3D ECM Stiffness Modulates Energy Metabolism to Drive Stem Cell Osteogenic Differentiation

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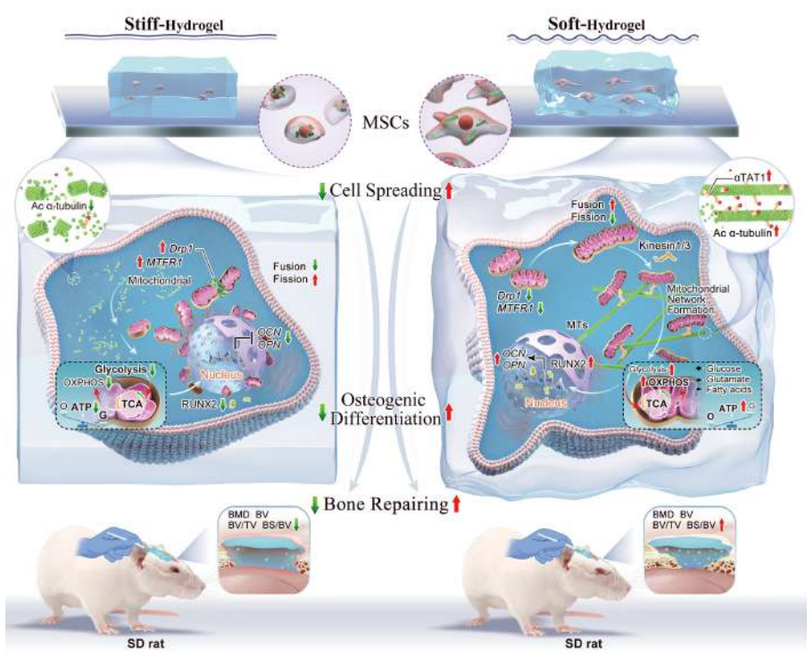
Highlights

