Institute for Biomechanics
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A self-assembling *in vitro* model for the bone-tendon interface

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Motivation

The enthesis connects tendon to bone and transfers loads between these tissues. High mechanical demands make the enthesis prone to inflammation-induced remodeling and tearing, with currently poor clinical outcomes. Current knowledge on enthesis regeneration mainly originates from rodent studies which limit translation towards humans. Although several human *in vitro* enthesis models have been developed[1-3], **bidirectional cell-matrix interactions remain largely overlooked** (figure 1)[4]. To enable studying mechanistic interactions *in vitro*, we developed biphasic miniature tissues by **integrating** biomimetic **collagen mineralization**, **collagen fibrillogenesis**, and constrained **cellular embedding**.

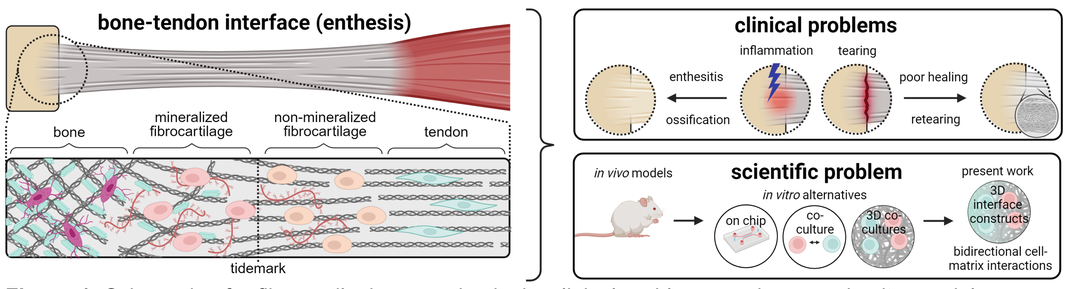


Figure 1. Schematic of a fibrocartilaginous enthesis, its clinical problems, and current *in vitro* model systems in relation to the present work.

Methods & Materials

A collagen mineralization premix containing NaOH and, for the bone compartment, $CaCl_2$ (low (L) = 5 mM, medium (M) = 7.5 mM, high (H) = 10 mM) and poly-aspartic acid (100 µg/ml) was prepared. Hyaluronic acid (HA; 2 mg/ml) was added to increase viscosity and enable formation of biphasic constructs. Acidic rat-tail collagen type I (1.5 mg/ml) was mixed into the premix, and for the bone-end a K_2HPO_4 solution (L = 2.5 mM, M = 3.75 mM, H = 5 mM) was added to induce *in situ* mineralization. Human tendon progenitor cells (TPCs) were mixed into the non-mineralized and mineralized hydrogel solutions (5 * 10^5 cells/ml). Cell-laden hydrogels were cast into custom-made molds with a separator in the center. After casting, separators were removed, posts were placed at both ends of the mold, and hydrogels were incubated for 30 minutes at 37 °C to allow fibrillogenesis (figure 2).

