Science Advances
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# Supplementary Materials for

## Immunomodulatory multicellular scaffolds for tendon-to-bone regeneration

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**The PDF file includes:**

Figs. S1 to S7
Table S1
Legend for movie S1
References

**Other Supplementary Material for this manuscript includes the following:**

Movie S1

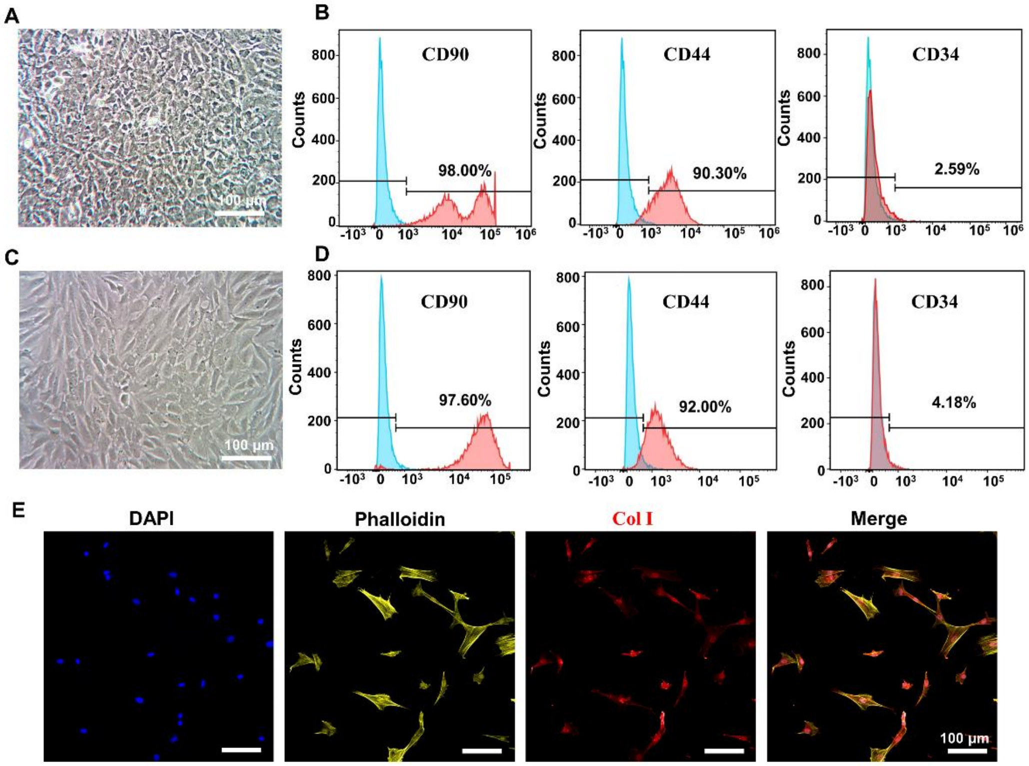



**Figure S1**. (A) The morphology and (B) surface marker expression (related to mesenchymal/hematopoietic stem cells) of bone marrow mesenchymal stem cells (BMSCs). (C) The morphology and (D) surface marker expression (related to mesenchymal/hematopoietic stem cells) of tendon stem/progenitor cells (TSPCs). (E) Immunofluorescence staining images of Col I protein expressed by primary TSPCs. **Both BMSCs and TSPCs adhered to the culture dish and possessed the morphological characteristics of mesenchymal stem cells. Besides, all TSPCs expressed high level of type I collagen, which was consistent with the previous work.(*80*) The above results demonstrated the successful isolation of BMSCs and TSPCs.**

