

Musculoskeletal Tissue Engineering

EunAh Lee, PhD

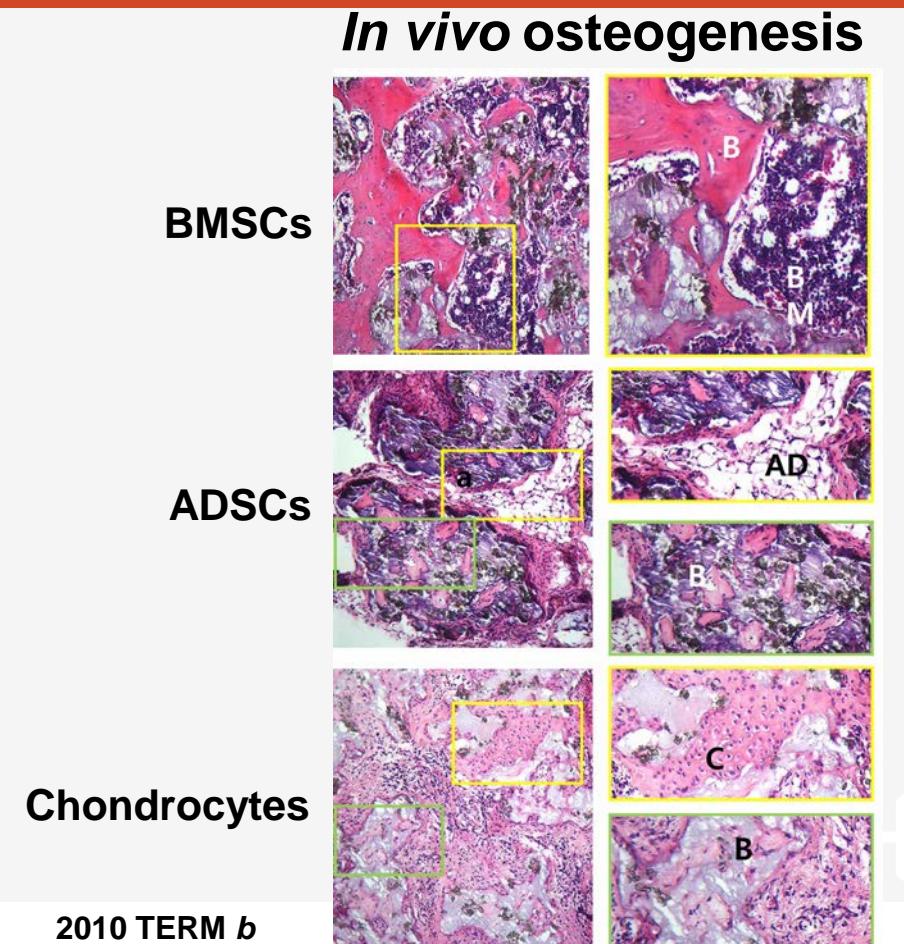
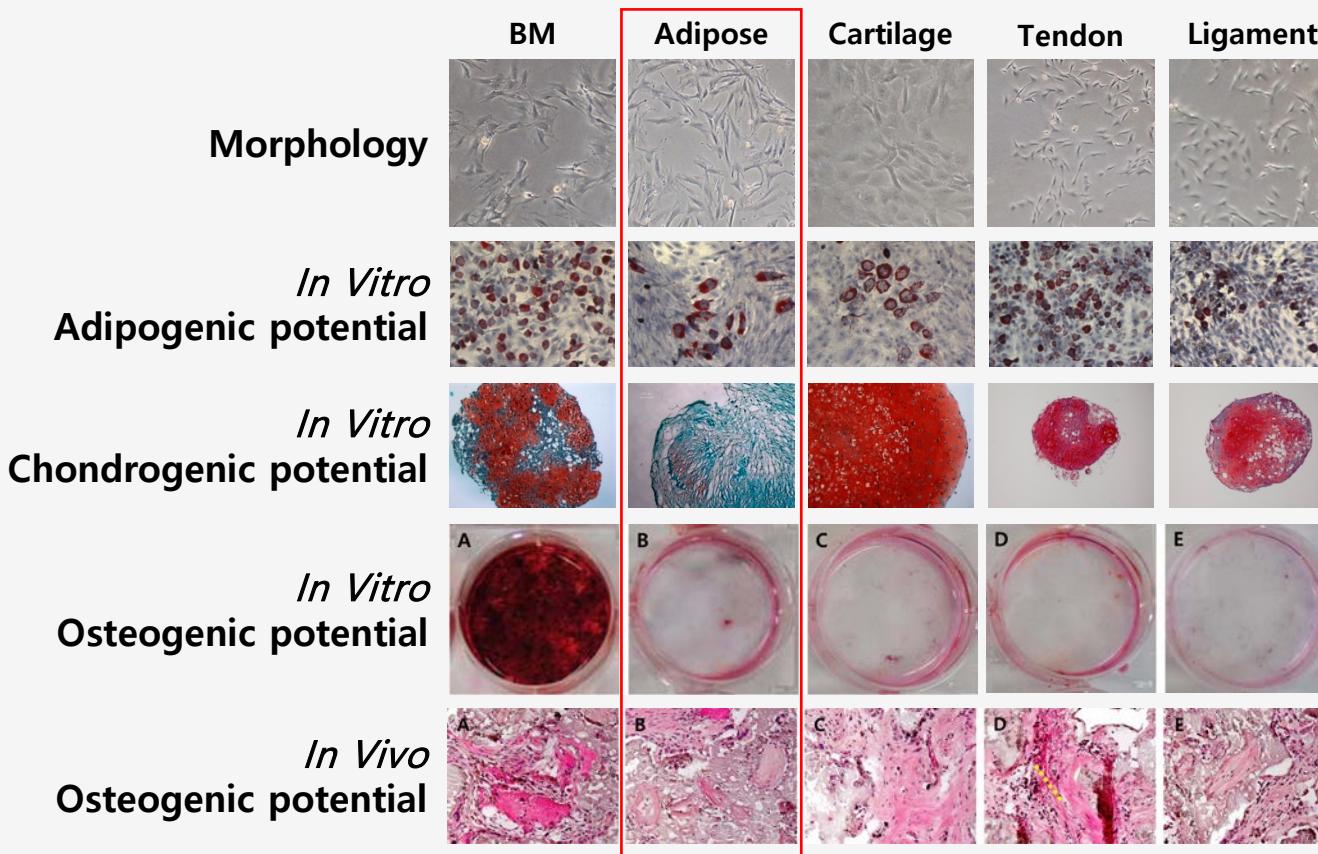
**Musculoskeletal Bioorgan Center/
Impedance Imaging Research Center
Kyung Hee University**



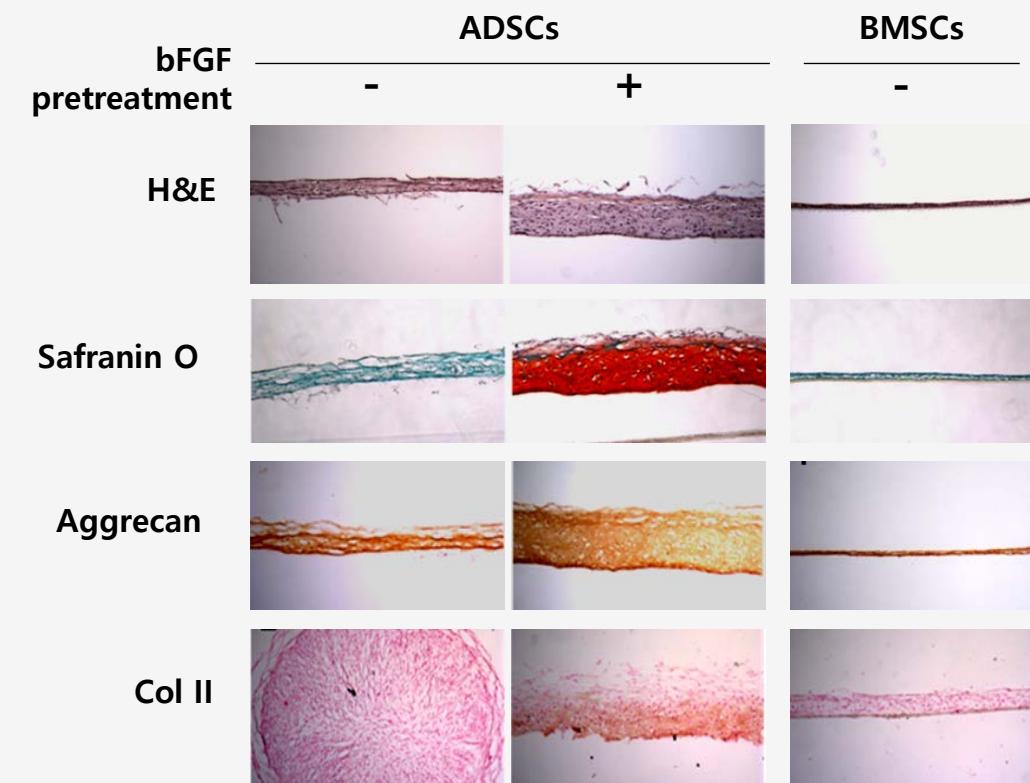
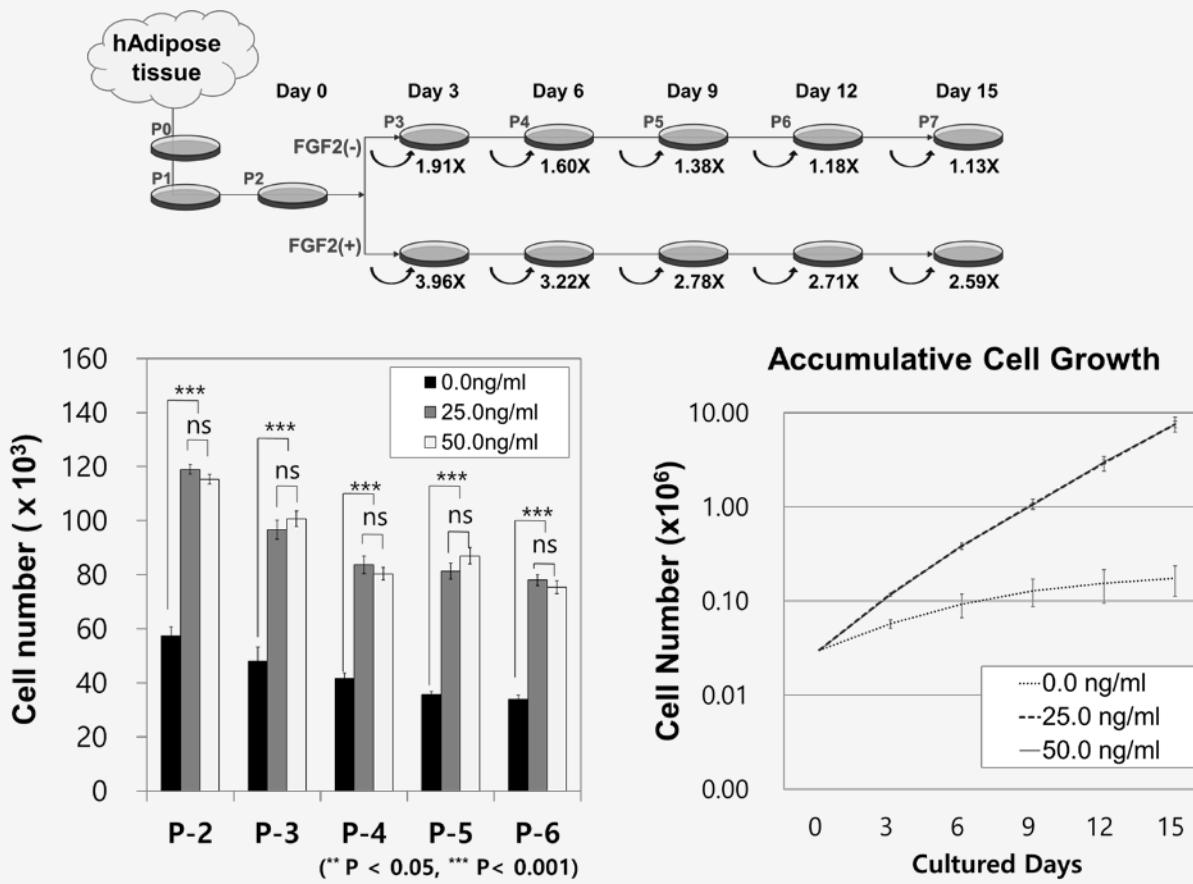
Stem Cell Alchemy

- Maximizing ADSCs Differentiation Activity

Sub-Optimal Differentiation Activity Exhibited by ADSCs

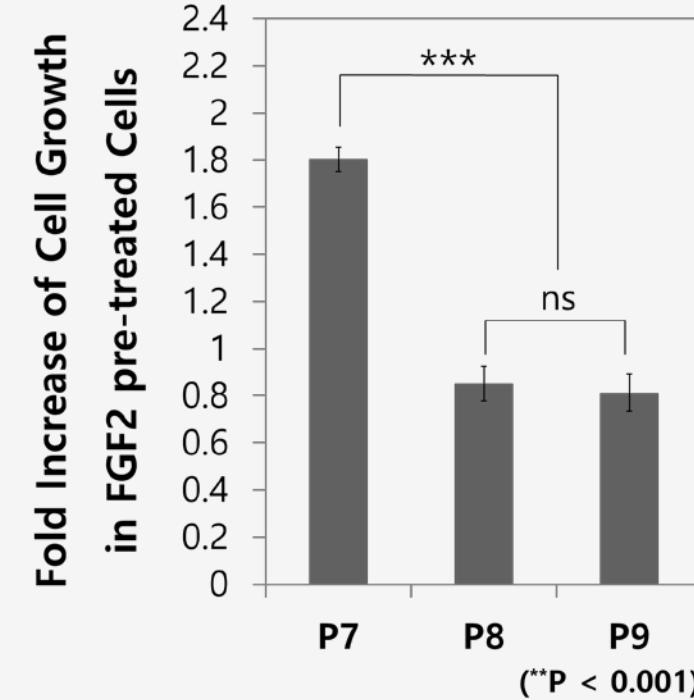
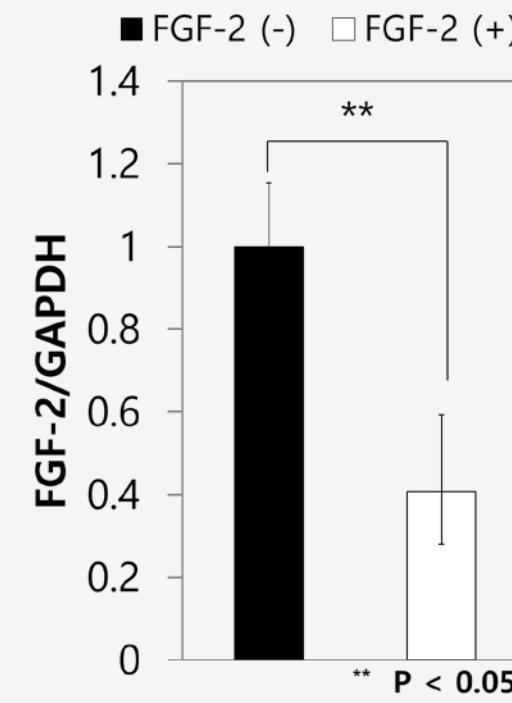
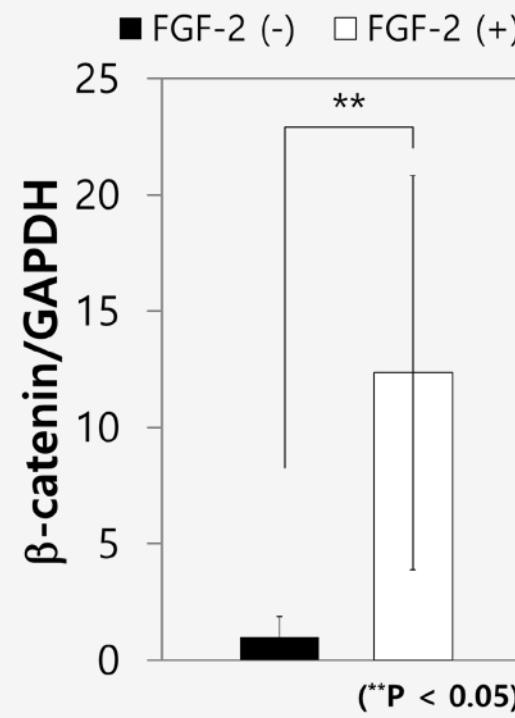


Optimized Chondrogenesis of Human ADSCs by FGF-2 Pretreatment

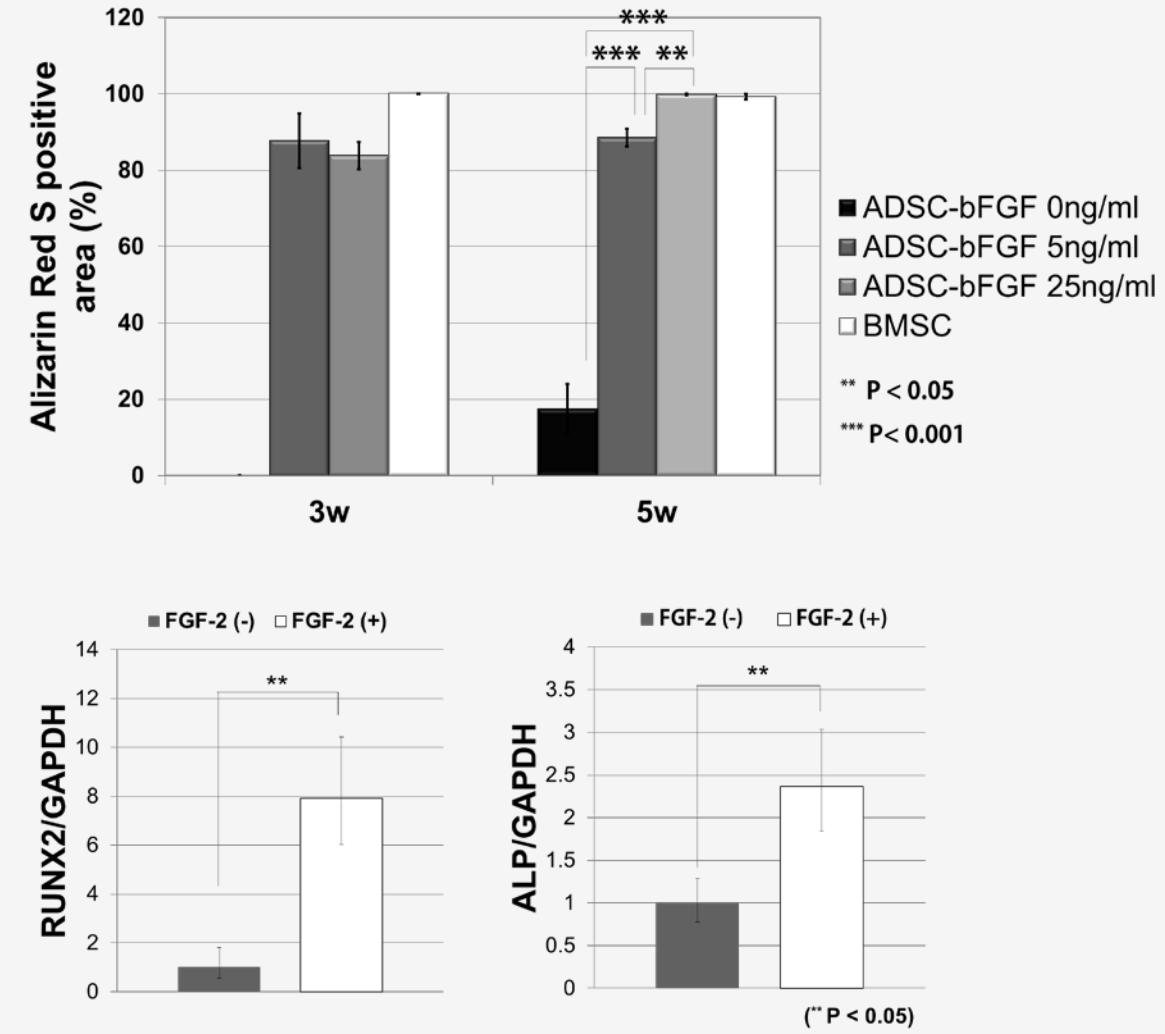
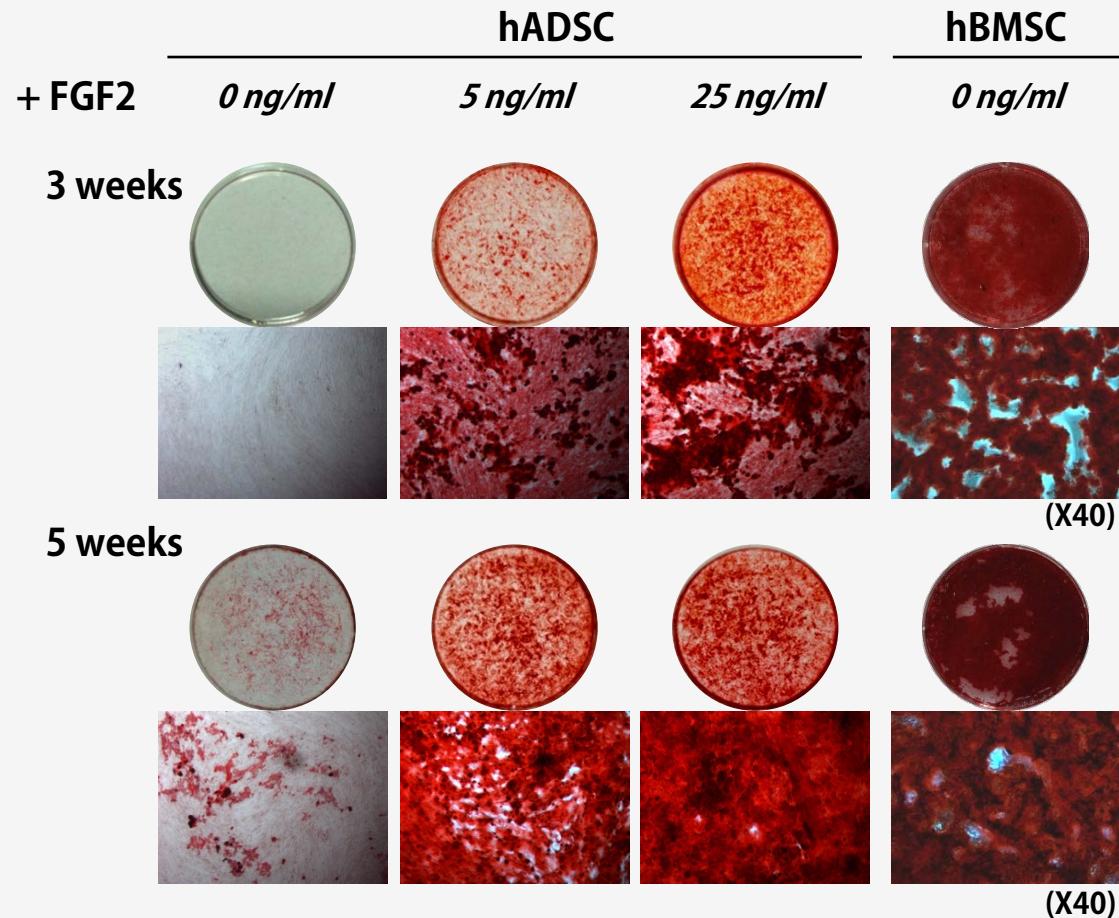


