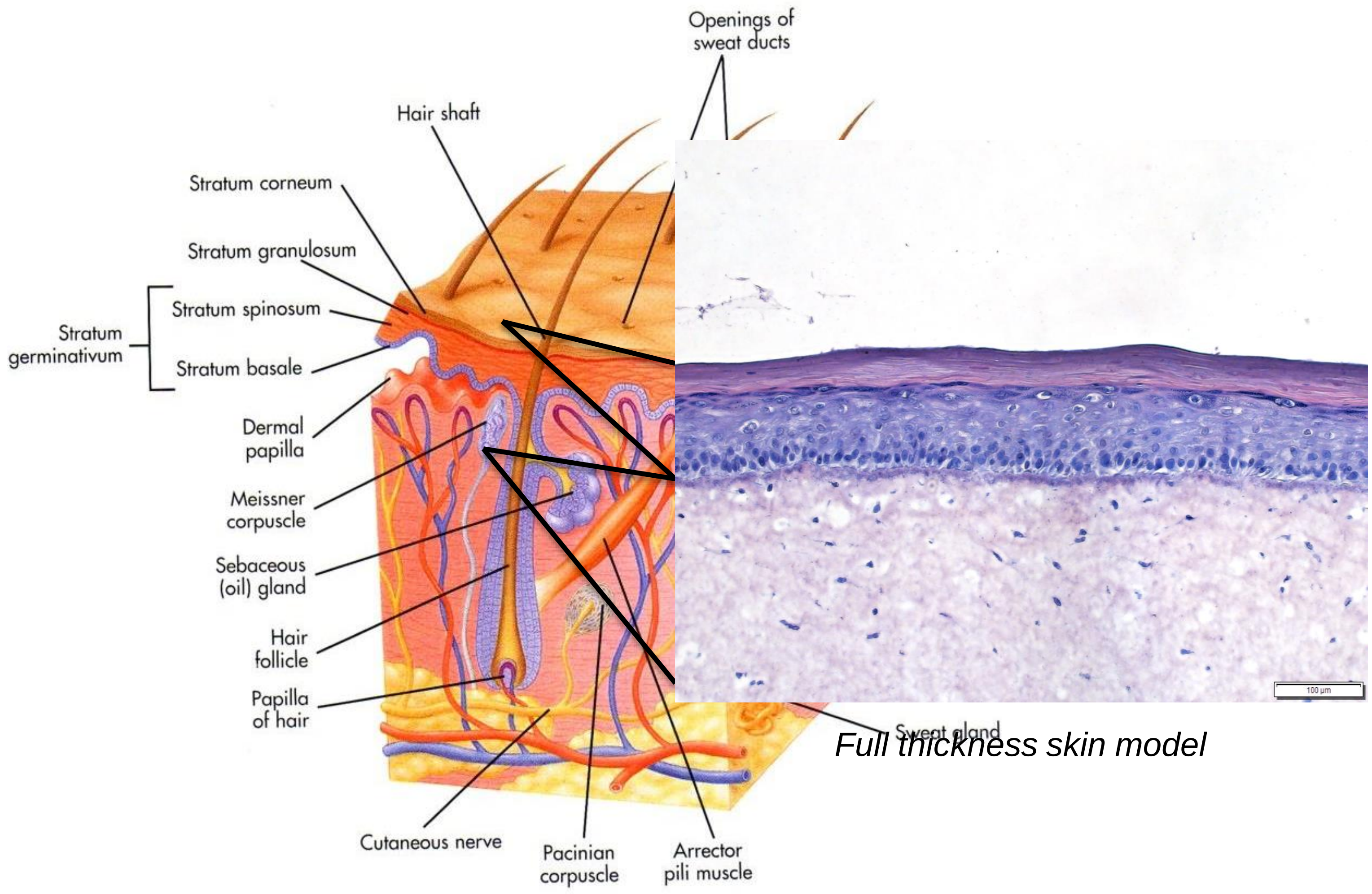
August, 2018

Human skin ➡ Reconstructed skin models

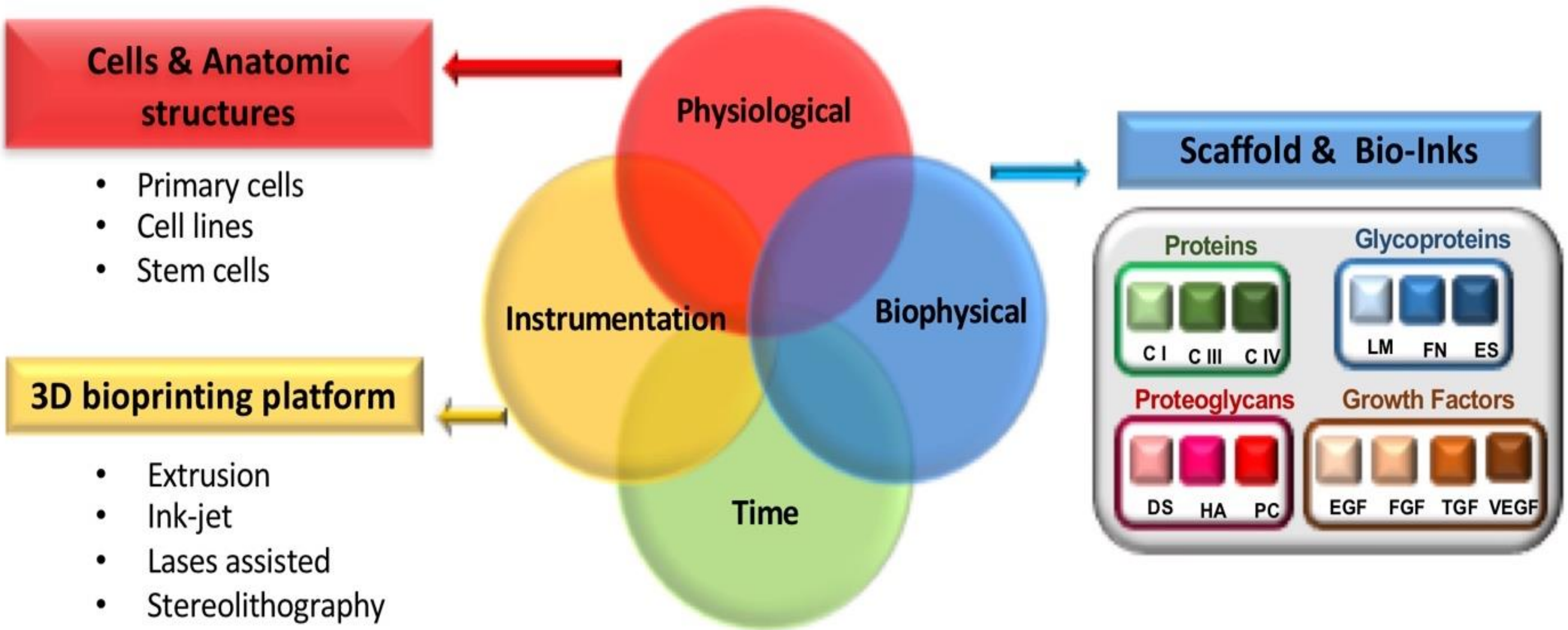


Full thickness skin model

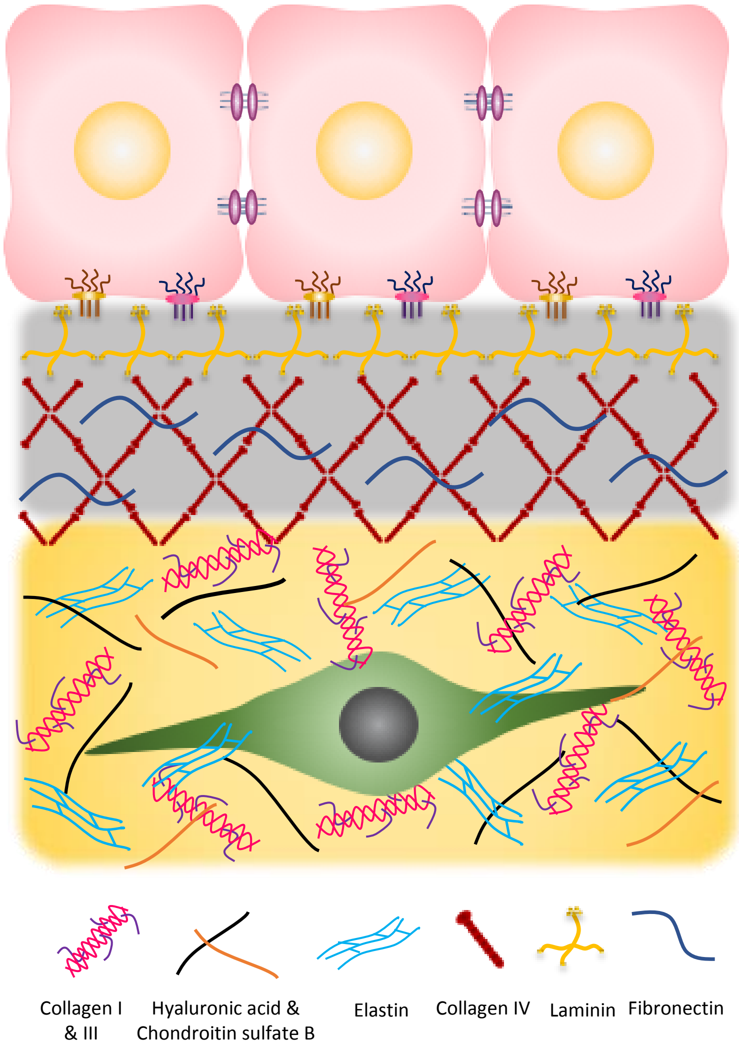
https://anatomybody-charts.us/skin-diagram-anatomy/skin-anatomy-diagram-worksheet/(Accessed: 16 August 2018)

COMPLEXITY ➡ 3D bioprinting

"Computer-aided transfer processes for patterning and assembly of living and non-living materials with a prescribed 2D or 3D organization to produce bio-engineered structures serving regenerative medicine, pharmacokinetics, and basic cell biology studies"



Moroni, L. *et al.* Biofabrication: A Guide to Technology and Terminology. *Trends Biotechnol.* **36,** 384–402 (2018)



Dermal- epidermal junction bioink

Basement membrane:

- Collagen IV
- Laminin
- Fibronectin

Dermal bioinks

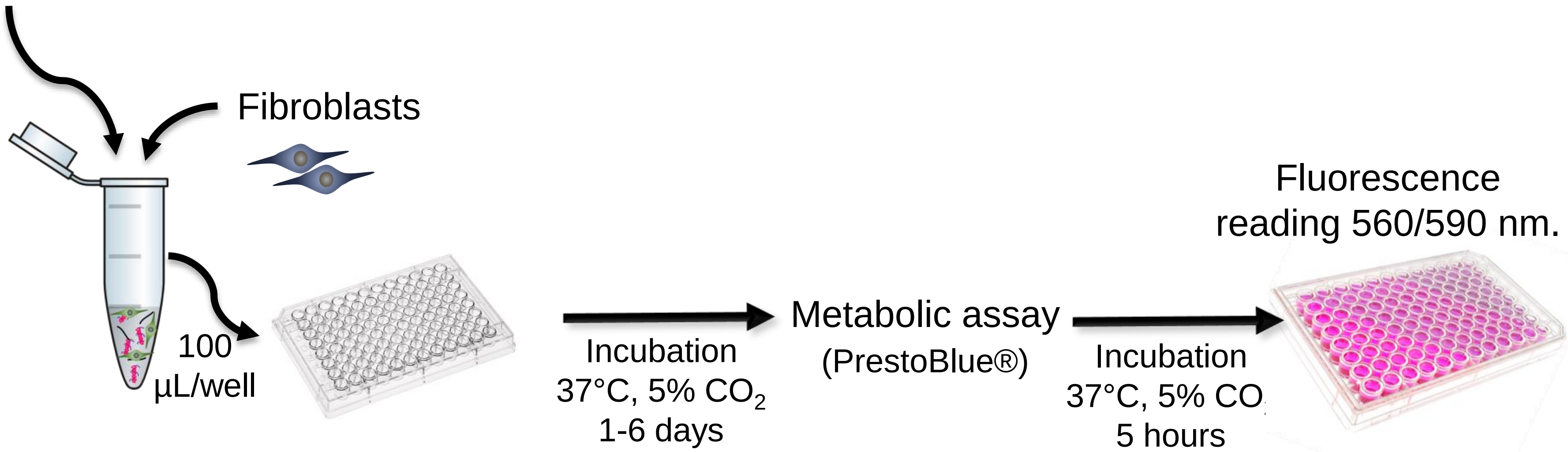
Interstitial extracellular matrix (IECM)

- Collagen I & III
- Hyaluronic acid
- Elastin
- Chondroitin sulfate B

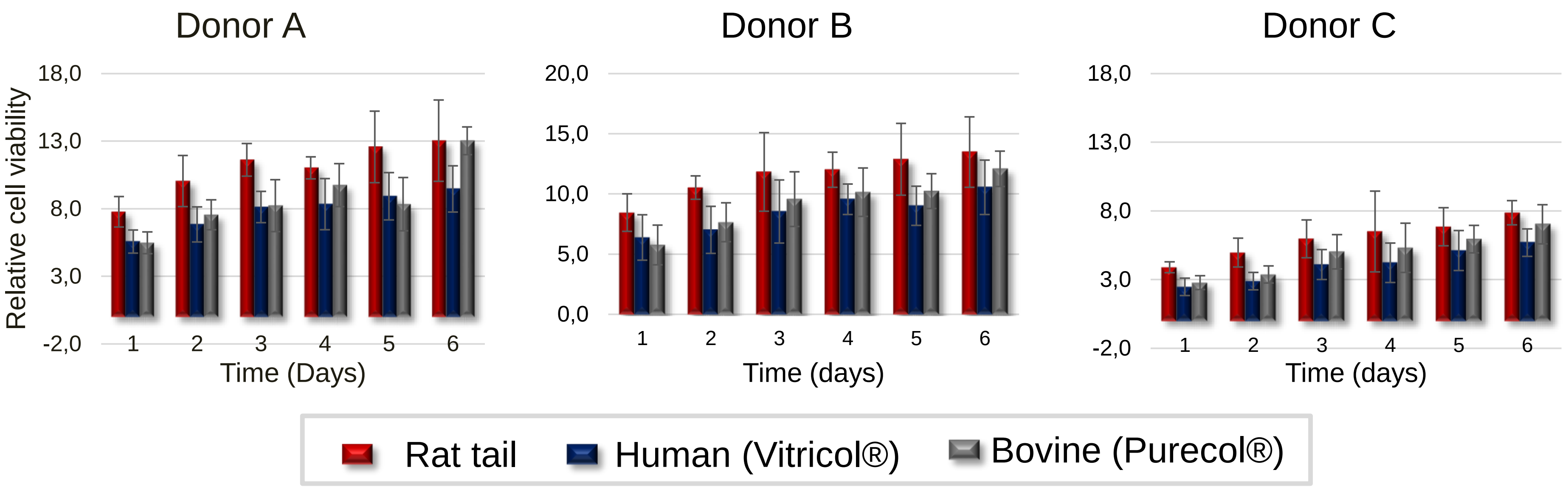
Design of dermal bioinks

Collagen type I:
- Rat Tail
- Human (Vitricol®)
- Bovine (Purecol®)

Collagen type III (human placenta)
Elastin (recombinant)
Hyaluronic acid sodium salt (recombinant)
Chondroitin sulfate B (CS) (porcine intestinal mucosa)