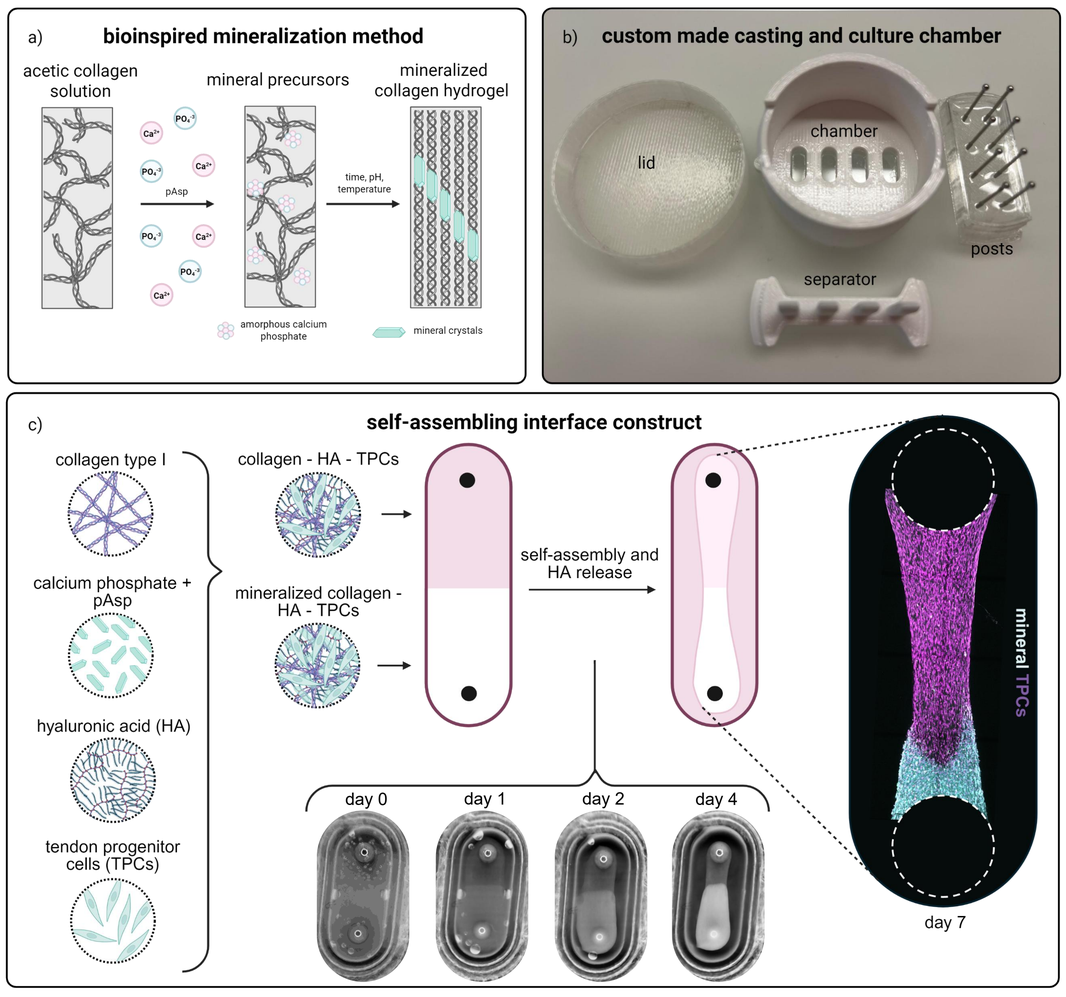
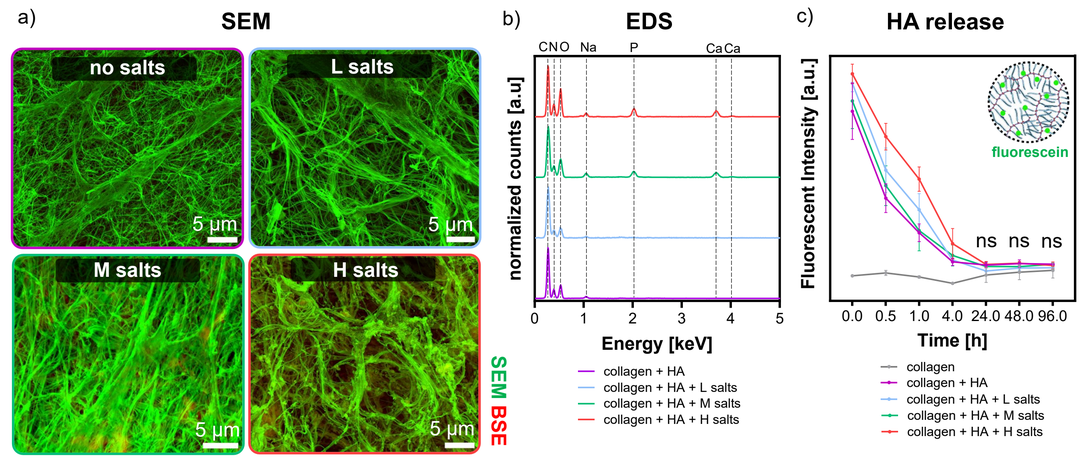


Figure 2. Experimental set-up. Polymer-induced liquid phase precursor mineralization was used to simultaneously achieve collagen fibrillogenesis and mineralization (a). Custom made chambers were used for hydrogel casting and culturing (b). Over time, cell-laden hydrogels self-assemble into biphasic constructs (c).