Osteogenic Stimulation of Human ADSCs by FGF-2 Pretreatment

Changes in basic cell activity

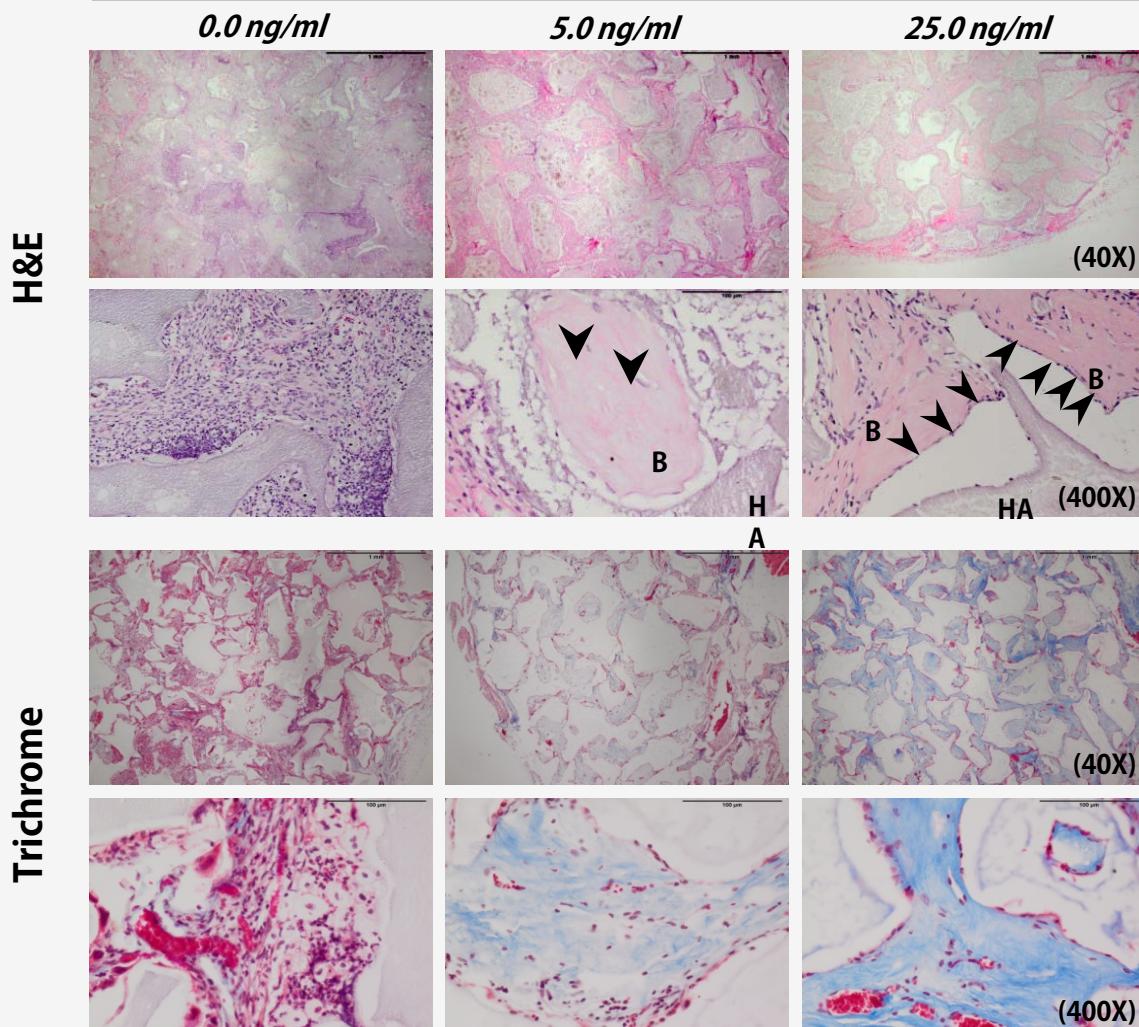


Improved Osteogenic Activity by FGF-2 Pretreatment

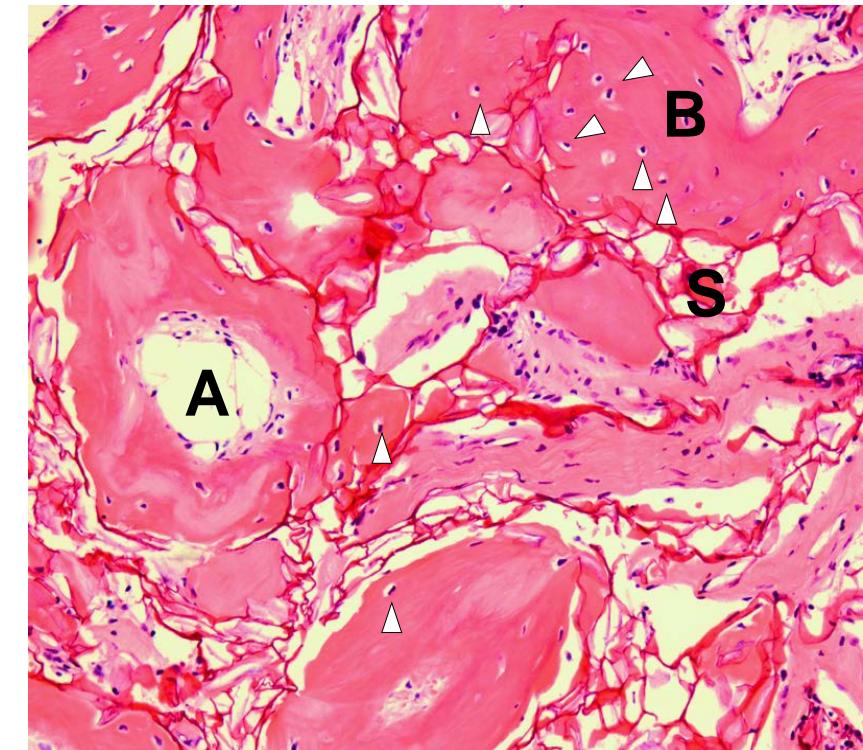


Increased In Vivo Matrix Production - No Real Bone Tissue

FGF2-pre-treatment dose



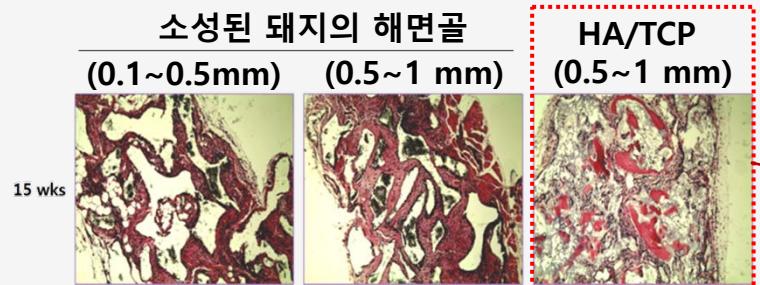
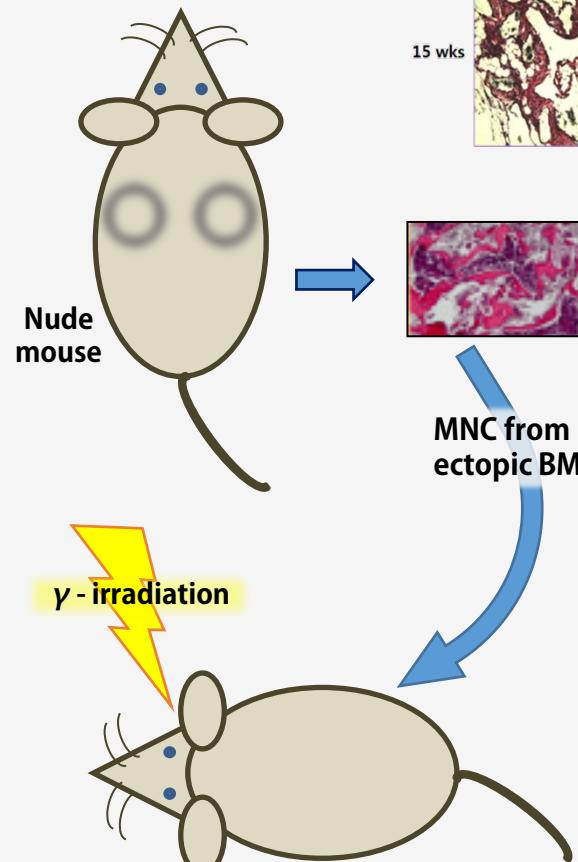
Ectopic bone marrow
(rBMSCs, 18 weeks upon transplant)



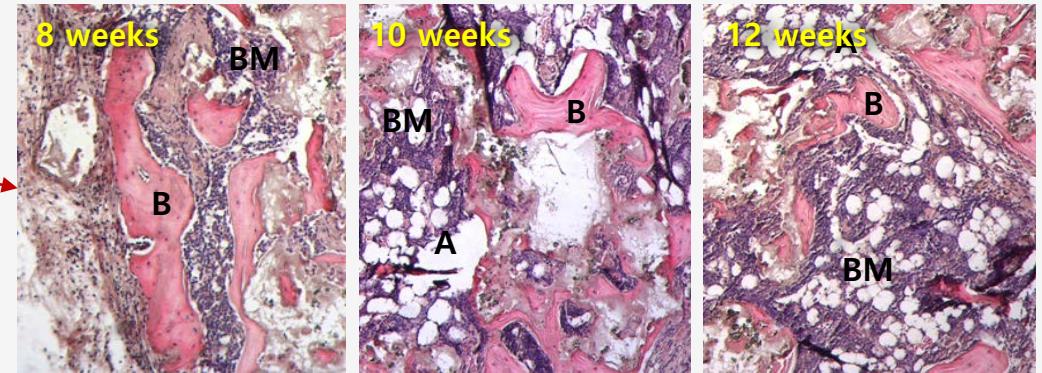
E Lee et al., unpublished data

Ectopic BM Model Study - Myelo-Supportive Activity

Screening of Bone substitutes

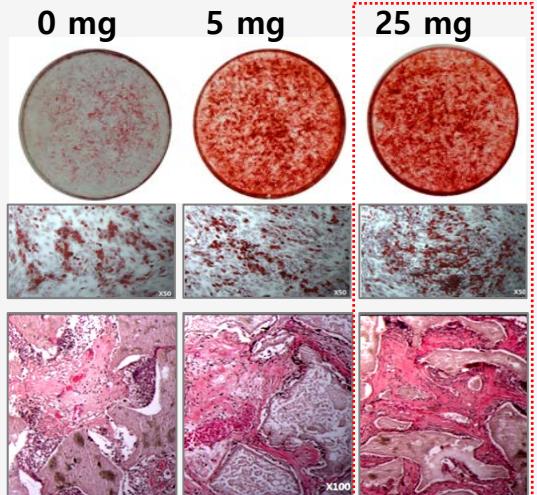


Histology of ectopic Bone & Marrow



Ectopic Osteoid Generation - ADSCs

bFGF pre-treatment



Type-Specific Engineered Cartilage

Contents

Three Cartilage Types

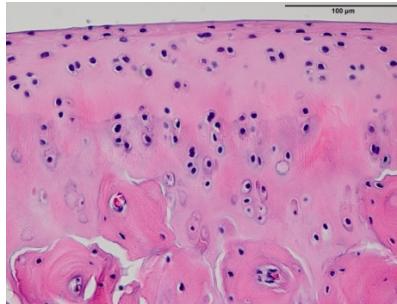
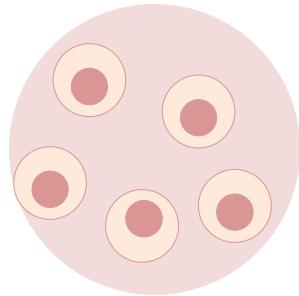
Challenges in Cartilage Tissue Engineering

- **Engineered Hyaline Cartilage**
- **Engineered Elastic Cartilage**
- **Engineered Fibro-Cartilage**

Conclusion

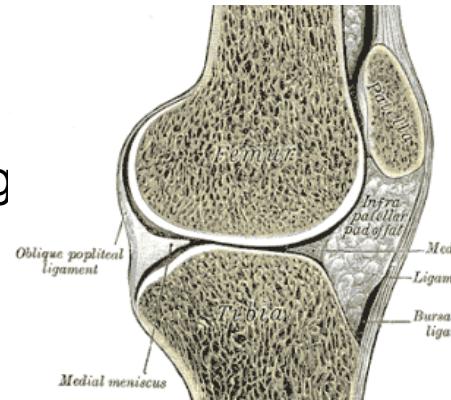
Cartilage Type

Hyaline cartilage

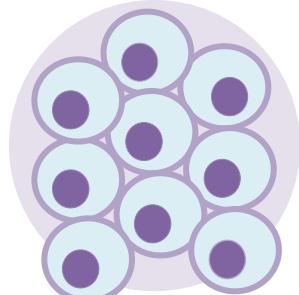


Articular cartilage

Various cell morphologies
Inter-territorial matrix
Type II collagen

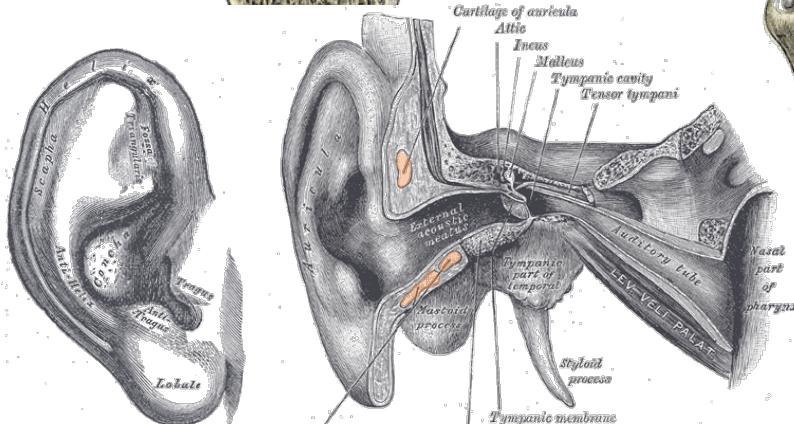


Elastic cartilage

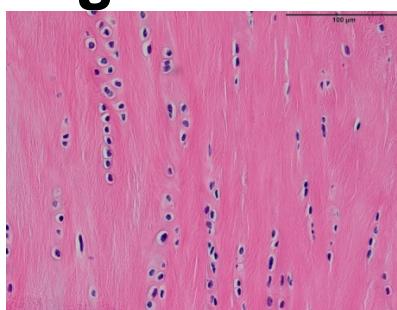
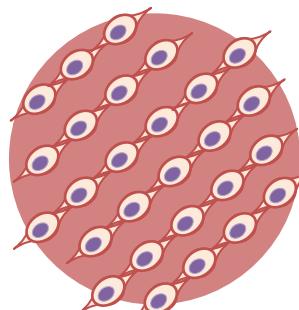


Auricular cartilage

Large lacunae
Peri-cellular matrix
Elastin

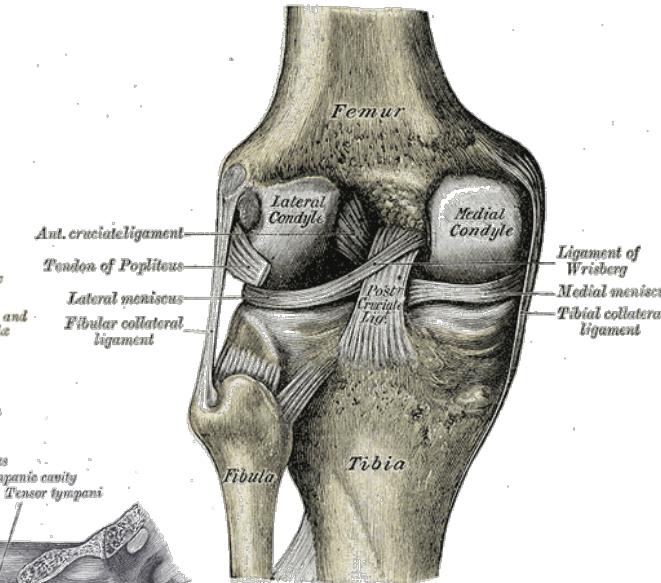
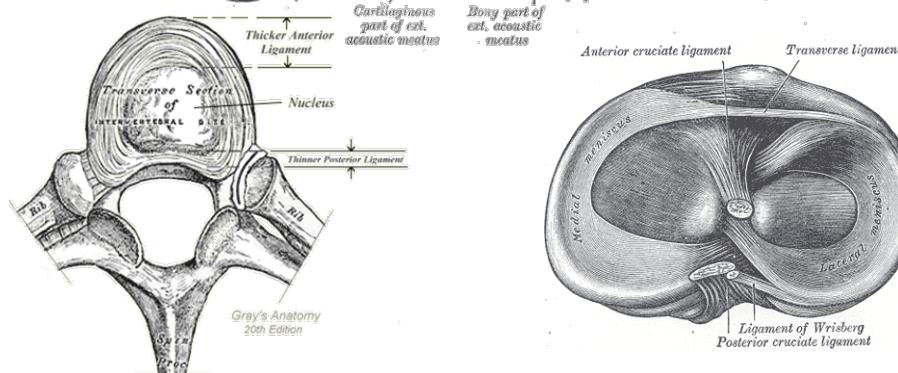


