Alginate-based Dynamic Hydrogels as Mechanically Adaptive Artificial ECM for 3D Cell Culture and Tissue Engineering

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Introduction

Hydrogels have evolved from static matrices toward dynamic networks that better mimic the native extracellular matrix (ECM) (Fig. 1). While degradable hydrogels enable cell remodeling and migration, dynamic hydrogels additionally provide reversible interactions and responsiveness to cellular behavior, creating a more biomimetic cellular microenvironment [1].

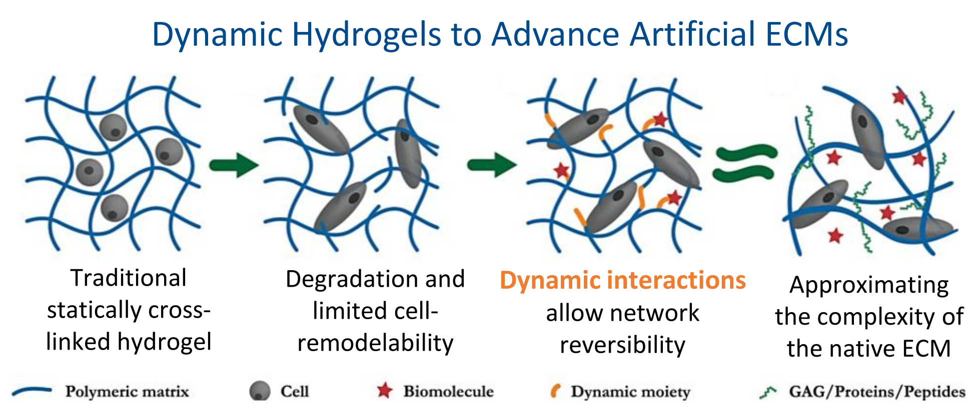

Fig. 1. Evolution of hydrogels toward biomimetic ECMs.

Dynamic Hydrogels

Dynamic covalent chemistries provide tunable hydrogel networks. Crosslink chemistry, including reaction rate kinetics (k_{on}, k_{off}) and thermodynamic equilibrium (K_{eq}), governs hydrogel mechanics and regulates cell–matrix interactions (Fig. 2) [2].

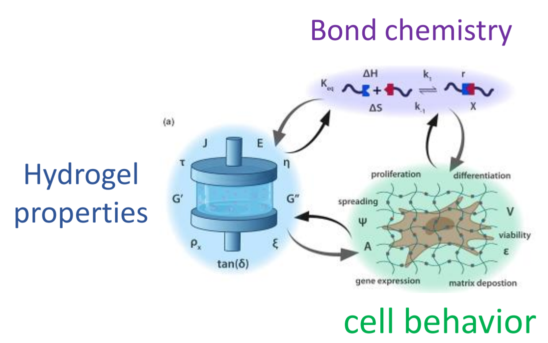

Fig. 2. Dynamic hydrogel design and structure–property relationships.

Experimental

Hydrogels were formed by crosslinking Alginate adipohydrazide (mAlg) and oxidized alginate (OxA) or oxidized dextran (OxD) (Fig. 3).

Hydrogel mechanics and dynamics were characterized by rheology and compression testing, while chondrocyte morphology was used to probe network dynamics at the cell–matrix level.

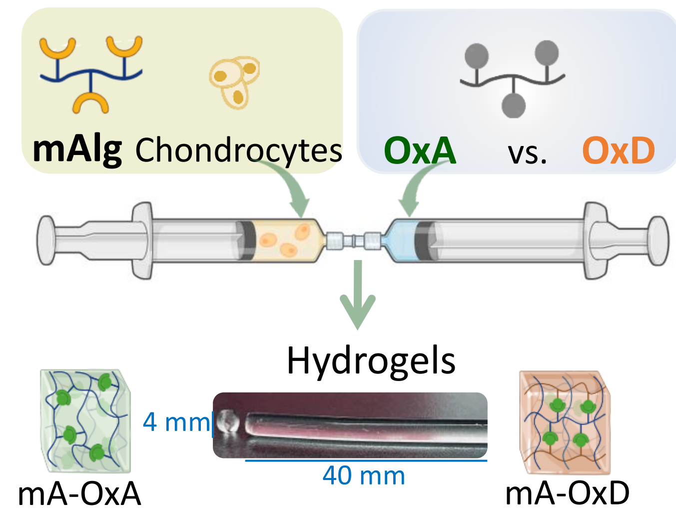

Fig. 3. Alginate-based dynamic hydrogels.

Results and Discussion

Network Dynamics and Mechanics

mA-OxA and mA-OxD hydrogels exhibit distinct formation kinetics while maintaining comparable stiffness and network dynamics (Fig. 4).