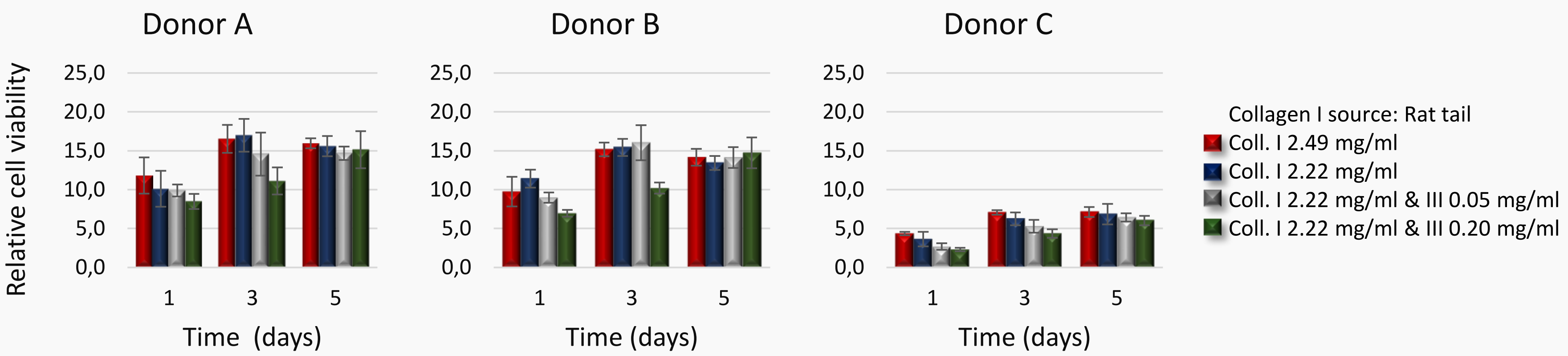


Effect of type I collagen source on proliferation of fibroblast

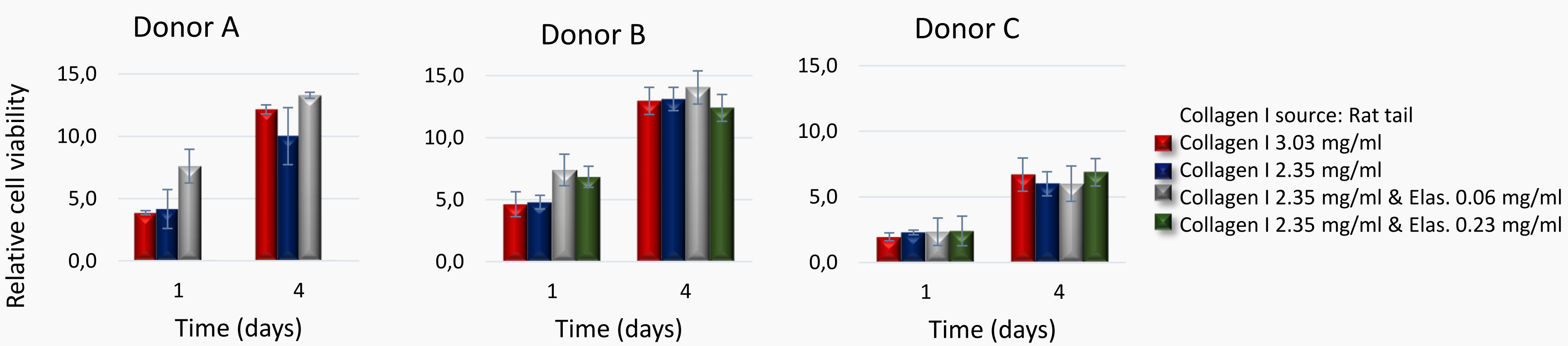


Collagen I concentration: 2.3 mg/ml

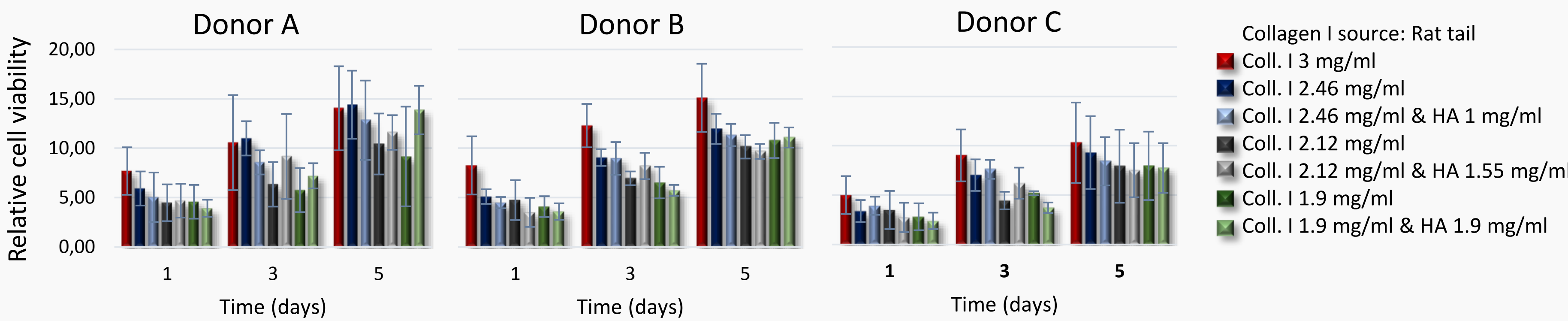
Effect of type I and **type III collagen** on proliferation of fibroblast



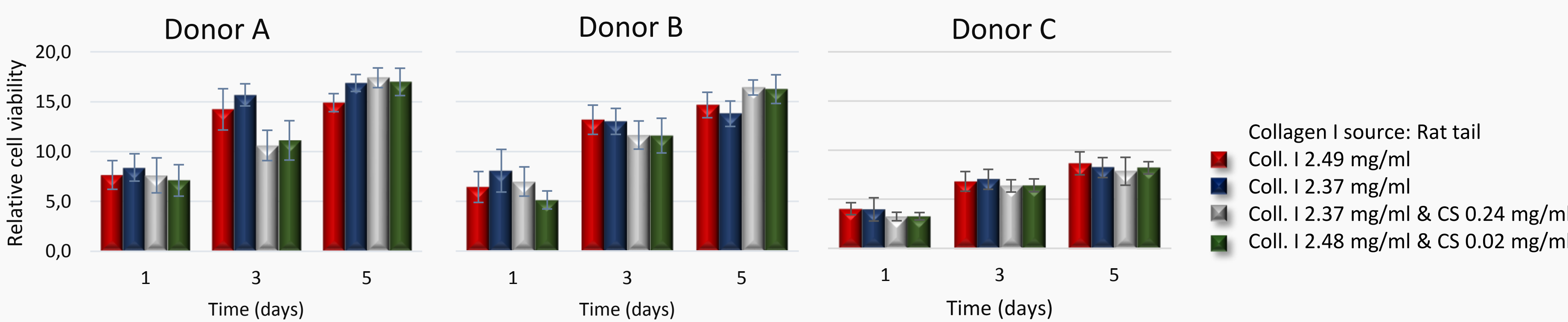
Effect of type I and **elastin** on proliferation of fibroblast



