

Supporting Information

TGF- β 1 Affinity Peptides Incorporated within a Chitosan Sponge Scaffold Can Significantly Enhance Cartilage Regeneration

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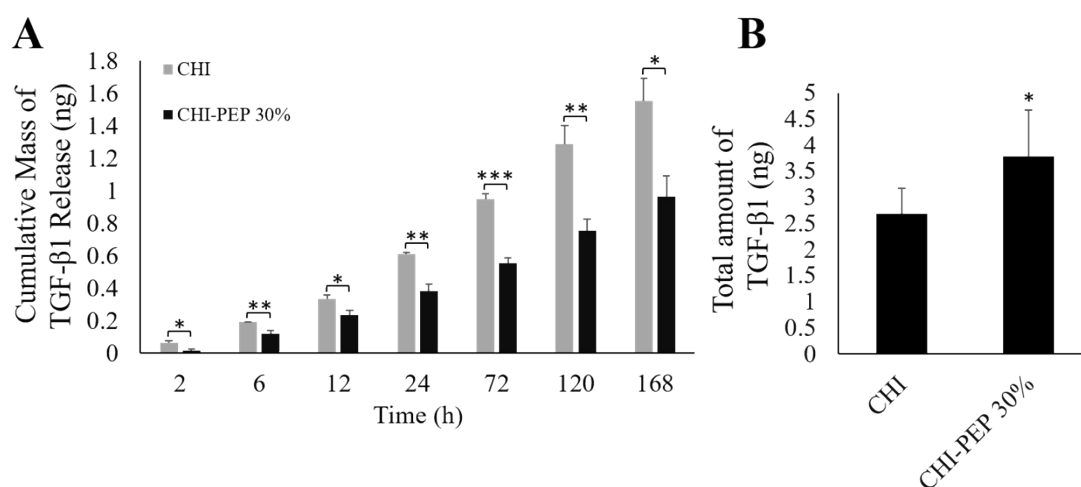


Fig. S1 (A) The difference analysis by SPSS about TGF- β 1 released from the CHI and CHI-PEP 30% at all time point in vitro. (B) The total amount of TGF- β 1 in scaffold.