Fibro-cartilage



Disc, Meniscus

Small cell body
Fibrous matrix
Type I collagen



Distinctive Characteristics of Cartilage Tissue

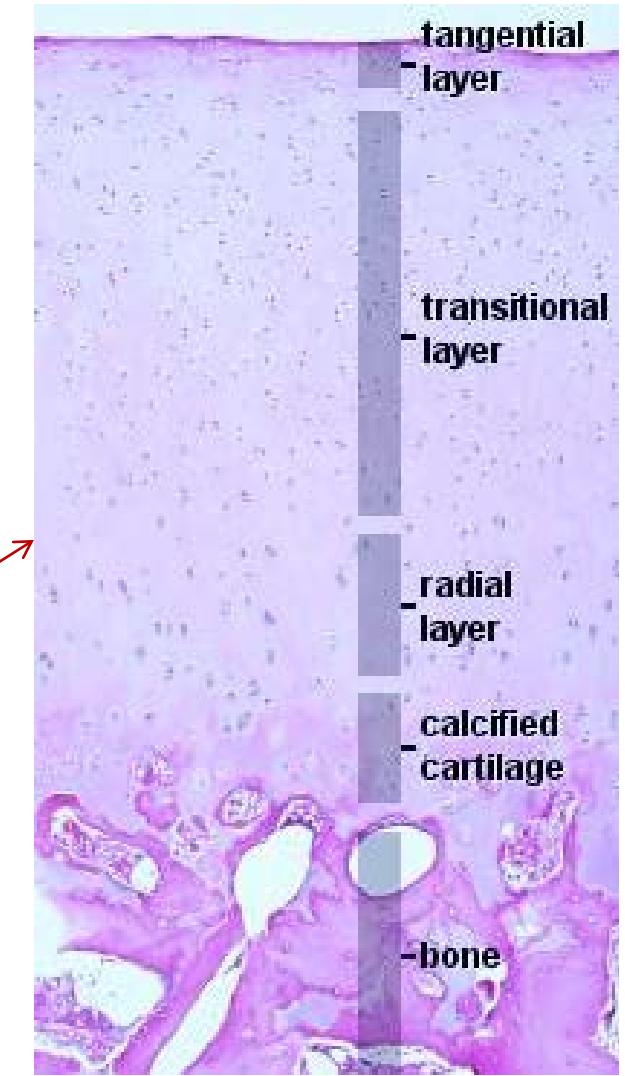
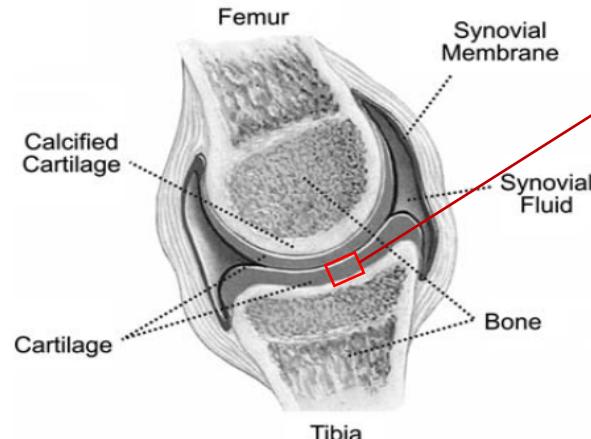
Comprised of Single Type of Cell

Chondrocyte

Limited Repair upon Damage

Cartilage is avascular tissue - lack of blood vessel.

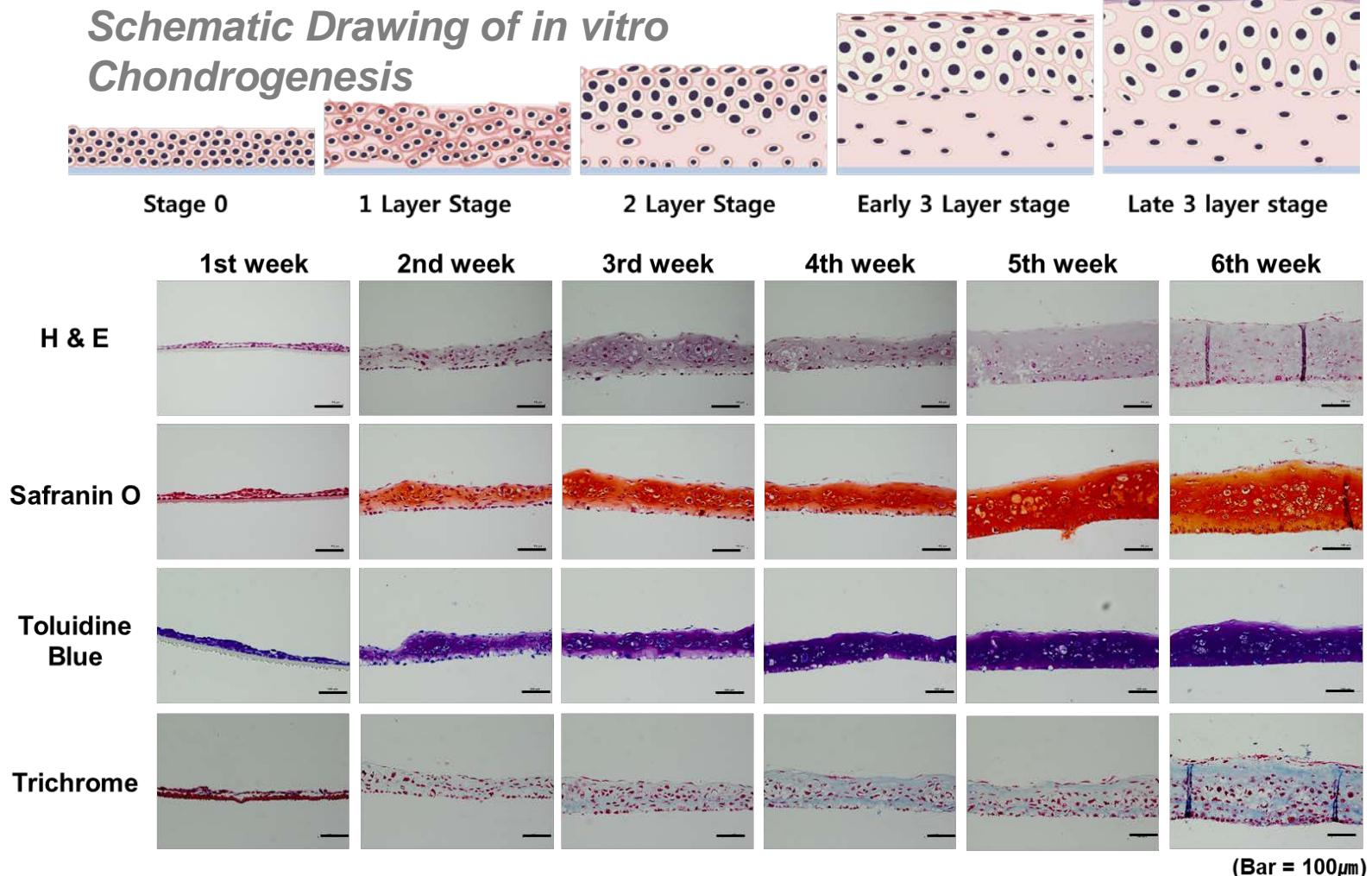
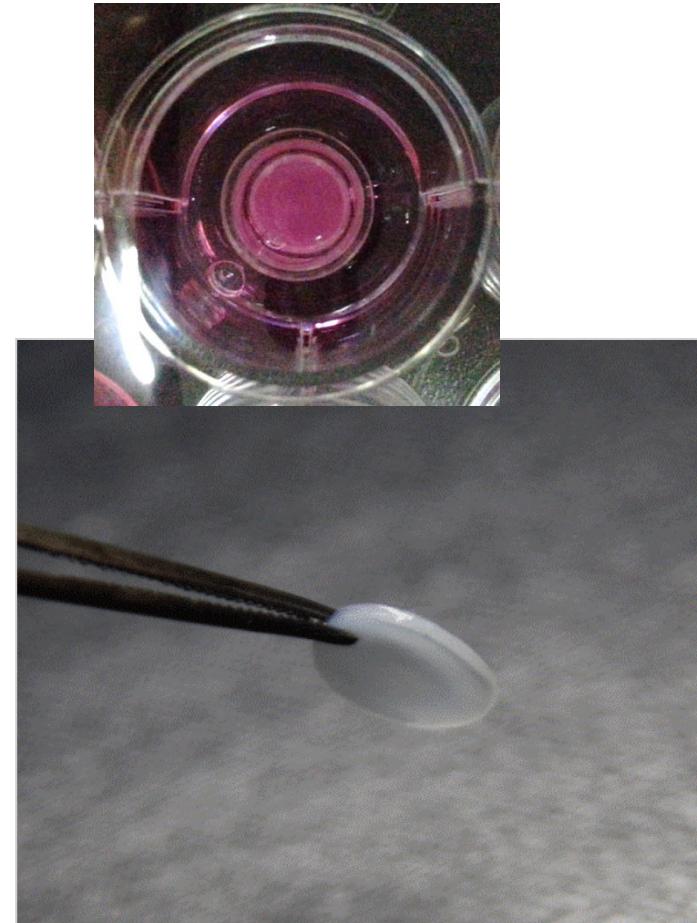
Low cell density - limited chondrocyte pool for regeneration



Cartilage - UWA Blue Histology

Advantage of *In Vitro* Chondrogenesis:

- Culture conditions can be precisely controlled.
- Material properties can be evaluated during culture.



Importance of Non-destructive Monitoring

Advantage of in vitro chondrogenesis:

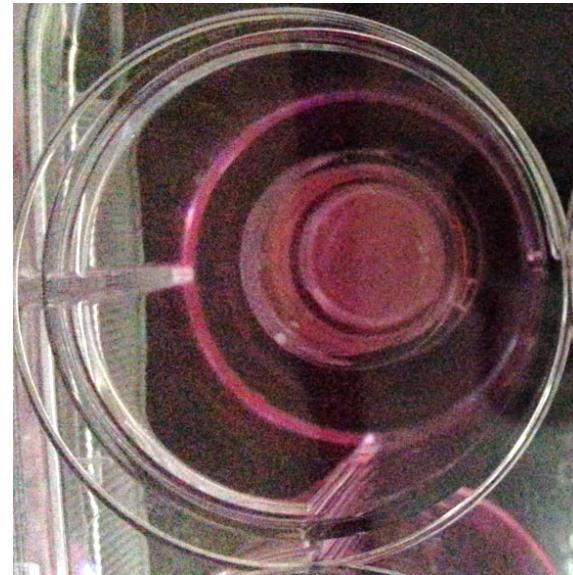
- Culture conditions can be precisely controlled.
- Material properties can be evaluated during culture.

Limitations of Current Tissue Monitoring Technologies:

- Destructive, Time consuming



Before cell seeding



After cell seeding

Challenges in Cartilage Tissue Engineering

Cell Source & Expansion

Similarity to Natural Tissue

- Low collagen content
 - *Hu & Athanasiou 2006a, Miot et al. 2006, Eyrich et al. 2007*
- Depth-dependent matrix organization
 - *Kim et al. 2003, Klein et al. 2003, Waldman et al. 2003,*
 - *Schuurman et al. 2009, Malda et al. 2010*

Tissue Integration

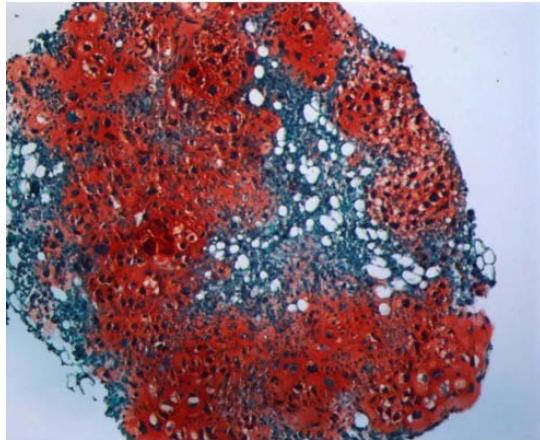
- *Kock L et al., 2011, Wang et al. 2007*

Non-Destructive Monitoring of Tissue Construction/Regeneration

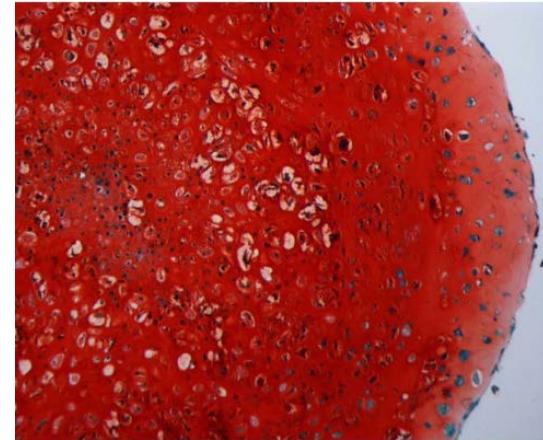
Importance of Cell Source

Red: Glycosaminoglycans (GAGs)

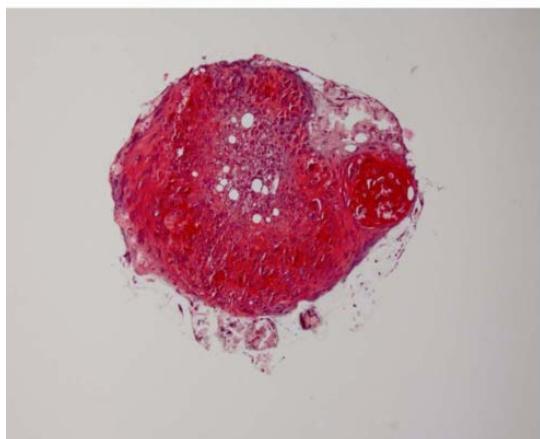
Bone Marrow



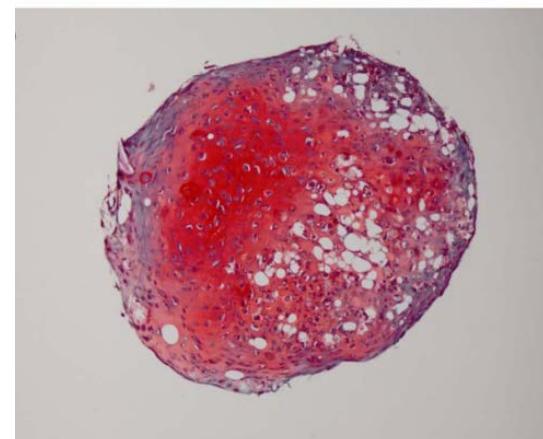
Cartilage



Tendon



Ligament



Cho HM et al., 2010 TERM

Characteristics of Chondrocytes from 3 Types of Cartilages

Chondrocytes
From
Hyaline
Cartilage

Chondrocytes
From
Elastic
Cartilage

Chondrocytes
From
Fibro-
Cartilage

GAGs Production (Toluidine Blue)

Col II (IHC)

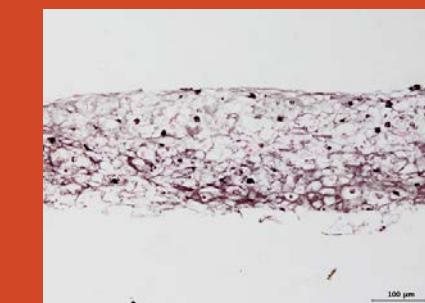
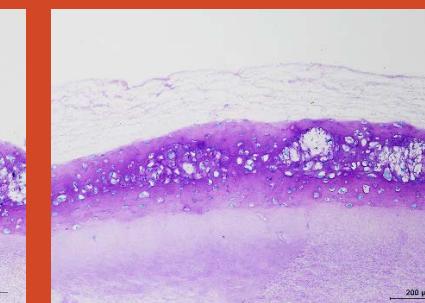
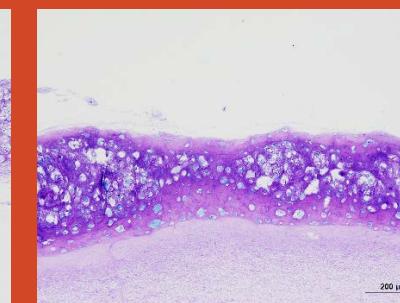
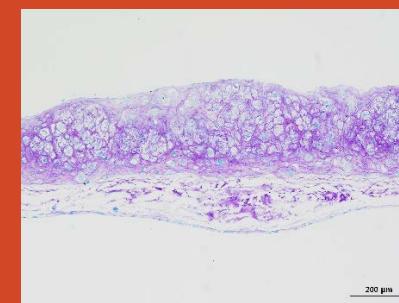
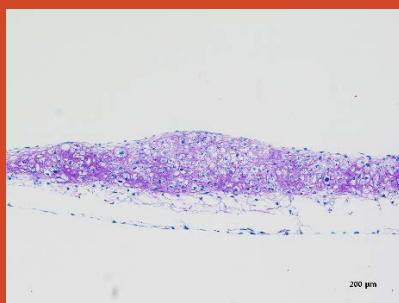
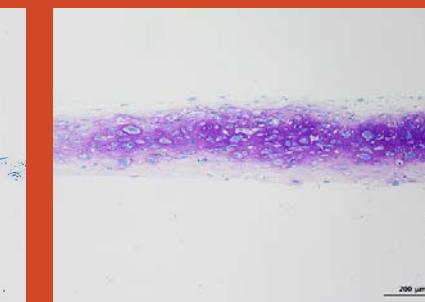
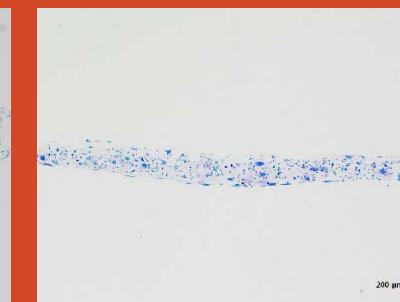
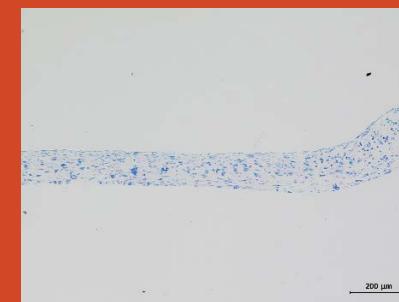
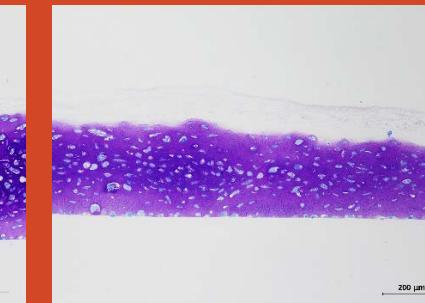
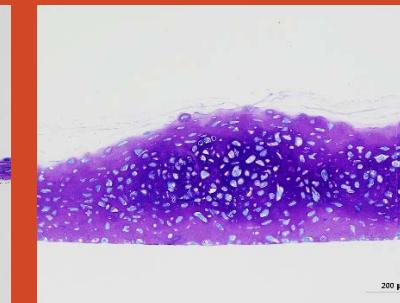
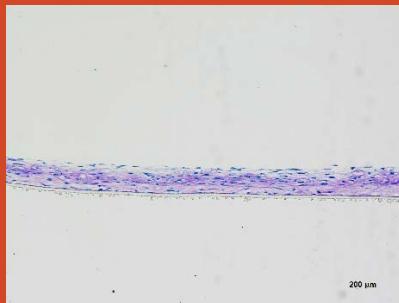
1w

