Sponsor Seminar

연조직 재생에 있어서 아텔로콜라겐의 역할

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Collagen

Main structural protein in the extracellular space in the **various connective tissues.**

Most abundant protein in mammals, making **25% to 35%** of the whole-body protein content.

Consists of **amino acid** wound together to form **triple-helices** of elongated fibrils.

Found in **fibrous tissues** such as **tendons, ligaments, and skin.**

Depending upon the **degree of mineralization**, may be **rigid (bone), compliant (tendon), or (cartilage).**

In **muscle tissue**, it serves as a major component of the **endomysium**.

- Type I: skin, tendon, vasculature, organs, bone (main component of the organic part of bone)
- Type II: cartilage (main collagenous component of cartilage)
- Type III: reticulate (main component of reticular fibers), commonly found alongside type I.
- Type IV: forms basal lamina, the epithelium-secreted layer of the basement membrane.
- Type V: cell surfaces, hair, and placenta

Collagen: Animal Sources and Biomedical Application *Journal of Applied Pharmaceutical Science 5 (03); 2015: 123-127*

History of collagen biomaterial

catgut in oil

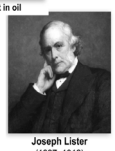
Greek word where
"kola" means gum and "gen" means producing

The use of collagen as a modern biomaterial began in 1881.

Biodegradable suture - "catgut", a collagen-rich biomaterial prepared.

- small intestine of a sheep

William Macewen
(1848–1924)

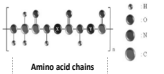


Joseph Lister
(1827–1912)

'Joseph Lister, who founded modern surgery'
his former student William Macewen reported independently on the advantages.

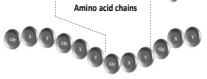
Collagen-Based Biomaterials for Wound Healing. *Biopolymers. 2014 Aug; 101(8): 821–833.*

Structure of collagen molecule

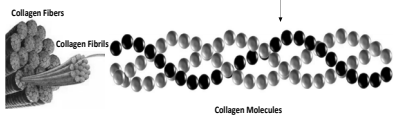


Amino acid chains

Repeating amino acid pattern of the Gly-X-Y and triple helical strucutre of collagen.



Gly : Glycine
X : Proline
Y : Hydroxyproline

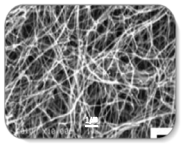


MW : 300,000
Length : 300nm
Diameter : 1.5nm

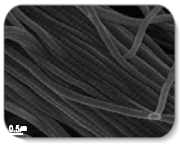
Collagen is one of the key structural proteins found in the extracellular matrices of many connective tissues in mammals, making up about 30% of the whole-body protein content (Friess, 2000; Muyonga et al., 2004).

Nature's hierarchical materials. Materials Science 52 (2007) 1263-1334

Features of collagen



Collagen fibrils (scale bar: 1μm) 2-1



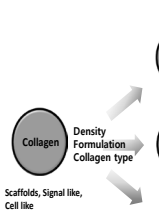
Collagen fibrils(scale bar: 0.5μm) 2-2

2-1 : Sci. Technol. Adv. Mater.11 (2010). 2-2 : PLOS journal.pone.020527

Collagen ; **biomaterials for wound healing**
structural integrity of the ECM
dynamic and flexible material
re-modeling to refine cellular behavior and tissue function
surface-active and is capable of penetrating a lipid-free interface
biodegradable and nontoxic
exogenous collagen is more biocompatible than other natural polymers

The effect of mean pore size on cell attachment, proliferation and migration in collagen-glycosaminoglycan scaffolds for bone tissue engineering. Biomaterials(2010)31, 461-466.

