Biomimetic thiol-ene-based hydrogels for photolithographic bioprinting and tissue fabrication

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1. Introduction

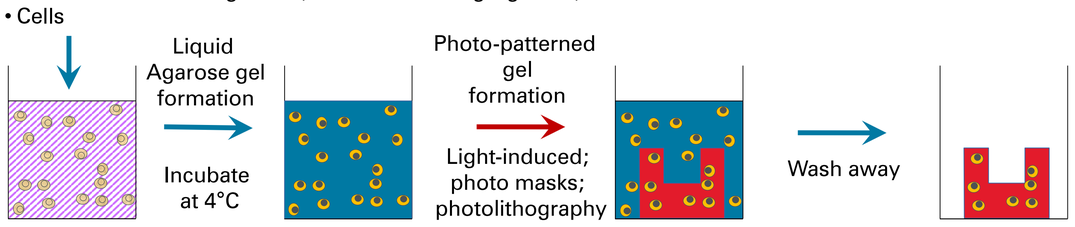
Light-inducible hydrogels are promising bioinks for the bioprinting of 3D tissues. The selection of wavelength, photoinitiator and chemistry are critical for robust gel formation and the preservation of cell viability to ensure an optimal formation of a physiological 3D matrix. The acrylate chemistry, which is most commonly used for the light-induced formation of hydrogels comprises several problems, such as detrimental side reactions of reactive oxygen species (ROS) with cells and inhibition of the crosslinking reaction by oxygen.

We developed a light-inducible biomimetic hydrogel system that is based on the norbornene chemistry. Compared to the acrylate-based systems, it provides a lower risk of cell toxicity, faster onset of gel formation and more uniform gel properties.

2. A photo-induced hydrogel system based on the thiol-ene chemistry

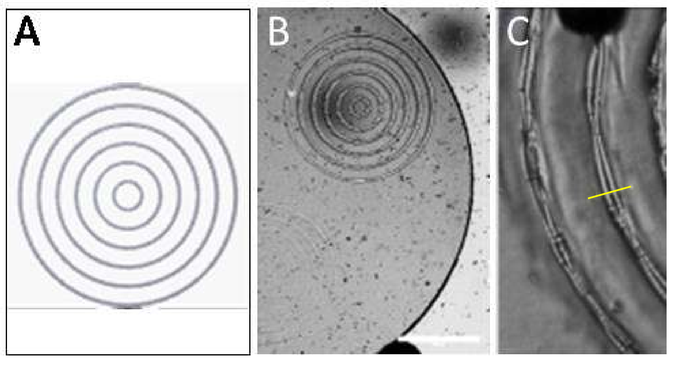
Gel system

- Norbornene polymer
- Thiol crosslinker (optional: MMP cleavable)
- Peptides (biomimetic modification)
- LAP (photoinitiator)
- Melted 0.15% LMP-Agarose (LMP: Low Melting Agarose)
- Cells



Incubation at 4°C induces gelation of LMP Agarose and keeps cells distributed in 3D while subsequent photo-induced gelation takes place. Non-illuminated parts are washed away to reveal the patterned gel. Hydrogel stiffness can be tuned by changing polymer and crosslinker concentration.

3. Fine structures can be obtained by laser-based bioprinting



Laser printing of hydrogel with LUMINATE (www.modulux3d.com)

A) 3D model: six circles.

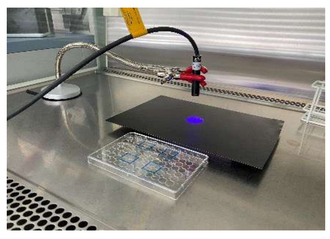
B) Overview image of 3D printed circles on surface of a glass cover slip.

C) Zoomed image of 3D printed circles. Yellow line: 9 μm.

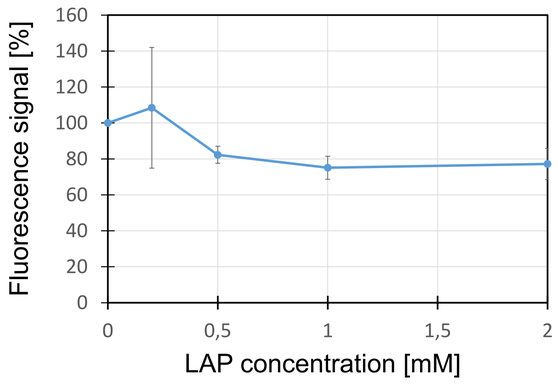
4. Cell viability is well maintained in photo-induced hydrogels

Experimental setup:

A LED pen connected to a power control and a mask was used to illuminate cells in gels of 4 wells of a 96 well plate.



Human fibroblasts (Hs27) were embedded in photo-induced hydrogels



Hydrogel composition:

Norbornene-Dextran, RGD Peptide, MMP-cleavable hyaluronic acid crosslinker.

Gelation was induced at increasing concentrations of LAP.