2w

3w

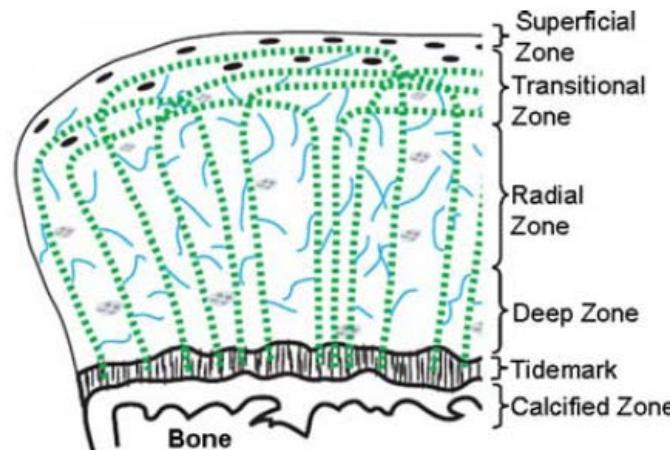
4w

2w

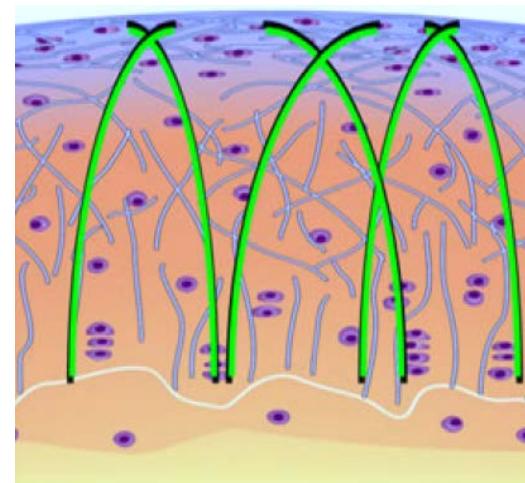


Hyaline Cartilage

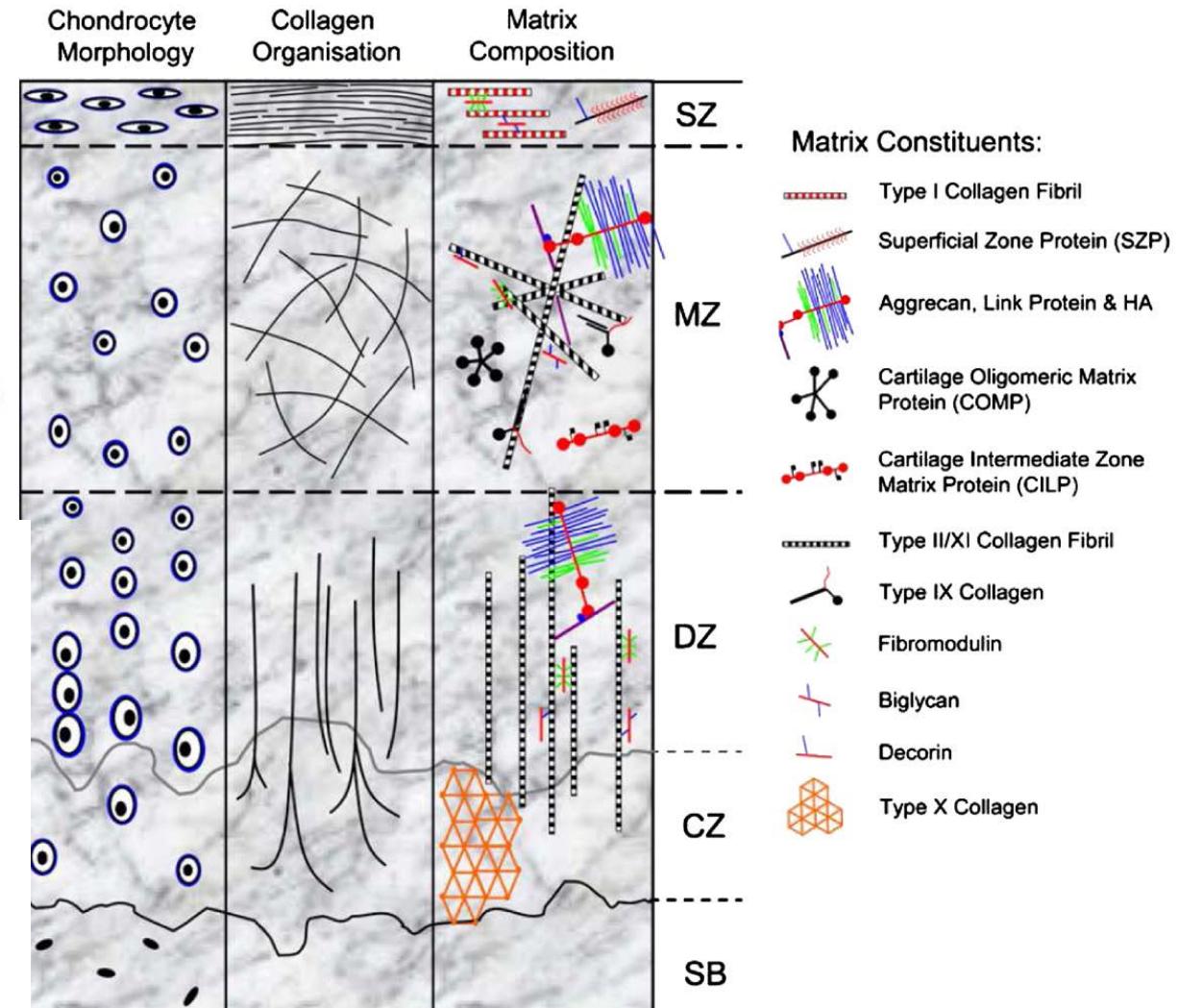
Structure & ECM organization of Articular Cartilage



Schulz RM and Bader A 2007 Eur Biophys J



Kock et al., 2011



Hayes AJ 2007 J Histochemistry & Cytochemistry

Engineering Cartilage with Zonal Structure

Cell-based methods

Kim et al., 2003; Waldman et al., 2003; Schuurman et al.,
2009; Malda et al., 2010; Ng et al., 2005

Scaffold-based methods

Woodfiled et al., 2005

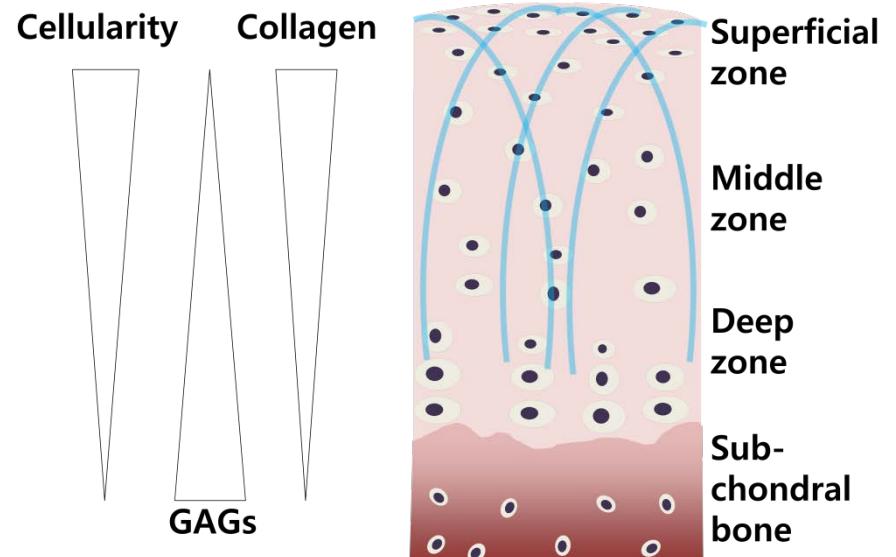
Combination of cells & scaffolds

Nguyen et al., 2011a; Nguyen et al., 2011b

Depth-dependent strain fields

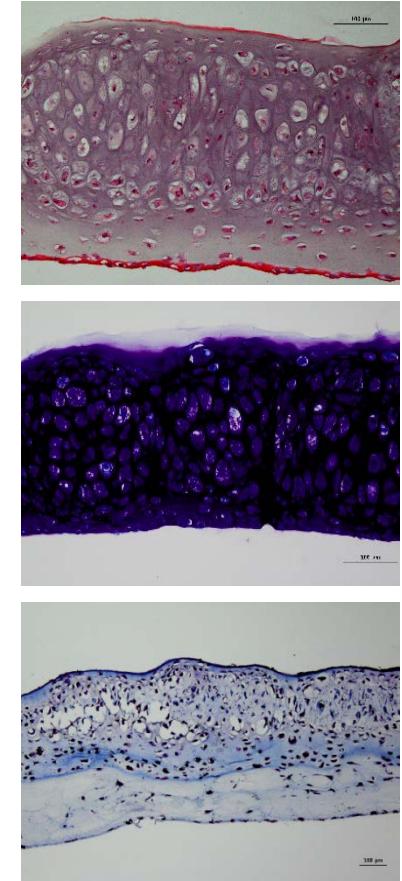
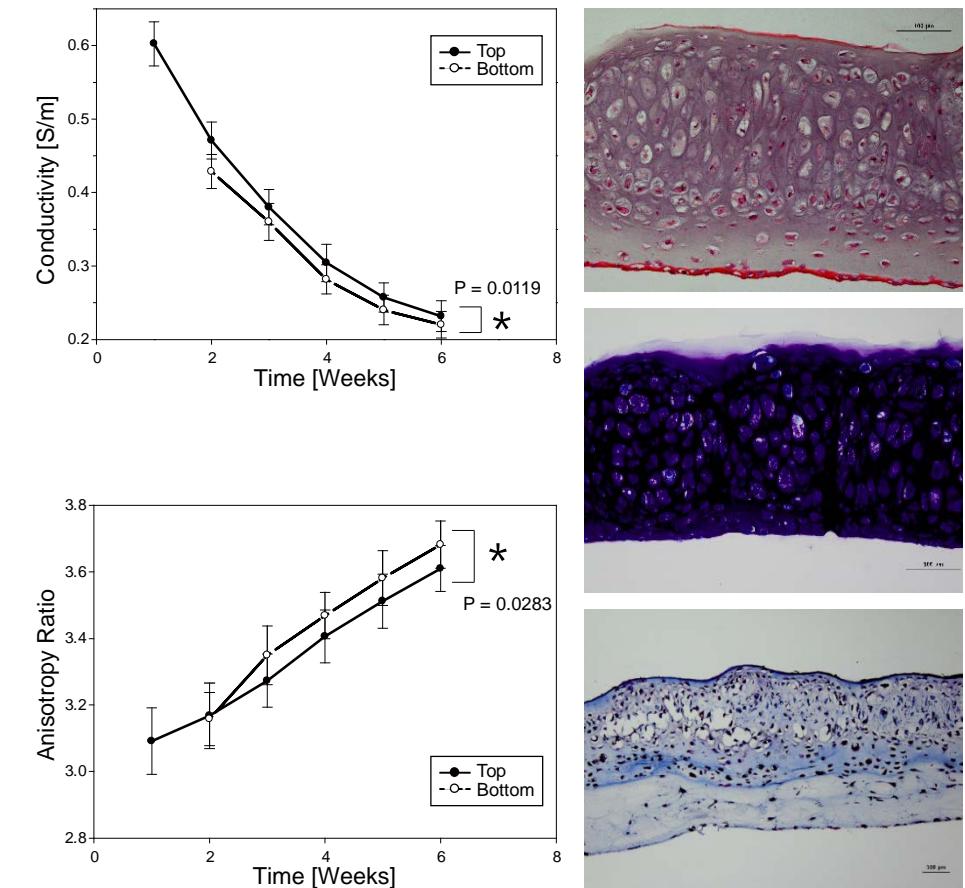
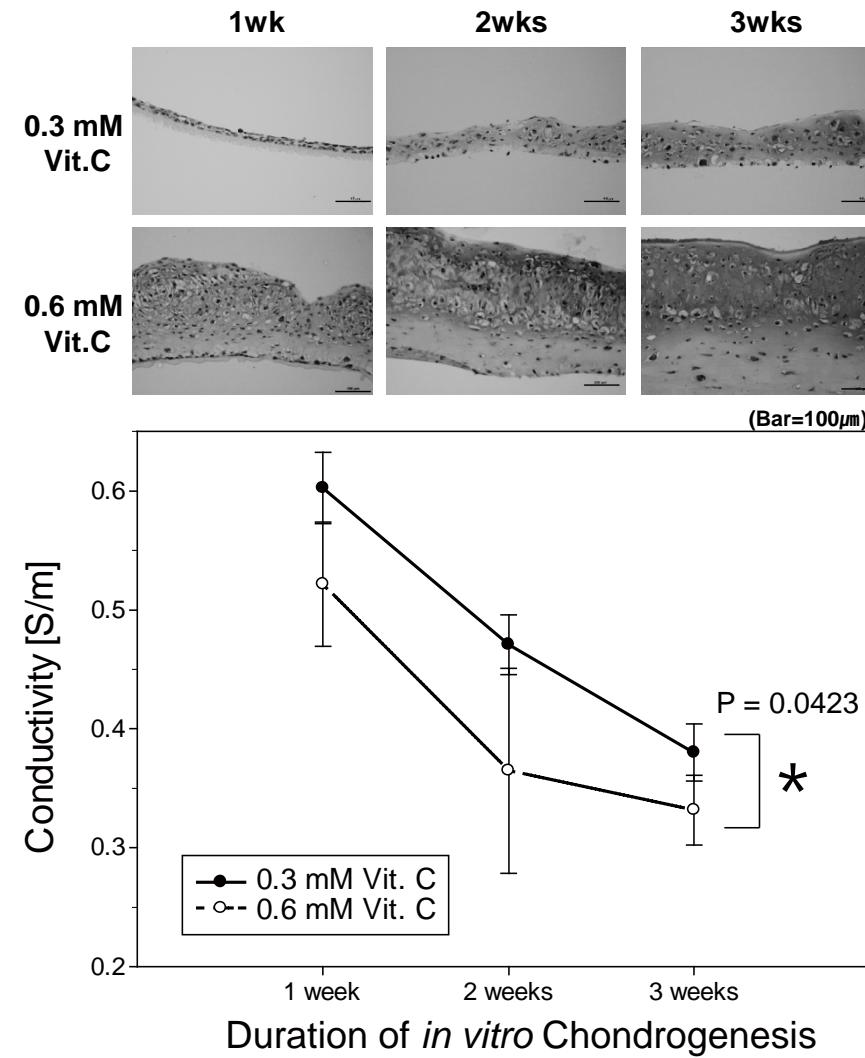
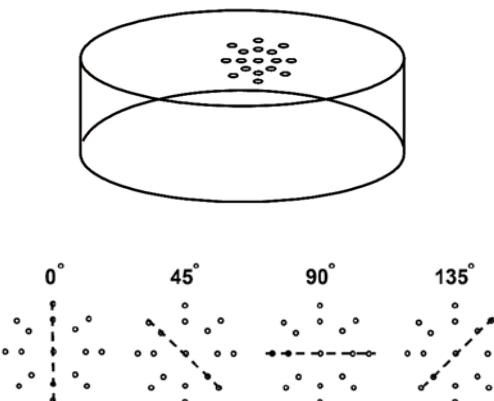
Kock et al., 2010; Khoshgoftar et al., 2011

Depth-Dependent Structure of Articular Cartilage

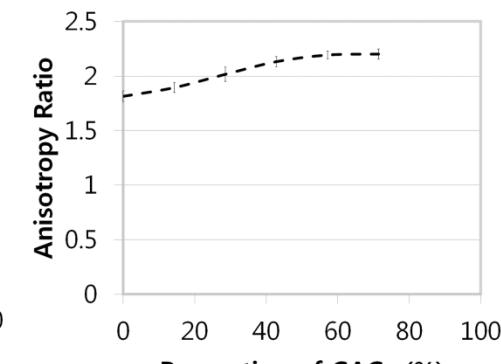
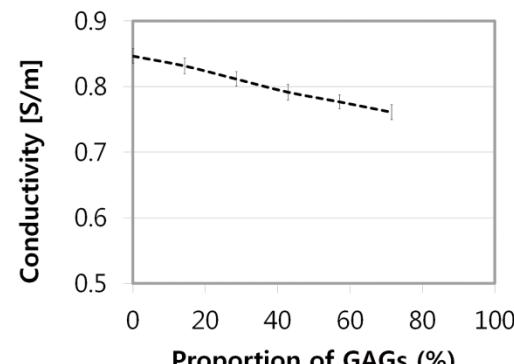
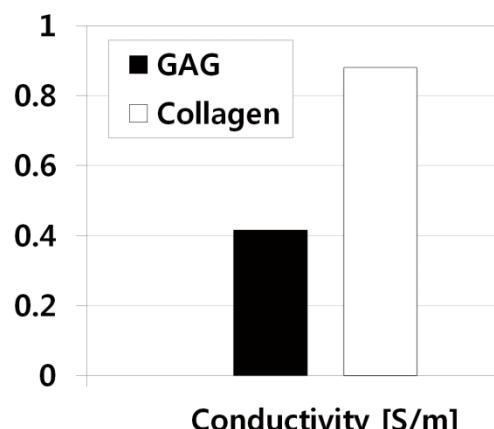
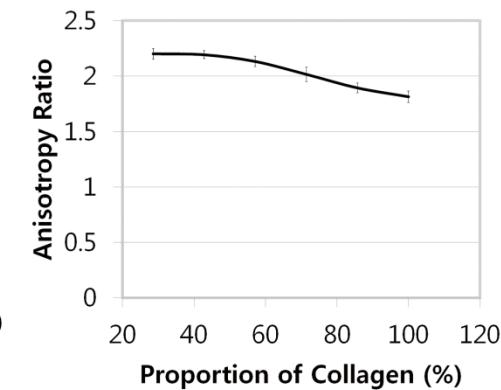
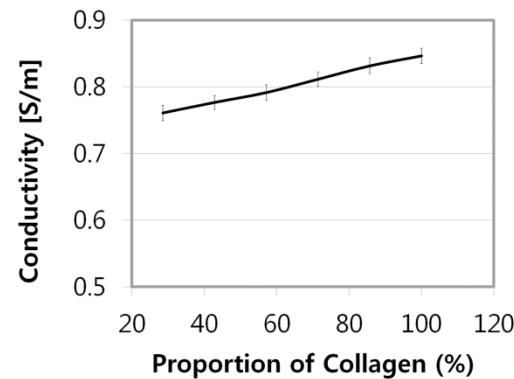
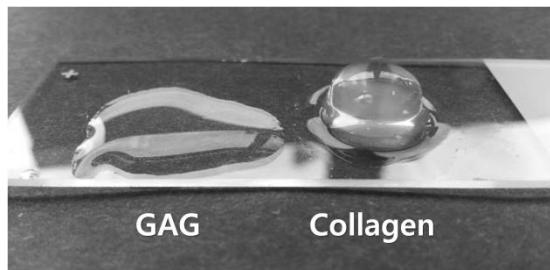
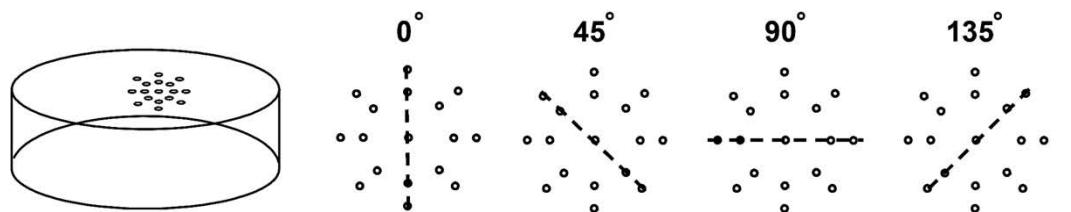


Bio-Impedance Spectra (BIS) - Based Monitoring of Tissue Regeneration

Bio-Impedance Spectra (BIS) Measurement System



Bio-Impedance Spectra (BIS) - Based Monitoring of Tissue Regeneration



Elastic Cartilage

Elastic Cartilage Deformity

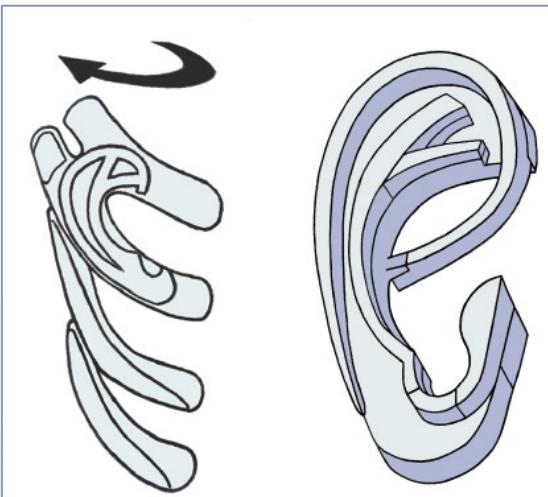
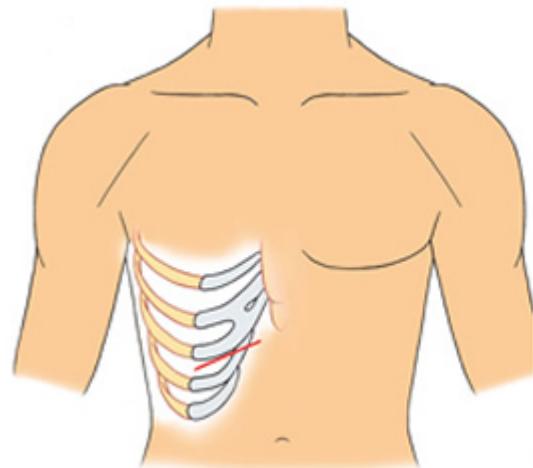
Microtia-anotia

- Unilateral or bilateral congenital deformity.
- Occurs in 1 out of about 8,000 births.
- Hearing loss is caused by inefficient conduction of sound to the inner ear.

