Tenogenic Differentiation of Mesenchymal Stem Cells and Their Applications in Tendon Tissue Engineering

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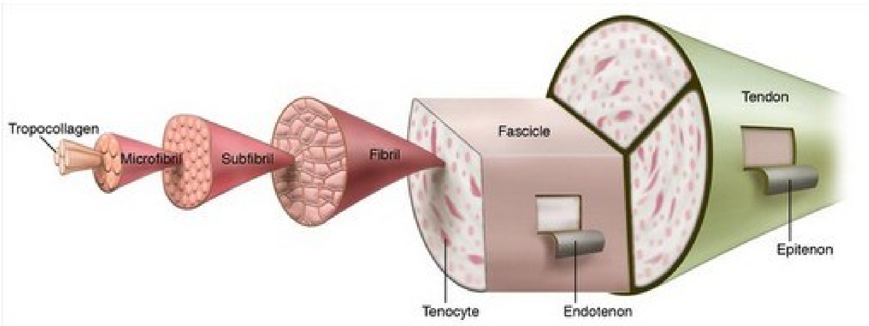
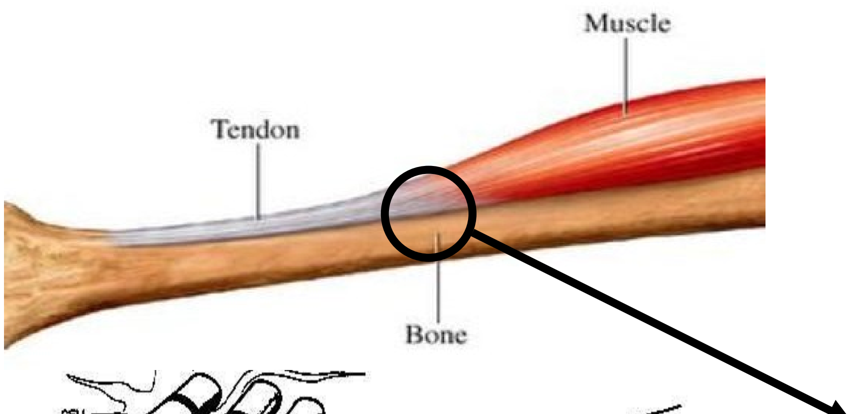
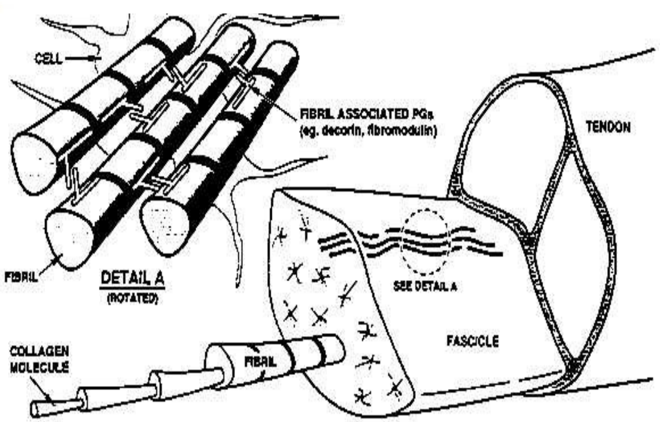
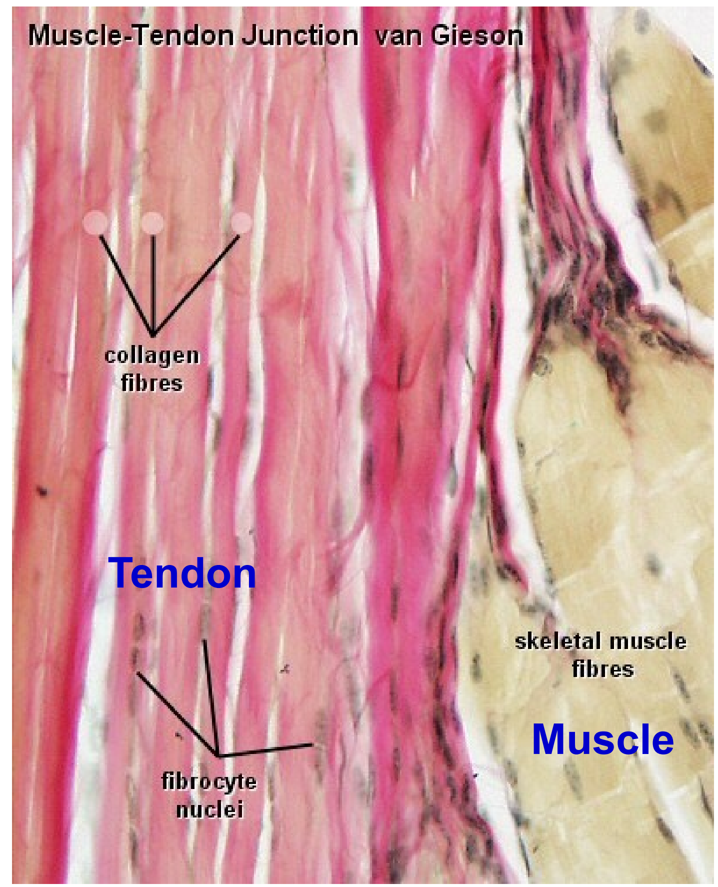
Anatomy of Tendon and Muscle Tendon Junction