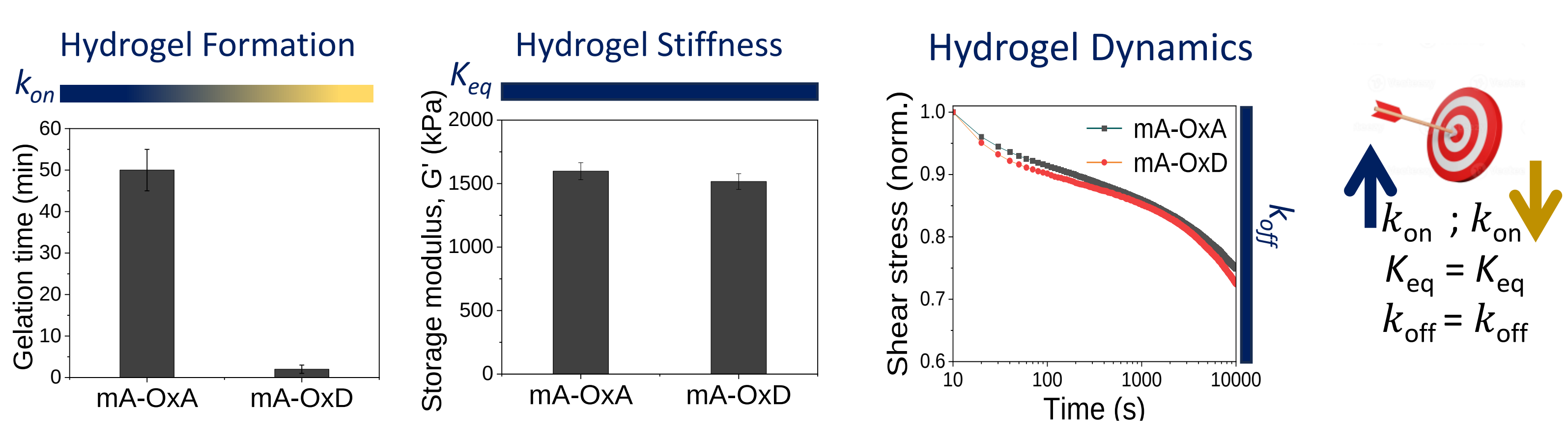

Fig. 4. Rheological characterization including hydrogel formation kinetics, stiffness, and stress relaxation behavior.

Dynamic Culture Conditions

Dynamic culture demonstrated distinct temporal mechanical responses in mA-OxA and mA-OxD hydrogels with different network formation kinetics (Fig. 5).

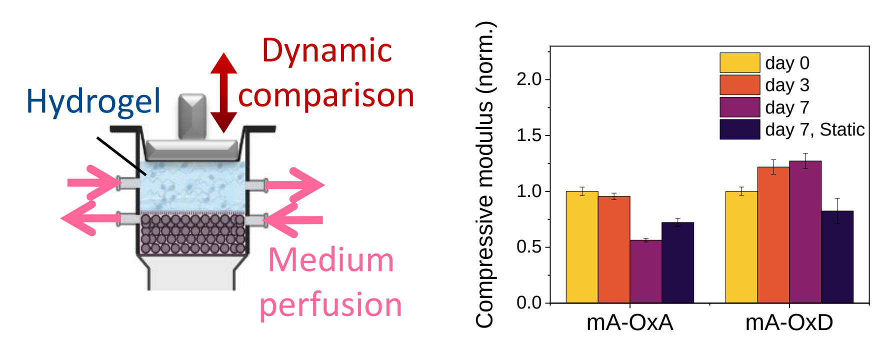

Fig. 5. Hydrogels behavior under dynamic culture conditions.

Hydrogel Performance

0.4 mm

Real-Time Label-Free Imaging

Early morphology differed between hydrogels: cells in mA–OxD were initially elongated, while cells in mA–OxA remained rounded. By day 21, chondrocytes in both hydrogels exhibited a predominantly rounded morphology (Fig. 6).