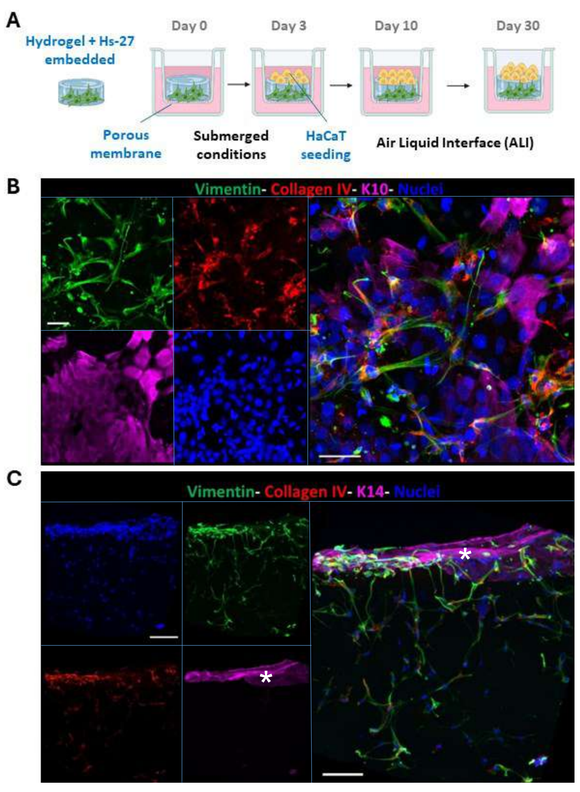
Light exposure: 25 mW/cm^2, 2 min

Viability assay: Alamar Blue

Results:

Cell viability stays > 80% even at high LAP concentrations.

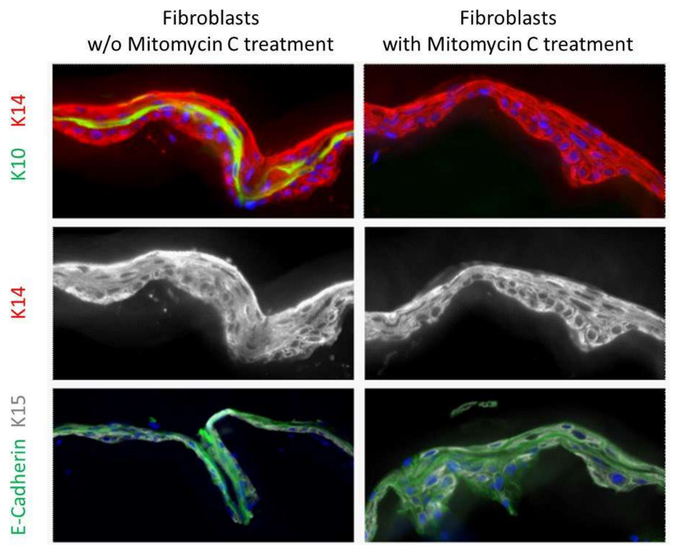
5. Skin tissue modeling with cell lines



Experimental setup:
A skin-like tissue was grown in the Air-Liquid Interphase (ALI) (A) in norbornene-pullulan hydrogels, modified with the adhesion peptide RGD and crosslinked with a MMP cleavable hyaluronic acid crosslinker. At day 14 tissue sections were immuno-stained for Vimentin (green) and Collagen IV (red), and the two keratin markers K10 and K14 (magenta). B: top view, C: lateral view. Individual channels (left) and merged (right). Nuclei were stained with Dapi. Scale bar=100 μm.

Results:
Fibroblasts (Hs27) are spread in the gel (green staining), forming long protrusions and an interconnected network below the keratinocytes (HaCaT, stained in magenta), secreting large amounts of the ECM protein Collagen IV. On top of the gel a dense keratinocyte multilayer structure is formed, marked with an asterisk * in C.

6. Bioengineered skin equivalent from human primary cells



Immunofluorescent staining of tissue sections of bioengineered skin. Stem/progenitor: Keratin K14 and K15, differentiation: Keratin K10

Hydrogel composition: N-Dextran, CD-HyLink (cell degradable hyaluronic acid), RGD Peptide.

Culture: A dermal-like hydrogel layer was created by growing NIH-3T3-J2 fibroblasts for seven days, followed by 2 weeks of Human Epidermal Foreskin stem cells cultivation on top of gel.

Fibroblasts are not captured in the sections as they were scarcely distributed in the culture.

Results:

- Very well organized epidermis, stratified in clear layers with the expected cuboidal basal and squamous suprabasal cell morphology that resembles native human skin.
- Expression of the differentiation marker K10 was evident only in tissues that contained NIH-3T3-J2 cells that were not treated with Mitomycin-C. This suggests that proliferation of fibroblasts and thus higher numbers of cells are required for proper differentiation of the epidermal layer.

7. Conclusions

- Photo-crosslinking of the norbonene-based hydrogel system is well compatible with cell culture.
- Hydrogels are endowed with versatile biomimetic features by modification with matrix protein-derived peptides and MMP cleavable crosslinkers.
- The norbornene chemistry can be integrated in the Cellendes *3-D Life* Biomimetic hydrogel platform and complements versatile features of the platform with photoinducibility.
- Using this hydrogel system tissue models can be developed as exemplified with skin-like tissues generated from human cell lines as well as human primary cells.
- The new hydrogel system can be used with various photoinducing techniques, including bioprinters, photomasks, photolithography and sound induced patterning. Structures as small as 9 μm can be produced.
- The norbornene based crosslinking chemistry is favored over the commonly used acrylate chemistry because a more homogenous polymer network is formed and cytotoxic reactive oxygen species are quenched.

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