Biomaterials Science

SUPPLEMENTAL MATERIAL

Cartilage regeneration using transforming growth factor beta-3-loaded injectable crosslinked hyaluronic acid hydrogel

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Supplemental Table: 2

Supplemental Schemes: 5

Supplemental Figures: 3

Supplemental Tables

Experimental group	Defect model	Transplantation materials
Control (No-defect)	—	—
Sham control (Only defect)	Chondral defect (circular shape, diameter: 4 mm, depth: 2 mm)	—
HA		HA hydrogel 100 μL
HAT		HAT hydrogel 100 μL

Supplemental Table 1. *In vivo* experimental groups, defect models, and transplantation material used for evaluating cartilage regeneration. HA, hyaluronic acid; HAT, hyaluronic acid loaded with transforming growth factor-beta 3.

(a)

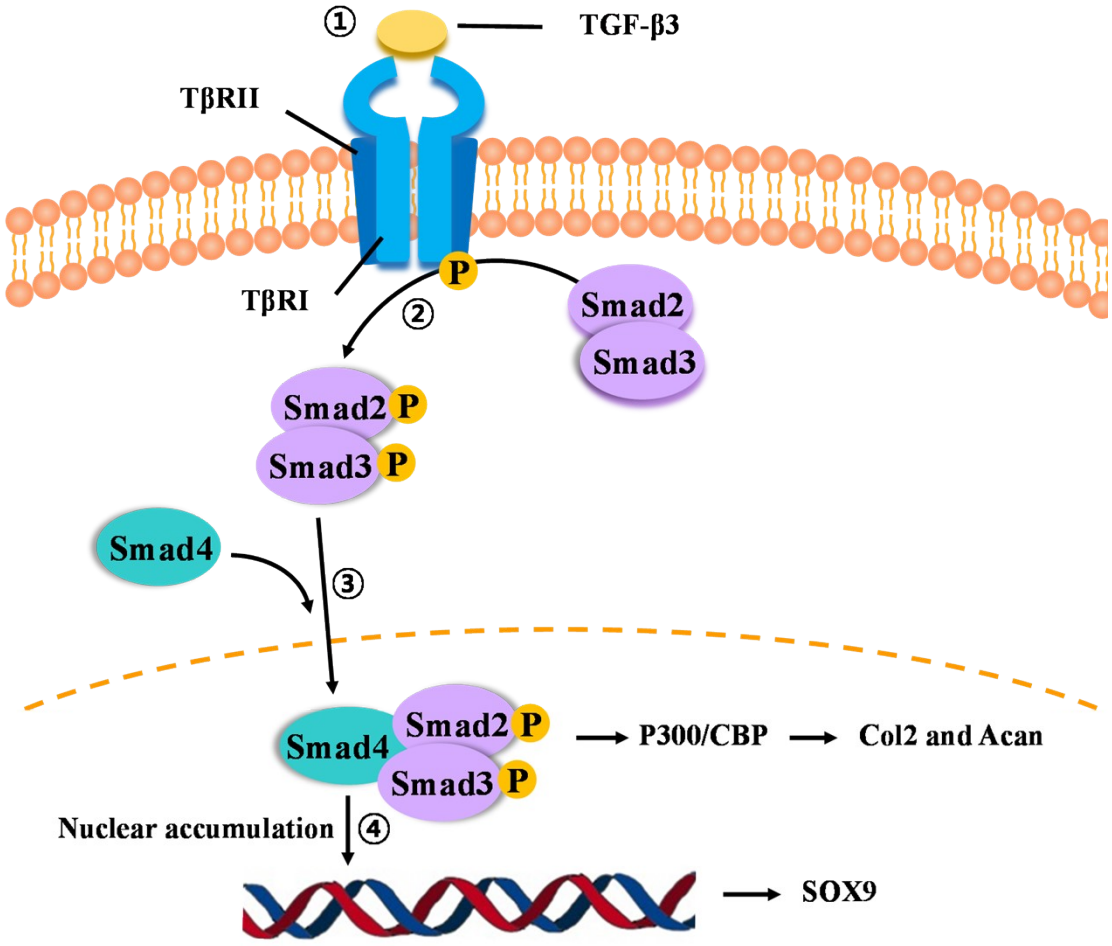
Defect model group	Defect condition
Chondral defect	Circular shape, diameter: 4 mm, depth: 2 mm
Osteochondral defect	Circular shape, diameter: 4 mm, depth: 2 mm + microfracture technique (circular shape, diameter: 1 mm, depth: 1 mm)

