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# BK21 §

## Tissue Engineered Intervertebral Disc by Atelocollagen Scaffolds and Growth Factors

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– Abstract –

**Study Design:** In vitro experimental study.

**Objectives:** To examine the cellular proliferation, synthetic activity and phenotypical expression of intervertebral disc (IVD) cells seeded on types I and II atelocollagen scaffolds, with the stimulation of TGF- 1 and BMP- 2.

**Summary of Literature Review:** Recently, tissue engineering is regarded as a new experimental technique for the biological treatment of degenerative IVD diseases, and has been highlighted as a promising technique for the regeneration of tissues and organs in the human body. Research on cell transplantation in artificial scaffolds has provided that the conditions for tissue engineering have to be equilibrated, including the cell viability and proliferation, maintenance of characteristic phenotype, suitable scaffolds in organisms and biologically stimulated growth factor.

**Material and Method:** Lumbar IVD cells were harvested from 10 New Zealand white rabbits, with the nucleus pulposus cells isolated by sequential enzymatic digestion. Each of 1% types I and II atelocollagen dispersions were poured into a 96- well plate (diameter 5 mm), frozen at -70 °C, and then lyophilized at -50 °C. Fabricated porous collagen matrices were made using the cross-linking method. Cell suspensions were imbibed by surface tension into a scaffold consisting of atelocollagen. The cell cultured scaffolds were then treated with TGF- 1 (10 ng/ml) or BMP- 2 (100 ng/ml) or both. After 1 and 2 week culture periods, the DNA synthesis was measured by [<sup>3</sup>H] thymidine incorporation, and newly synthesized proteoglycan by incorporation of [<sup>35</sup>S] sulphate. Reverse transcription- polymerase chain reactions for the mRNA expressions of type I and II collagen, aggrecan and osteocalcin were performed. The inner morphology of the scaffolds was determined by scanning electron microscopy (SEM).

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(6-2004-1084),

, Brain Korea 21

**Results:** The IVD cultures in collagen type II with TGF- 1 demonstrated an increase in proteoglycan synthesis and up regulation of aggrecan and types I and II collagen mRNA expressions compared to the control. IVD cultures in the type I atelocollagen scaffold with growth factors exhibited an increase in DNA synthesis and up regulation of the type II atelocollagen mRNA expression. With all combinations of growth factor, the IVD cultures in types I and II atelocollagen scaffolds showed no up regulation of the osteocalcin mRNA expression. Furthermore, there was no synergistic effect of TGF- 1 and BMP-2 in the matrix synthesis or for the mRNA expression of the matrix components.

**Conclusions:** Nucleus pulposus cells from rabbit were viable in atelocollagen types I and II atelocollagen scaffolds. The type I atelocollagen scaffold was suitable for cell proliferation, but the type II atelocollagen scaffold was more suitable for extracellular matrix synthesis. The IVD cells in both scaffolds were biologically responsive to growth factors. Taken together, nucleus pulposus cells in atelocollagen scaffolds, with anabolic growth factors, provide a mechanism for tissue engineering of IVD cells.

**Key Words:** Intervertebral disc, Atelocollagen, TGF- 1, BMP- 2, Tissue engineering

가 1, 2, , TGF- 1 BMP-2 1-7) , 가 10 (3.5 kg, ) 8,9) , Hams F-12 1% (v/v) penicillin, streptomycin, nystatin (all antibiotics from Gibco-BRL, Grand Island, NY) 0.4% (w/v) protease, 0.004% (w/v) DNase (Sigma, St. Louis, MO, USA) 가 37 60 . Hams F-12 0.025% (w/v) collagenase type II, 0.004% (w/v) DNase (Sigma, St. Louis, MO, USA) 가 37 3 Hams F-12 2 , Nylon (Falcon, Franklin Lakes, NJ, pore size 100 μm) 10% (Fetal bovine serum, Gibco-BRL, Grand Island, NY), 1% (v/v) antibiotic-antimycotic, 25 μg/ml ascorbic acid가 Dulbecco's Modified Eagle Medium and Hams F-12 medium (DMEM/F-12, Gibco-BRL, Grand Island, NY) 10-13) 14-17) 18-21) TGF- 1 (Transforming growth factor-1) BMP-2 (Bone morphogenetic protein-2)가 가 가 , 가 가

T-25 flask (NUNC, Rockilde, Denmark) 37 ,  
5% CO<sub>2</sub> . 3  
3 .