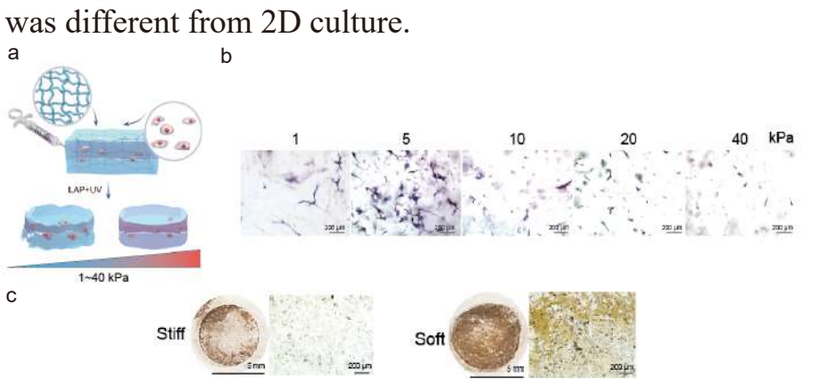


Introduction

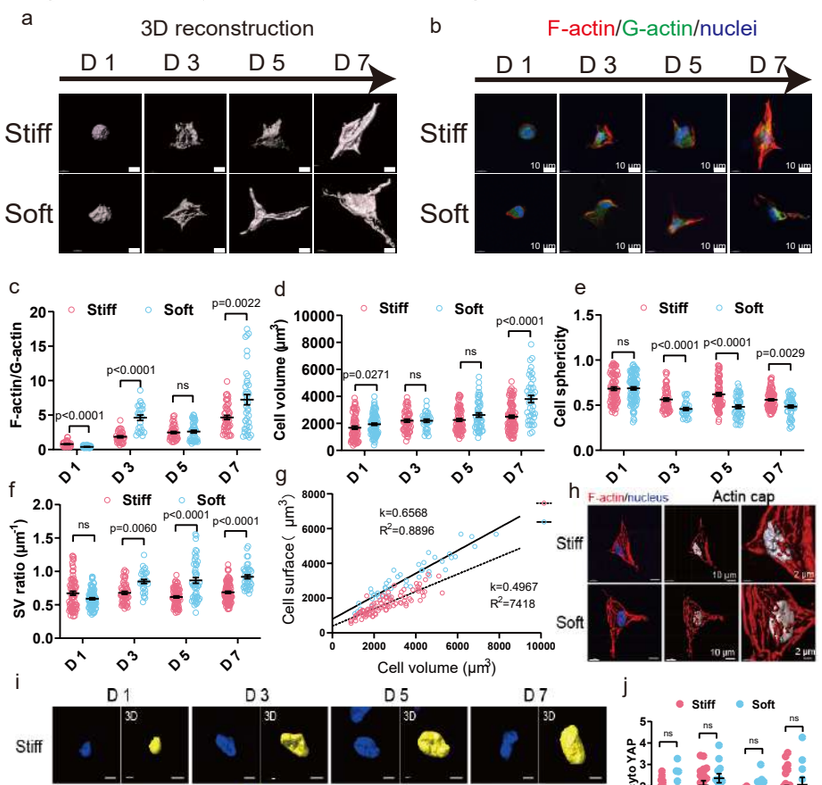
The early stage of bone reforming contains low stiffness ECM. Matrix stiffness is crucial to regulating stem cell behavior. However, the mechanism of stem cells respond to matrix stiffness of 3D culture enviroment was still unrevealed. Our study aims to explore the effect of ECM stiffness on the differentiation of MSCs in 3D cultures and the potential mechanism.

Results

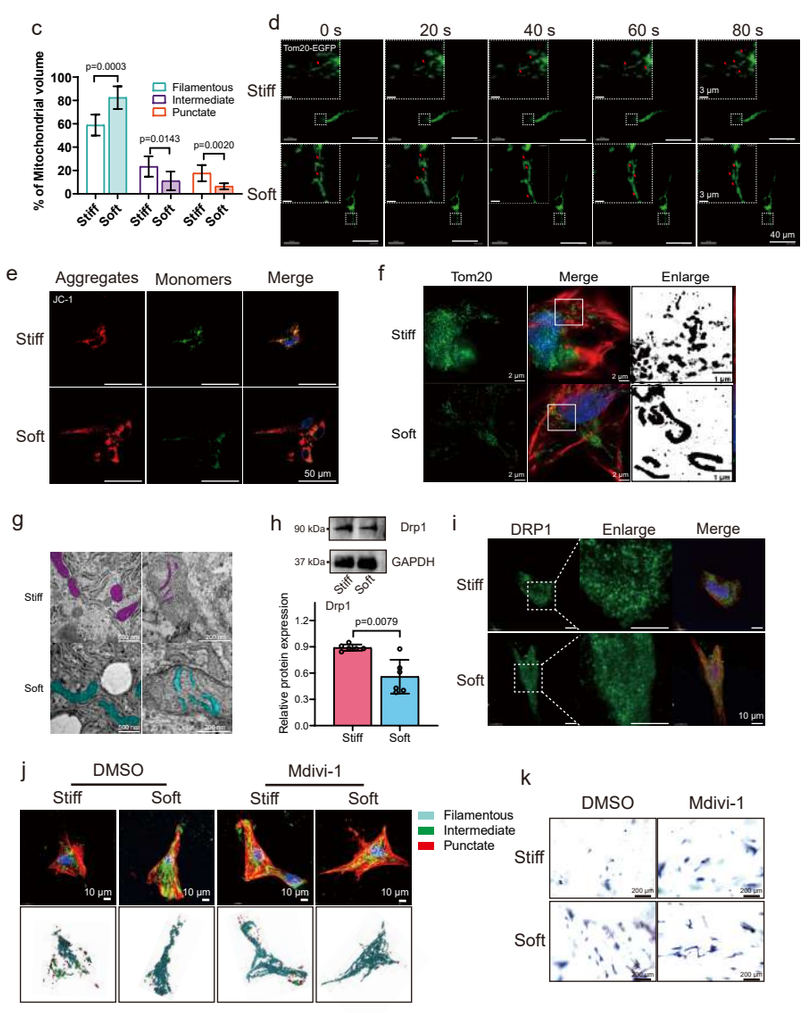
For 3D culture, hMSCs and GelMA were used to construct a 3D culture system with various matrix stiffness. In our studies, it was found that the softer (1-5 kPa) matrix promoted osteogenic differentiation both in vitro, ALP staining, compared to the stiffer (10-40kPa) matrix, which was different from 2D culture.