(b)

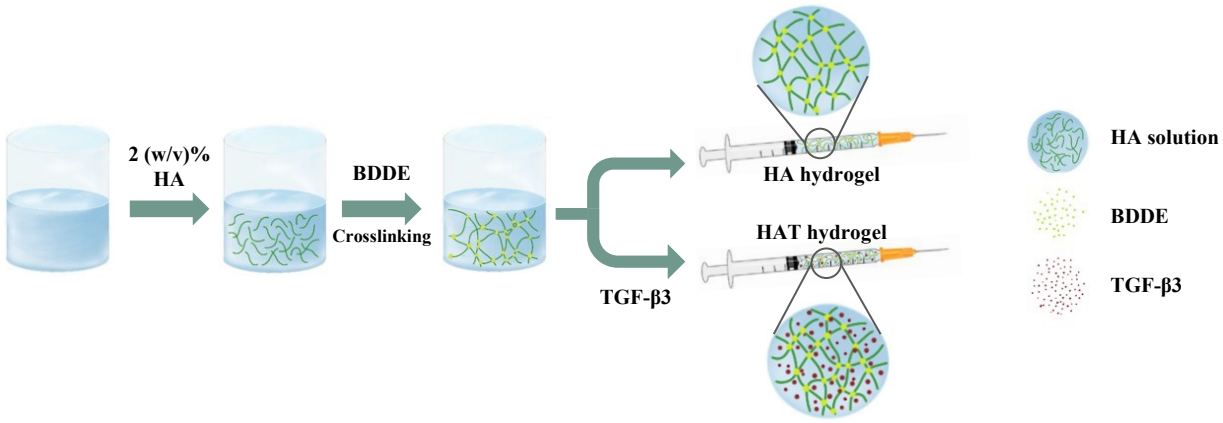
Experimental group	Defect model	Transplantation materials	Induction materials
Control (No-defect)	—	—	—
Sham control (Only defect)	Chondral	—	—
HA	Chondral	HA 100 μL	—
HAB	Osteochondral		BMSCs
HAT	Chondral	HAT 100 μL	—
HATB	Osteochondral		BMSCs

Supplemental Table 2. (a) *In vivo* defect model group and (b) experimental groups for evaluation of preclinical conditions to regenerate cartilage. HA, hyaluronic acid; HAT, hyaluronic acid loaded with transforming growth factor-beta 3; HAB, bone marrow mesenchymal stem cell induction and HA transplantation; HATB, bone marrow mesenchymal stem cell induction and HAT transplantation.