2.

1% 1 , 2 (Regenmed,  
Seoul, Korea) 56 μ 96-well plate (Falcon, Franklin  
Lakes, NJ) -70 , -50  
50  
mM 1-ethyl-(3-3-dimethylaminopropyl) carbodiimide  
hydrochloride (EDC, Sigma Chemical Co., St. Louis, MO,  
USA) 24 .  
, -50 <sup>22,23)</sup>.

3.

Trypsin/EDTA  
(Calbiochem, La Jolla, CA) Culture flask  
, 96-well plate (Falcon, Franklin Lakes, NJ)

, DMEM/F-12  
,  
5 × 10<sup>5</sup> /ml  
10% FBS, 25 μg/ml ascorbic acid, 1% anti-  
otics가 DMEM/F-12 37 , 5% CO<sub>2</sub>  
4 , 5% FBS가  
10 ng/ml TGF- 1, 100 ng/ml BMP-2  
1:1 100 μ 가  
가 2  
2 .

4. TGF- 1 BMP-2

TGF- 1 R&D(R&D Systems, Inc. USA)  
, BMP-2 <sup>24)</sup>.  
plasmid DNA pcDNA3.1/hygro vector  
BMP-2 , Chinese  
hamster ovary (CHO-K1) lipofectamine (Gibco  
BRL) pcDNA3.1/hygro/BMP-  
2 CHO-K1 hygromycin  
(CHO-K1/BMP-2) .

BMP-2 , Heparin  
sepharose A chromatography , YM10  
-70

5.

가  
[methyl-<sup>3</sup>H] thymidine 5 μCi/ml (Amersham  
Phamacia Biotech, Uppsala, Sweden) 가  
24 , 가  
DPBS (Dulbecco's Phosphate Buffered Saline, Gibco-BRL,  
Grand island, NY) scintillation  
vial liquid scintillation cocktail 3 ml 가  
, 24 beta  
scintillation counter(Packard, Downers Grove, IL)  
DNA .

6.

[<sup>35</sup>S] sulfate 20 μCi/ml  
(Amersham Phamacia Biotech, Uppsala, Sweden) 가  
4 가  
-70 , 4M guanidine  
hydrochloride, 50 mM sodium acetate (pH5.8)  
0.1M 6-aminohexanoic acid, 10 mM EDTA (Ethylenedi-  
aminetetraacetic Acid Disodium Salt), 5 mM Benzamidine  
hydrochloride, 10 mM N-ehylmaleimide, 0.5 mM pheynyl-  
methyl-sulfonyl fluoride가 proteinase inhibitor  
가 4 48  
[<sup>35</sup>S]  
Sephadex G-25M PD-10 column  
(Pharmacia Biotech, Uppsala, Sweden) -70  
scintillation vial

. 2, 3, 4 6 ml  
liquid scintillation cocktail (Beckman, Fullerton, CA)  
가 , 24  
, Beta scintillation counter

7. Aggrecan, 1 , 2 , osteocalcin mRNA  
RNeasy mini kit (QIAGEN, Maryland, USA)  
Total RNA .

Total RNA 1 µg oligo (dT) 1 µl (Invitrogen, USA, 0.5 µg/ µl) 9.  
 3 50 µl  
 AccuPower RT-premix (Bioneer, ) 5 × 10<sup>5</sup>  
 Reverse Transcription Polymerase Chain Reaction (RT-PCR) , TGF- 1 (10 ng/ml), BMP-2 (100  
 . 42 60 , 94 5 ng/ml) 1:1  
 cDNA , cDNA 2 가  
 , 20 µl cDNA 1 µl 2  
 , sense primer antisense primer 10pmole, 3 , aggrecan, 1 , 2  
 10 µl Sapphire , osteocalcin mRNA  
 PCR-premix (Sapphire, USA) , RT(Real Time)-PCR , DMEM/F-12(5% FBS, 1% v/v  
 (Table 1,2). PCR 2% agarose antibiotics, 25 µg/ml ascorbic acid)  
 가  
 RT-PCR beta actin  
 TINA 2.0e program 10.  
 3  
 8. , ( ±  
 가 ) , SPSS (SPSS Inc. Chicago IL)  
 7 14 , . One-way Analysis of variance Fisher's pro-  
 , tected LSD post-hoc test, power analysis  
 , p<0.05 .

