Biocompatibility and functional performance of hydrogels made from recombinant ULD-ELP fusion proteins for sealing of deep corneal defects

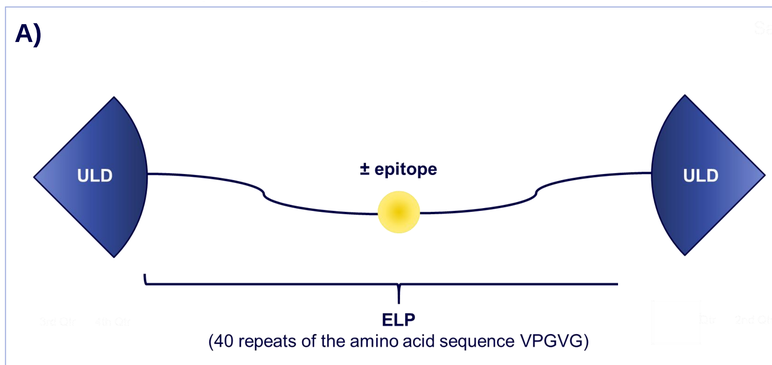
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Background

- Previously, we introduced a tunable hydrogel platform based on recombinant ubiquitin-like domains (ULD) and elastin-like polypeptides (ELP; **Fig. 1**).[1]
- Hydrogels made from ULD-V40-ULD are elastic, mechanically stable, and wet-adhesive, and have been used successfully for the sealing of deep corneal defects *ex vivo* (V40 = 40 repeats of the pentapeptide VPGVG).[1]
- Here, we report hydrogel cytocompatibility and functionalization of ULD-V40-ULD with cell-instructive epitopes, assessing effects on mechanical and adhesive performance.



Cell-adhesive epitope (AA sequence)	Epitope derived from	Protease-sensitive epitope (AA sequence)	Epitope derived from
RGD	Fibronectin	PANLVA	Pan-MMP cleavage site
KGD	Collagen	PAALVGA	MMP-2/9 cleavage site
IKVAV	Laminin		

Figure 1: (**A**) Schematic representation of the hydrogel building blocks, composed of ubiquitin-like domains (ULD) flanking an elastin-like polypeptide (ELP) sequence with an optional cell-instructive epitope (see Table).[2,3] (**B**) Protein structure of the ULD homotetramer with tyrosines at the interface enabling covalent photocrosslinking. Abbr.: AA – amino acid; MMP – matrix metalloproteinase

Materials and methods

- Recombinant protein production in *E. coli* BL21(DE3), purification, mechanical hydrogel characterization, and *ex vivo* leakage pressure testing were performed as previously described.[1]
- Hydrogel preparation: Lyophilized protein aliquots were reconstituted at 20% (w/w) in water with riboflavin-5'-phosphate (riboflavin; 2.5 mM) and ammonium or sodium persulfate (APS/SPS; 30 mM), then photocrosslinked under blue light (460 nm; 5.8 J/cm²).[1]
- Cytocompatibility of the hydrogel components was evaluated based on OECD Test Guideline No. 492 using a Reconstructed human Cornea-like Epithelium model (RhCE; SkinEthic™) (**Fig. 2, A**). Tissue viability was assessed relative to the negative control.
- Residual APS leaching was measured using the Pierce™ Quantitative Peroxide Assay Kit. Crosslinked hydrogels were incubated in 2 mL PBS + antibiotics for 14 days.

Results

Cytocompatibility testing based on OECD Test Guideline No. 492

- The RhCE test method identifies chemicals not requiring classification for eye irritation or serious eye damage, based on cytotoxicity to the corneal epithelium.[4]
- Mean tissue viability was comparable for illuminated and non-illuminated hydrogel precursor solutions, as well as for the negative control (PBS; 100% tissue viability; **Fig. 2, B**).