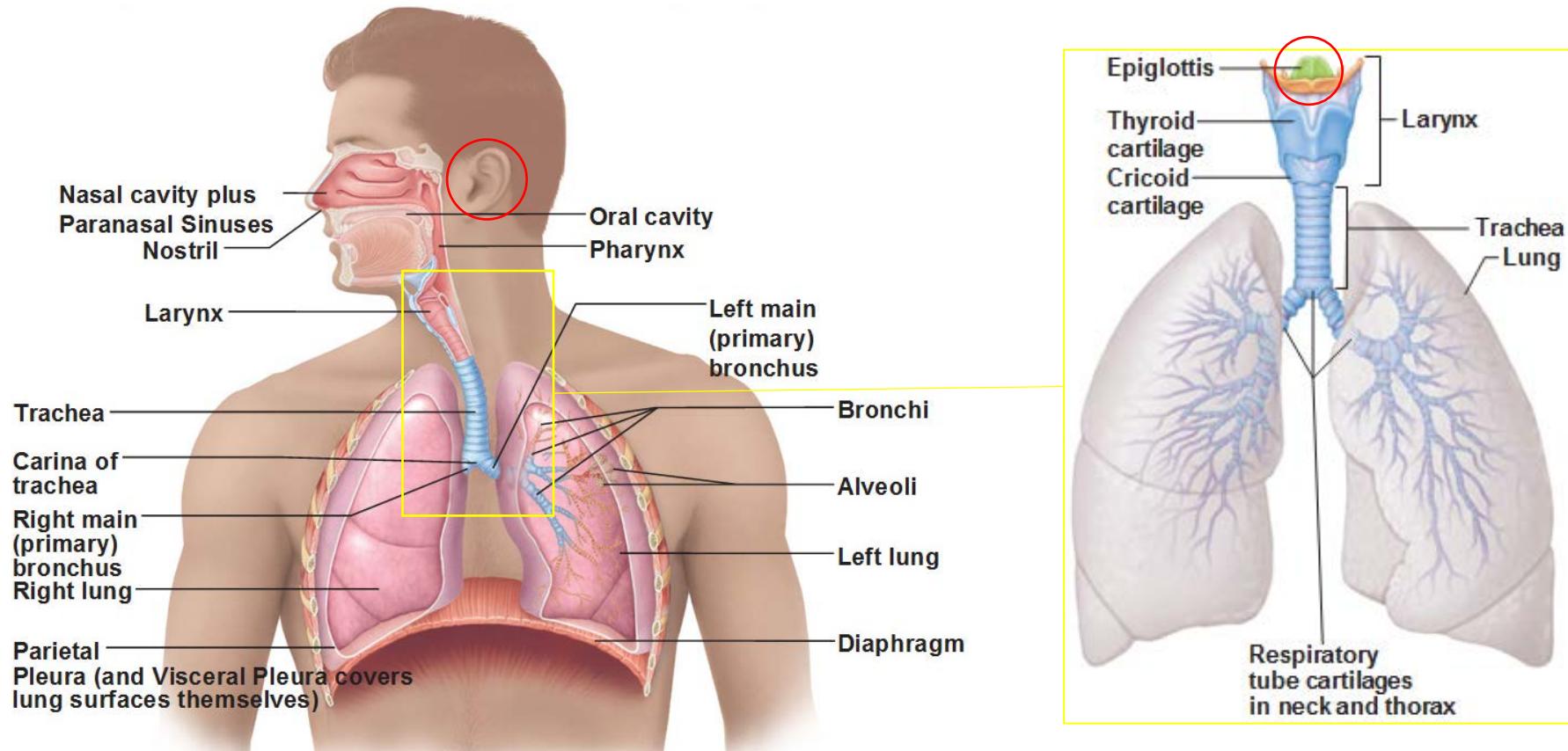
Treacher Collin Syndrome

- Craniofacial deformity
- Occurs in 1 out of about 50,000 births.

Injury

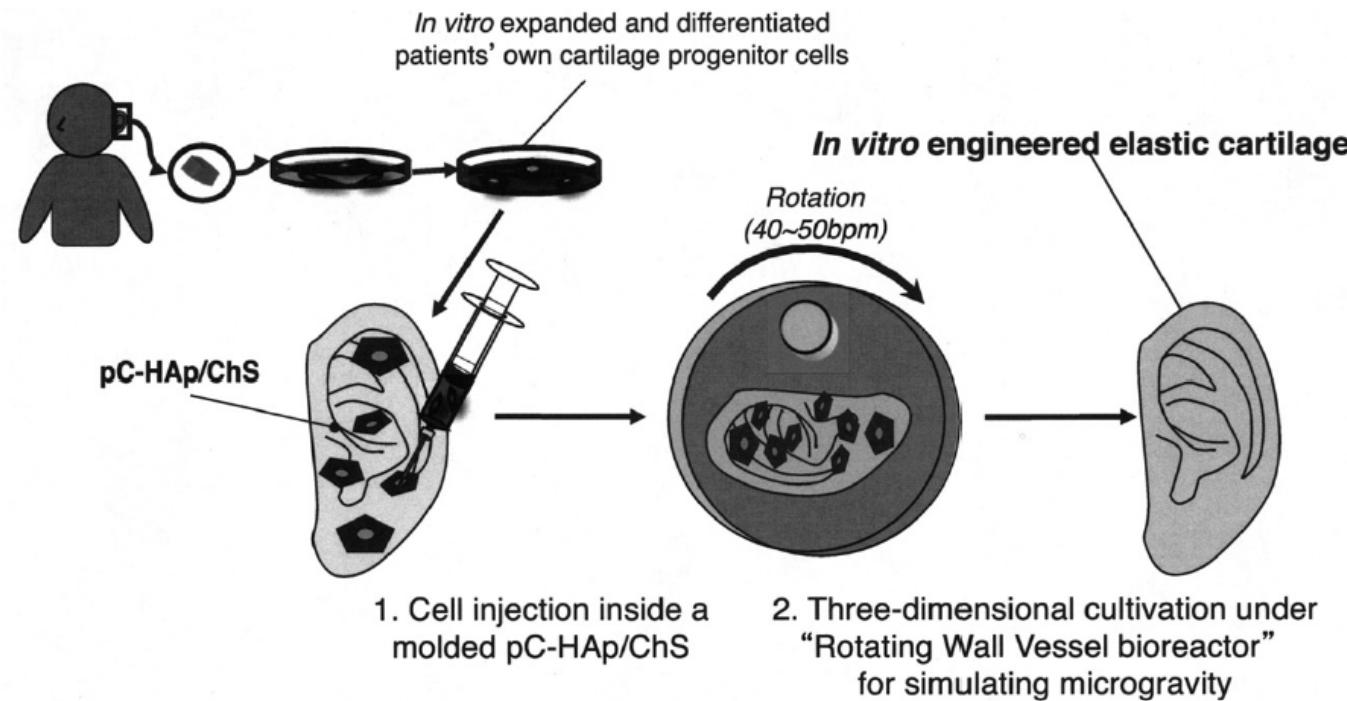


Distribution of Elastic Cartilage in the Body



Conventional Cell Sources for Elastic Cartilage Regeneration

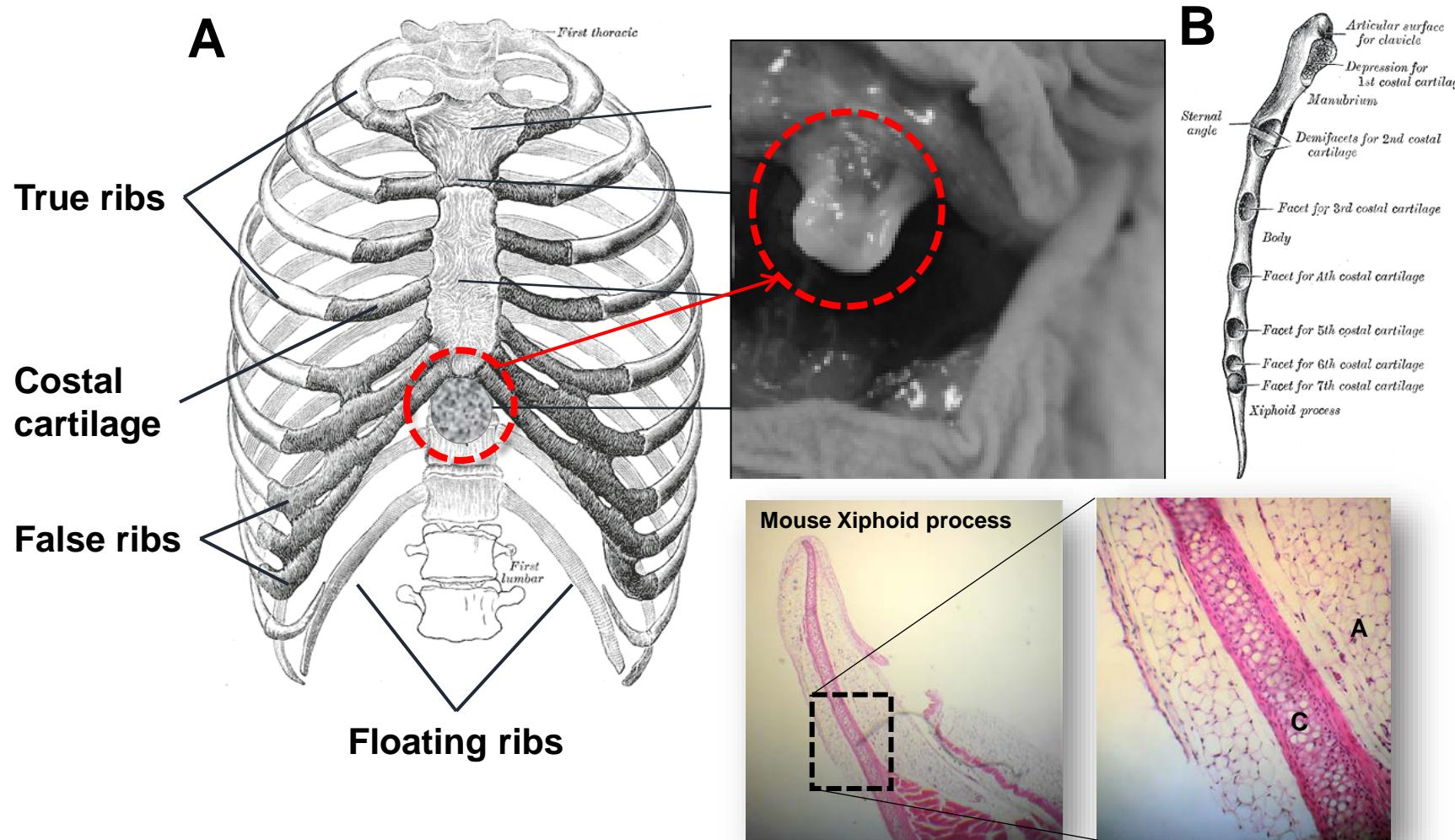
- BMSCs subjected to chondrogenic differentiation: Eventually turns to calcified tissue
- Chondrocytes from hyaline cartilage: Exhibit different mechanical property
- Chondrocytes from remnant elastic cartilage of damaged site: Limited availability



Takebe T 2012 Transplantation Proc

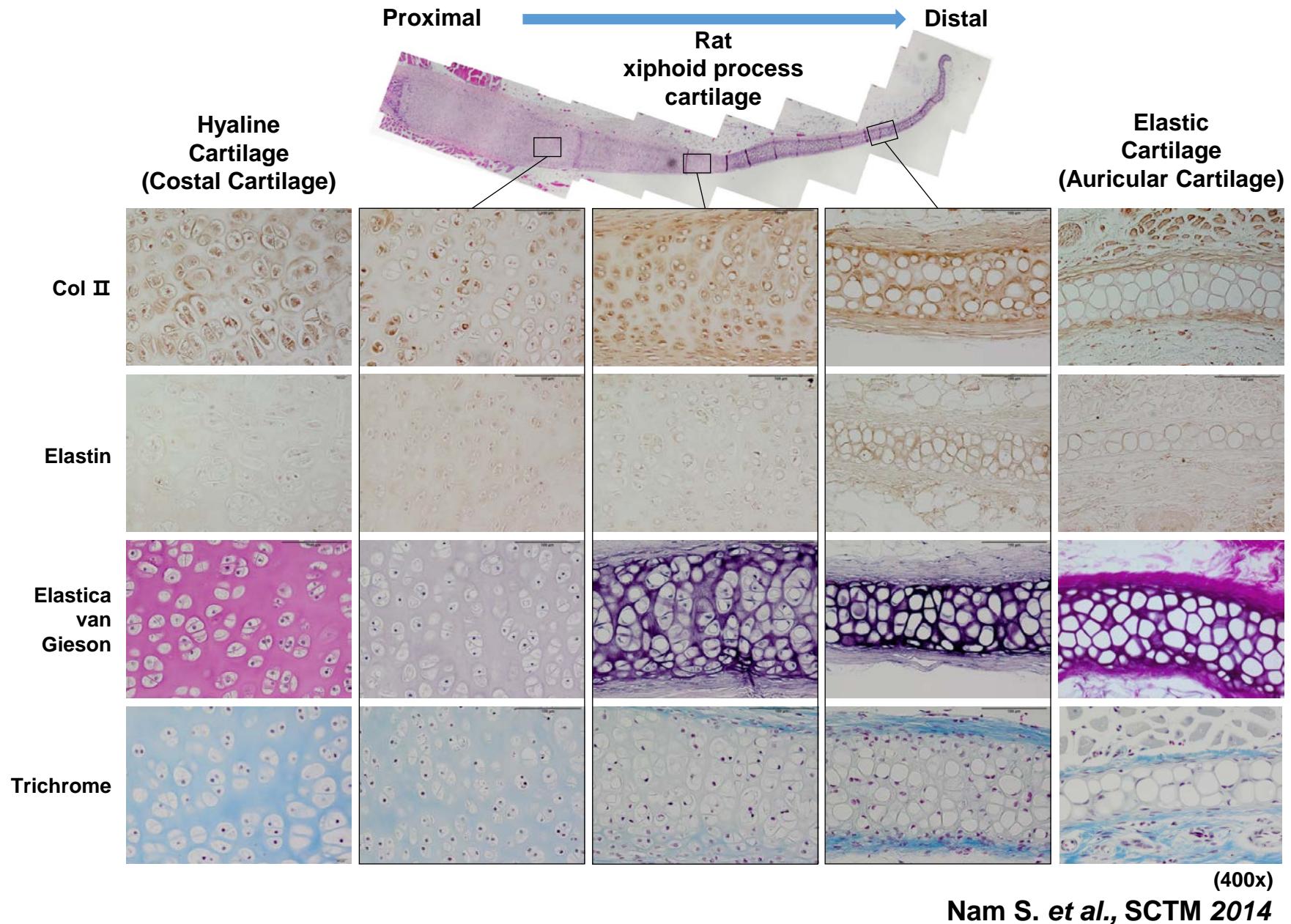
Xiphoid process

Xiphoid process, also known as the Xiphisternum
Usually ossified in the adult human