**Table 1.** Sequences of primers used for PCR amplification of cDNA

Gene	Sequence (5' 3')	Length	Size (bp)
Aggrecan	AGG TGT TGT GTT CCA CTA TC	20	378 bp
	GTC ATA GGT CTC GTT GGT GT	20	
Collagen type I	AGA AGG AGT AAC CTC CAA GG	20	321 bp
	ATG ACC AAA GGT GCA ATA TC	20	
Collagen type II	GCA CCC ATG GAC ATT GGA GG	20	367 bp
	GAC ACG GAG TAG CAC CAT CG	20	
Osteocalcin	AAG AGA TCA TGA GGA GCC TG	20	420 bp
	AGG AAA CAA GCA CTG TGC AT	20	
Beta-actin	GCC ATC CTG CGT CTG GAC CT	20	228 bp
	GTG ATG ACC TGG CCG TCG GG	20	

**Table 2.** PCR conditions

Primer	Conditions						
	Denaturation		Annealing		Polymerization		Cycles
Aggrecan	94	30sec	50	30sec	72	90sec	30
Collagen type I	94	5sec	50	5sec	72	30sec	26
Collagen type II	94	5sec	50	5sec	72	30sec	30
Osteocalcin	94	5sec	46	5sec	72	30sec	25
Beta-actin	94	5sec	58	5sec	72	30sec	30

1. DNA synthesis

TGF-1 가 1 238% 가

가 TGF-1 BMP-2 가 276% 가

(Fig. 1A).

1

2 TGF-1 BMP-2 가 1 가 2

TGF-1 BMP-2 가 1,2 가 (Fig. 2A).

1 가 2 535%

가 TGF-1 가 524% 가

2 BMP-2 가

(Fig. 2B).

가 (Fig. 1B).

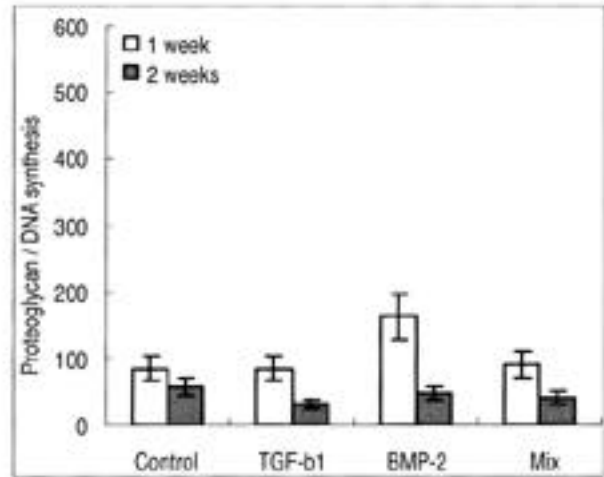
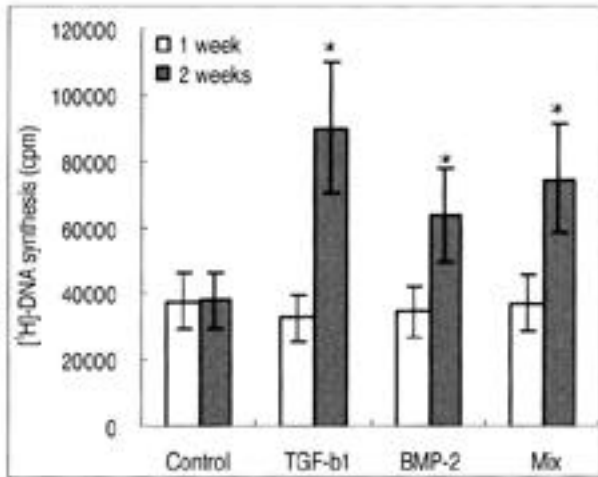


