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Semi-Interpenetrating Polymer Networks of Hyaluronan and Chitosan Self-Healing Hydrogels for Tissue Engineering

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Introduction Self-healing hydrogels with reversible crosslinks can recover the original morphologies and functions after repeated damages. In recent years, self-healing hydrogels have been extensively investigated for cell therapy and drug delivery through local injection. In the current study, self-healing hydrogel with semi-interpenetrating polymer network (SIPN) was prepared by incorporation of hyaluronan (HA) into chitosan-based self-healing hydrogel. As the HA content increased, the hydrogel showed a more packed nanostructure and a more porous microstructure verified by coherent small-angle X-ray scattering (CSAXS) and scanning electron microscopy (SEM). The unique structure of SIPN hydrogel enhanced the spreading, migration, proliferation, and differentiation of encapsulated cells.

Rheological analysis Chitosan (CS) hydrogels and chitosan-HA (CH) hydrogels were prepared via dynamic imine bonds. As HA content in hydrogel increased, the G' value decreased, and the G'' oscillation occurred at a later time (**Fig. 1a**). The addition of HA induced the rearrangement of network that delayed the dynamic crosslink formation. Moreover, each hydrogel displayed excellent self-healing efficiency (~100%), as revealed by the experiment of damage-healing cycles at alternate low and high dynamic strains (**Fig. 1b**).

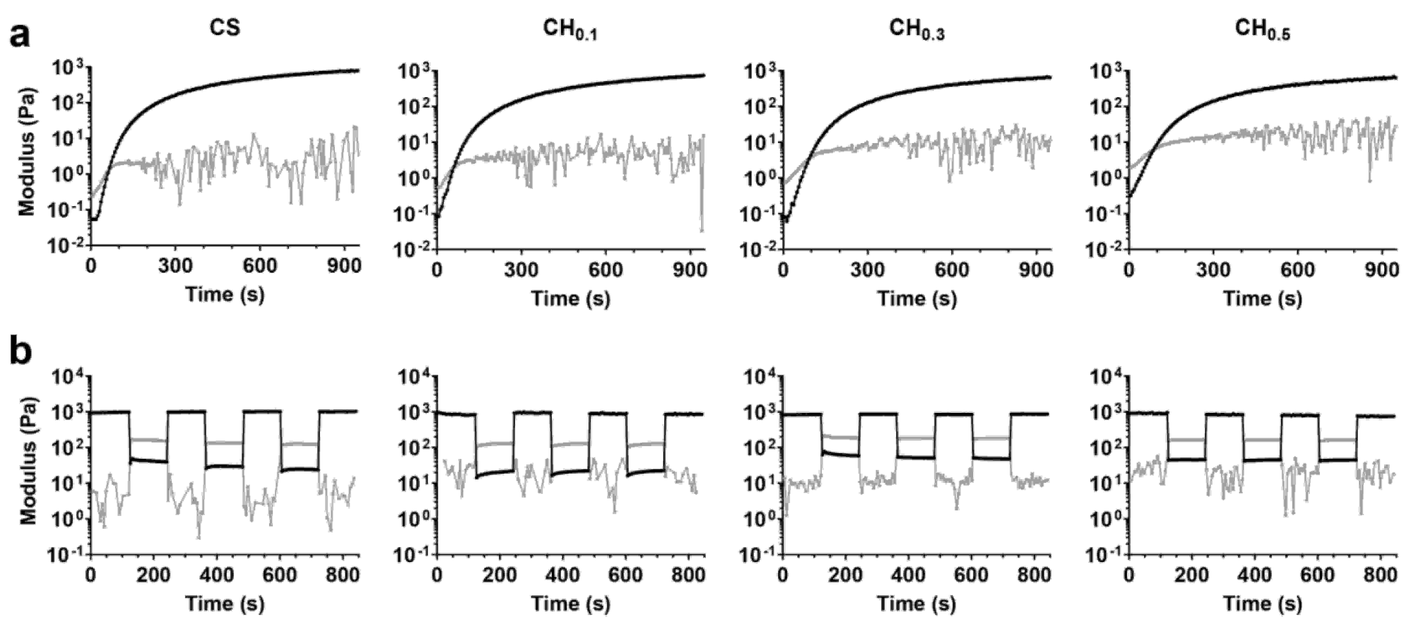

Fig. 1 Rheological properties of the hydrogels, including a) time sweep and b) damage-healing cycles. x represents to percent content of HA in CH_x. storage modulus (G', ●), loss modulus (G'', ●).

Structural Analysis CSAXS profiles indicated the hydrogels exhibited more packed nanostructure with increasing HA content (**Fig. 2a**). SEM images showed the larger hole size of CH hydrogel indicating more porous microstructure (**Fig. 2b**). A denser nanostructure and a looser microstructure in CH hydrogel suggested that HA polymer chains may be entangled in the network and generate steric repulsion forces that could produce pores in microscale but densify network in nanoscale.