Effect of type I and **Hyaluronic acid** on proliferation of fibroblast



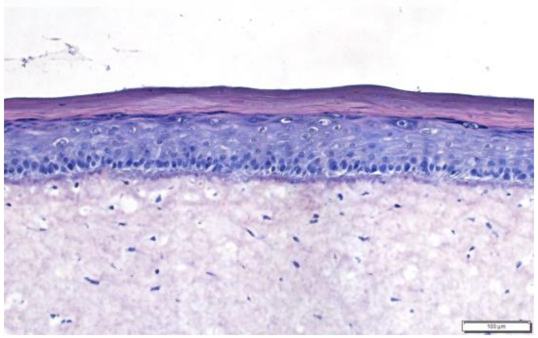
Effect of type I and **chondroitin sulfate B** on proliferation of fibroblast



Reconstructed skin models with dermal bioinks

Control

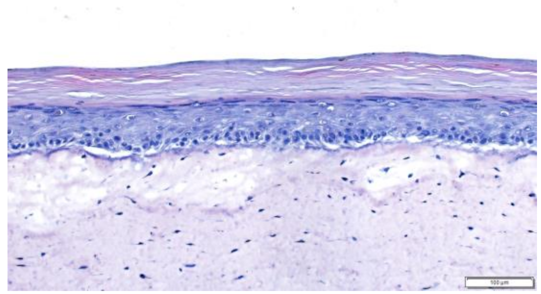
Contraction: 55.3±7.2%



Coll. I (2.62 mg/ml)

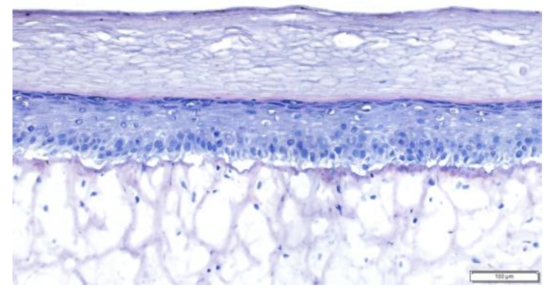
Hyaluronic acid

Contraction: 59.0±7.2%



Coll. I(2.1 mg/ml) & HA (1.0 mg/ml)

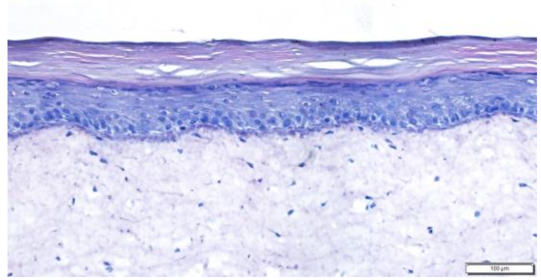
Contraction: 68.2±6.5%



Coll. I (1.62 mg/ml) & HA (1.91 mg/ml)

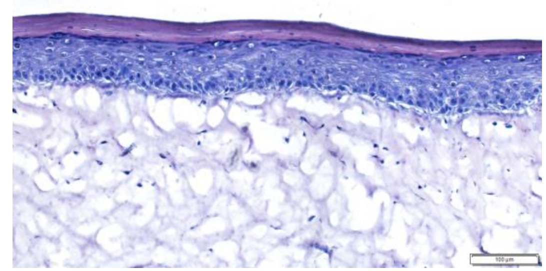
Chondroitin sulfate B

Contraction: 48.0±9.0%



Coll. I (2.61 mg/ml) & CS (0.026 mg/ml)

Contraction: 42.8±7.7%

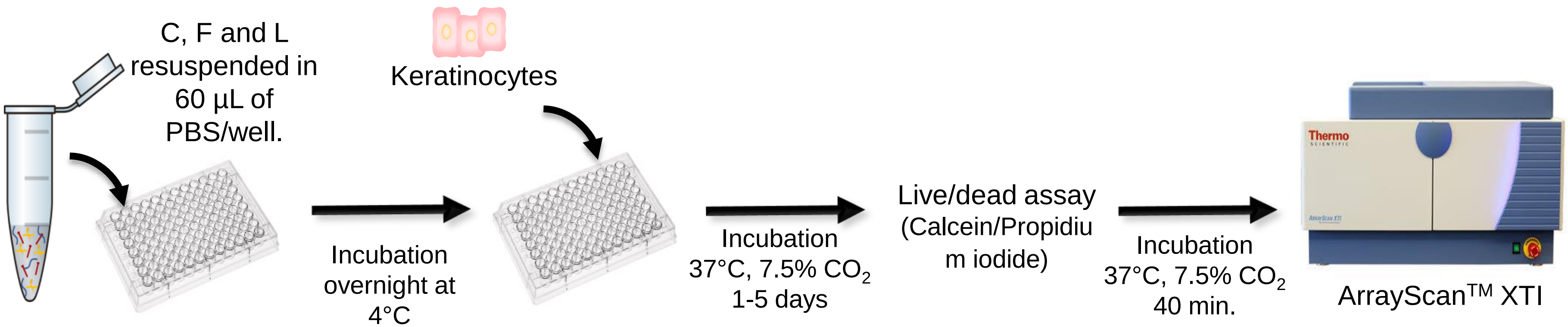


Coll. I (2.49 mg/ml) & CS (0.25 mg/ml)

Design of Dermal-Epidermal Bioinks:

Collagen IV (C), Fibronectin (F) and Laminin (L) at 2 different concentrations were screened alone and in combination to mimic the basement membrane. The protein concentrations used for coating are:

C1 = 2 μg/cm²	**F1 = 1 μg/cm²**	**L1 = 0.5 μg/cm²**
C2 = 8 μg/cm²	**F2 = 4 μg/cm²**	**L2 = 2 μg/cm²**



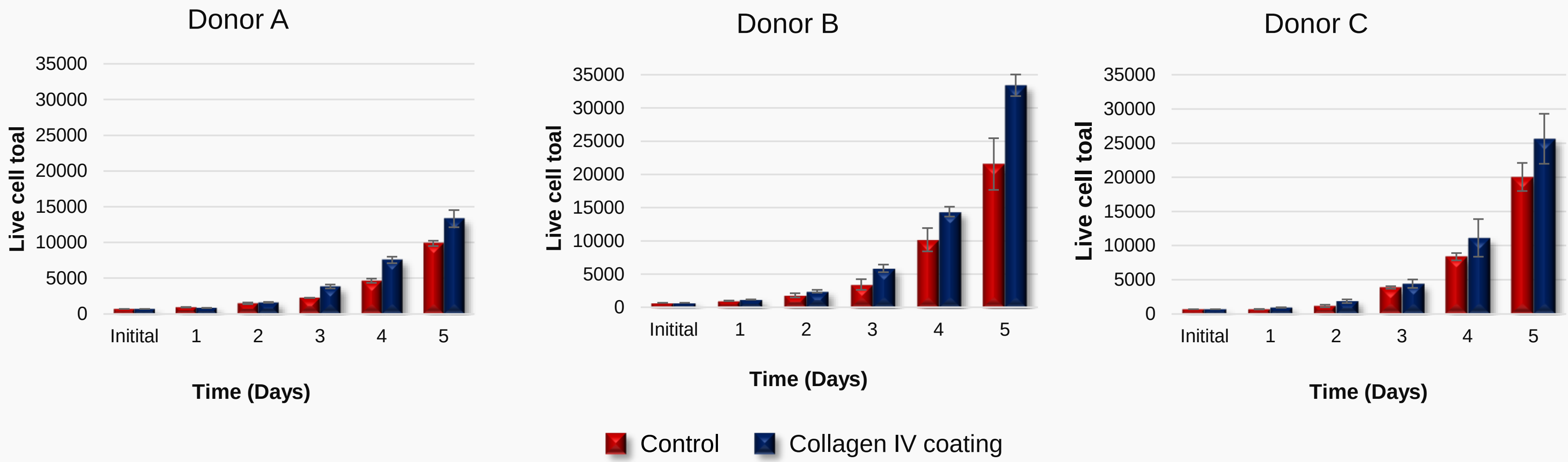
Effect of basement membrane proteins on proliferation of keratinocytes

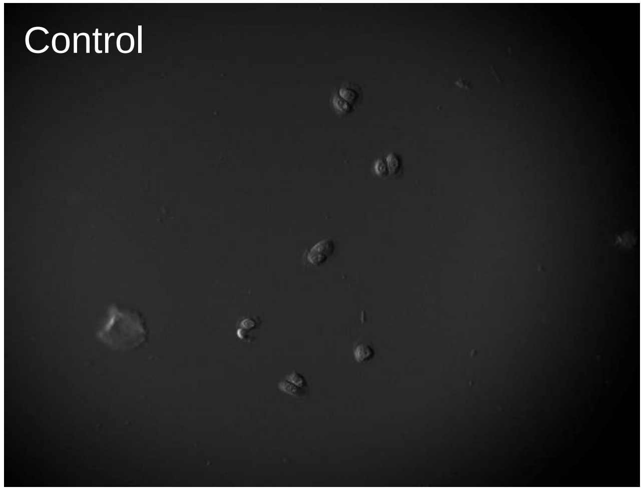
	Donor A	Donor B	Donor C
control	0.44	0.63	0.81
C1	0.04	0.04	0.04
C2	0.22	0.22	0.19
F1	1.00	0.89	0.93
F2	0.59	0.33	0.63
L1	0.11	0.19	0.26
L2	0.89	0.74	0.89
C1F1	0.70	0.30	0.22
C1L1	0.30	0.15	0.44
F1L1	0.19	0.26	0.37
C2F2	0.37	0.11	0.11
C2L2	0.93	0.78	0.74
F2L2	0.85	0.93	0.96
C1F2	0.07	0.07	0.15
C1L2	0.74	0.81	0.85
F1L2	0.96	1.00	1.00
C2F1	0.48	0.37	0.41
C2L1	0.15	0.41	0.07
F2L1	0.26	0.48	0.52
C1F1L1	0.52	0.59	0.48
C2F2L2	0.81	0.85	0.70
C1F1L2	0.67	0.70	0.67
C1F2L2	0.56	0.96	0.78
C1F2L1	0.78	0.44	0.56
C2F1L1	0.63	0.52	0.59
C2F1L2	0.41	0.67	0.30
C2F2L1	0.33	0.56	0.33

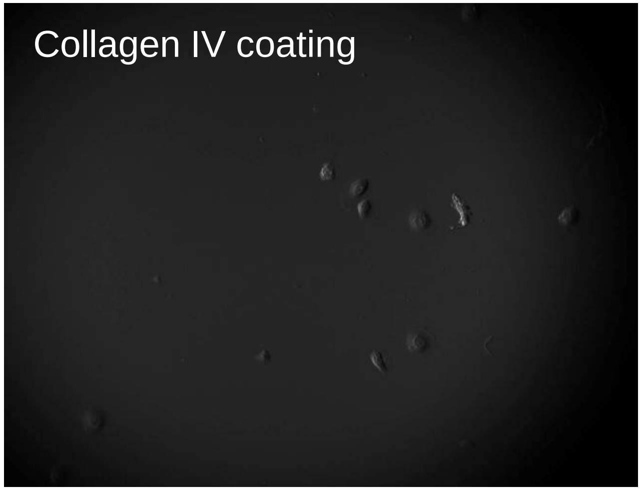
Heat Map (day 4)