Results and Discussion

Mineralization of the bone-end was confirmed with scanning electron microscopy (SEM) and energy dispersive x-ray spectroscopy (EDS) in the M and H salt conditions, detecting calcium (Ca) and phosphorus (P). HA temporarily increased viscosity to enable biphasic casting and was released after 24 hours from all conditions. Cells were viable after 3 days in the L and M salt conditions, as shown by Calcein AM/Propidium Iodide staining. Therefore, the composition with a M salt concentration **enabled collagen mineralization while maintaining cell viability**. Cell tracking in constructs created with this salt concentration indicated minimal TPC migration across the bone-tendon interface on day 3 (figure 3).



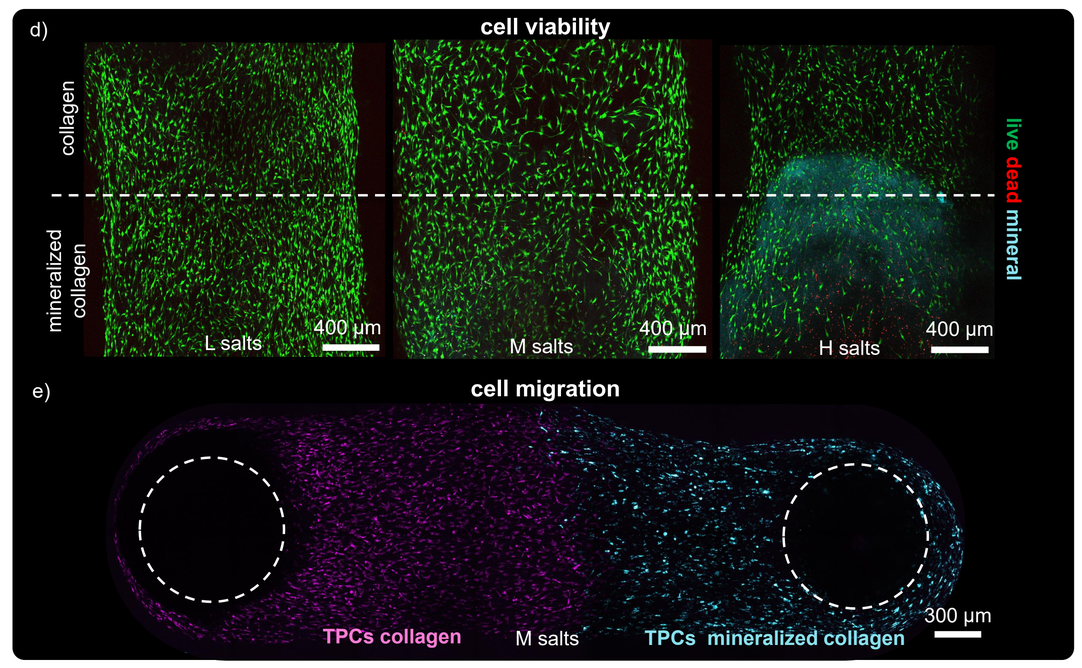


Figure 3. SEM images of (mineralized) hydrogels (a). EDS spectra of (mineralized) hydrogels (b). Quantification of HA release over time measured by the hydrogel fluorescent intensity after combining it with fluorescein-conjugated HA. *ns* = *non-significant* (c). Cell viability (Calcein AM/Propidium Iodide) after 3 days of culture, the interfaces were created with different salt concentrations (d). Cell migration after 3 days of culture across the interface (e).

Conclusion

This **one-step methodology** enables simultaneous **collagen fibrillogenesis, mineralization, and cell embedding**, generating a self-assembling **biphasic bone-tendon-mimetic construct**. This platform will facilitate mechanistic studies of cell-matrix interactions in future and may ultimately support the development of therapies for enthesis injuries.

References

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