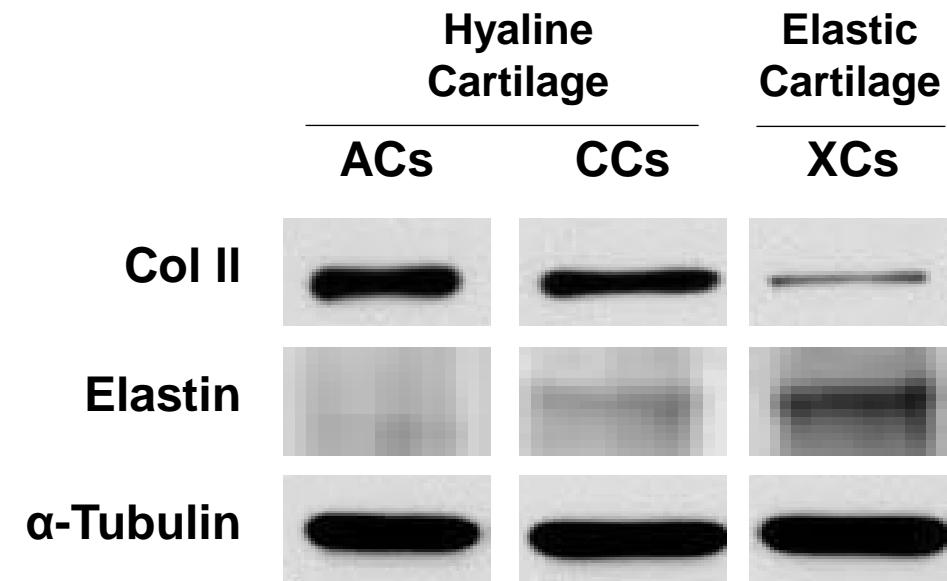


A. The human rib cage. B. Lateral border of sternum

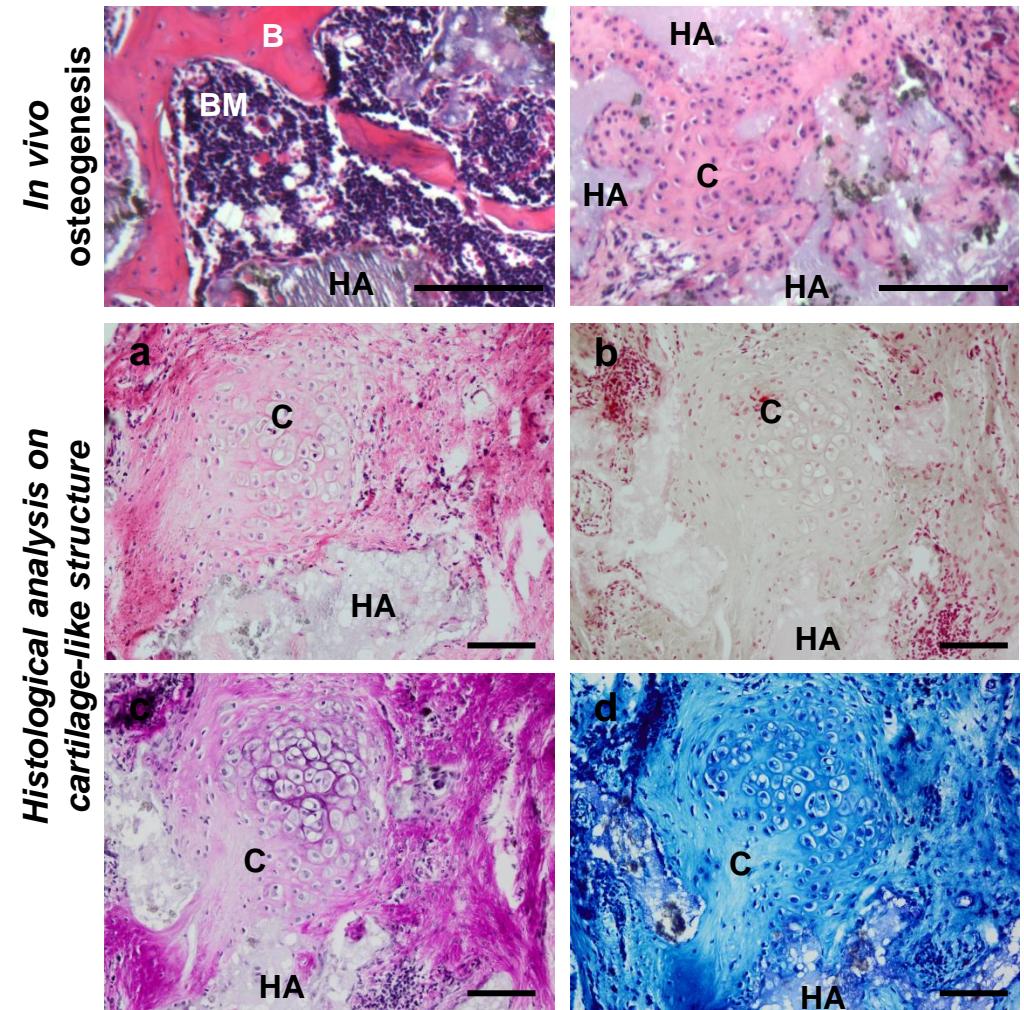
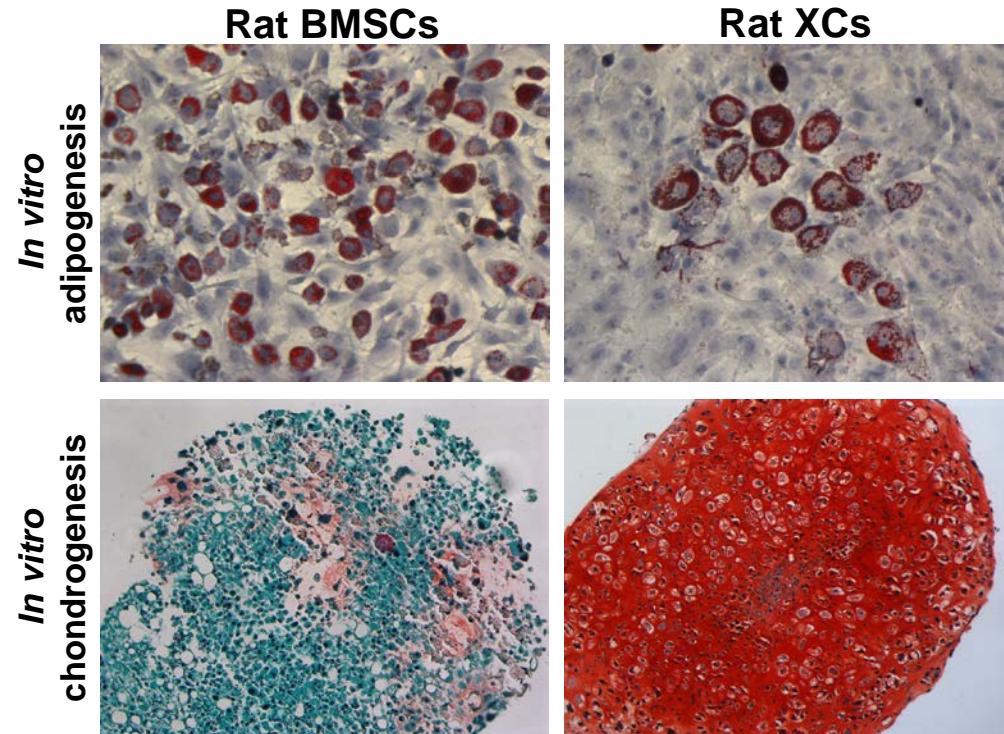
(Figures from Gray's Anatomy : The Anatomical Basis of Medicine and Surgery (British Edition. 38th Ed) (Hardcover))



ECM expression - Chondrocytes from Hyaline vs Elastic Cartilage

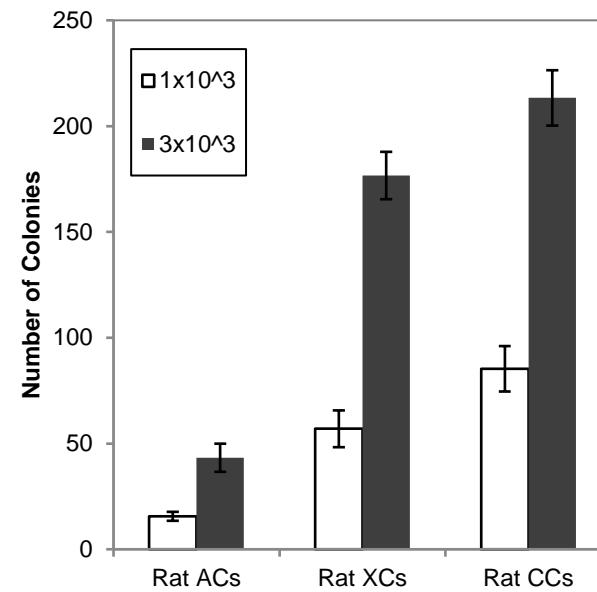


Differentiation - BMSCs vs Elastic Chondrocytes

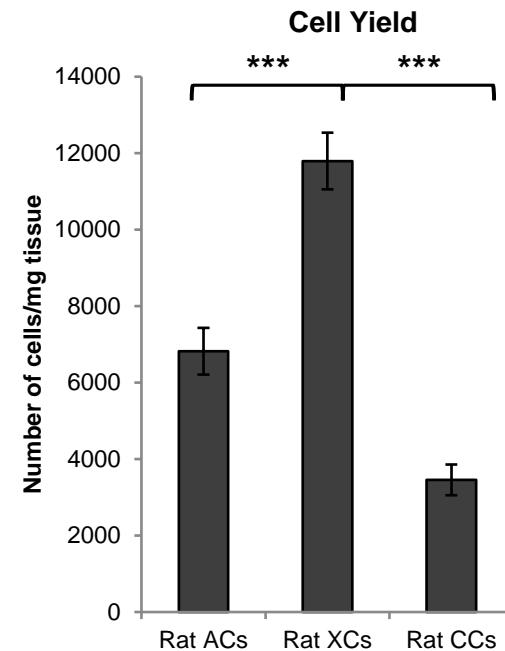


In Vitro Expansion Capability

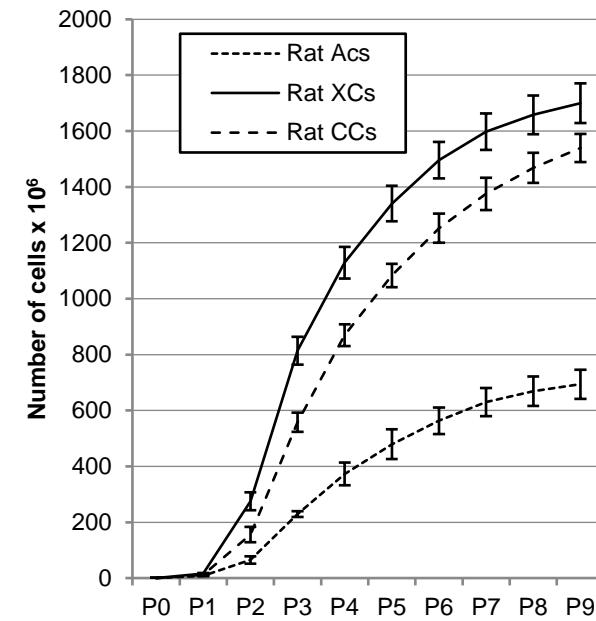
CFU-F assay



Cell Yield

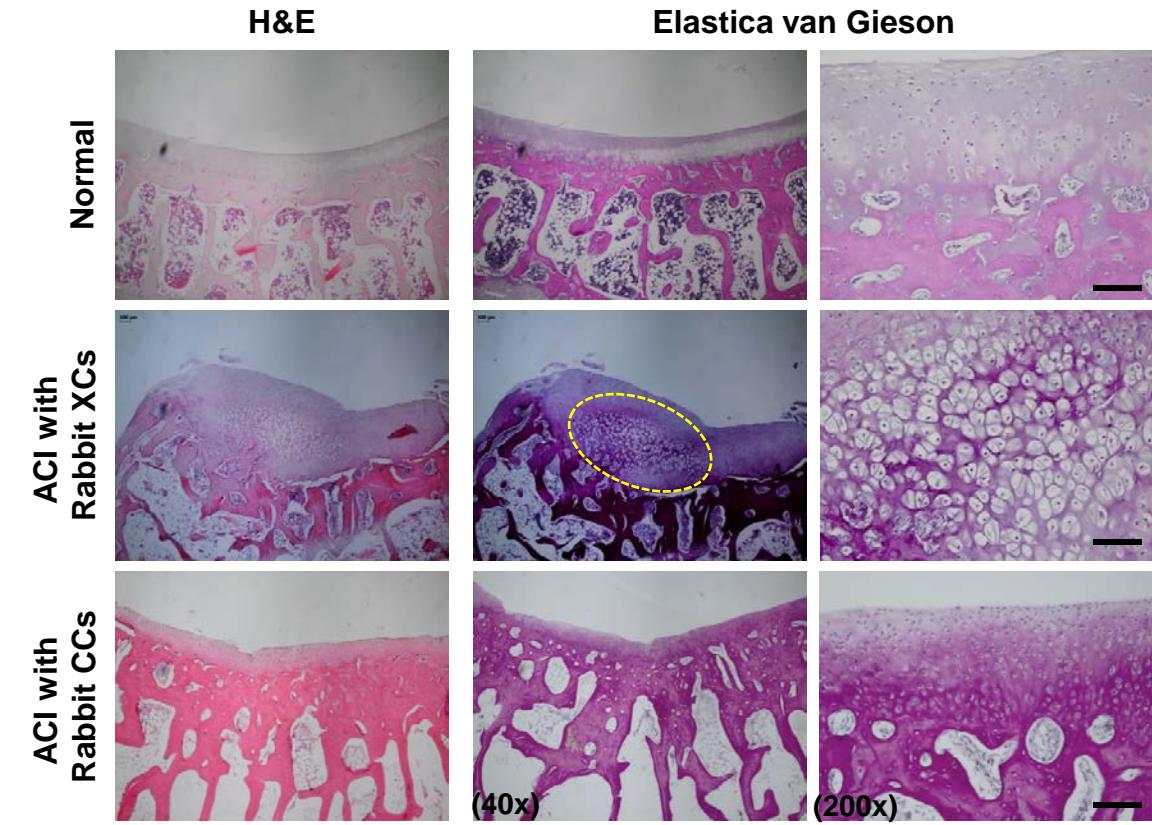
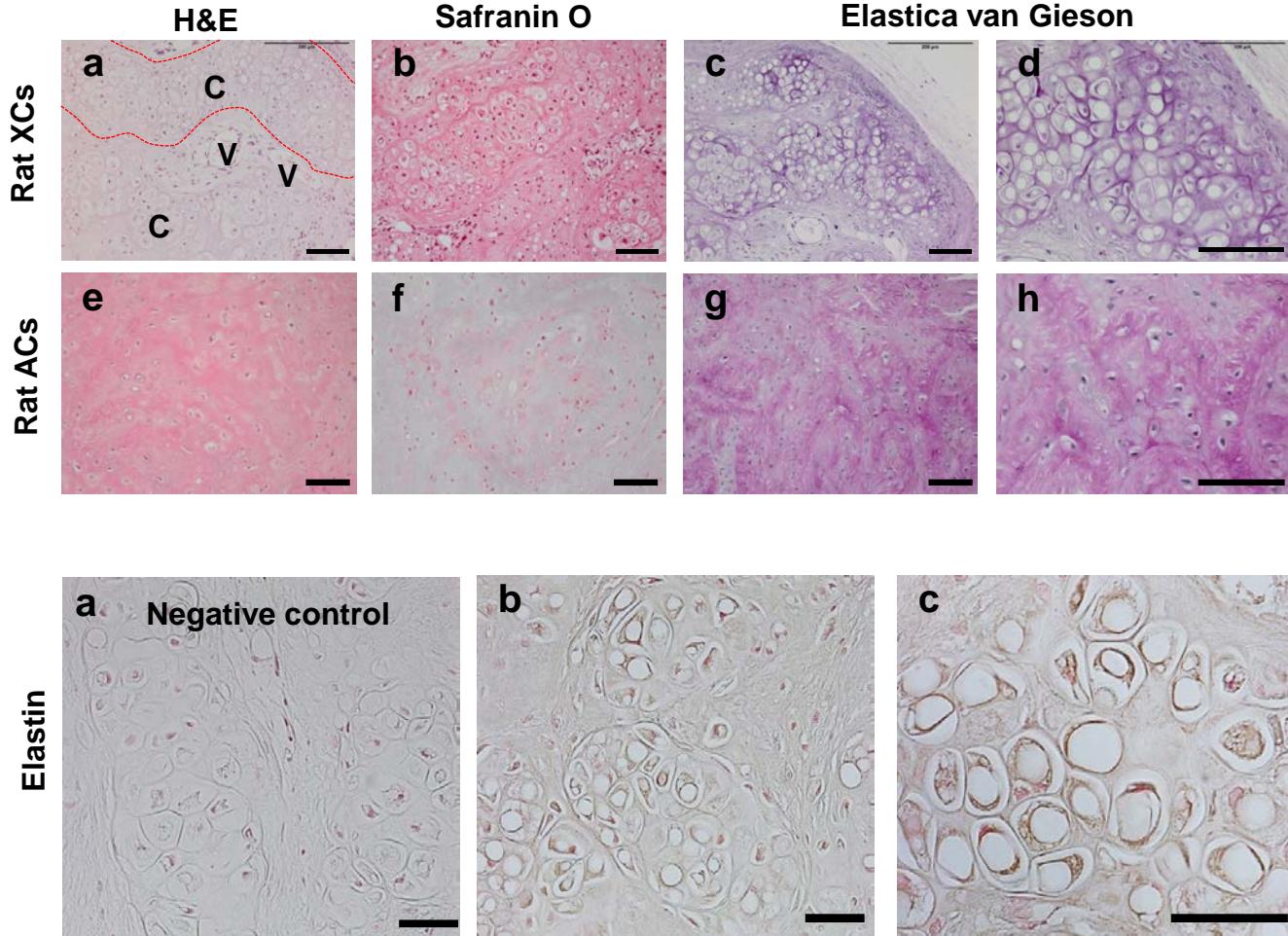


In vitro Cell Expansion



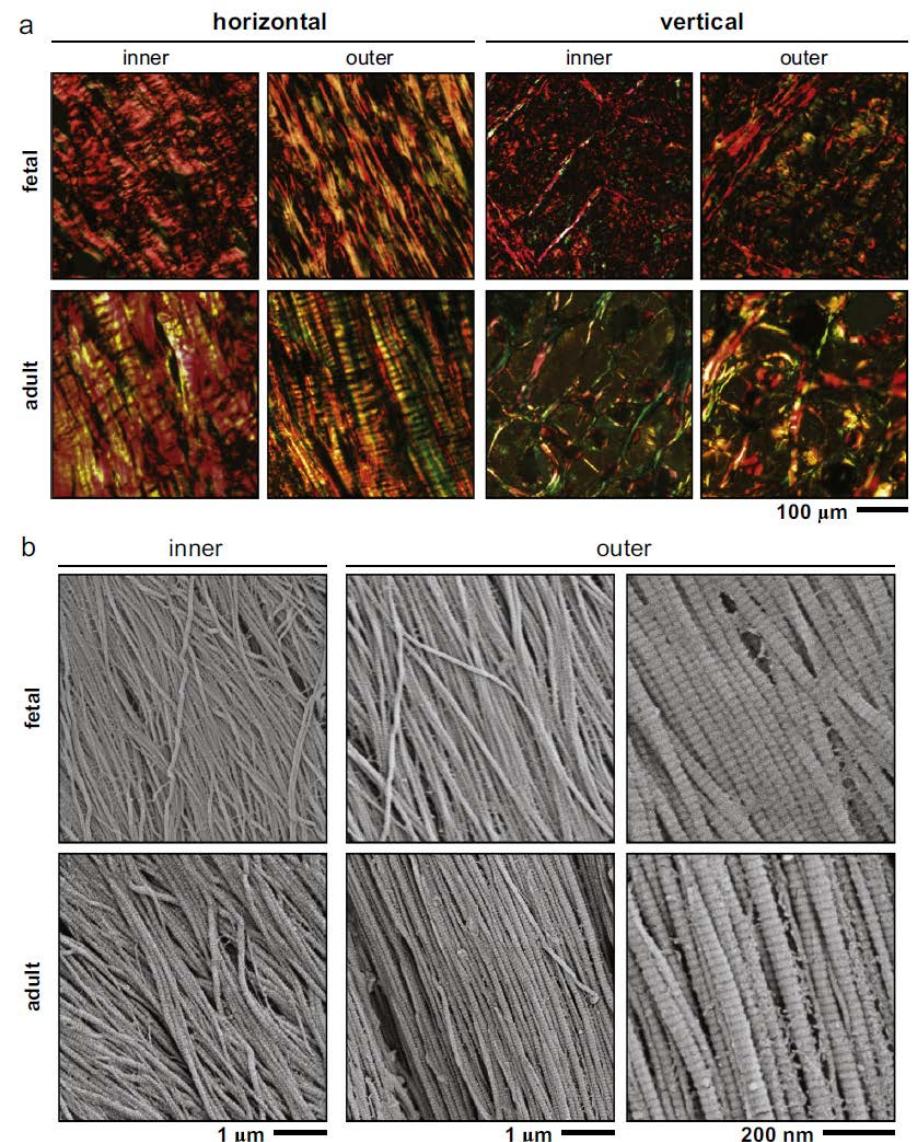
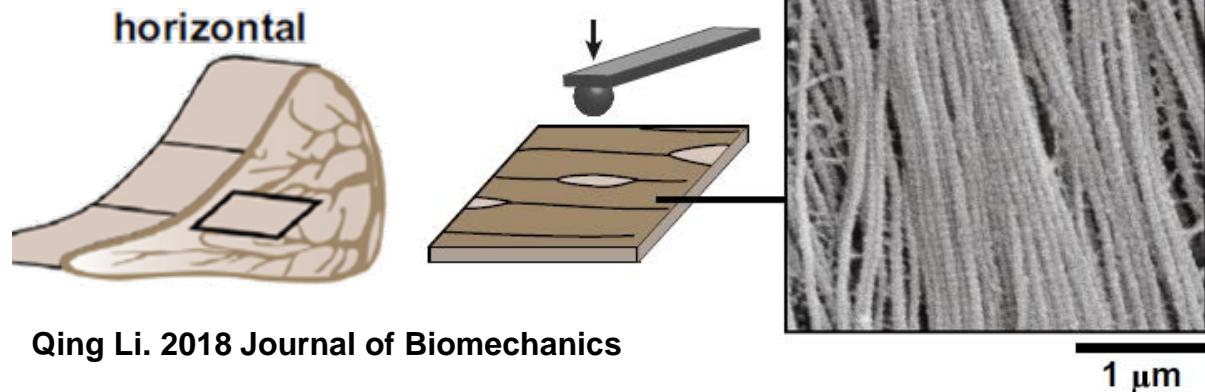
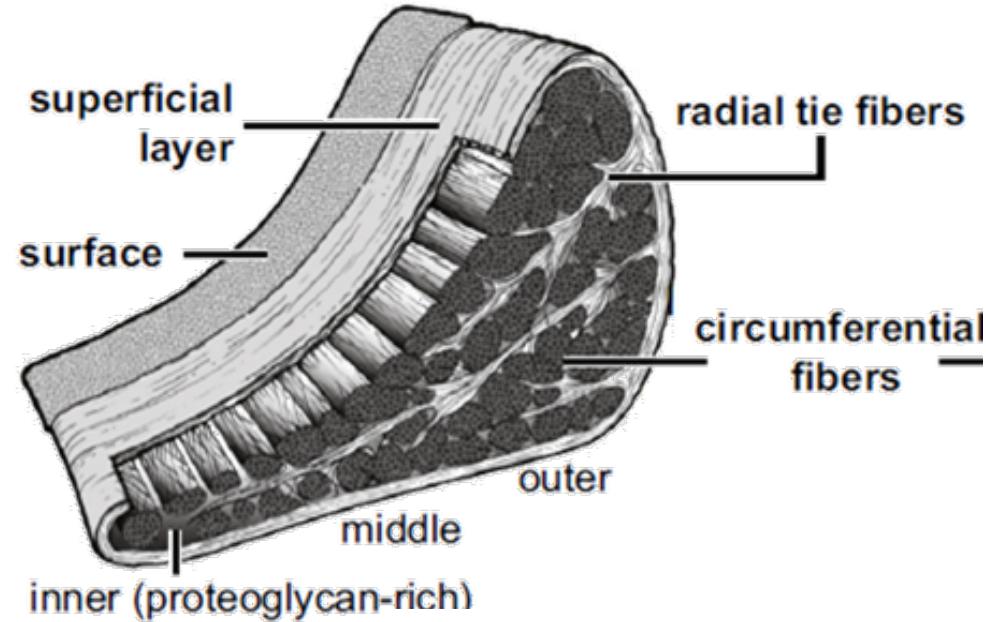
Nam S. et al., SCTM 2014