Fig. 1. Rabbit nucleus pulposus cells seeded on atelocollagen type I scaffold. (A) DNA synthesis, (B) Proteoglycan synthesis (\*p>0.05)

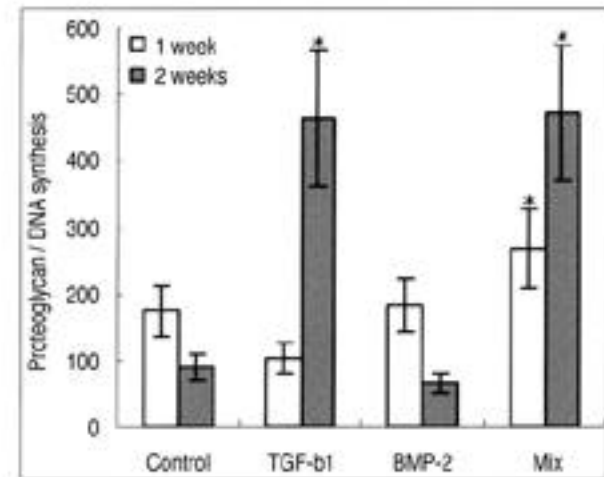
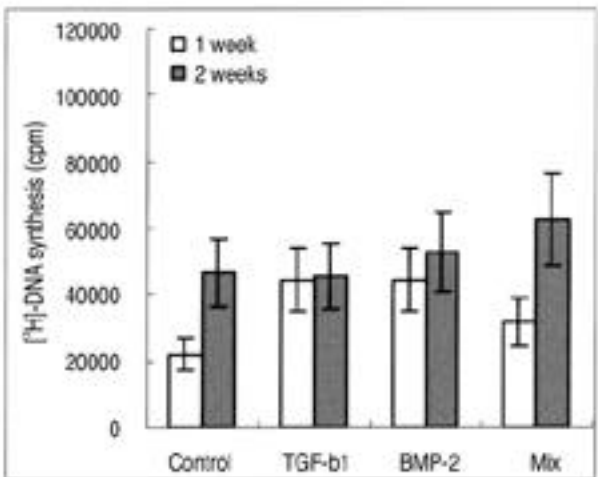


Fig. 2. Rabbit nucleus pulposus cells seeded on atelocollagen type II scaffold. (A) DNA synthesis, (B) Proteoglycan synthesis (\*p>0.05)

2. Aggrecan, I, II, osteocalcin mRNA

2 가 1

7, 14

RT-PCR

BMP-2 (osteocalcin mRNA)

, 2 가 1

densitometry

TGF-1 BMP-2가 mRNA

, 2 가 1

513%가 가, 1 2 196% 가 가

(Fig. 6).

TGF-1 mRNA (Fig. 4A-C).

TGF-1 BMP-2

가 2

(fibrous protein)

1, 2 mRNA 가 1 2 가 가 TGF-1 가

(antigen) (antibody)

aggrecan, 1, 2 mRNA 가

(antigen)

(antigenic com-ponent)

BMP-2 가 mRNA 가 (Fig. 5A-C).

(automatic immune response)

가

3.

25,26)

DNA가

(gene transfer method)