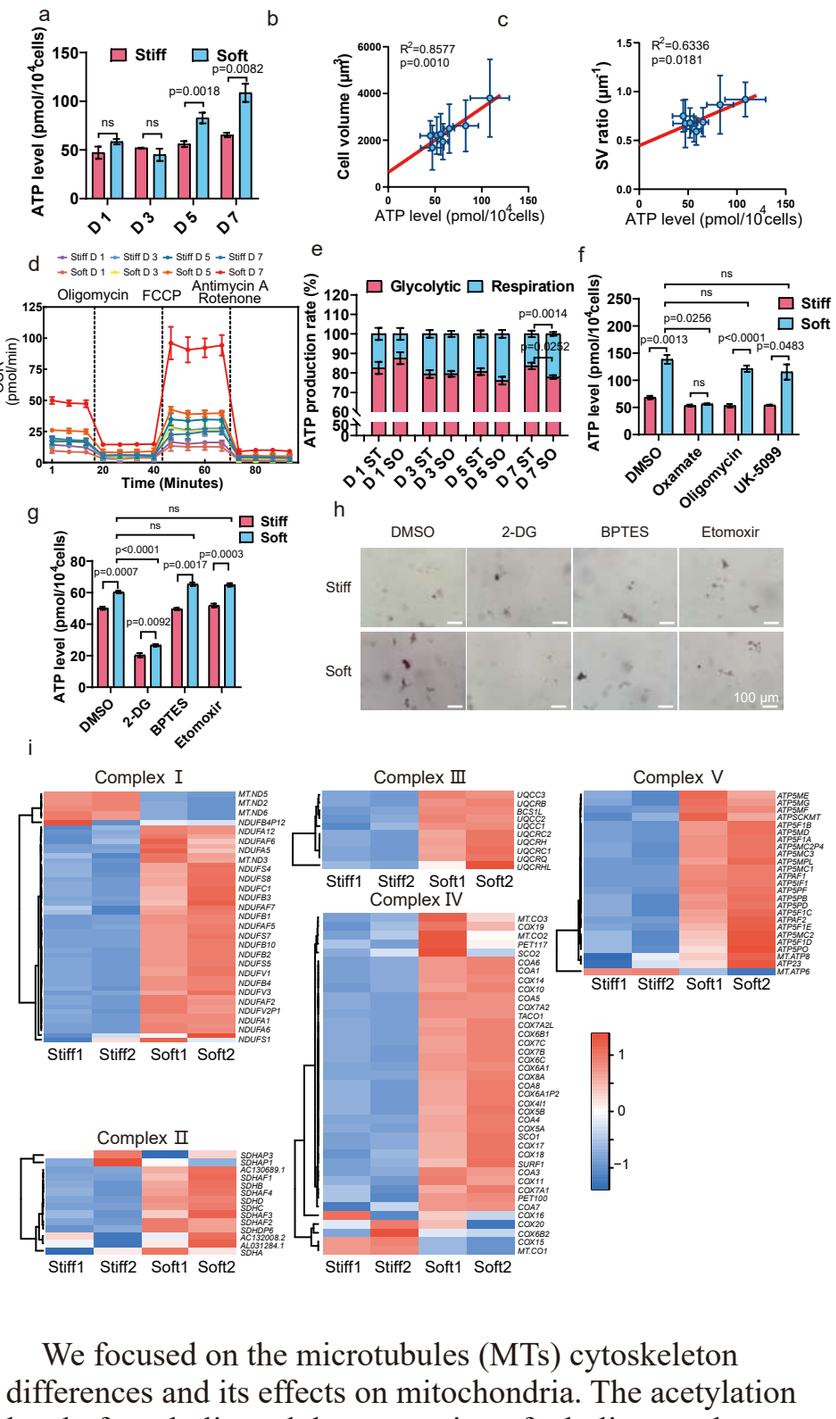
Compared with the stiff matrix (~ 20 kPa), the cells in the soft matrix spread faster and showed larger volume (~3000 μm3), with higher sueface/volume ratio, indicating higher efficacy of nutrient exchange.



