Approaches for controlling contraction in tissue engineered skin

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Motivation

3D skin models are an improvement compared to **2D**

Challenge: fibroblast-mediated contraction prevents the manufacture of reproducible, validated and up-scalable models

Main current approach: Increase mechanical properties of the collagen scaffold by crosslinking the network to counter contraction

Crosslinkers such as **glutaraldehyde** are **cytotoxic**, cells can only be seeded after washing the crosslinked scaffold

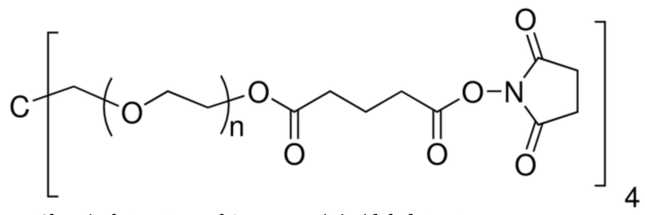
➔ **Need for a crosslinker which allows for cell seeding prior to crosslinking**

This Project

Use of a molecule which allows **crosslinking with cells already embedded** in the collagen: **Succinimidyl Glutarate-terminated Polyethylene Glycol (SG-PEG)**[1]

Study influence of SG-PEG on **mechanical properties** and **cell behaviour** by varying concentration and chain length of SG-PEG added to the collagen scaffold

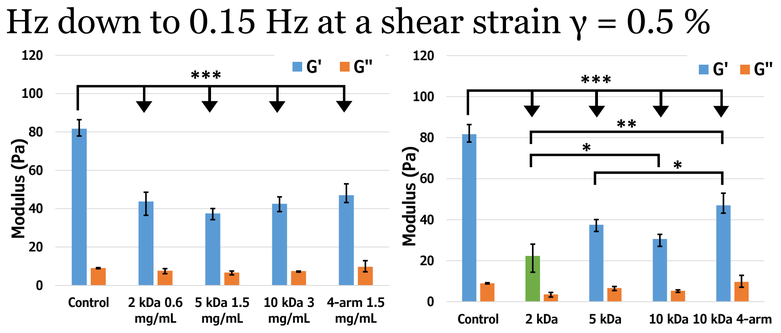
Use of **4-arm** as well as bifunctional **2 kDa, 5 kDa and 10 kDa SG-PEG** as an additive to type I collagen gels



Chemical structure of 4-arm succinimidyl glutarate.

Rheology

Method: Oscillatory frequency sweep from 100 Hz down to 0.15 Hz at a shear strain γ = 0.5 %



Left G' and G" st 10 Hz for 3 mg/mL collagen gels with added SG-PEG at a theoretical crosslinking density of 50 %. **Right** G' and G" st 10 Hz for 3 mg/mL collagen gels with 1.5 mg/mL SG-PEG of different molecular weights. ANOVA: *P < 0.05, **P < 0.01, ***P < 0.005

Very low G" for all samples → foremost elastic material

The addition of SG-PEG **decreased G'** for all molecular weights & concentrations

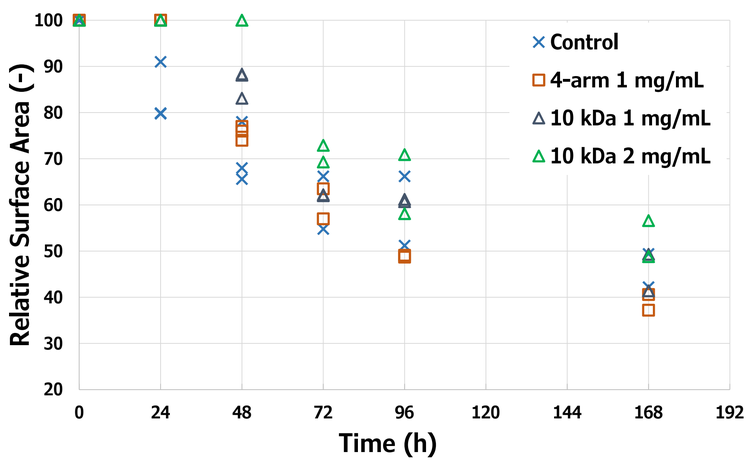
At **equal termination group concentrations**: no statistically relevant difference in G' for different SG-PEG chain lengths

G' varied with termination group concentration

The produced materials are softer by up to **3 orders of magnitude** compared to real skin tissue[2]

Contraction Assessment

Method: Macroscopic imaging of seeded samples every 24 hours for one week



Contraction assessment of 2 mg/mL collagen gels seeded with $6.6*10^4$ cells/mL BJ5TA dermal fibroblasts for a period of one week.