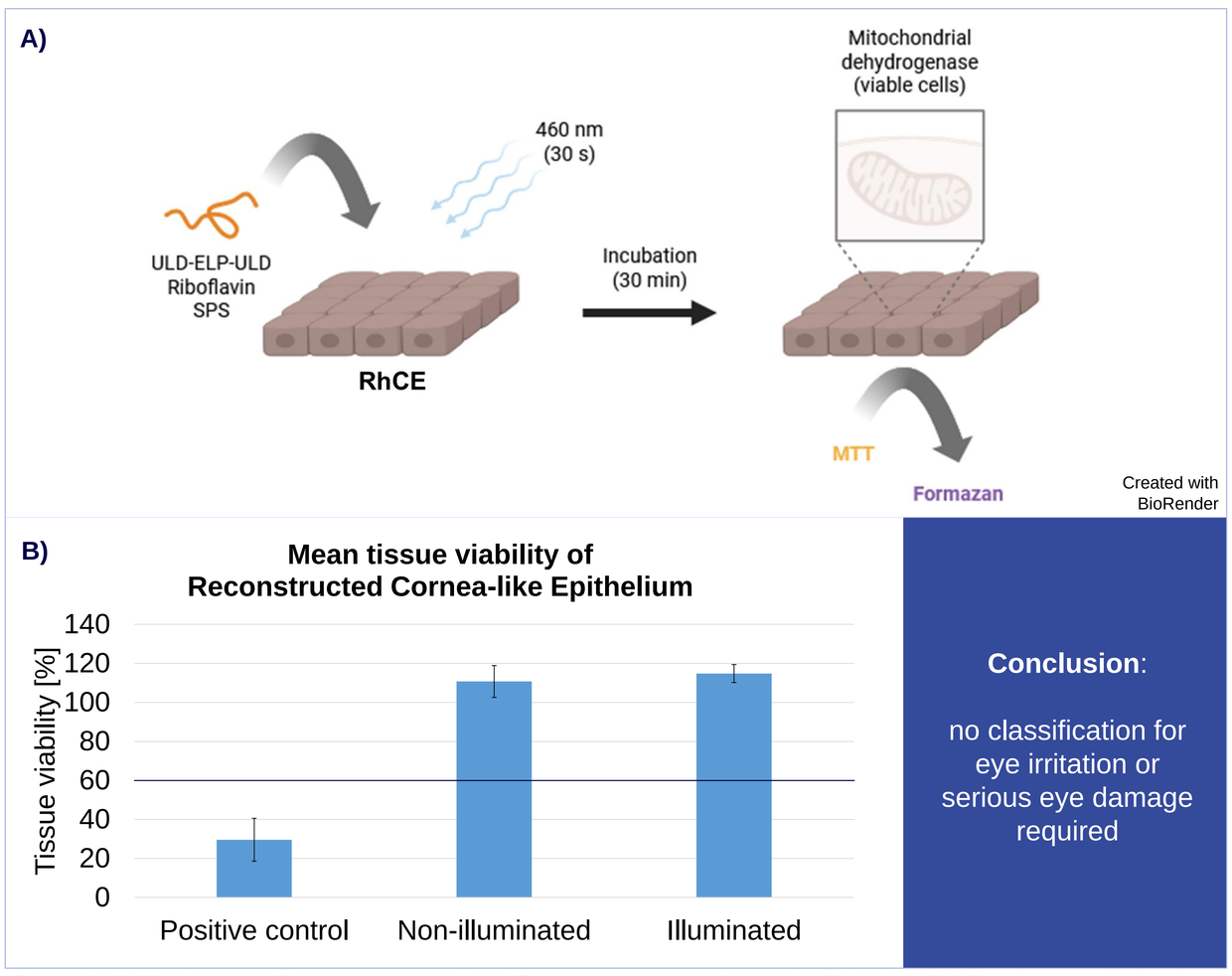


Figure 2: (**A**) Workflow and (**B**) mean tissue viability of Reconstructed human Cornea-like Epithelium (RhCE) using a modified protocol based on OECD Test Guideline No. 492. Test quantities were adjusted to reflect a typical application volume of 10 µL hydrogel precursor solution. Positive control: methyl acetate. Measured in triplicates, error bars depict standard deviation.

Residual APS leaching

- Residual APS leaching from crosslinked ULD-V40-ULD hydrogels remained <15 µM over a course of 14 days, well below cytotoxic concentrations (>300 µM[5]).

Acknowledgements

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Mechanical hydrogel characterization

- Epitope incorporation did not significantly affect the hydrogels' mechanical properties (**Fig. 3**).
- ULD-based hydrogels exhibited high elasticity in uniaxial tensile stretch tests (maximum strain: 133 ± 33 %); GelMA was not measurable in this setup.