Compared with the stiff matrix, the cells in the soft matrix had more filamentous-structure-like mitochondria (~80 %), more mature crests. The mitochondria networking was mediated by restrain the expression of Dynamin-related protein (Drp1), the mitochondrial fission factor. Treatment with Drp1 inhibitor Mdivi-1significantly promoted mitochondrial networking and osteogenesis in stiff matrix.

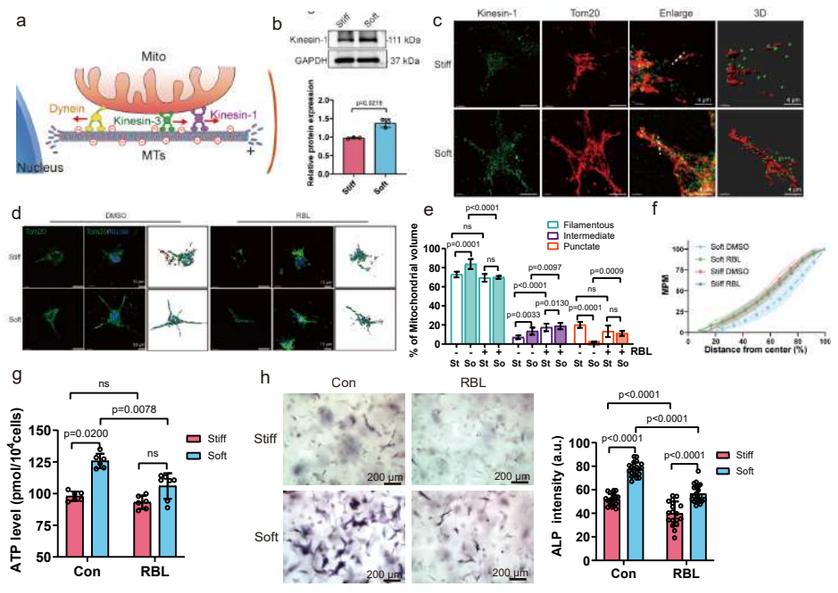


Compared with the stiff matrix, the cells in the soft matrix had more active metabolism. We further discovered that inhibition of glucose-based, amino acid-based and fatty acid-based metabolism inhibited osteogenic differentiation,while only glucose-based metabolism significantly inhibited ATP production, suggesting the existence of regulatory other than energy.