All samples contracted over a 7 day period

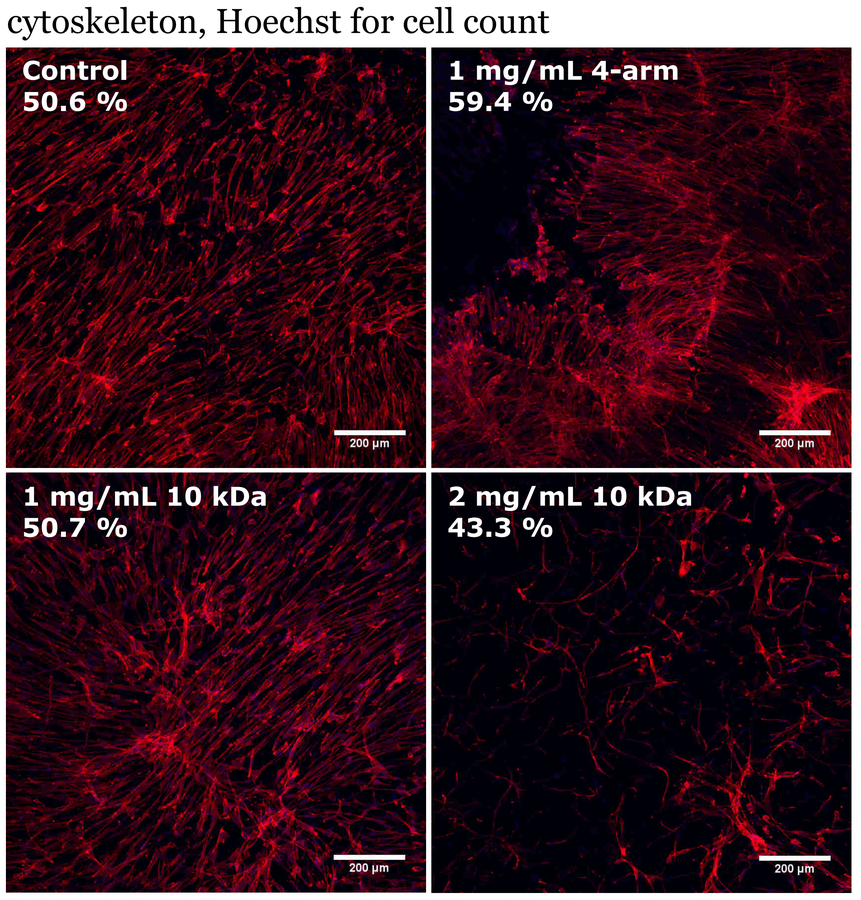
SG-PEG slowed down contraction, did **NOT prevent it**

Only **2 mg/mL 10 kDa SG-PEG** showed some reduction in contraction

Large differences in contraction within sample groups

Imaging

Method: Fluorescence Confocal Microscopy
Staining: Live-dead for viability, Phalloidin for cytoskeleton, Hoechst for cell count



Phalloidin (red) and Hoechst (blue) staining of 2 mg/mL collagen gels seeded with $6.6*10^4$ cells/mL BJ5TA dermal fibroblasts 7 days after seeding. The contraction percentage is given.

Rounded cells (phalloidin staining) for high SG-PEG concentration. Those gels **contracted the least** after a 7 day period

Conclusions & Future Work

SG-PEG is not a suited crosslinker for preventing contraction in 3D tissue engineered skin

SG-PEG decreases the shear modulus of the gels due to **its hydrophilic nature** attracting water molecules to the scaffold, thus **lowering the friction** between collagen chains

No substantial **prevention in contraction** even at high SG-PEG concentrations for periods of **one week**

High concentrations of SG-PEG in collagen gels **slowed down** fibroblast-mediated **contraction** not by improving the gel's mechanical properties, but by **preventing the fibroblasts to attach** to the scaffold

Future work: Find a non hydrophilic molecule suitable as a collagen crosslinker and characterize the new system

References

[1] C. Lotz, F. F. Schmid, E. Oechsle, M. G. Monaghan, H. Walles, and F. Groeber-Becker, 'Cross-linked Collagen Hydrogel Matrix Resisting Contraction To Facilitate Full-Thickness Skin Equivalents', *ACS Appl. Mater. Interfaces*, vol. 9, no. 24, pp. 20417–20425, Jun. 2017, doi: 10.1021/acsami.7b04017.

[2] H. Joodaki and M. B. Panzer, 'Skin mechanical properties and modeling: A review', Proc. Inst. Mech. Eng. [H], vol. 232, no. 4, pp. 323–343, Apr. 2018, doi: 10.1177/0954411918759801