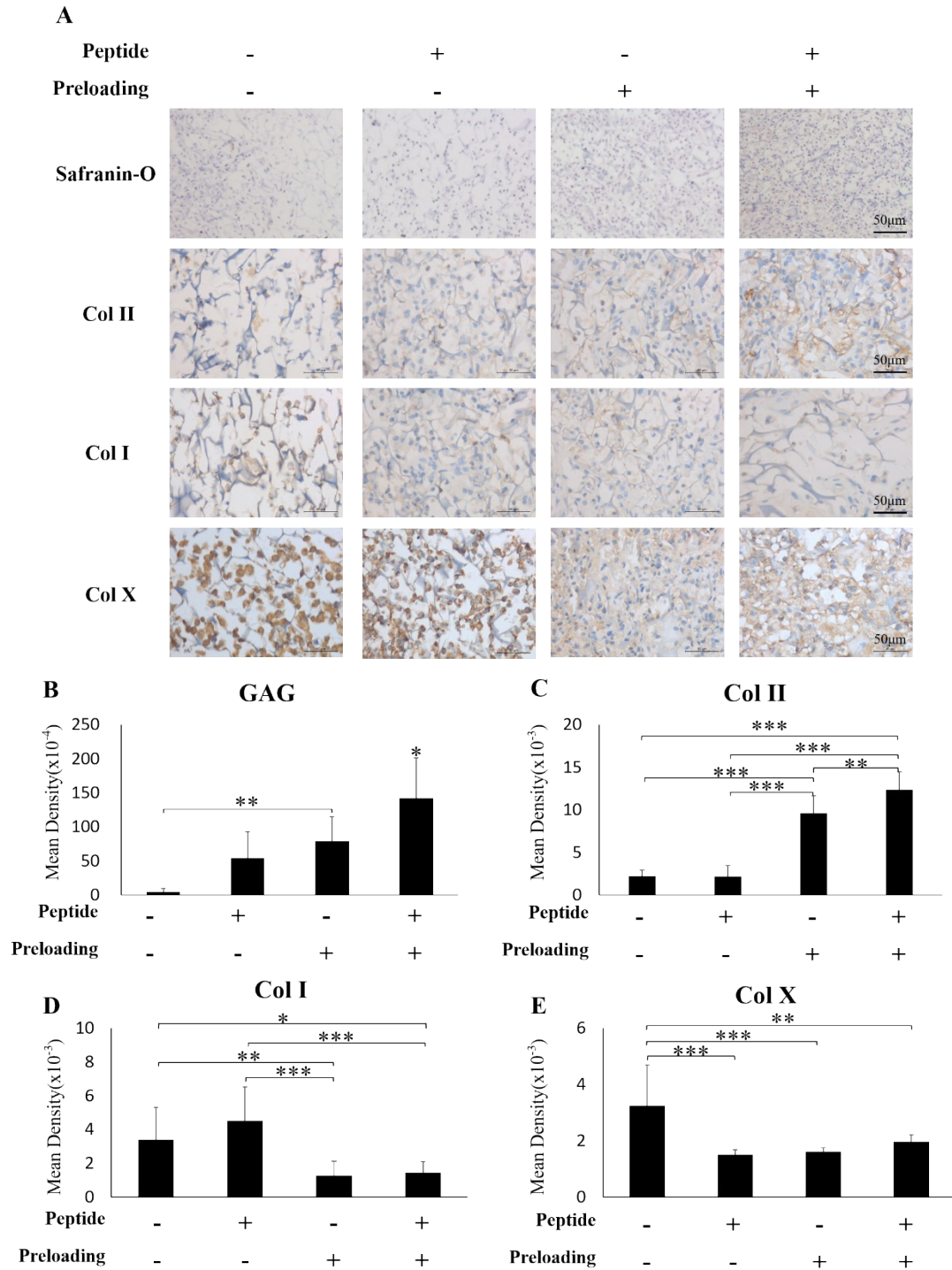


Fig. S2 Implants in nude mice model at 4 weeks post-surgery with a seeding density of 5×10^5 cells/sponge. (A) Results of safranin-O staining and immunohistochemical staining of collagen II, I and X at a seeding density of 10^6 cells/sponge. (B) GAG content in sponges. (C) Collagen II content in sponges. (D) Collagen I content in sponges. (E) Collagen X content in sponges ($n=9$, mean values \pm SD, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Scale bar represents $50 \mu\text{m}$.

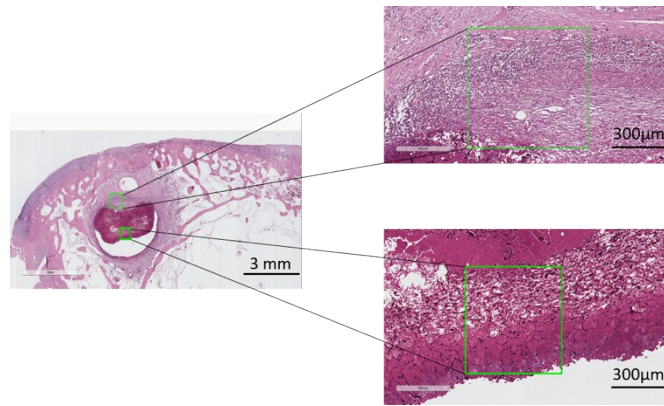


Fig. S3 The connective fibrous tissues and minor inflammation in one of the CHI-PEP sponge scaffolds implanted in the rabbit after 6 months.



Fig. S4 The illustration of different section of the defect from the lateral to Interior regions.

Table S1 qRT-PCR primer sequences for chondrogenic marker genes

Target	Primer sequence forward and reverse (5'-3')
GAPDH	GTC ATC CAT GAC AAC TTC GG GCC ACA GTT TCC CAG AGG
COL I	CAG AAC GGC CTC AGG TAC CA CAG ATC ACG TCA TCG CAC AAC
COL II	GAG AGG TCT TCC TGG CAA AG AAG TCC CTG GAA GCC AGAT
COL X	CAG GTA CCA GAG GTC CCA TC CAT TGA GGC CCT TAG TTG CT
AGG	CGA AAC ATC ACC GAG GGT GCA AAT GTA AAG GGC TCC TC

Sox9

ATC AGT ACC CGC ACC TGC AC

CTT GTA ATC CGG GTG GTC CTT

Table S2 ICRS macroscopic evaluation of cartilage repair

Cartilage repair assessment ICRS	Score
I. Degree of defect repair	
In level with surrounding cartilage	4
75% repair of defect depth	3
50% repair of defect depth	2
25% repair of defect depth	1
0% repair of defect depth	0
II. Integration to border zone	
Complete integration with surrounding cartilage	4
Demarcating border < 1 mm	3
3/4th of graft integrated, 1/4th with a notable border > 1 mm width	2
1/2 of graft integrated with surrounding cartilage, 1/2 with a notable border > 1 mm	1
From no contact to 1/4th of graft integrated with surrounding cartilage	0
III. Macroscopic appearance	
Intact smooth surface	4
Fibrillated surface	3
Small, scattered fissures or cracs	2

Several, small or few but large fissures	1
Total degeneration of grafted area	0
Overall repair assessment	
Grade I: normal	12
Grade II: nearly normal	11–8
Grade III: abnormal	7–4
Grade IV: severely abnormal	3–1

Table S3 ICRS Visual Histological Assessment Scale

Feature	Score
I. Surface	
Smooth/continuous	3
Discontinuities/irregularities	0
II. Matrix	
Hyaline	3
Mixture: hyaline/fibrocartilage	2
Fibrocartilage	1
Fibrous tissue	0
III. Cell distribution	
Columnar	3
Mixed/columnar-clusters	2
Clusters	1

Individual cells/disorganized	0
IV. Cell population viability	
Predominantly viable	3
Partially viable	1
<10% viable	0
V. Subchondral Bone	
Normal	3
Increased remodeling	2
Bone necrosis/granulation tissue	1
Detached/fracture/callus at base	0
VI. Cartilage mineralization (calcified cartilage)	
Normal	3
Abnormal/inappropriate location	0