Biomedical Applications of Collagen



Composition	Biomaterial Form	Applications
Collagen	Gel	Cosmetic Skin Defects Drug Delivery Vitreous Replacement Surgery Coating of Bioprostheses
	Sponge	3D Cell Culture Wound Dressing Hemostatic Agent Skin Replacement Drug Delivery
	Hollow Fiber Tubing	Cell Culture Nerve Regeneration
	Sphere	Micro-carrier for Cell Culture Drug Delivery
	Membrane	Wound Dressing Dialysis Tissue Regeneration Corneal Shields Skin Patches
	Rigid Form	Bone Repair
	Sponge	3D Cell Culture Wound Dressing Skin Replacement
Collagen + GAG	Membrane	Tissue Regeneration Skin Patches
Collagen + Hydroxyapatite	Powder Sponge	Bond-Filling and Repair Drug Delivery (BMP)

Acta Neurochir (Wien) (2008) 150: 803–810
DOI 10.1007/s00701-008-1617-7
Printed in The Netherlands

Acta Neurochirurgica

Experimental Research

The AdLMP-1 transfection in two different cells; AF cells, chondrocytes as potential cell therapy candidates for disc degeneration

S. U. Kuh[1], Y. Zhu[2], J. Li[2], K.-J. Tsai[2], Q. Fei[2], W. C. Hutton[2], S. T. Yoon[2]

[1] Department of Neurosurgery, The Spine and Spinal Cord Institute, Yonsei University Medical Colleg
[2] Veterans Affairs Medical Center, Department of Orthopaedics, Emory University School of Medicin

Table 3. *mRNA expressions for the AF cells and the chondrocytes (given for 20 MOI AdLMP1-GFP infection) compared to the control group*

	AF cells	Chondrocytes
Aggrecan	1.73* ± 0.41	1.68* ± 0.14
Type I collagen	1.86* ± 0.33	1.31* ± 0.06
Type II collagen	1.34* ± 0.07	1.35* ± 0.12
LMP-1	51.09* ± 21.05	48.78* ± 9.73
BMP-2	3.01* ± 1.01	2.14* ± 0.36
BMP-7	7.67* ± 5.50	2.50* ± 0.40

Mean value ± standard error.
* Indicates $p < 0.05$.

Table 2. *Increased sGAG production compared to the control*

	AF cells			Chondrocytes		
	10 MOI	20 MOI	30 MOI	10 MOI	20 MOI	30 MOI
sGAG	1.17 ± 0.07	1.30* ± 0.05	1.23 ± 0.34	1.05 ± 0.10	1.33* ± 0.09	1.14* ± 0.06

Available online at ScienceDirect www.sciencedirect.com
Elsevier Masson France EM|consulte www.em-consulte.com
JOINT BONE SPINE
Joint Bone Spine 76 (2009) 70–74

Original article

A comparison of three cell types as potential candidates for intervertebral disc therapy: Annulus fibrosus cells, chondrocytes, and bone marrow derived cells

Sung Uk Kuh[a,*], Yerun Zhu[b], Jun Li[b], Kai-Jow Tsai[b], Qiuming Fei[b], William C. Hutton[b,c], Tim S. Yoon[b]

[a] Department of Neurosurgery, Yonsei University Medical College, Spine and Spinal Cord Institute, Seoul, South Korea
[b] Department of Orthopaedics, Emory University School of Medicine, Veterans Affairs Medical Center, Atlanta, GA, USA

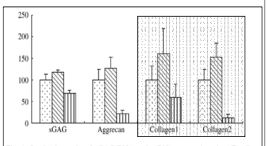


Fig. 1. Production ratio of sGAG/DNA and mRNA expressions in AF cells, chondrocytes, and BMDCs. 100 on the Y-axis scale represents the value for the AF cells. The values for the other two cell types are compared to the value for the AF cells.

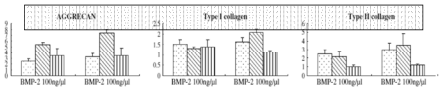
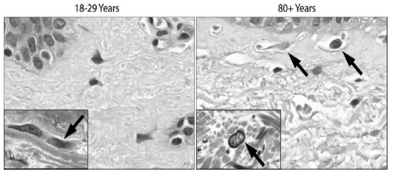
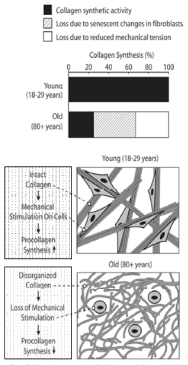
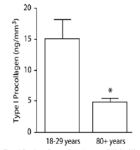


Fig. 3. The mRNA expression of AF cells, chondrocytes, and BMDCs after rhBMP-2 treatment. Y-axis scale represents the increased ratio compared to each of untreated AF cells, chondrocytes, and BMDCs.