Reconstruction of Elastic Cartilage Tissue

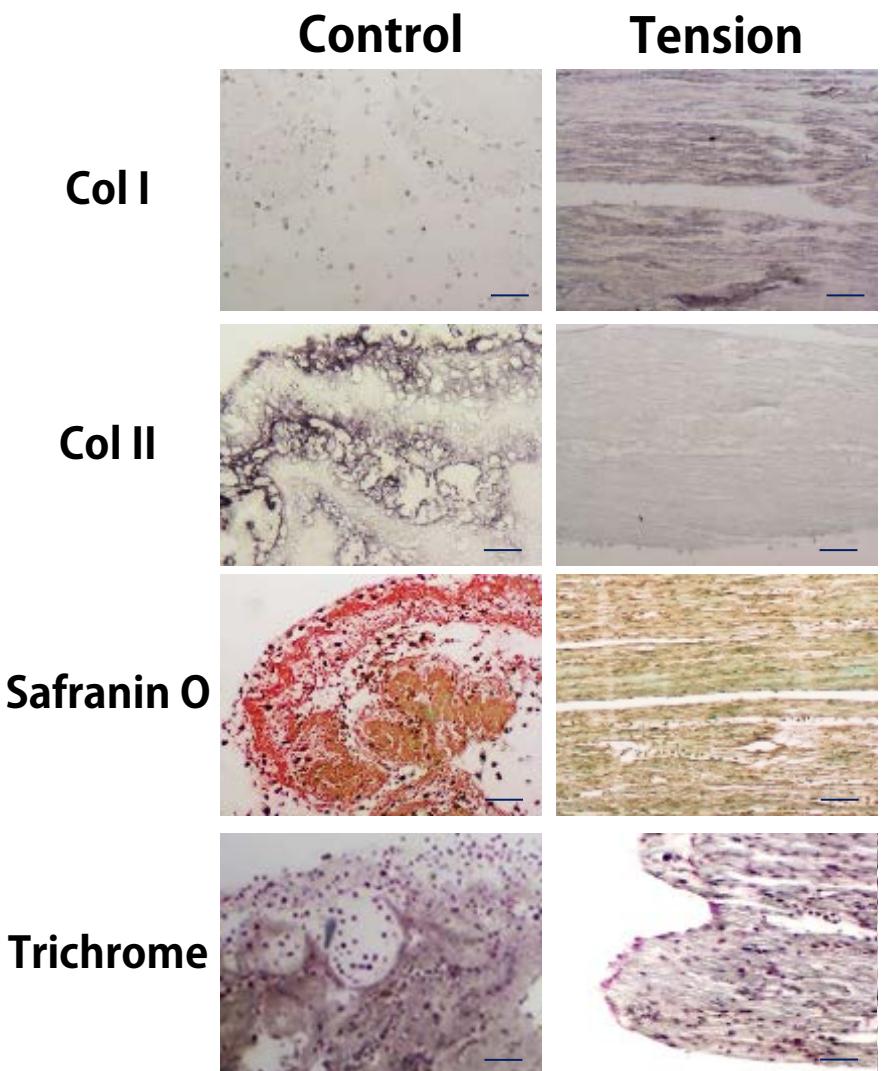
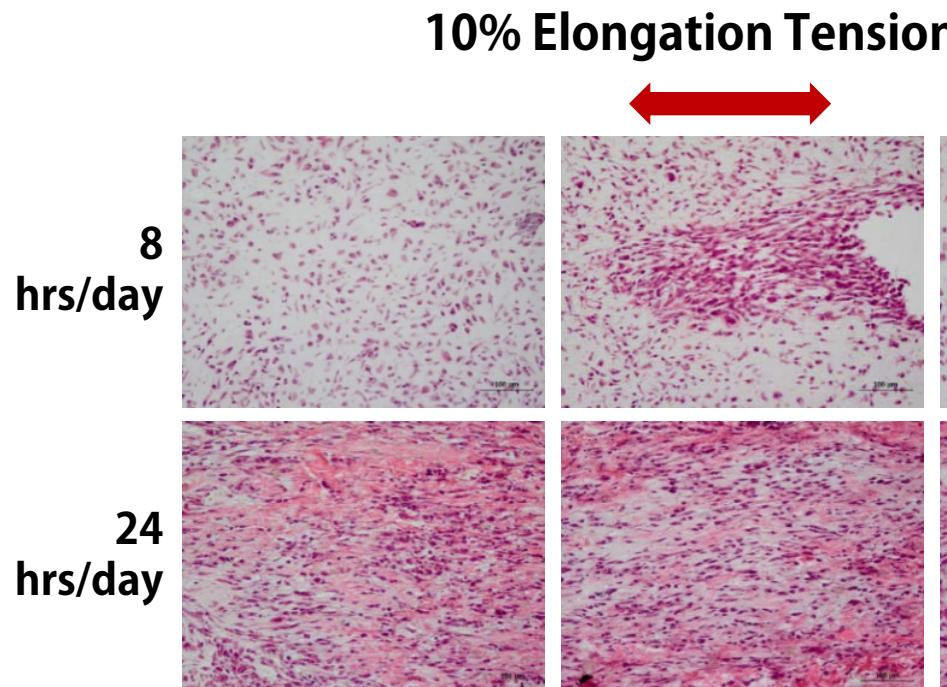


Fibrocartilage

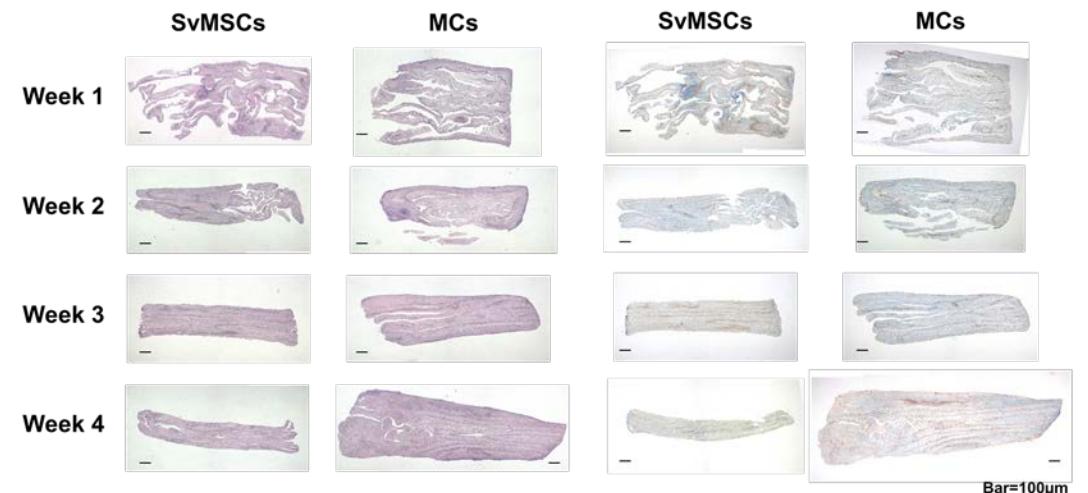
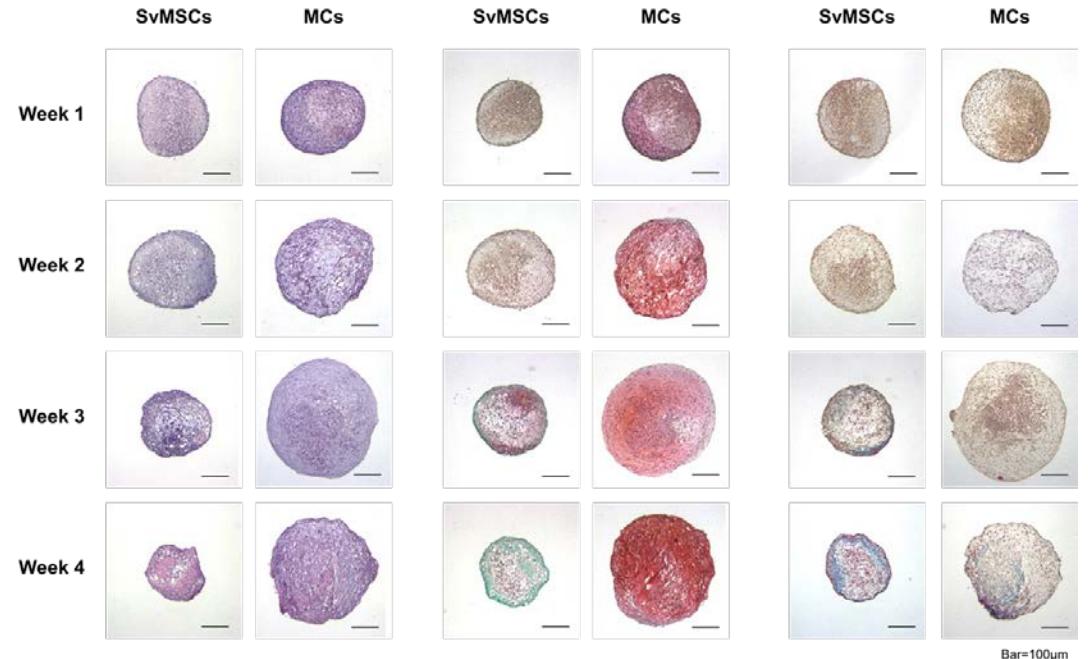
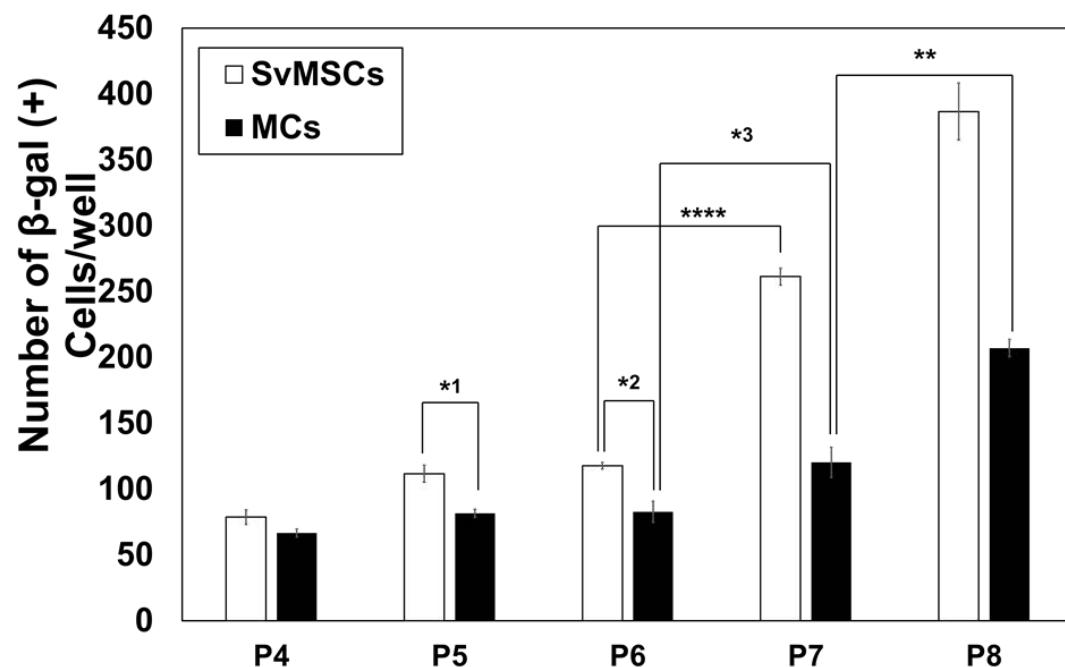
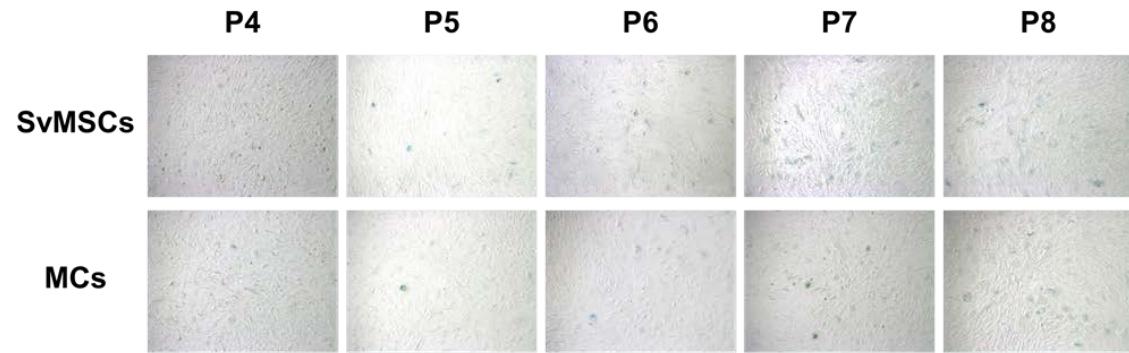
Microstructure of Meniscus - Fibrocartilage

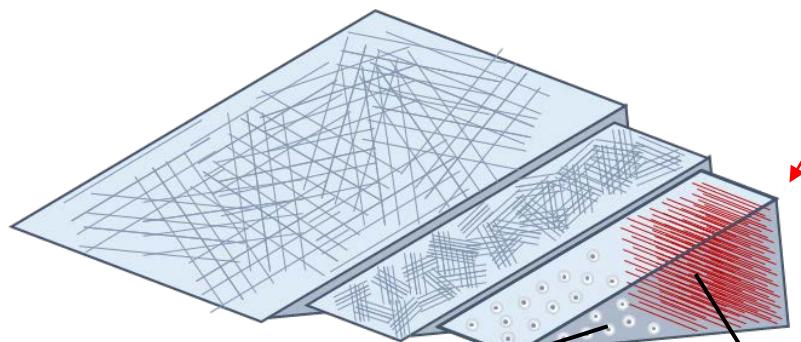
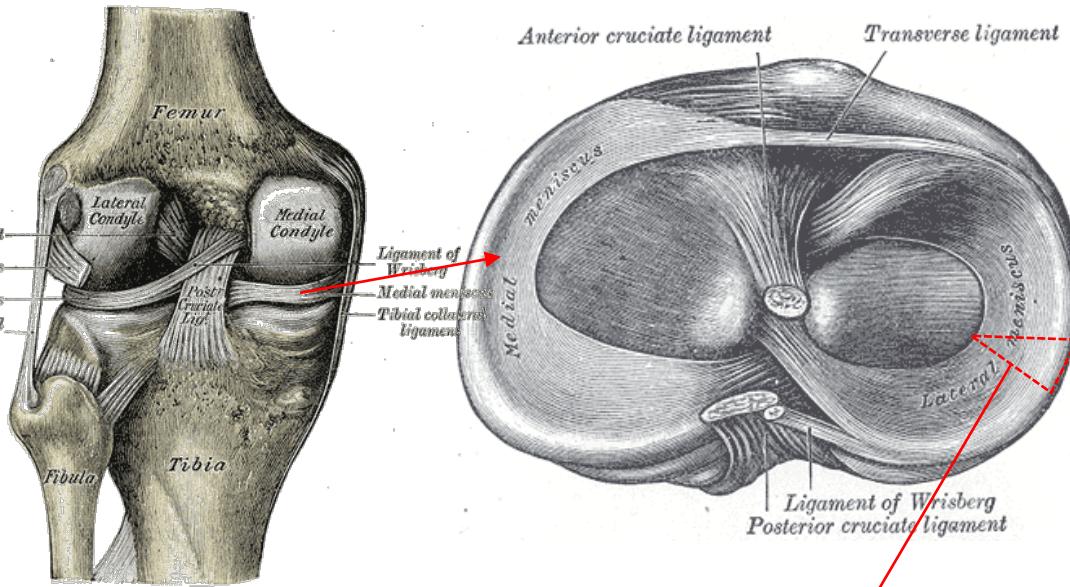


Tensile Stimulation during In Vitro Chondrogenesis



Meniscal Chondrocytes vs Synovial MSCs





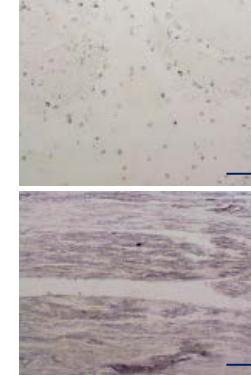
Inner part
– Load bearing strength
(**Hyaline cartilage**)

Outer part
– Resistant to tensile strain
(**Fibro-cartilage**)

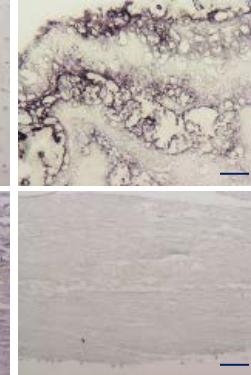
No Tension

Tension

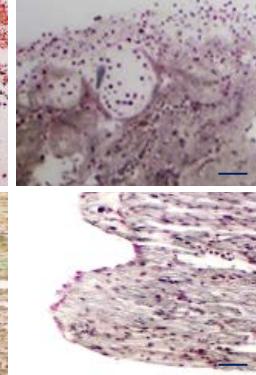
Col I



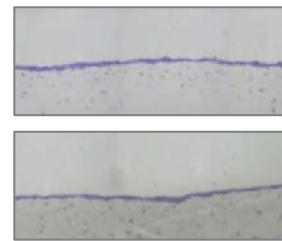
Col II



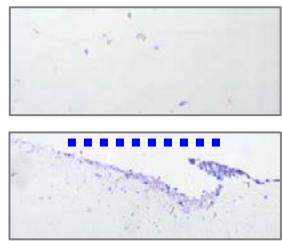
Safranin O Trichrome



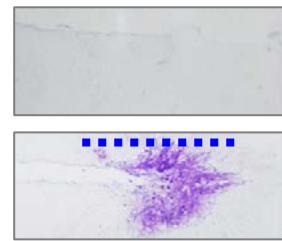
0 wk



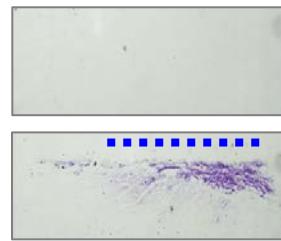
1 wk



2 wk



3 wk



Factor 1

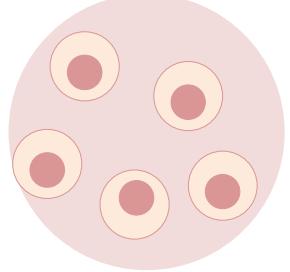
Factor 1 + Factor 2

.....