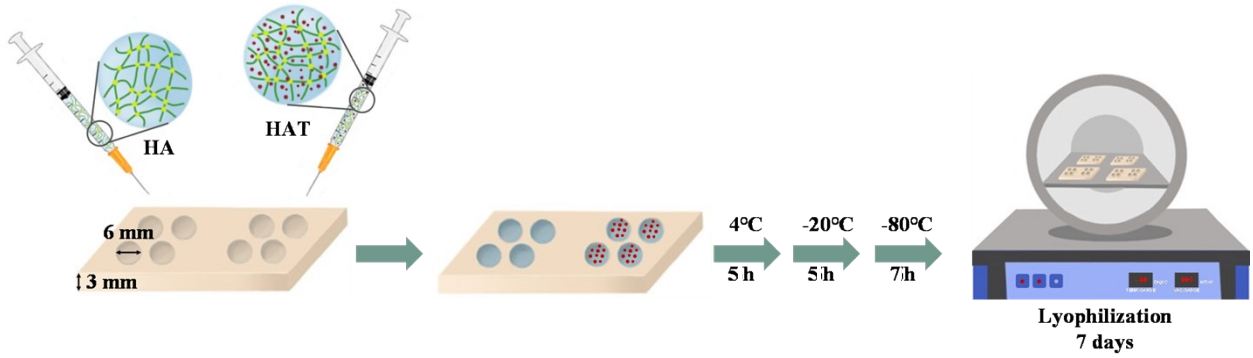
Supplemental Schemes



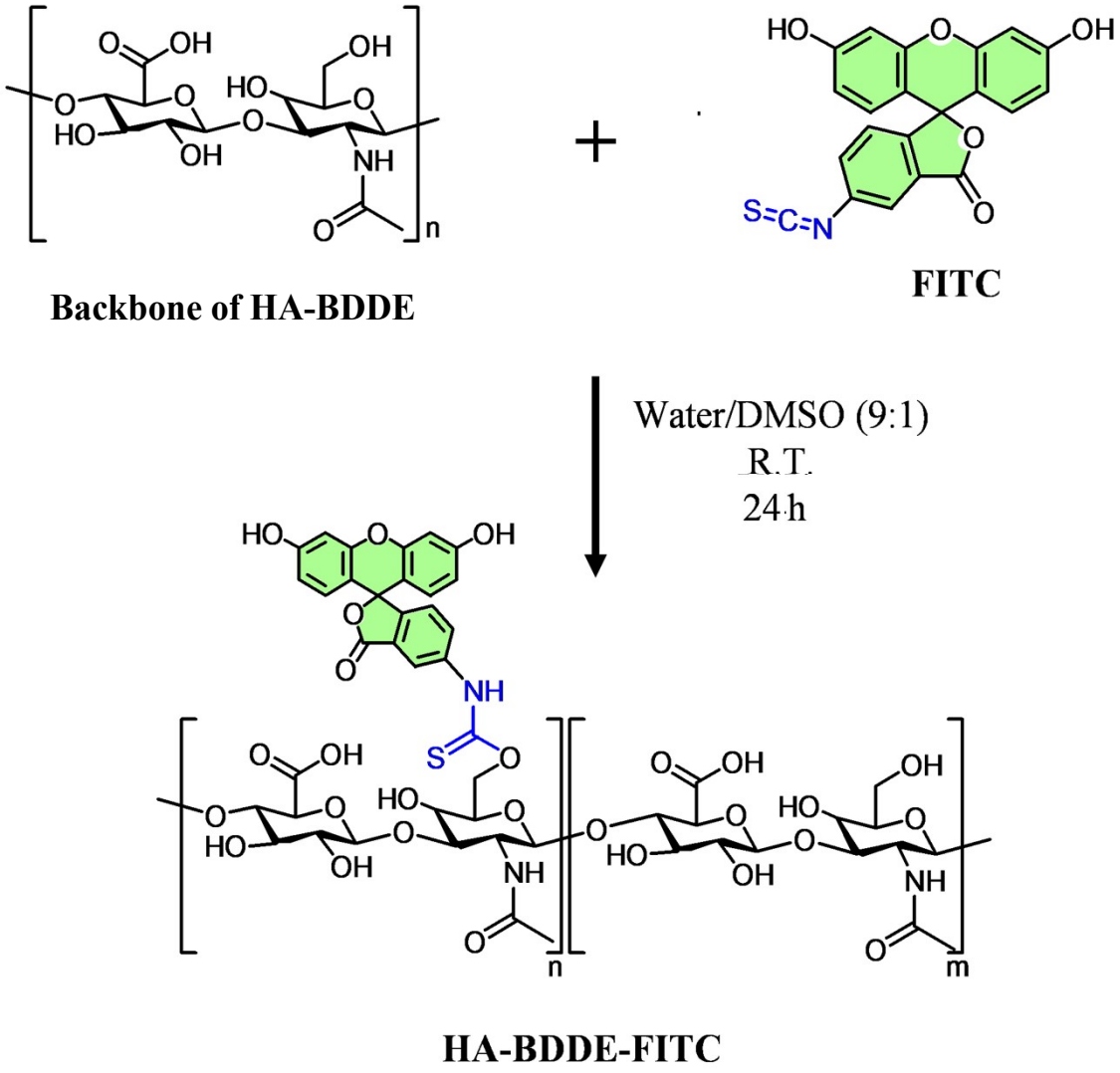
Supplemental Scheme 1. Signaling pathways and proteins involved in chondrogenic differentiation of mesenchymal stem cells through transforming growth factor-beta 3 (TGF-β3).