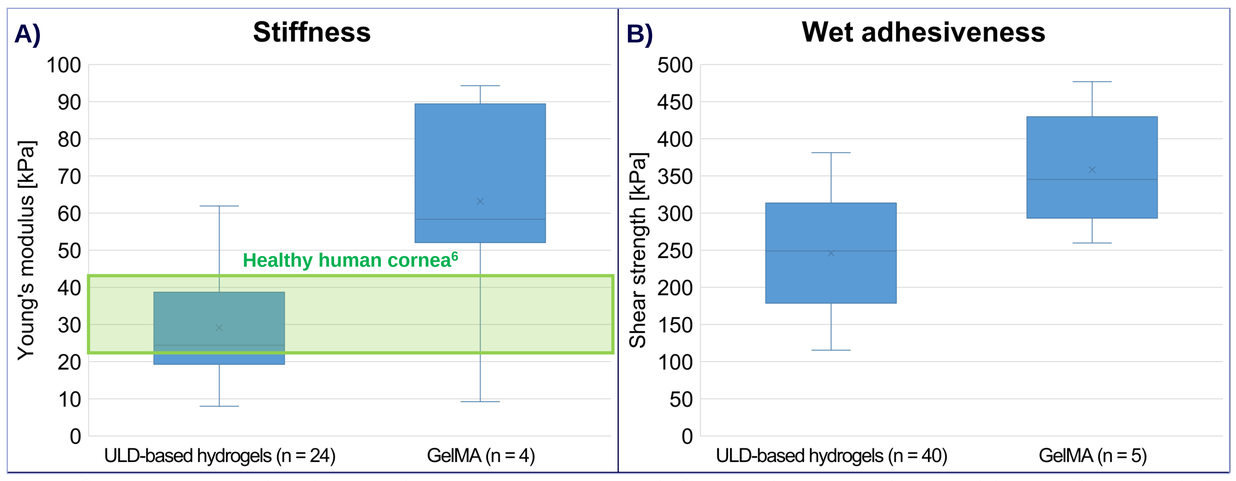


Figure 3: Mechanical characterization of ULD-based hydrogels compared to GelMA, determined by A) bioindentation and B) lap shear test. Data from all ULD-V40-ULD hydrogel variants (no epitope, RGD, KGD, IKVAV, PANLVA, and PAALVGA) were pooled. n = number of technical replicates; for evaluation of stiffness, data from all single measurements were pooled.

Ex vivo leakage pressure testing

- ULD-based hydrogels, GelMA, and cyanoacrylate glue (CA) resulted in successful sealing of full-thickness corneal perforations in cadaveric porcine corneas in all cases, withstanding anterior chamber pressures well above physiological levels (**Fig. 4, A**).
- ULD-based hydrogels yielded elastic, smooth, near-transparent sealings (**Fig. 4, B**), whereas CA formed rough, brittle patches (**Fig. 4, C**).