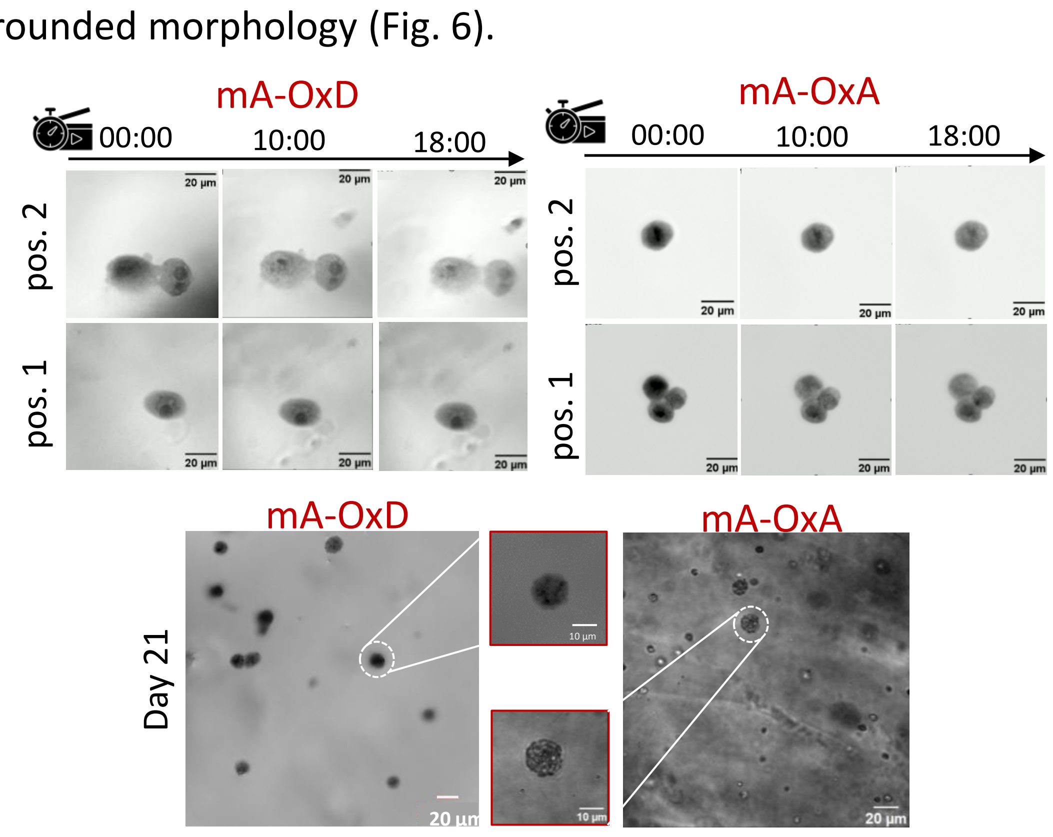

Fig. 6. Time-lapse bright-field microscopy images showing early (immediately after encapsulation) and late (day 21) chondrocyte morphology.

Fluorescence Imaging

Live/Dead confocal imaging confirmed high cell viability in both hydrogels. On day 1, chondrocytes in mA–OxD exhibited an elongated morphology, whereas cells in mA–OxA remained rounded. By day 14, cells in both hydrogels displayed a predominantly rounded phenotype.

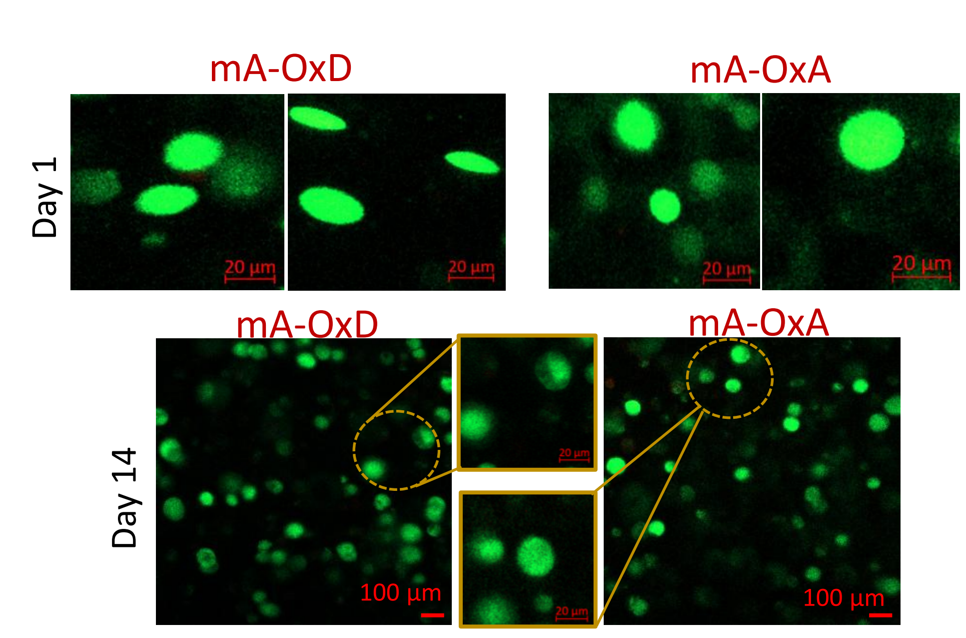

Fig. 7. Confocal fluorescence images of Live/Dead-stained chondrocytes showing chondrocyte morphology on days 1 and 14.

Key Finding and Conclusions

- Rapid gelation was achieved without compromising network dynamics, demonstrating decoupling between gelation kinetics and network dynamics.
- The recovery of rounded morphology over prolonged culture indicates that the dynamic acylhydrazone network retains sufficient stress-relaxation capability to permit cellular adaptation and morphological recovery over time.

Collectively, these results highlight the potential of dynamic acylhydrazone alginate hydrogels as advanced scaffolds that recapitulate native ECM adaptive dynamics.

References and Acknowledgments

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