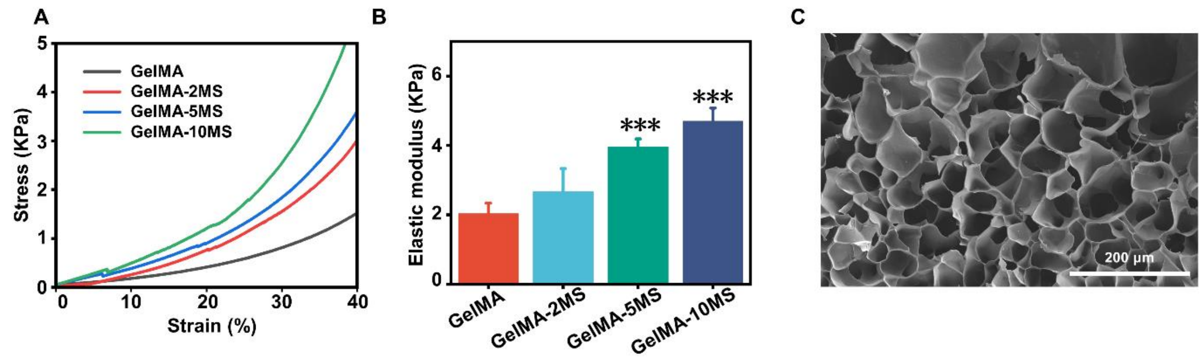



**Figure S2.** (A) The stress-strain curve and (B) elastic modulus of scaffolds with different contents of MS nanoparticles ($n = 4$). (C) SEM images of GelMA-5MS bioink. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. **The incorporation of MS nanoparticles increased the mechanical strength of GelMA hydrogel but did not affect their porous structure.**

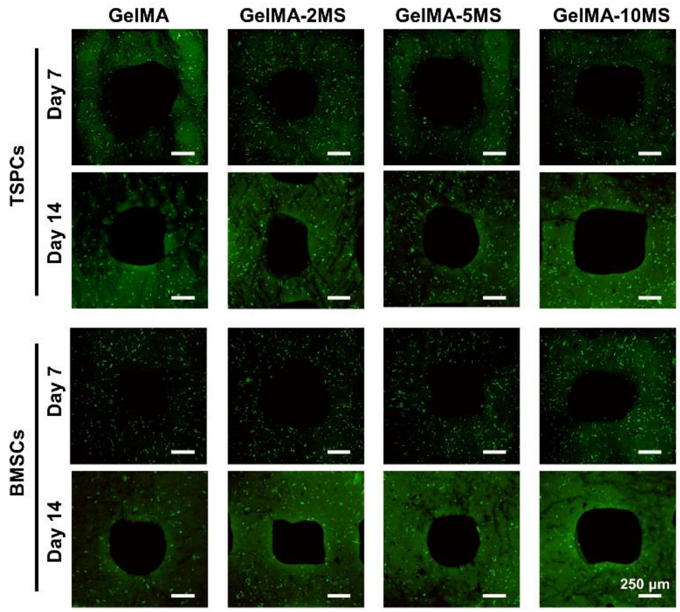



**Figure S3.** Live/dead staining images of TSPCs and BMSCs in multicellular scaffolds containing different concentrations of MS nanoparticles after cultured for 7 and 14 days.

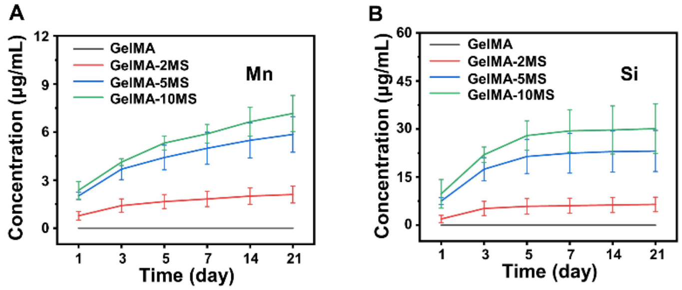



**Figure S4.** (A) Mn ions and (B) Si ions release curves of multicellular scaffolds containing different concentration of MS nanoparticles ($n = 4$) **indicated that multicellular scaffolds based on MS nanoparticles could release Mn and Si ions stably during 21-day culture period.** $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