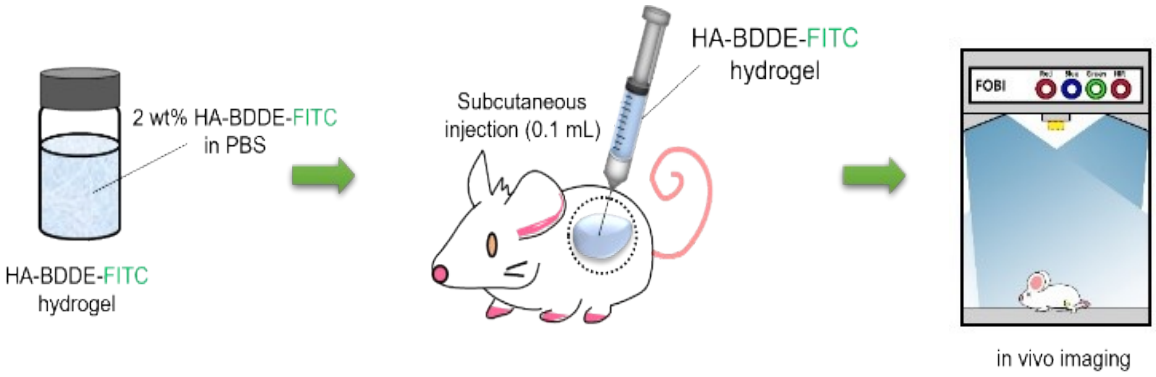
Supplemental Scheme 2. Schematic representation of the method used to prepare hyaluronic acid (HA) and HA gel-loaded TGF-β3 (HAT) gels. BDDE, 1,4-butanediol diglycidyl ether; TGF-β3, transforming growth factor-beta 3.



Supplemental Scheme 3. Preparation method of lyophilized hyaluronic acid (HA) and HA gel-loaded TGF-β3 (HAT) gel for SEM and sol fraction analysis. SEM, scanning electron microscope; TGF-β3, transforming growth factor-beta 3.



Supplemental Scheme 4. The procedure for synthesizing isothiocyanate conjugated hyaluronic acid (HA) gel. BDDE, 1,4-butanediol diglycidyl ether.



Supplemental Scheme 5. Schematic diagram illustrating the *in vivo* biodegradability assessment. BDDE, 1,4-butanediol diglycidyl ether; HA, hyaluronic acid; PBS, phosphate-buffered saline