Red (=1): lowest proliferation rate
Green (=0) highest proliferation rate

Effect of collagen IV coating on proliferation of keratinocytes

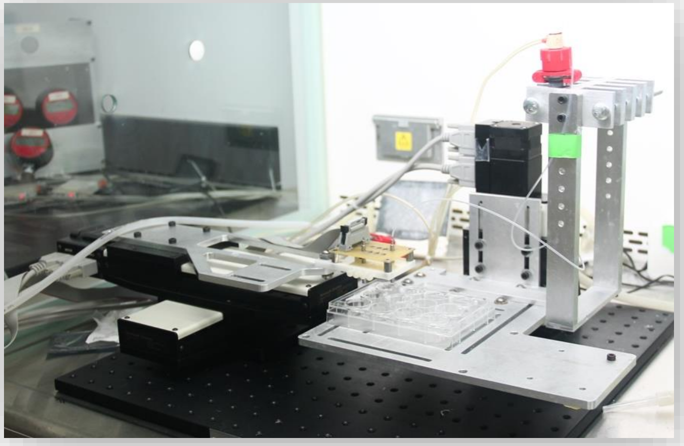






3D Bioprinting

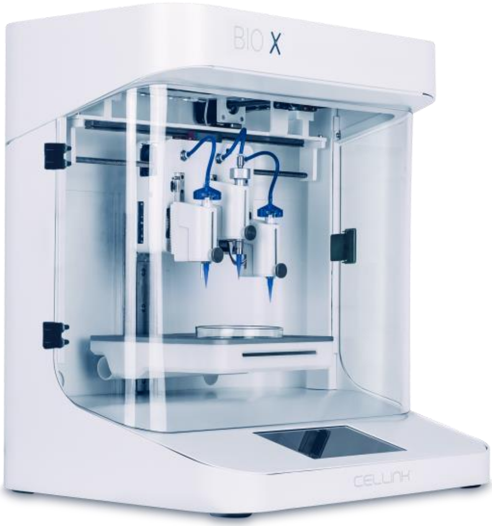
RPI 3D bioprinting platform



Bioprinter features:

- 8 independently controlled channels.
- Pneumatic dispensing system.
- Resolution: ~100 µm
- High cell viability

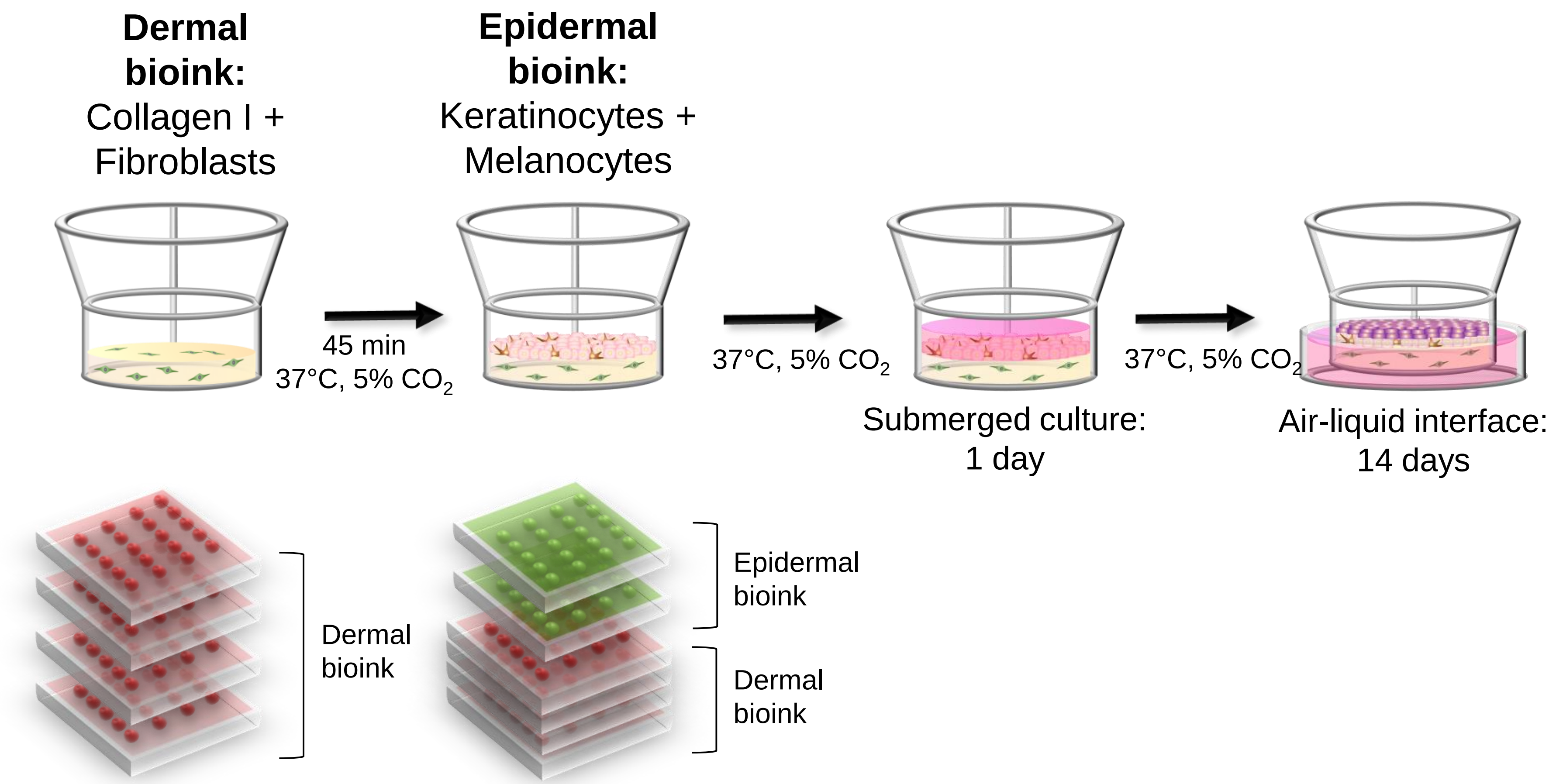
BioX^{TM} 3D bioprinting platform (CellInk).



