## **Supporting Information**

## Injectable and degradation heterogeneous microgel assembly capable of forming "micro-nest group" for cell condensation and cartilage regeneration

Zequ Lin<sup>1,2,7</sup>, Qingtao Li<sup>2,3,7</sup>, Xiyuan Han<sup>1,2</sup>, Huitong Luo<sup>1,2</sup>, Zetao Wang<sup>1,2</sup>, Zhihao Qin<sup>1,2</sup>, Yue Huang<sup>6</sup>, Qi Feng<sup>1,2,4,6\*</sup>, Xiaodong Cao<sup>1,2,4,5\*</sup>

<sup>1</sup>Department of Biomedical Engineering, School of Materials Science and Engineering, South China University of Technology, Guangzhou, 510006, China <sup>2</sup>National Engineering Research Center for Tissue Restoration and Reconstruction (NERC-TRR), South China University of Technology, Guangzhou, 510006, China <sup>3</sup>School of Medicine, South China University of Technology, Guangzhou 510006, P. R. China.

<sup>4</sup>Key Laboratory of Biomedical Materials and Engineering of the Ministry of Education, South China University of Technology, Guangzhou 510006, China

<sup>5</sup>Key Laboratory of Biomedical Engineering of Guangdong Province, South China University of Technology, Guangzhou, 510641, China

<sup>6</sup>School of Stomatology, Jinan University, Guangzhou, 510641, China

<sup>7</sup>These authors contributed equally

\*Correspondence:

caoxd@scut.edu.cn (X.D.C)

feng2119@scut.edu.cn (Q.F.)

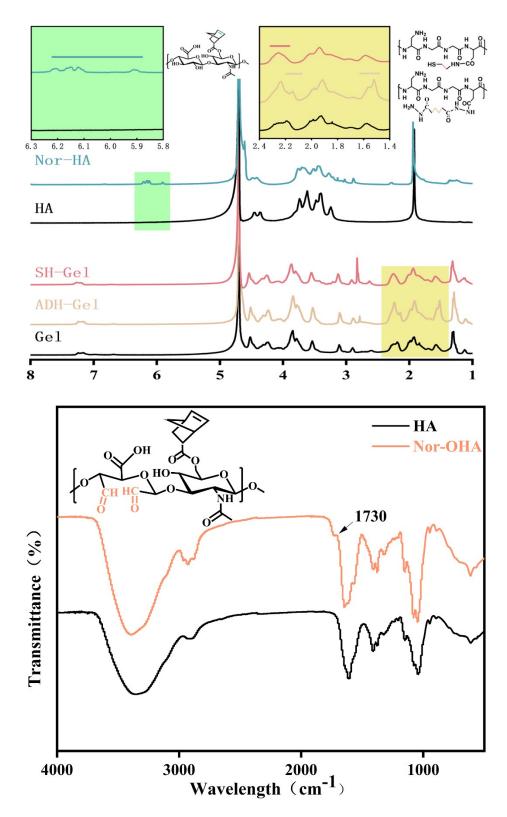


Figure S1. <sup>1</sup>H NMR spectra of Nor-HA, ADH-Gel and SH-Gel, and IR spectra of Nor-OHA.

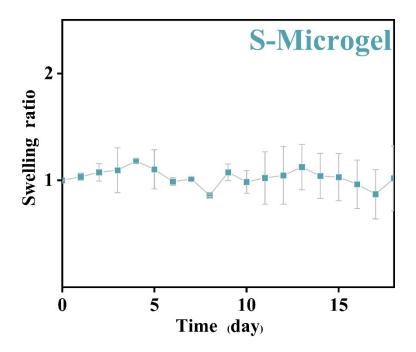


Figure S2. Swelling of S-microgel.