Fibroblast aging에 의해 80세 이상은 세포의 활성화가 감소되어 **67% 콜라겐 합성이 감소**



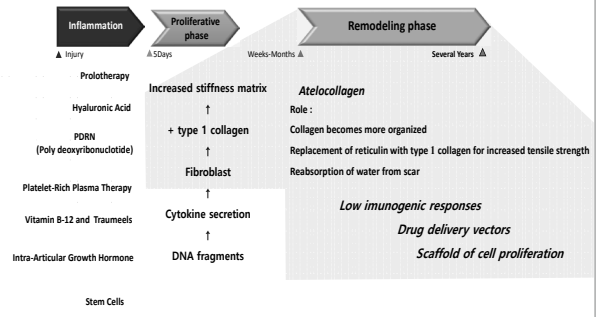
Collagen : Type Ⅰ collagen 중요성

손상 된 조직의 치유과정 완성

(1) 염증 단계와 (2) 세포 및 세포외 기질의 증식 단계 이후에는 (3) **Type I collagen 생성**이 수개월에서 수년에 걸쳐 진행

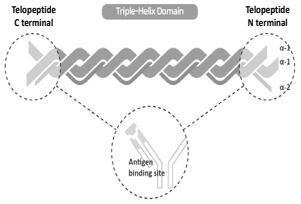
From mechanical loading to collagen synthesis, structural changes and function in human tendon. Scand J Med Sci Sports. (2009) Aug;19(4):500-10

Atelocollagen of outpatients cases



Structure of Atelocollagen molecule

The significance of 'Atelocollagen', concerns regarding collagen-induced autoimmunity.



Pepsin treatment →

Provides any clinically significant immunological benefits.

Atelocollagen

Low Immunogenic responses

Drug delivery vectors

Scaffold of cell proliferation

Atelocollagen-mediated systemic delivery prevents immunostimulatory adverse effects of siRNA in mamals. Molecularttherapy.org vol. 20 no. 2, 2012

Original Article

Tissue Engineering of the Intervertebral Disc with Cultured Nucleus Pulposus Cells Using Atelocollagen Scaffold and Gene Therapy

Hak Sun Kim, M.D.[1], Kwang Il Lee, M.D.[2], Hyung Kim, M.D.[2], Un-Hyo Kwon, M.D.[3], Mi Ran Nam, M.D.[4], Ju-Woong Jang, M.D.[1], Ji-Ji Cho[1], Beom Kim, M.D.[1], Hwan Mo Lee, M.D.[1], Seung-Hwan Moon, M.D.[1]
[1]Department of Orthopaedic Surgery, Yonsei University College of Medicine/[2]Korea Bone Bank Co., Ltd./[3]Bucm Korea Co./[4]Medical Korea Graduated School

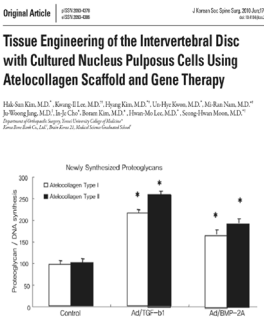


Fig. 1. Proteoglycan synthesis[S35] incorporation) normalized by DNA synthesis[H3] incorporation) in rabbit nucleus pulposus cells seeded on atelocollagen type I and II scaffolds for culture period of 1 week. Ad/TGF-b1, Ad/BMP-2 (75MOI) were administered. Control is only cell seeded scaffold without virus infection. (* P<0.05)

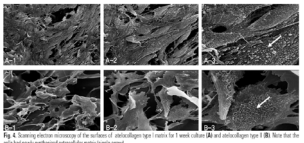


Fig. 4. Scanning electron microscope of the surfaces of atelocollagen type I matrix for 1 week culture (**A**) and atelocollagen type II (**B**). Note that the cells had newly synthesized extracellular matrix (single arrow).
Magnification is ×500 (A-1, B-1), ×1,000 (A-2, B-2), ×3,000 (A-3, B-3).