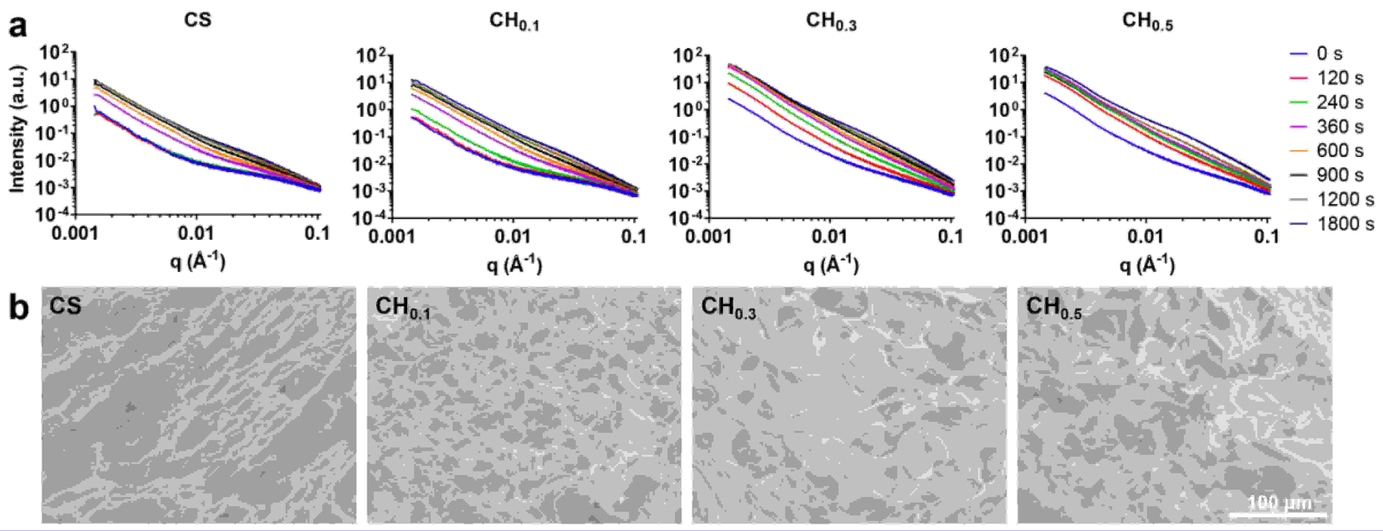

Fig. 2 a) CSAXS profiles of each hydrogel using in situ measurements against gelling time. b) SEM images of the freeze-dried hydrogels.

Live-Cell Imaging The microscopic photographs demonstrated more extension and migration of cells in CH compared to those in CS (**Fig. 3**). The cells remained circular shapes in CS which limited the cell growth.

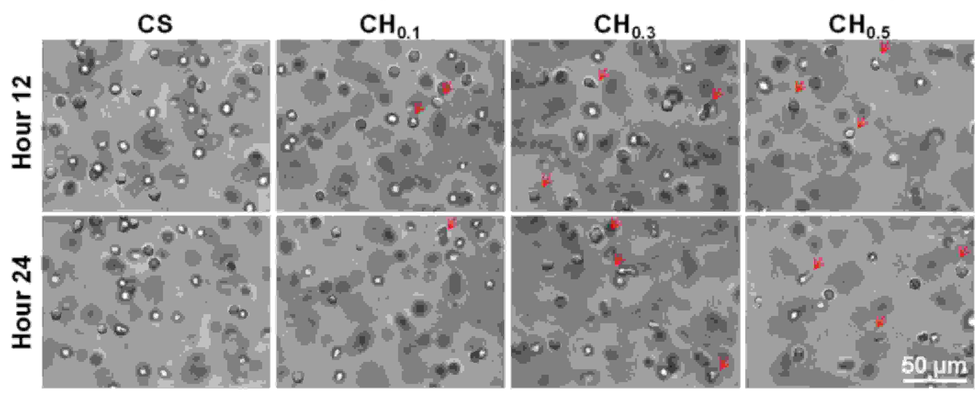

Fig. 3 Morphology of cells encapsulated in CS and CH hydrogels after 12 and 24 hours of cultivation. Filopodia and lamellipodia were indicated by arrows (↑).

Live/Dead Staining Apoptotic bodies of cells showed in CS, while cells had healthy growth in CH after 3-day culture (**Fig. 4**).

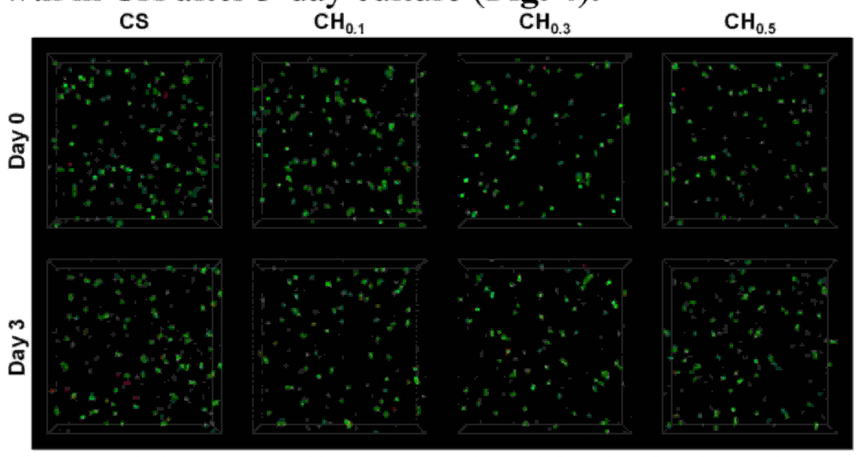

Fig. 4 Live/dead staining for cells encapsulated in the hydrogels. Red and green indicate dead and live cells, respectively. Grid: 100 μm.

Conclusion SIPN CH hydrogel had greater microporosity but was more densely packed in nanoscale. The unique hydrogel structure was favorable for cell spreading, migration, proliferation, and differentiation.