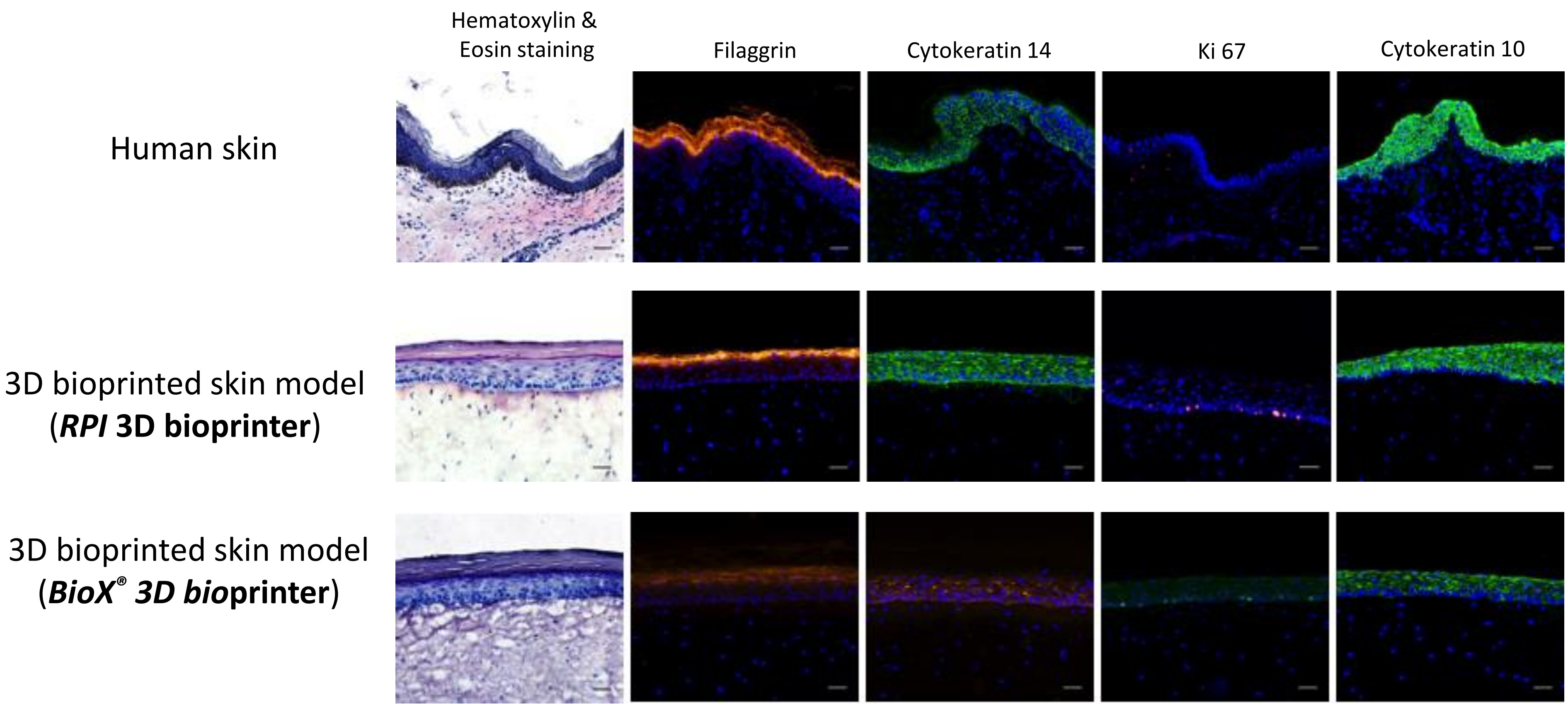
Bioprinter features:

- 3 independently controlled printheads.
- Temp. control (printhead and print bead)
- Resolution: ~1 µm
- Pneumatic extrusion system.
- High cell viability

3D Bioprinting human skin



3D Bioprinted human skin



Conclusions

The specific epidermal and dermal components (matrix molecules and cells) and their compositions exhibit significant effects on cell proliferation.

We have designed and identified epidermal and dermal bioinks that support the growth and proliferation of individual cell types

Cells from different origins (donor, anatomical location, age, gender) exhibit differential behavior, highlighting the importance of optimizing bioinks in tissue engineering.

We have demonstrated the feasibility of using different 3D bioprinting platforms for engineering a human skin tissue that mimics the native human skin.

Future Work

Design of new bioinks:
-Recombinant human collagen I

Inclusion of dermal papilla cells:
- Hair follicle 3D bioprinting

Acknowledgements

Nicholas Vecchiarello
Akshat Mullerpatan
Divya Shastry
Ronit Ghosh
Luiza Motter Catarino
Tânia Baltazar

Dr. Brigitte Arduini (RPI Stem Cell Core facility director)
Dr. Silvya Stuchi Maria-Engler (University of São Paulo, Brazil)

Dr. Pankaj Karande

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