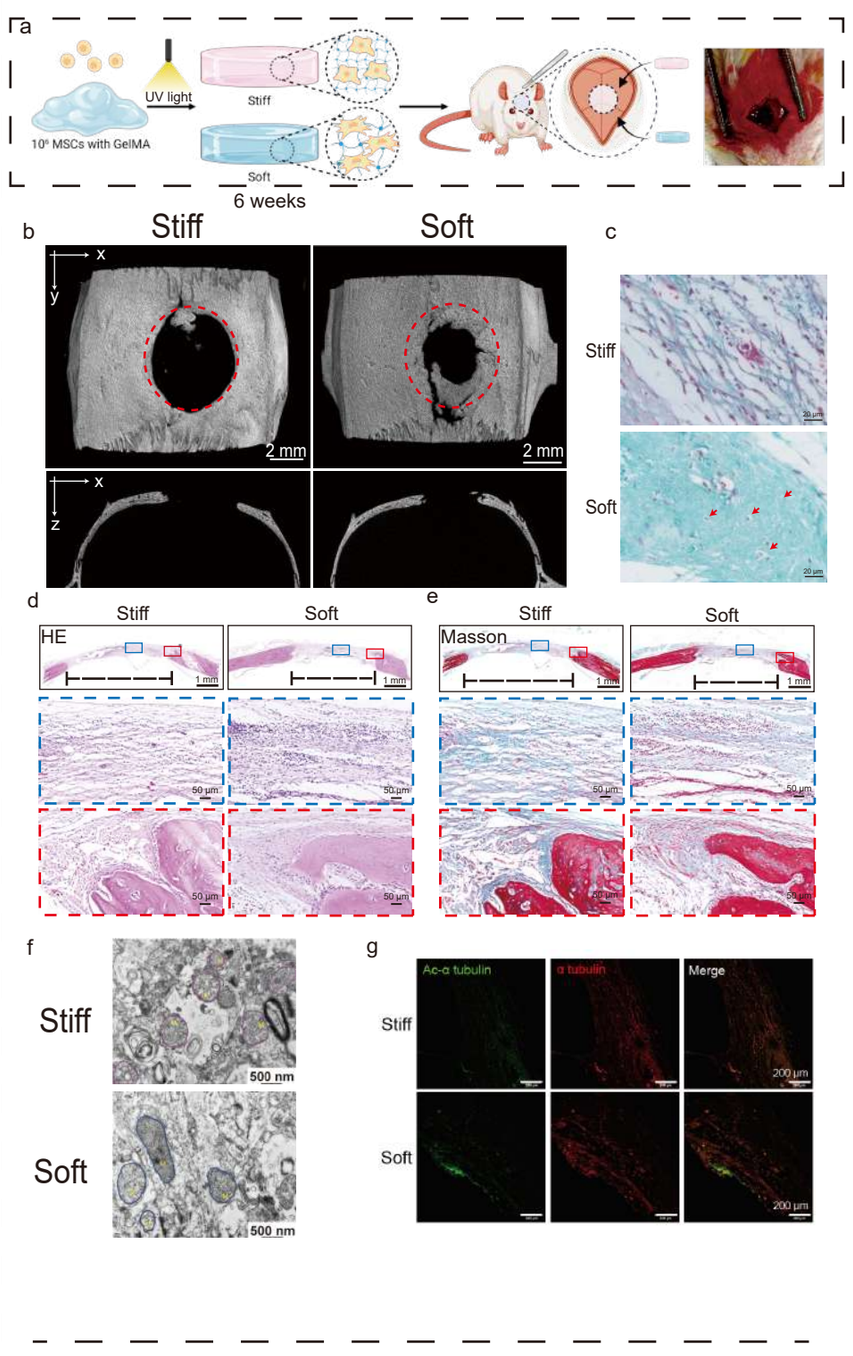


We focused on the microtubules (MTs) cytoskeleton differences and its effects on mitochondria. The acetylation level of α-tubulin and the expression of tubulin acetylase αTAT1 were significantly up-regulated in the soft matrix group. The mitochondrial networking, and ALP activity was inhibited by si-αTAT1 or eliminating microtubule homeostasis with Taxol.

Kinesins and Dynein are charged for organelle transporting along the MTs. In 3D soft ECM, Kinesin-1/3 were found upregulated, among which Kinesin-1 directly mediated mitochondrial transporting and network formation. The inhibitor RBL were performed to test the function of Kinesin-1.



For evaluating the effect of 3D matrix stiffness on hMSCs osteogenic differentiation in vivo, we preformed the rats' skull defect and repairing experiment. The results showed that the soft-matrix-MSCs hydrogel significantly promoted the repair of rat calvarial defects. Furthermore, we observed that the larger mitochondria size and more mature crest in the soft-matrix-MSCs hydrogel implanted area with higher acetylation level of α-tubulin, which again proved our above points.



Summary

In 3D culture, **soft matrix** was proved promoted cell expanding and osteogenesis and bone regeneration. Soft 3D ECM increased the activities of **glycolysis and OXPHOS**, promoted **metabolic mode shifting**. The mitochondria of soft 3D ECM exhibited **more mature network and more uniform distribution**. In 3D culture, **YAP was found not participating** in mediating 3D matrix stiffness regulation**.** Soft 3D ECM promoted mitochondrial network formation and distribution by **restraining DRP1-depended mitochondrial fission** and enhancing **microtubule acetylation** and its motor protein **Kinesin-1** expression to support mitochondrial transport, further influenced MSCs differentiation. Our findings present an effective metabolic target for stem cells clinic application and provide new insights for 3D biomaterial design.

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