Supplemental Figures

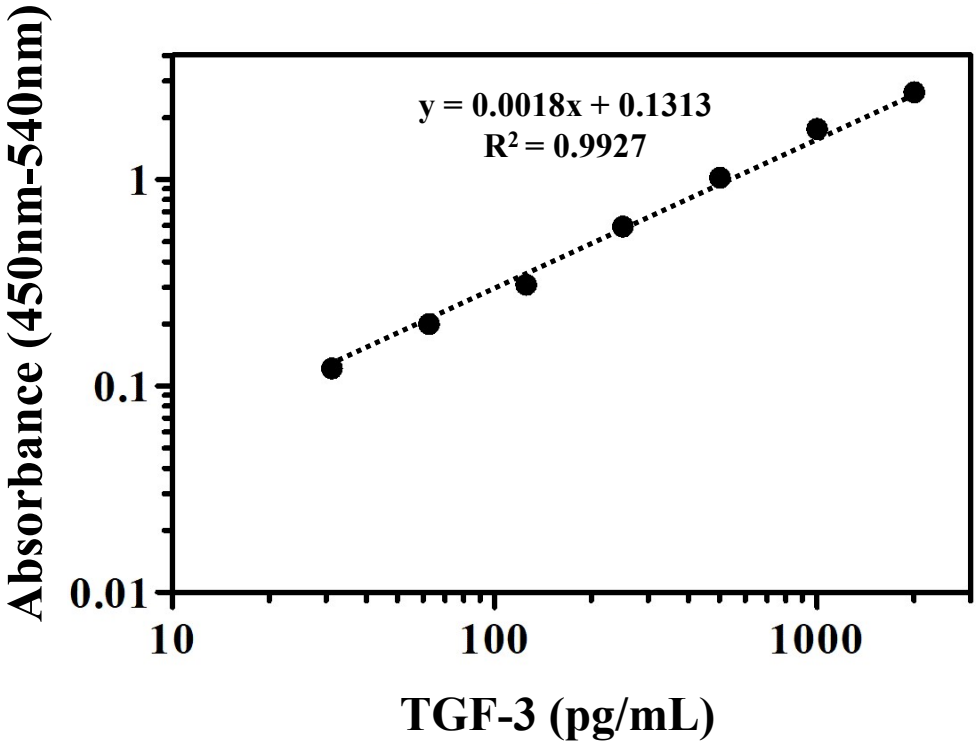


Fig. S1. Standard curve of TGF-β3 standard solution for the release profile analysis of TGF-β3 loaded into HA hydrogel. HA, hyaluronic acid; TGF-β3, transforming growth factor-beta 3.