가

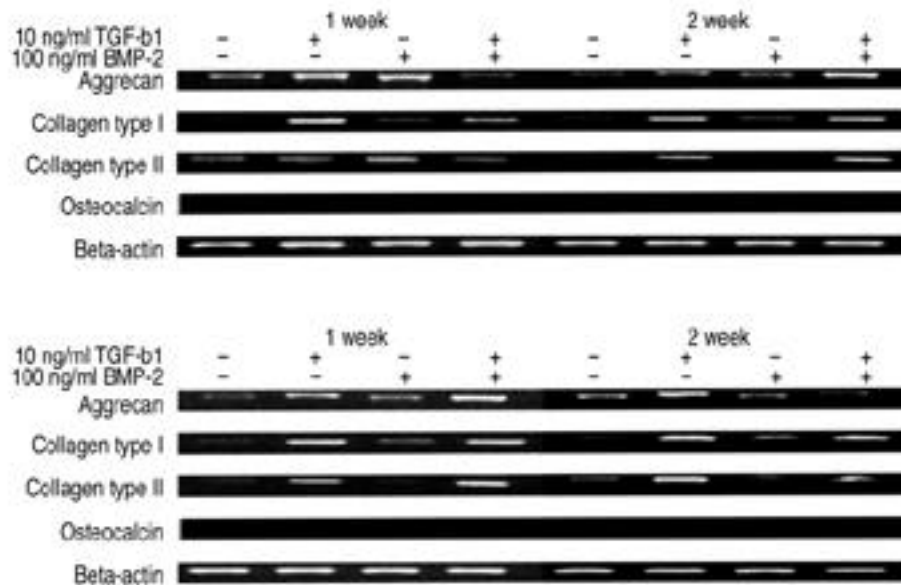


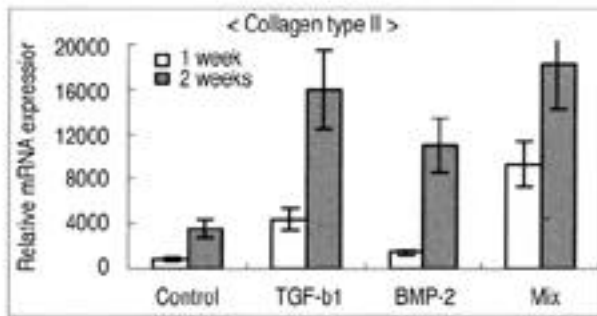
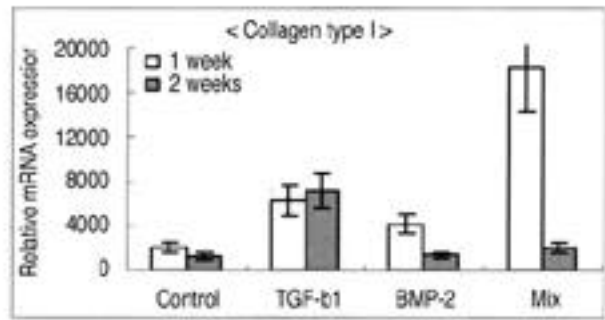
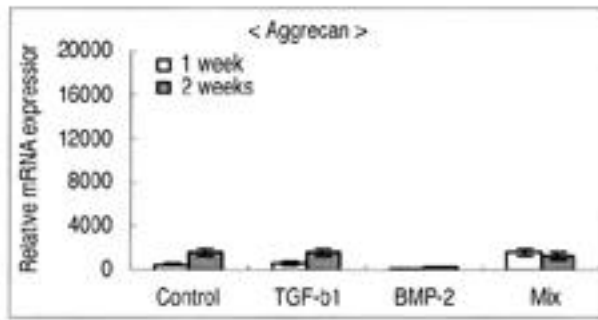
Fig. 3. RT-PCR of beta-actin, aggrecan, collagen type I and type II, osteocalcin. (A) Atelocollagen type I scaffold. (B) Atelocollagen type II scaffold.