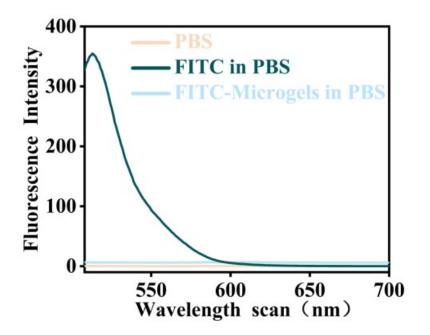


Figure S3. Fluorescence spectrum of FITC-PEG-SH loaded S-microgels.

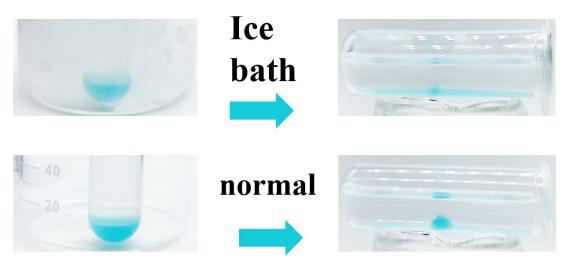


Figure S4. State of F-microgels at room temperature and in ice bath.

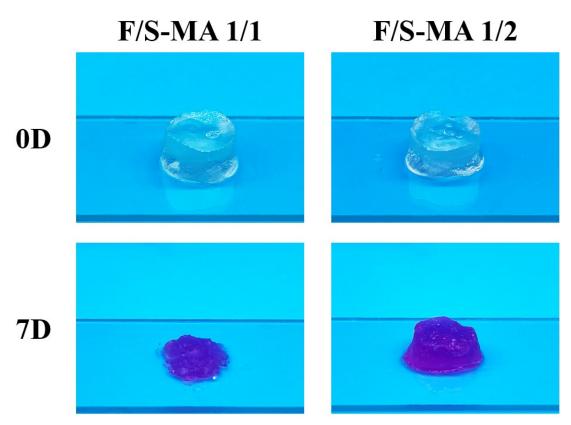


Figure S5. Macroscopic images of F/S-MA 1/1 and F/S-MA 1/2 after 1 and 7 days of degradation.

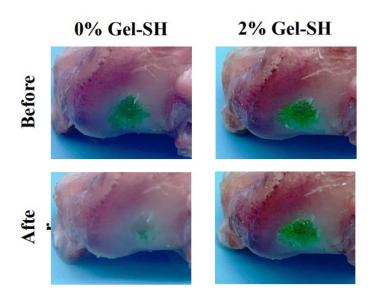


Figure S6. Adhesion of microgel assemblies, adhesion before and after PBS flushing.

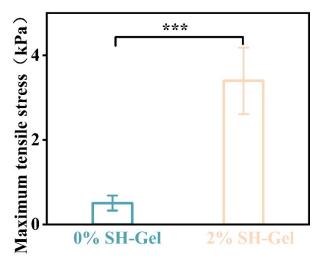
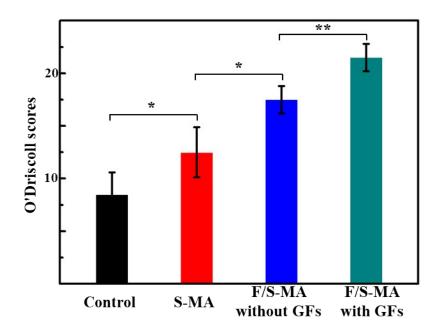


Figure S7. Maximum tensile stress of microgel assembles. (Without and with assembly agent.)



Figure S8 Surgical procedures for the treatment of articular cartilage injuries



**Figure S9** O'Driscoll scores of Control (microfracture), S-MA, F/S-MA without GFs and F/S-MA with GFs.

| Table S1 Lists of primers using in RT-PCR |                            |  |  |
|-------------------------------------------|----------------------------|--|--|
| Target gene                               | Sequence (5'-3')           |  |  |
| ACAN                                      | F: AGTGGATCGGTCTGAATGACAGG |  |  |
|                                           | R: AGAAGTTGTCAGGCTGGTTTGGA |  |  |
| Sox9                                      | F: AGCACAAGAAAGACCACCCC    |  |  |
|                                           | R: ATGTGAGTCTGTTCCGTGGC    |  |  |
| Col 2                                     | F: AGGGCAACAGCAGGTTCACATAC |  |  |
|                                           | R: TGTCCACACCAAATTCCTGTTCA |  |  |
| Col 1                                     | F: ATGCCGCGACCTCAAGATG     |  |  |
|                                           | R: TGAGGCACAGACGGCTGAGTA   |  |  |
| Gapdh                                     | F: GTCGGTGTGAACGGATTTGG    |  |  |
|                                           | R: CTGGAAGATGGTGATGGGCT    |  |  |

