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The inductive effect of BMP-4 protein on chondral-lineage differentiation and in-situ cartilage repair

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Objectives: Recent studies suggest that bone morphogenetic protein-4 (BMP-4) and BMP-7 are promising cartilage differentiation factors, so this study aimed to evaluate their efficacy for articular cartilage repair *in vitro* and *in vivo*.

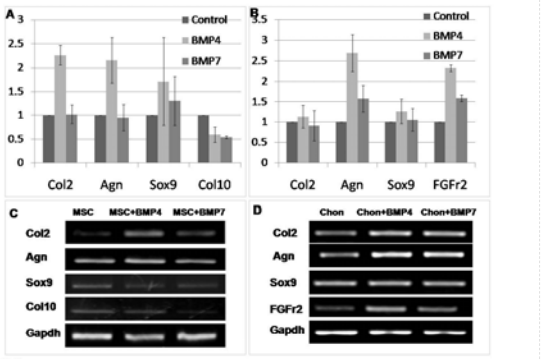


Figure 1. (A) mRNA expression in rabbit MSCs after culture with BMP-4 (BMP4; 10 ng/ml) or BMP-7 (BMP7; 10 ng/ml) or without treatment (Control) for 3 days. (B) Col2, Agn, Sox9 and FGFr2 mRNA expression levels in rabbit chondrocytes after culture with BMP-4 (BMP4; 10 ng/ml) or BMP-7 (BMP7; 10 ng/ml) or without treatment (Control) for 3 days. (C) PCR bands of Col2, Agn, Sox9, and Col10 in rabbit MSCs. (D) PCR bands of Col2, Agn, Sox9, and FGFr2 expression in rabbit chondrocytes.

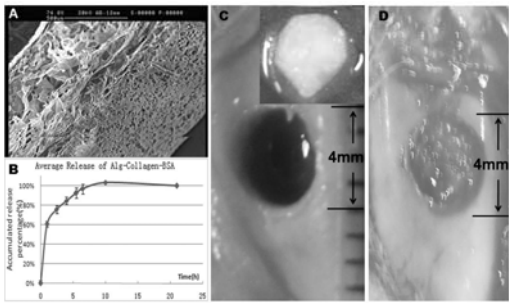


Figure 2.

(A) SEM photograph of a cross-section showing the bi-layer scaffold. (B) Average cumulative BSA release from the alginate-collagen scaffold. (C) Full osteochondral defect in rabbit patellar groove. Inset: BMP-4 protein delivery system, collagen sponge, and growth factor in alginate capsule (25 µl system). (D) Scaffold and protein implanted in defect area.

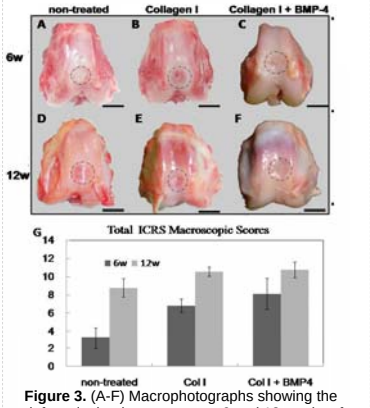


Figure 3. (A-F) Macrophotographs showing the defects in the three groups at 6 and 12 weeks after surgery. Scale bars, 5 mm.
(G) ICRS scores of groups I, II, and III at 6 and 12 weeks after surgery (maximum score = 14). Values are mean ± standard deviation.

Methods: Rabbit mesenchymal stromal cells (MSCs) and articular chondrocytes were treated with 10 ng/ml hrBMP-4 or hrBMP-7. The expression of cartilage-specific genes (Col II, Aggrecan, and Sox9) and FGF receptor genes were tested by real-time PCR *in vitro*. Also, full-thickness cartilage defects (diameter 4 mm, thickness 3 mm) were created in New Zealand white rabbits and treated with a bi-layer collagen scaffold (group II), BMP4 with scaffold (group III) or not treated (group I) (n=12/group). The repaired tissues were harvested for histology and mechanical testing at 6 or 12 weeks after operation.