TGF- $\beta$ 1 BMP-2

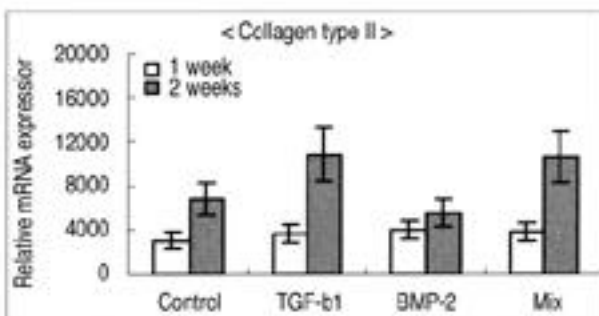
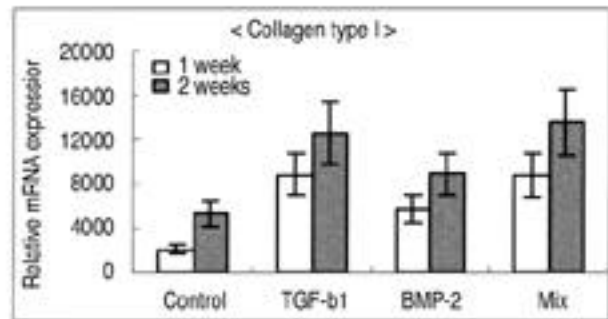
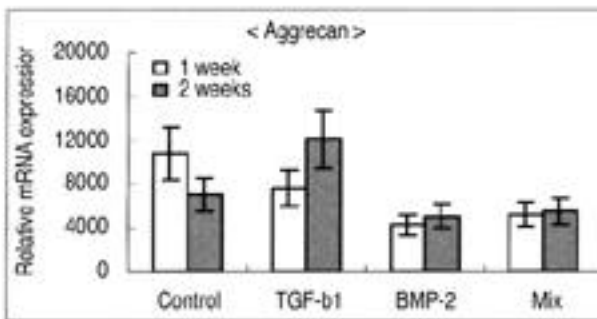
가 , 가

TGF- $\beta$ 1 BMP-2

가 , 가

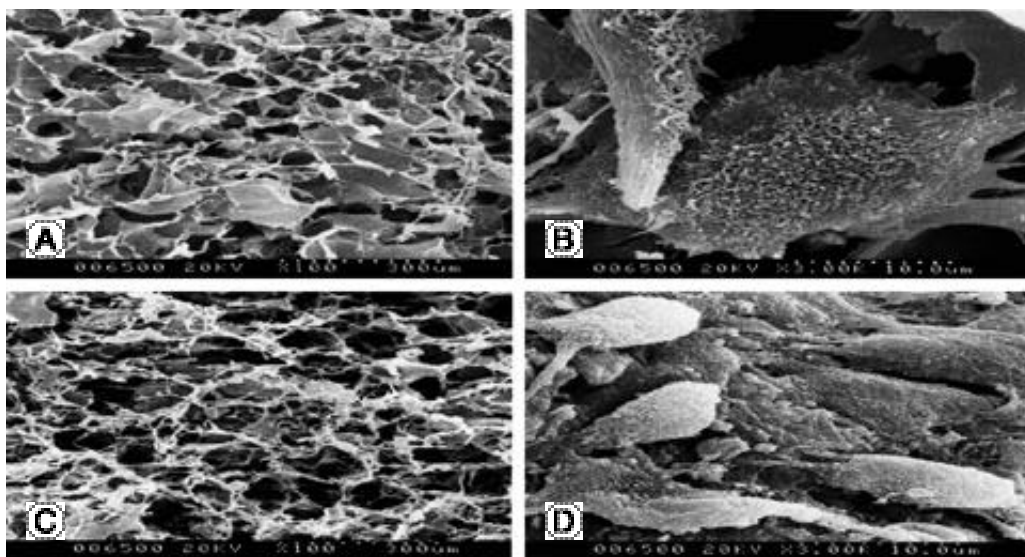


**Fig. 4.** Densitometry of mRNA expression in atelocollagen type I scaffold. (A) Aggrecan, (B) Collagen type I, (C) Collagen type II.



**Fig. 5.** Densitometry of mRNA expression in atelocollagen type I scaffold. (A) Aggrecan, (B) Collagen type I, (C) Collagen type II.

가 , mRNA 가  
 , 2 29.30 가  
 1  
 가 TGF- 1 가  
 TGF- 1 BMP-2 가  
 aggrecan, 1 ,2 mRNA 가  
 1 가 1 2  
 , 2 mRNA BMP-2 TGF- 1  
 가 2 TGF- 1, BMP-2 가  
 가 BMP-2 (osteocal-  
 , 1 cin) 2  
 , 2 가 1  
 , 가  
 가 가 가



**Fig. 6.** Morphology of porous atelocollagen scaffolds on scanning electron microscopy (SEM). (A, B) Atelocollagen type I scaffold ( $\times 100$ ,  $\times 3,000$ ), (C, D) Atelocollagen type II scaffold ( $\times 100$ ,  $\times 3,000$ ).



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