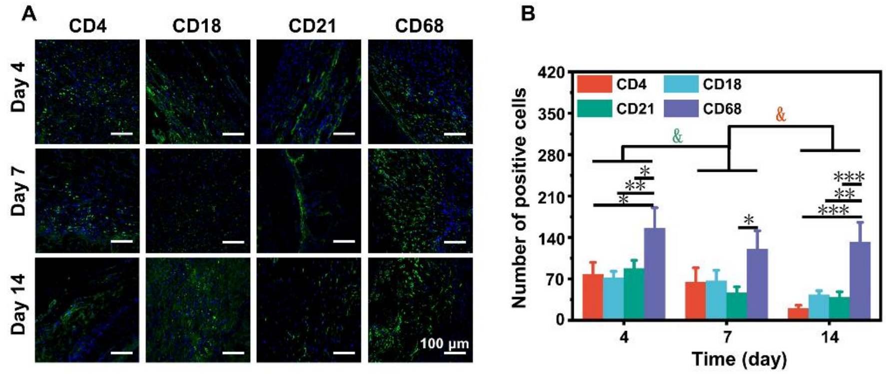
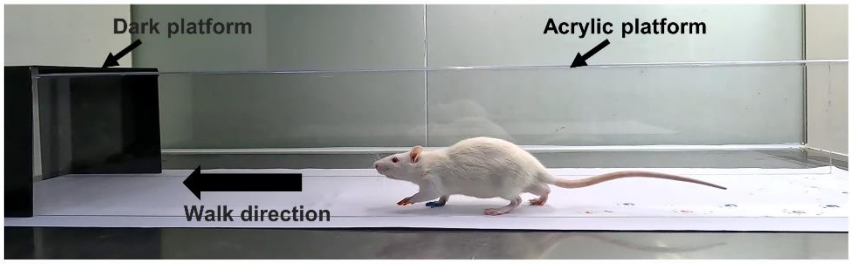



**Figure S5. The number statistics of immune cells surrounding the damaged regions after implantation of GelMA-cells-MS scaffold.** (A) Immunofluorescence staining images of CD4 (T cells marker), CD18 (neutrophils marker), CD21 (B cells marker) and CD68 (macrophages marker) at day 4, 7 and 14 after surgery. (B) The corresponding number statistics of T cells, neutrophils, B cells and macrophages ($n$ = 3). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ (comparing the expression of different marks at the same time points). &$p < 0.05$, &&$p < 0.01$, &&&$p < 0.001$ (comparing the expression of the same mark at different time points. Red: CD4; Blue: CD18; Green: CD21; Purple: CD68).