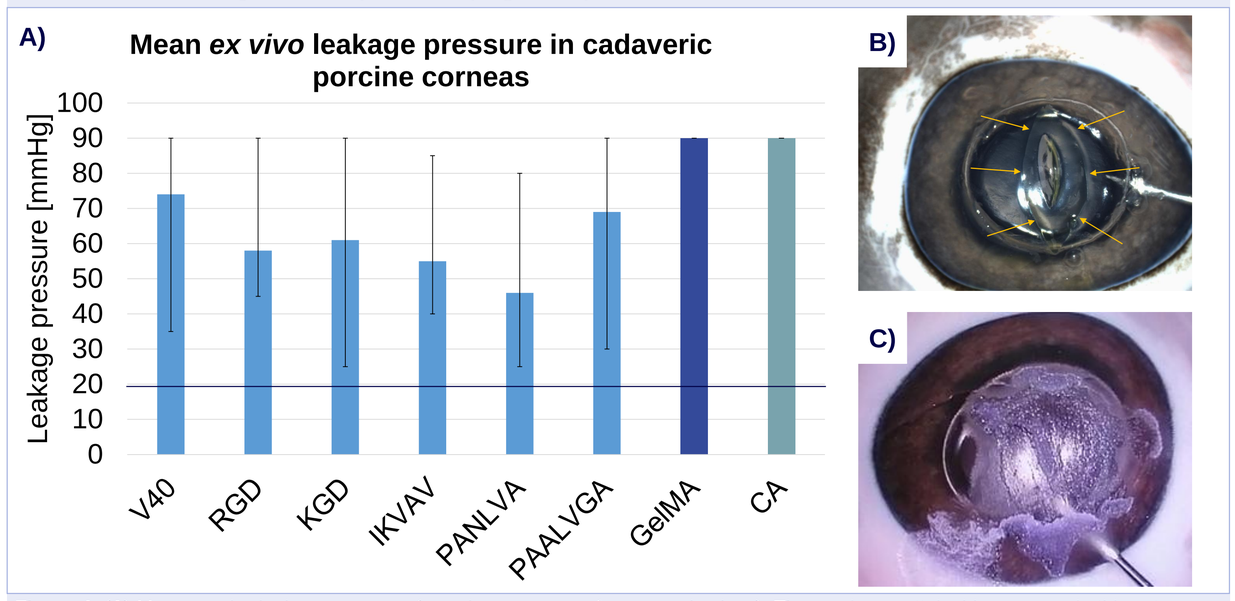


Figure 4: (**A**) Mean ***ex vivo*** leakage pressure (error bars = range of measured values). The maximum attainable pressure in this setup was 90 mmHg. (**B, C**) Full-thickness perforation in a cadaveric porcine cornea, sealed with ULD-based hydrogel (**B**) or cyanoacrylate glue (**C**).

Formulation optimization

- ELP reversibly form coacervates above their transition temperature (T_t).[7] Arginine-HCl and Poloxamer 188 can potentially increase T_t and thereby reduce aggregation.[8,9]
- Protein reconstitution in 0.1 M arginine-HCl and 0.1% Poloxamer 188 yielded a homogeneous, more porous hydrogel structure compared to water (**Fig. 5**).

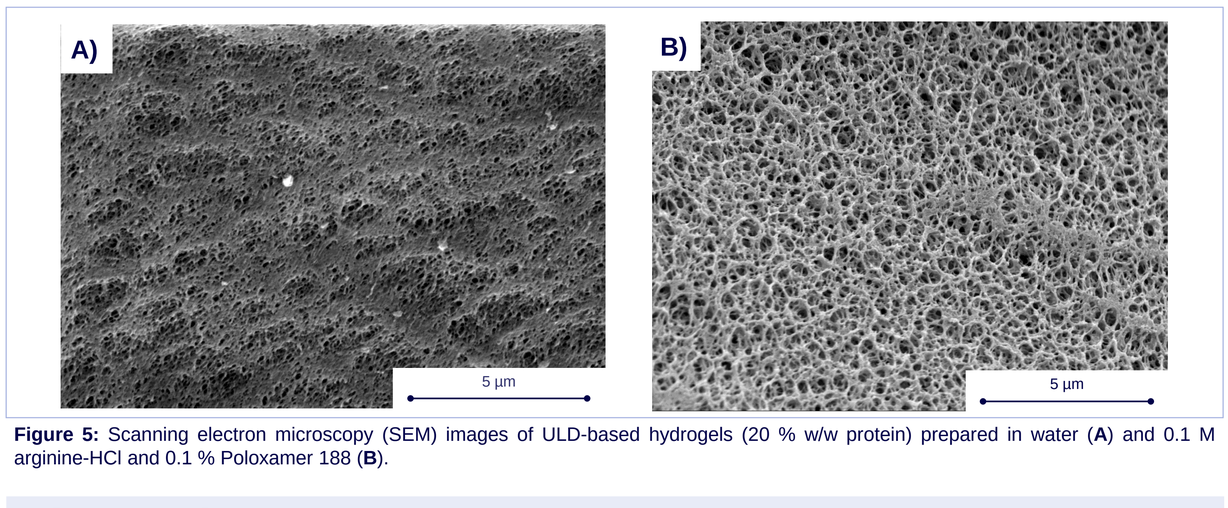


Figure 5: Scanning electron microscopy (SEM) images of ULD-based hydrogels (20 % w/w protein) prepared in water (**A**) and 0.1 M arginine-HCl and 0.1 % Poloxamer 188 (**B**).

Discussion

- Epitope-functionalized hydrogels showed no significant differences in mechanical properties and successfully sealed corneal perforations.
- ULD-based hydrogels in arginine/Poloxamer demonstrated a more porous structure, potentially enhancing cell infiltration for tissue engineering applications.
- Further studies will assess corneal cell viability and differentiation across hydrogel variants and formulations to identify the most favorable composition for sealing of corneal defects.

Conclusion

Cytocompatibility of the hydrogel system for corneal applications was demonstrated. Functionalization of hydrogel building blocks with cell-instructive epitopes enables modulation of biochemical cues without affecting mechanical or adhesive performance, thereby enhancing the hydrogel's potential as a corneal sealer.

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