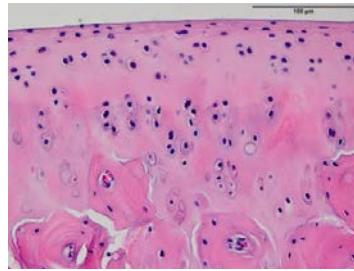
Position of 2nd stimulus

Construction of 3 Different Cartilage Types

Hyaline Cartilage



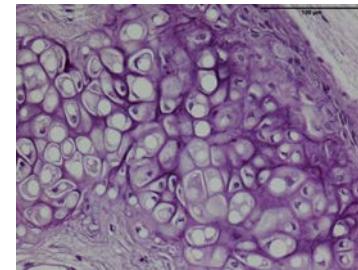
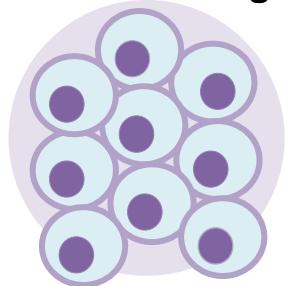
Natural Tissue



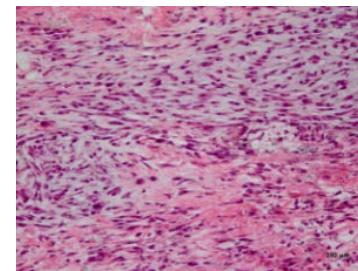
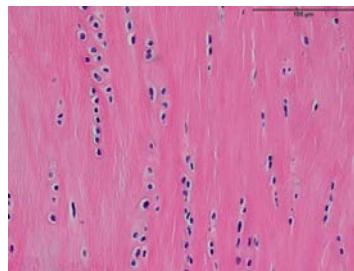
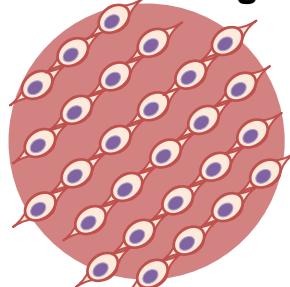
Engineered Tissue



Elastic Cartilage



Fibro-Cartilage





Acknowledgement

Hyaline Cartilage

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Elastic Cartilage

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Seungwoo Nam **Life Science Kyung Hee Univ.**

Materialization of Tissue Unit Structures

Prof. Tong In Oh **BME Kyung Hee Univ.**

Hyunji Cho **BT Kyung Hee Univ.**

Seoyoung Jang **BME Kyung Hee Univ.**

Jungsun Lee Ph.D. DVM, CEO Biosolution Inc.



Fibrocartilage

Prof. Tong In Oh **BME Kyung Hee Univ.**

Seoyoung Jang **BME Kyung Hee Univ.**

JunPyo Kwon **BME Kyung Hee Univ.**

Stem Cell Biology BioMedical Engineering Electronic Engineering Biomaterials

Grants



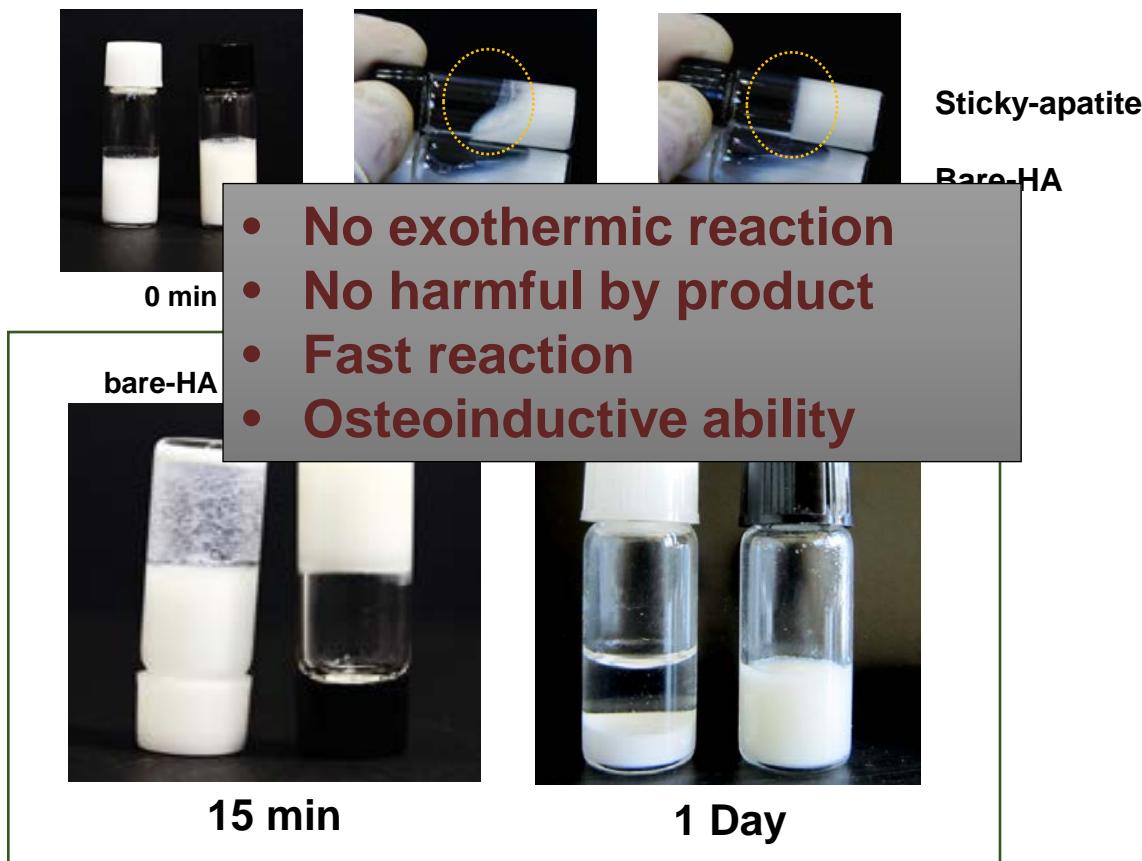
R&D Re-Discovery



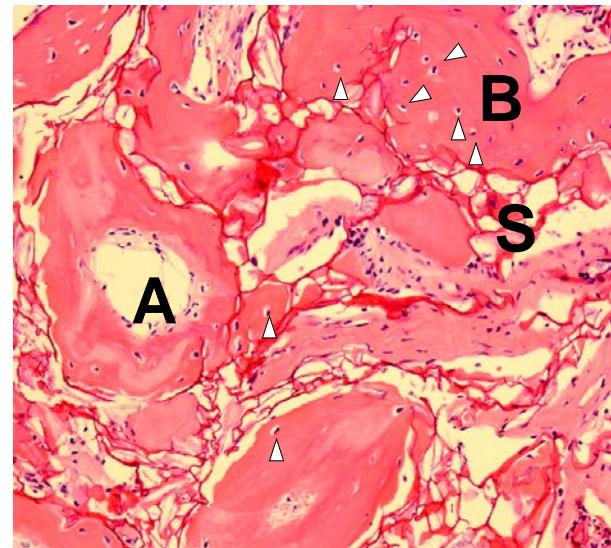
**Thank you
for your attention!**

Core Technology (II)

Reaction between Sticky-HA and organic polymer



Ectopic bone marrow
(rBMSCs, 18 weeks upon transplant)

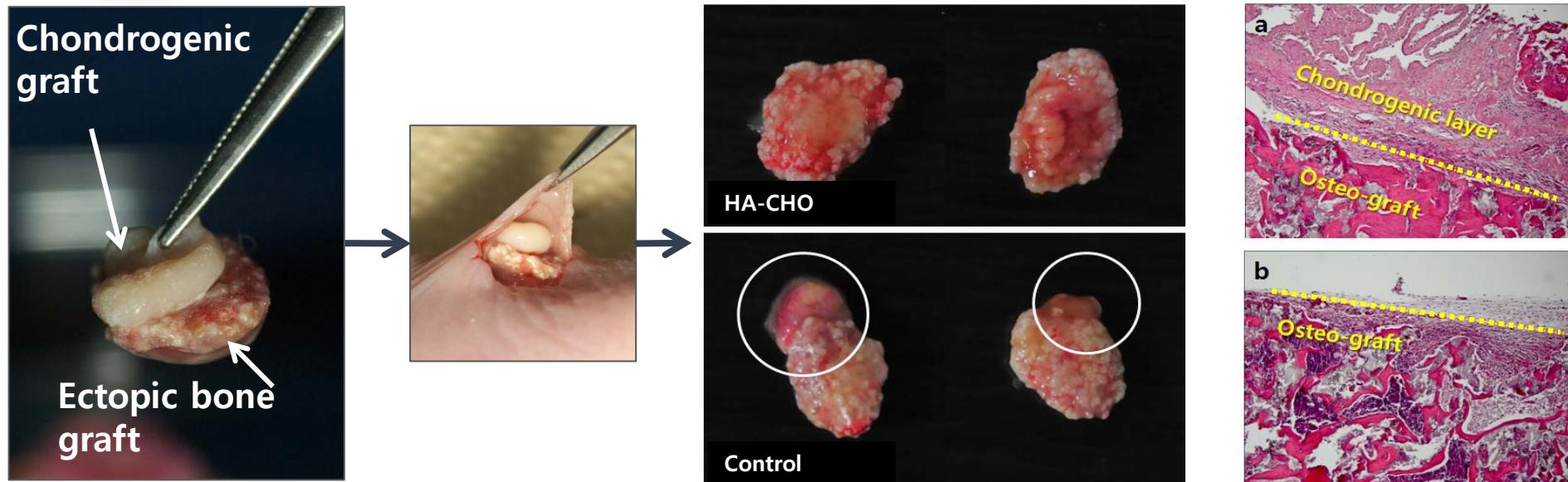
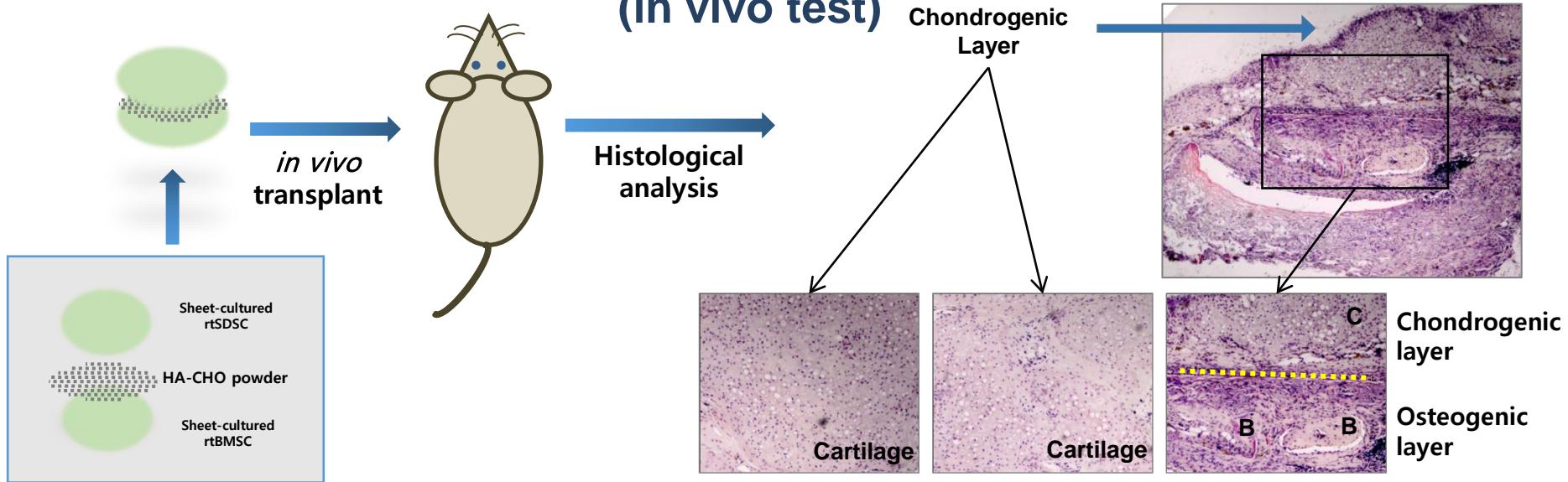


E Lee et al., unpublished data

PCT Application 2011 Bio-adhesive agent comprising surface modified hydroxyapatite and use thereof

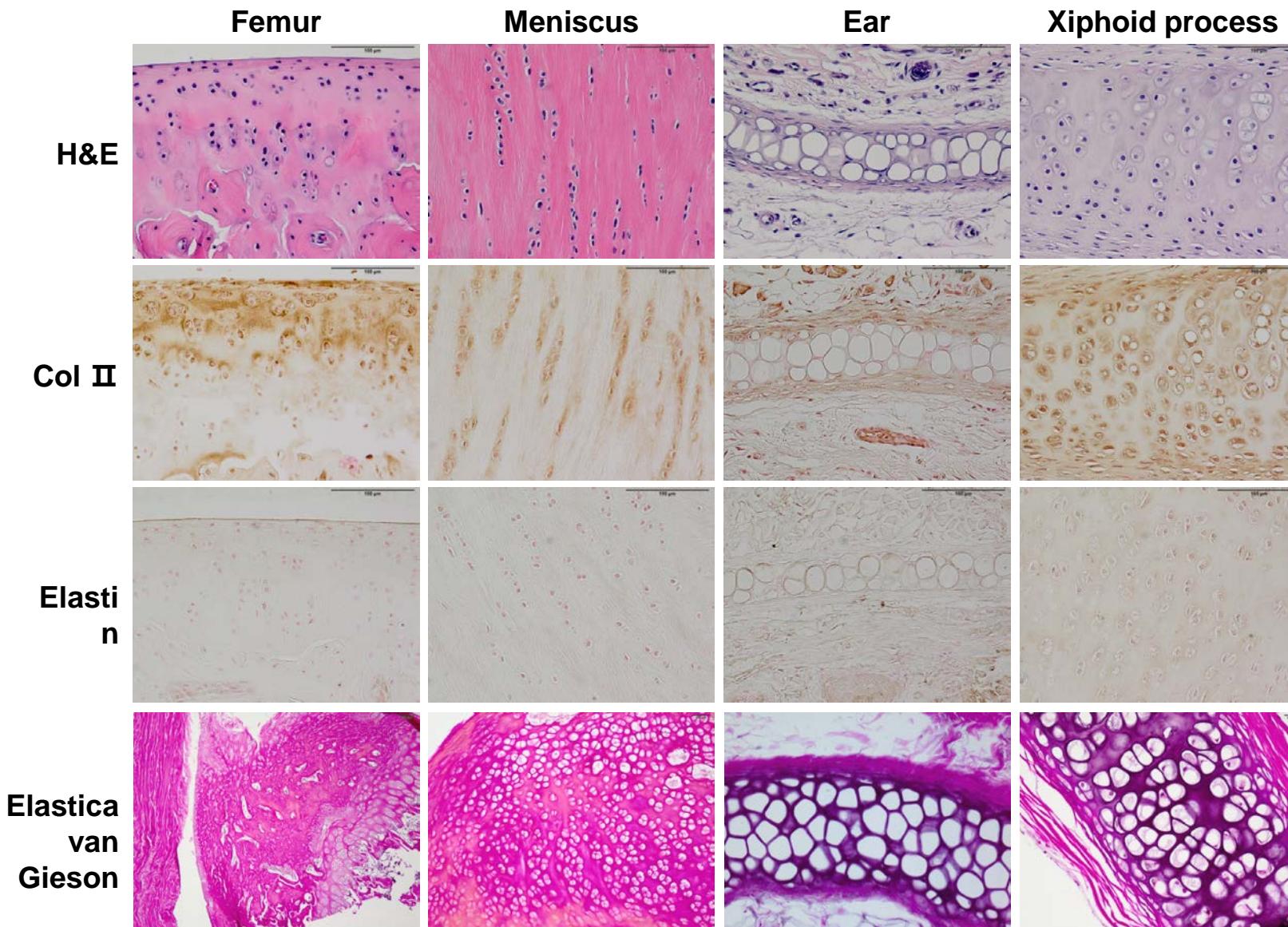
대한민국 특허 출원번호(2010) : 10-2010-0114232. “표면개질된 하이드록시아파타이트를 함유하는 생체접착제 및 그의 용도”

Bone-Cartilage Interfacing (in vivo test)



[Supplement Figure 3]

Immunohistochemical analysis of Col II & Elastin expression in various types of cartilage.



(400x)

EunAh Lee - Tissue Engineer

Tissue Engineering = Biomaterials + Stem Cells + α

Biomaterials

Surface modification of biomaterials by nanotechnology

- Center for Microcrystal Assembly (CRI), Sogang Univ.

Post-natal stem cells

Skeletal stem cells

- NIDCR/CSDB, NIH (US)
- Musculoskeletal Bioorgan Center, KHU

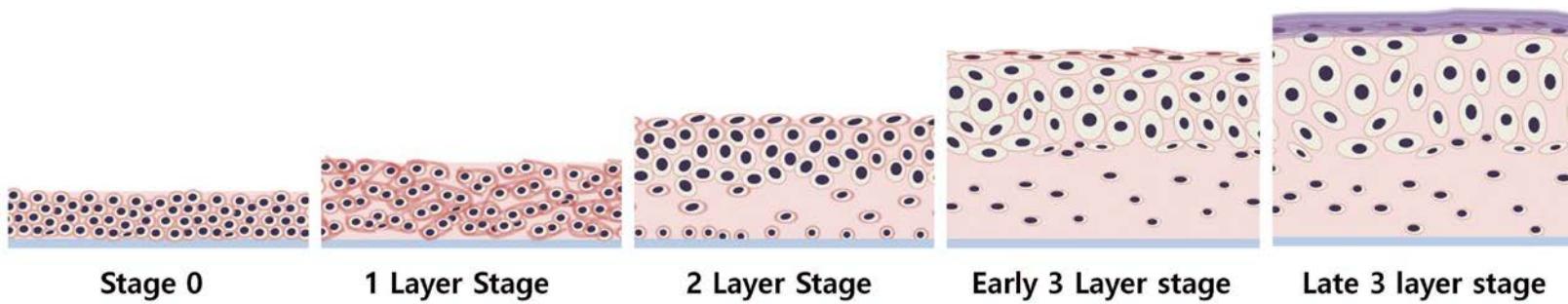
• Alpha

Tissue integration and non-destructive monitoring

Non-epigenetic modulation of stem cell activity - Cell surface modification

Deep learning for cell therapy scale-up manufacturing process

- IIRC, KHU



Supplemental Figure 2. Schematic drawing of *in vitro* chondrogenesis.

Supplemental Table 1. Characteristics of each stage in construction of disc-type cartilage *in vitro*

| | Stage 0 | 1 Layer Stage | 2 Layer Stage | Early 3 Layer stage | Late 3 layer stage⁴⁾ |
|--|-----------------------------------|---------------------------------|-------------------------------------|------------------------------|--|
| Distinct feature | Cell condensation ^{2,3)} | Initiation of matrix production | Two layers of different cellularity | Superficial layer | Highly fibrous superficial layer |
| Matrix Characteristics | - | Fibrous | Hyaline/slightly fibrous | Hyaline | Hyaline/fibrous layer |
| Peri-cellular matrix | - | + | ++ | ++ | ++ |
| Inter-territorial matrix | - | - | + | +/- | +++ |
| Fibrous superficial layer | - | - | - | + | ++ |
| Cell size | Small, Homogeneous | Small, Homogeneous | Small + Medium, Heterogeneous | Small + Large, Heterogeneous | Small + Large, Heterogeneous |
| Anisotropy in vertical cell distribution | - | - | + | ++ | +++ |
| Cell proliferation ³⁾ | +++ | ++ | + | +/- | +/- |

Characteristics shown by three distinct type of chondrocytes

| | 1 week | 2 weeks | 3 weeks | 4 weeks | Overall Characteristics |
|--------------------------|-----------------|----------------|--------------------------|---------------------------|---|
| Hyaline Cartilage | 1~2 Layer stage | 2 Layer stage | 2 Layers + Fibrous layer | 2 Layers + Fibrous layer | <ul style="list-style-type: none">Thickness gradually increased |
| Elastic Cartilage | Stage 0 | 1 Layer Stage | Thick 1 Layer Stage | 1 Layer + Granular Tissue | <ul style="list-style-type: none">Tissue formation starts at around 3 weeksAlmost no GAGs accumulation |
| Fibro Cartilage | 1~2 Layer | Thick 2 Layers | Early 3 Layer Stage | Thick 3 Layer Stage | <ul style="list-style-type: none">Upper layer showing hyper-cellularity and acellular low layer |

- No elastic fiber was shown in any type of chondrocytes
- No type II collagen expression was shown in elastic cartilage
- Low expression of fibrous collagen
- High expression of glycosaminoglycans (GAGs)