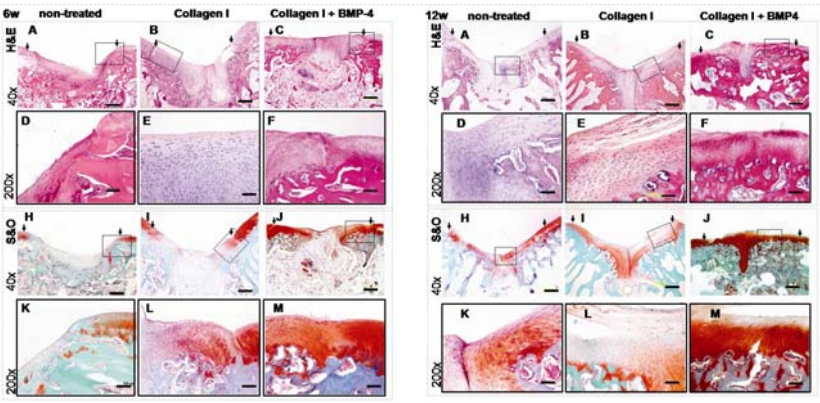
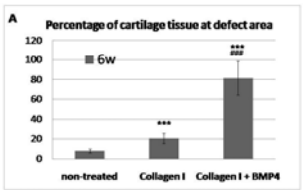
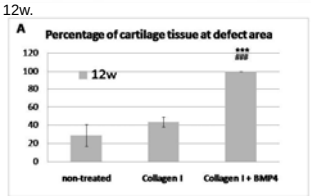


Fig3, Fig5. Histological sections of HE and SO staining for 6w and 12w.





B ICRS Visual Histological Assessment Scale

6week	Sample	I	II	III	IV	V	VI	Total	AVNE ±SD
Non-treated	S1	0	0	0	1	1	0	2	3.33±1.154
	S2	0	1	0	1	2	0	4	
	S3	0	1	0	0	0	3	4	
Col I scaffold	S1	3	1	0	3	3	3	13	11±5.291
	S2	0	0	0	0	2	3	5	
	S3	3	2	2	3	2	3	15	
Col I scaffold +BMP-4	S1	3	2	3	3	2	3	16	13±4.358
	S2	3	2	2	3	2	3	15	
	S3	3	2	1	1	1	0	8	

B ICRS Visual Histological Assessment Scale

12week	Sample	I	II	III	IV	V	VI	Total	AVNE ±SD
Non treated	S1	0	1	0	3	1	3	8	10±2.828
	S2	3	1	0	3	2	3	12	
Col I scaffold	S1	3	1	1	3	2	3	13	12.25±3.304
	S2	0	1	2	3	2	0	8	
	S3	3	2	2	3	2	0	12	
	S4	3	3	2	3	2	3	16	
Col I scaffold +BMP-4	S1	3	3	3	3	3	3	18	15.25±1.893
	S2	3	2	2	3	2	3	15	
	S3	3	2	2	3	1	3	14	
	S4	3	2	1	3	2	3	14	

Fig.4, Fig6. (A)Percentage of cartilage tissue in the area of defect from the three groups after surgery (B) ICRS scores for all samples of 6w and 12w.

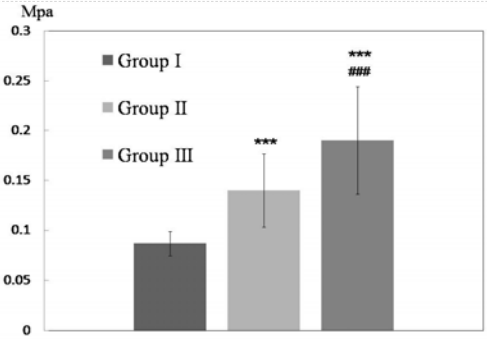


Fig.8 Biomechanical analysis of repaired tissues at 12 weeks after surgery. (n = 5).

Results: Cartilage differentiation of MSCs was more apparent after BMP-4 treatment, as evidenced by the higher expression of type II collagen and aggrecan genes. Also, BMP-4 induced higher aggrecan and FGFr2 gene expression in chondrocytes, while BMP-7 had no effect. In the *in vivo* experiments, group III treated with BMP4 protein had the largest amounts of cartilage tissue, which restored a greater surface area of the defect and achieved higher International Cartilage Repair Society scores. Moreover, Young's modulus, which indicates the mechanical properties of the repaired tissue, was markedly higher in group III than in groups I and II ($p < 0.05$), but lower than in normal tissue.

Conclusion: BMP-4 is more potent than BMP-7 for cartilage differentiation. The delivery of BMP-4 protein in a bi-layer collagen scaffold stimulates the formation of cartilage tissue.

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