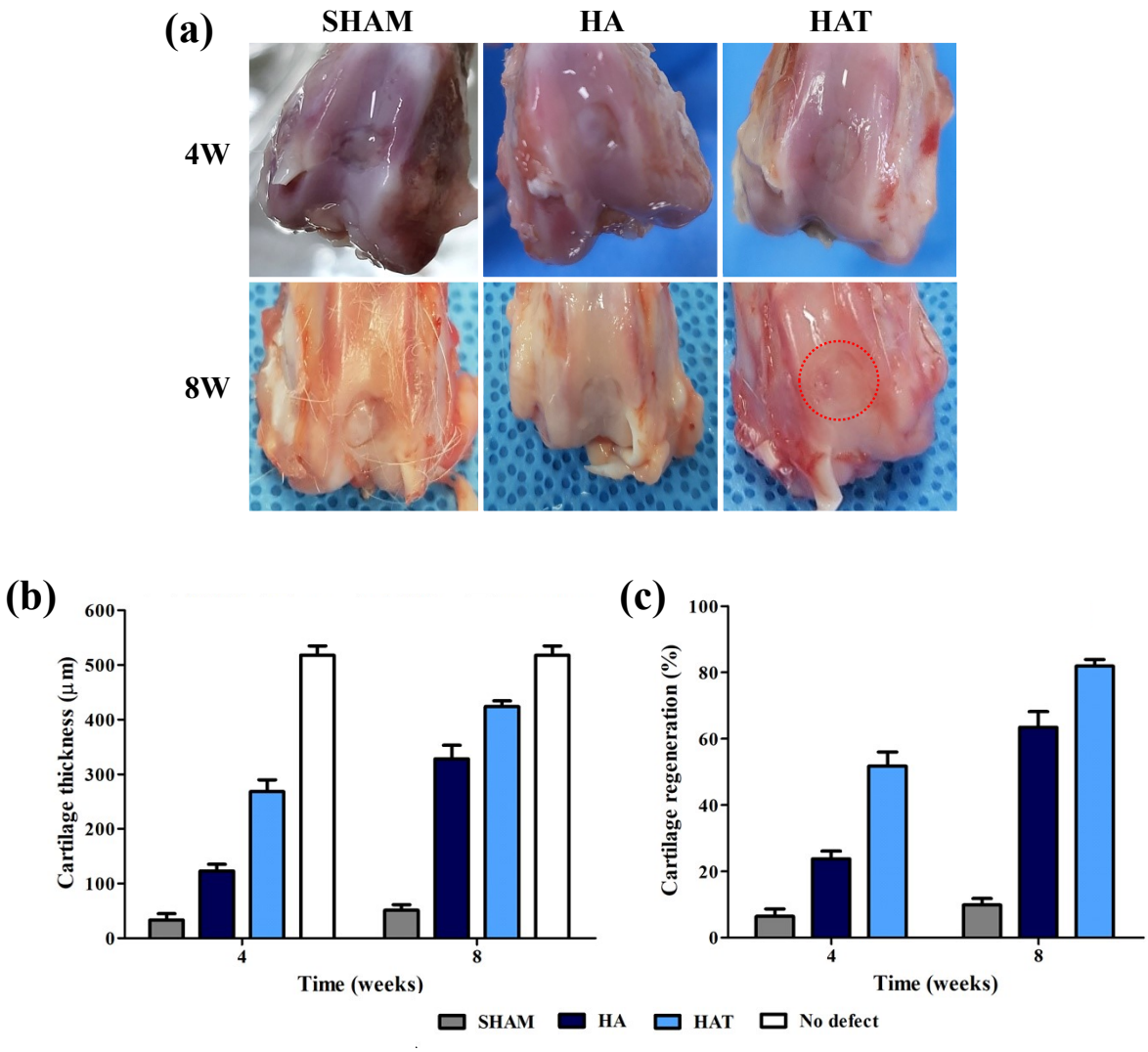


Fig. S2. (a) Macroscopic observation of cartilage defects in a rabbit model at 4 and 8 weeks post-transplantation. Graph showing (b) the thickness of cartilage from the subchondral bone to the surface of the cartilage and (c) the percentage of regenerated cartilage. HA, hyaluronic acid; HAT, hyaluronic acid loaded with transforming growth factor-beta 3.

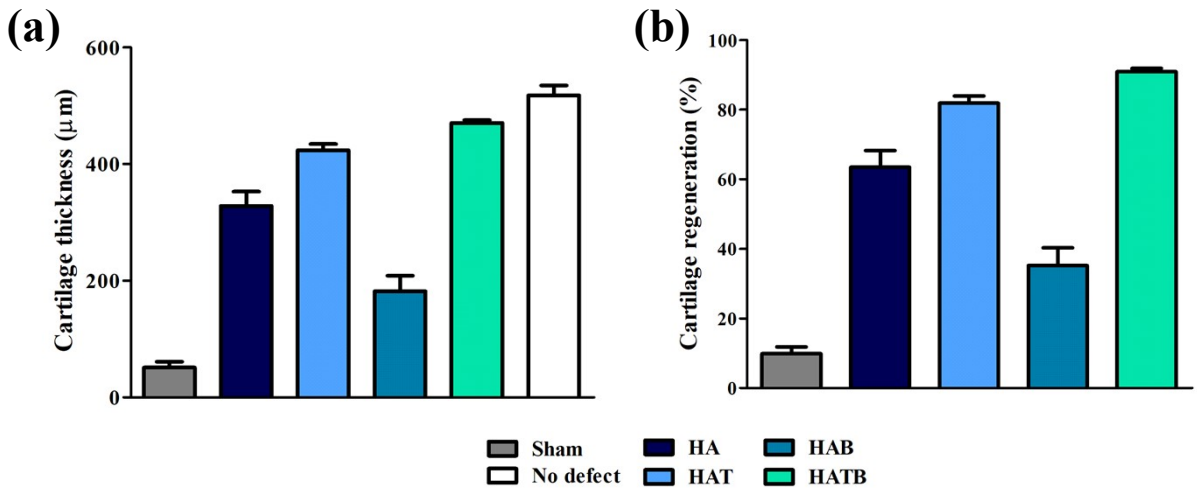


Fig. S3. Graph showing (a) the thickness of cartilage from the subchondral bone to the surface of the cartilage and (b) the percentage of regenerated cartilage. HA, hyaluronic acid; HAT, hyaluronic acid loaded with transforming growth factor-beta 3; HAB, bone marrow mesenchymal stem cell induction and HA transplantation; HATB, bone marrow mesenchymal stem cell induction and HAT transplantation.