A soft-hearted approach to healing

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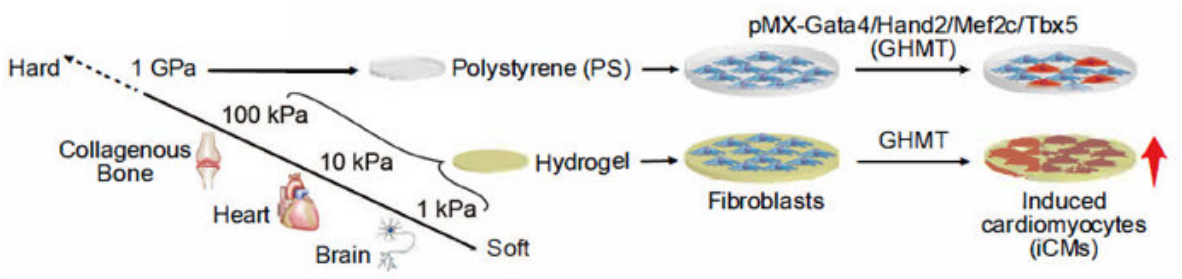

Credit: University of Tsukuba

Cardiovascular disease is the leading cause of death in humans. Worldwide, as the population ages, the burden of treating heart failure is increasing; opportunities for heart transplantation cannot keep pace. As adult cardiomyocytes (heart muscle cells) are terminally differentiated and do not proliferate, regeneration may be the answer. Support cells like fibroblasts can be directly reprogrammed in vitro, but these induced cardiomyocytes (iCMs) are less mature than those in vivo. Now, researchers at the University of Tsukuba have identified the roles of matrix stiffness and mechanotransduction in cardiac reprogramming, showing that matrices with softness comparable to native myocardium enhance the efficiency of this transformation by about 15%.

The mammalian myocardium is essentially incapable of regeneration following injury. Instead, a non-contractile fibrous scar forms to

maintain structural integrity; compensatory hypertrophy is often inadequate to prevent eventual cardiac failure. Though cell transplantation holds potential, regeneration of damaged myocardium by direct reprogramming of cardiac fibroblasts, present in abundance, is a viable alternate strategy.

The researchers sought to investigate the signaling pathways for cardiac reprogramming as well as the underlying mechanotransductive processes whereby cells convert physical stresses into electrochemical signals. They first prepared Matrigel-coated polystyrene dishes and Matrigel-based hydrogels to replicate extracellular matrices (ECM) with elasticities ranging from that of brain tissue to bone. These substrates were plated with transgenic mouse fibroblasts which were later transduced with four cardiac transcription factors, Gata4, Mef2c, Tbx5 and hand2 (GHMT), to generate iCMs.

"After four weeks, we found that soft substrates showed significantly more spontaneously beating iCMs, maximally on those matching native myocardium," says senior author Professor Masaki Ieda. "Additionally, using innovative high-speed video microscopy and motion vector analysis, we demonstrated increased iCM contraction/relaxation velocities on those substrates, thus illustrating their functional maturation."

Further immunocytochemistry, western blot analysis, and fluorescence-activated cell sorting analysis helped elucidate the underlying mechanisms and signaling pathways. The researchers showed that soft ECM promotes cardiac reprogramming by inhibiting two related transcriptional co-activators, YAP and TAZ, thus suppressing fibroblast signatures. The upstream mechanotransduction pathway was also elucidated; suppression of YAP/TAZ was mediated by inhibition of integrins (transmembrane receptors that facilitate and signal ECM adhesion), Rho/ROCK (a kinase that modulates cell shape and

movement), and actomyosin (a contractile protein-complex).

Professor Ieda explains the implications of their results: "Following a heart attack, the healing myocardium stiffens due to fibrosis. Understanding how matrix softness and mechanobiology affect cardiac reprogramming could inform clinical research. Direct reprogramming of cardiac fibroblasts may allow replacement of non-contractile scar by functional muscle in patients recovering from myocardial infarction."

More information: Shota Kurotsu et al. Soft Matrix Promotes Cardiac Reprogramming via Inhibition of YAP/TAZ and Suppression of Fibroblast Signatures, *Stem Cell Reports* (2020). DOI: 10.1016/j.stemcr.2020.07.022

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