A




B

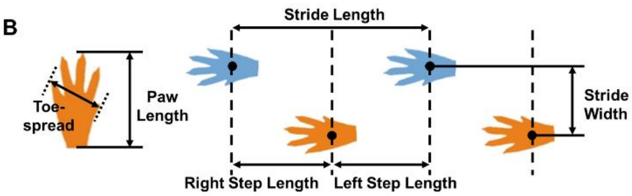



**Figure S6.** (A) Walking apparatus for recording rat pawprints. (B) Schematic diagram of paw and gait parameters.

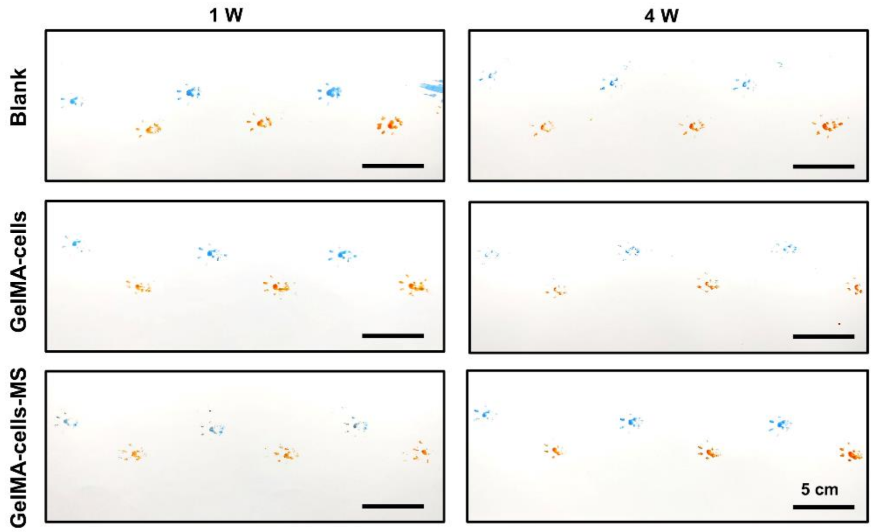



**Figure S7.** Pawprints of rats in the Blank, GelMA-cells, and GelMA-cells-MS groups at 1 and 4 weeks postoperatively.

**Table S1. The primer sequences used for RT-qPCR assays.**

The primer sequences of tenogenic and osteogenic genes.

| Gene | | Primer sequence |
|---|---|---|
| *GAPDH* | Forward | TCACCATCTTCCAGGAGCGA |
| *GAPDH* | Reverse | CACAATGCCGAAGTGGTCGT |
| *Runx2* | Forward | CCTCTGACTTCTGCCTCTGG |
| *Runx2* | Reverse | GATGAAATGCCTGGGAACTG |
| *OCN* | Forward | ACAAGTCCCACACAGCAACTC |
| *OCN* | Reverse | CCAGGTCAGAGAGGCAGAAT |
| *OPN* | Forward | GAGACCGTCTGAAACAGCGT |
| *OPN* | Reverse | AACCACTGCCAGTCTCATGG |
| *BMP2* | Forward | GAGGAGAAGCCAGGTGTCT |
| *BMP2* | Reverse | GTCCACATACAAAGGGTGC |
| *BSP* | Forward | GAATCCACATGCCTATTGC |
| *BSP* | Reverse | AGAACCCACTGACCCATT |
| *TNC* | Forward | CGTGAAAAACAATACCCGAGGC |
| *TNC* | Reverse | GCCGTAGGAGAGTTCAATGCC |
| *DCN* | Forward | ACTGGGCACCAACCCTCTGA |
| *DCN* | Reverse | ATCTGAAGGTGGATGGCTGGA |
| *BGN* | Forward | GATGGCCTGAAGCTCAA |
| *BGN* | Reverse | GGGTTGTTGAAGAGGCTG |

The primer sequences of macrophage phenotype-related genes.

| Gene | | Primer sequence |
|---|---|---|
| *GAPDH* | Forward | TCACCATCTTCCAGGAGCGA |
| *GAPDH* | Reverse | CACAATGCCGAAGTGGTCGT |
| *CCR7* | Forward | CCATGACGGATACCTACCTGCT |
| *CCR7* | Reverse | CCCTTACACAGGTAGACGCCAA |
| *iNOS* | Forward | ACGCTTCACTTCCAATGCAAC |
| *iNOS* | Reverse | CAGCCTCATGGTAAACACGTTC |
| *IL-6* | Forward | ATAGTCCTTCCTACCCCAATTTCC |
| *IL-6* | Reverse | GATGAATTGGATGGTCTTGGTCC |
| *IL-1β* | Forward | CTACCTGTGTCTTTCCCGTG |
| *IL-1β* | Reverse | TTTGTTGTTCATCTCGGAGC |
| *TNFα* | Forward | CTGTAGCCCACGTCGTAGCAA |
| *TNFα* | Reverse | TGTCTTTGAGATCCATGCCGTT |
| *CD206* | Forward | ATCCACGAGCAAATGTACCTCA |
| *CD206* | Reverse | TAGCCAGTTCAGATACCGGAA |
| *Arg-1* | Forward | ATCAACACTCCCCTGACAACC |
| *Arg-1* | Reverse | TCGCAAGCCAATGTACACGAT |
| *IL-10* | Forward | GAGAAGCATGGCCCAGAAATC |
| *IL-10* | Reverse | GAGAAATCGATGACAGCGCC |
| *IL-4* | Forward | AGATGGATGTGCCAAACGTCCTCA |
| *IL-4* | Reverse | AATATGCGAAGCACCTTGGAAGCC |

**Video S1. Video of gait analysis of rats in all groups at different time points.**

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