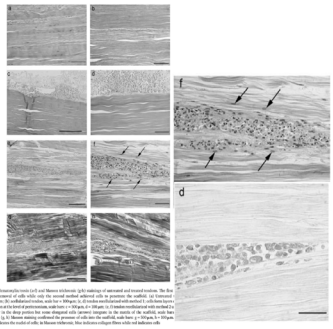
결론

"척추 추간판 세포는 제 1형, 2형 아텔로콜라겐 지지체 내에서 생존 가능하였고, 제 1형, 제 2형 아텔로콜라겐 지지체에서 아데노바이러스를 이용한 TGF-β1, BMP-2 cDNA 전달로 추간판 세포의 단백다당 생성, 연골형 표현형의 발현 증가가 있었다. 이는 조직 공학, 세포 치료, 유전자 치료를 함께 적용하여 추간판 조직 재생을 시도한 것이며 추간판 질환 치료에 새로운 접근법을 제시하였다."

Atelocollagen : Scaffold of cell proliferation

Successful recellularization of human tendon scaffolds using adipose-derived mesenchymal stem cells and collagen gel

J Tissue Eng Regen Med (2012)



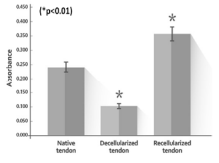
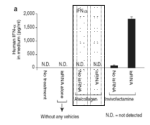


Figure 4. Colorimetric assay to determine the survival of cells. The values shown are the average ± deviation standard of absorbance of native, decellularized and recellularized tendon. The Anova test was used to set statistical significativity (*p< 0.01)

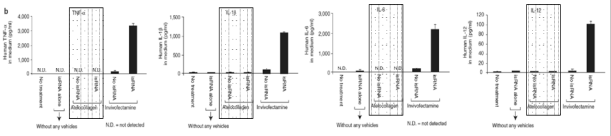
- 방법 : 외상 환자 수술 중에 flexor tendon을 기증 받아 탈세포화 과정 후 증식 과정 연구
- 분석 : 건(tendon)의 착색(Hematoxylin / eosin, Masson trichromic)을 통해 세포의 증식을 비교분석

Atelocollagen-mediated Systemic Delivery Prevents Immunostimulatory Adverse Effects of siRNA in Mammals

Shinichiro Inaba[1,2], Shunji Nagahara[3], Naoki Makita[3], Yuzo Tarumi[4], Takuji Ishimoto[1,2], Seiichi Matsuo[2], Kenji Kadomatsu[1] and Yoshifumi Takei[1,3]
[1]Department of Biochemistry, Nagoya University Graduate School of Medicine, Nagoya, Japan; [2]Department of Nephrology, Nagoya University Graduate School of Medicine, Nagoya, Japan; [3]Formulation Laboratories Technology Research and Development Center, Daiichigan Sumitomo Pharma, Ibaraki, Japan; [4]Koken Co., Ltd., Tokyo, Japan; [5]Division of Disease Models, Center for Neurological Diseases and Cancer, Nagoya University Graduate School of Medicine, Nagoya, Japan



Atelcollagen은 Antigenic biding site를 제거하여 면역반응을 최소화하였기 때문에 Mammal에서 IFN α, IFN β, IL-1β, TNF-γ, IL-6, IL-12 염증성 사이토카인을 유도하지 않음.



Collagen ➡ Atelocollagen

; biomaterials for wound healing

structural integrity of the ECM

dynamic and flexible material

re-modeling to refine cellular and tissue function

; exogenous collagen

non-allergenic

biodegradable and nontoxic

more biocompatible than other natural polymers