## Table S2 Modified cartilage repair score system

|             |                 | category                    | score |
|-------------|-----------------|-----------------------------|-------|
|             |                 | Hyaline articular cartilage | 4     |
|             | Cellular        | Incompletely differentiated | 2     |
| Nature of   | morphology      | mesenchyme                  | 2     |
| predominant |                 | Fibrous tissue or bone      | 0     |
| tissue      | Safranin O      | Normal or nearly normal     | 3     |
|             | staining of the | Moderate                    | 2     |
|             | matrix          | Slight                      | 1     |

|                                                        |                                                                     | None                                              | 0  |  |
|--------------------------------------------------------|---------------------------------------------------------------------|---------------------------------------------------|----|--|
|                                                        | Collagen<br>IIstaining of the<br>matrix                             | normal or nearly normal                           | 3  |  |
|                                                        |                                                                     | Moderate                                          | 2  |  |
|                                                        |                                                                     | Slight                                            | 1  |  |
|                                                        |                                                                     | None                                              | 0  |  |
|                                                        | Surface<br>regularity                                               | Smooth and intact                                 | 3  |  |
|                                                        |                                                                     | Superficial horizontal lamination                 | 2  |  |
|                                                        |                                                                     | Fissures: 25–100% of the thickness                | 1  |  |
|                                                        |                                                                     | Severe disruption, including fibrillation         | 0  |  |
|                                                        | Structural integrity                                                | Normal                                            | 2  |  |
|                                                        |                                                                     | Slight disruption, including cysts                | 1  |  |
| Structural                                             |                                                                     | Severe disintegration                             | 0  |  |
| characteristics                                        | Thickness                                                           | 100% of normal adjacent cartilage                 | 2  |  |
|                                                        |                                                                     | 50–100% of normal cartilage                       | 1  |  |
|                                                        |                                                                     | 0–50% of normal cartilage                         | 0  |  |
|                                                        | Bonding to the adjacent                                             | Bonded at both ends of graft                      | 2  |  |
|                                                        |                                                                     | Bonded at one end, or partially at both ends      | 1  |  |
|                                                        | cartilage                                                           | Not bonded                                        | 0  |  |
| Freedom from<br>cellular<br>changes of<br>degeneration | Hypocellularity                                                     | Normal cellularity                                | 3  |  |
|                                                        |                                                                     | Slight hypocellularity                            | 2  |  |
|                                                        |                                                                     | Moderate hypocellularity                          | 1  |  |
|                                                        |                                                                     | Severe hypocellularity                            | 0  |  |
|                                                        | Chondrocyte<br>clustering                                           | No clusters                                       | 2  |  |
|                                                        |                                                                     | < 25% of the cells                                | 1  |  |
|                                                        |                                                                     | 25–100% of the cells                              | 0  |  |
|                                                        | Freedom from<br>degenerative<br>changes in<br>adjacent<br>cartilage | Normal cellularity, no clusters, normal staining  | 3  |  |
|                                                        |                                                                     | Normal cellularity, mild clusters,                | 2  |  |
|                                                        |                                                                     | moderate staining                                 | -  |  |
|                                                        |                                                                     | Mild or moderate hypocellularity, slight stainung | 1  |  |
|                                                        |                                                                     | Severe hypocellularity, poor or nostaining        | 0  |  |
| total score                                            |                                                                     |                                                   |    |  |
|                                                        |                                                                     |                                                   | 27 |  |