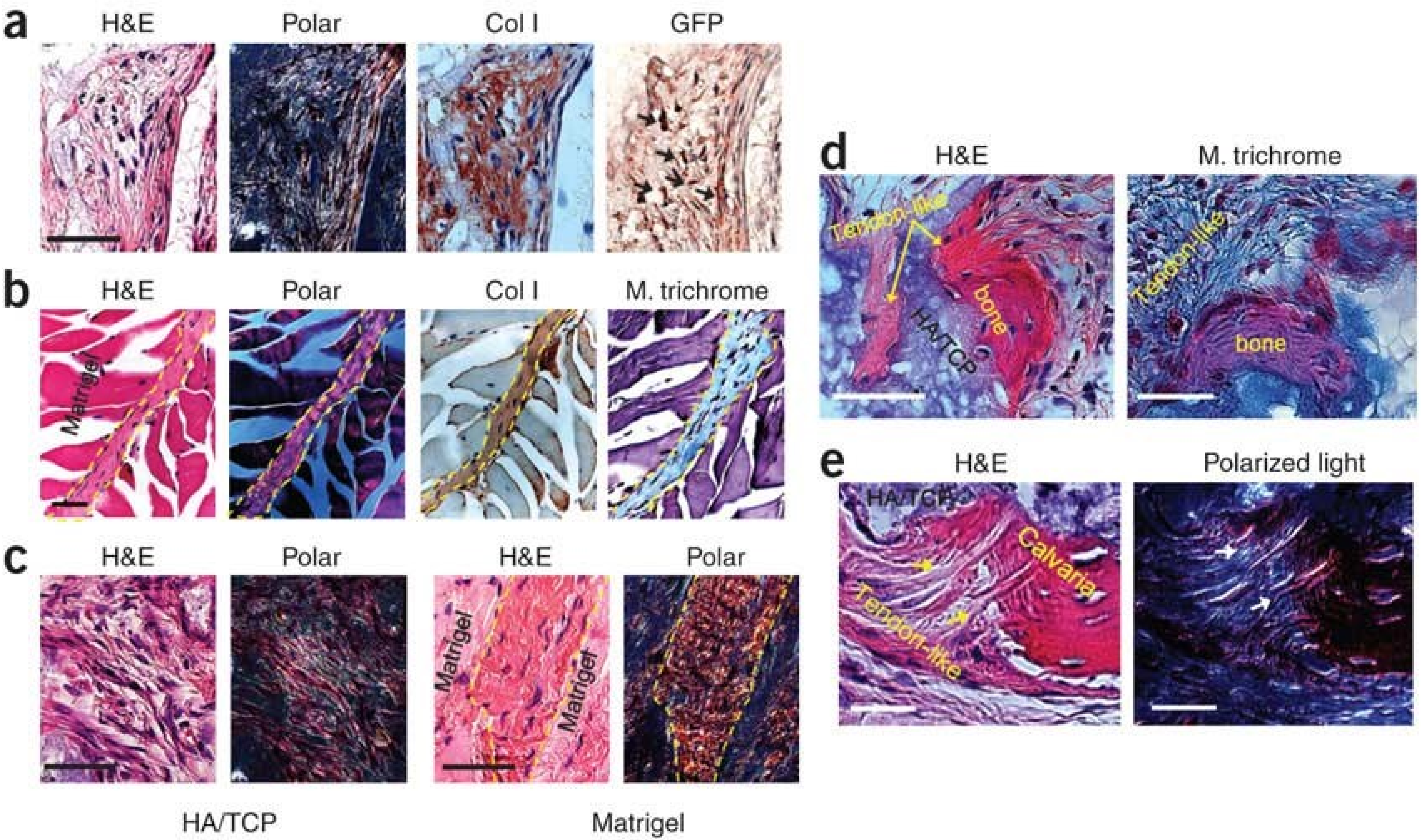




Tendon has unique composition

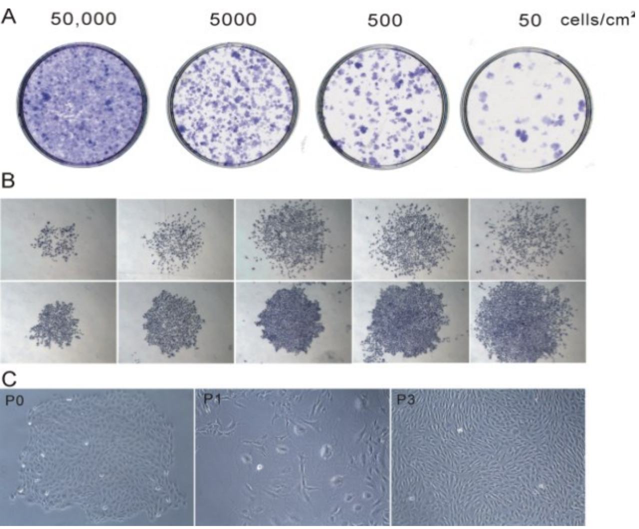
Composition	Ligament	Tendon	Bone
Water (w/w)	60%	55%	9%
Mineral (w/w)	0	0	69%
Collagen (% dry weight)	75% (90% type I; 10% type III)	85% (>90% type I, <10% types III, V. etc.)	20% (80% type 1 and 20% others)
Fribroblasts (% volume)	20%	15%	10%
Extra-cellular matrix (% volume)	80%	85% (predominant proteoglycan is decorin)	91% (mainly calcium and phosphate)
Collagen fibrils diameter (nm)	40-75	60-175	70-100

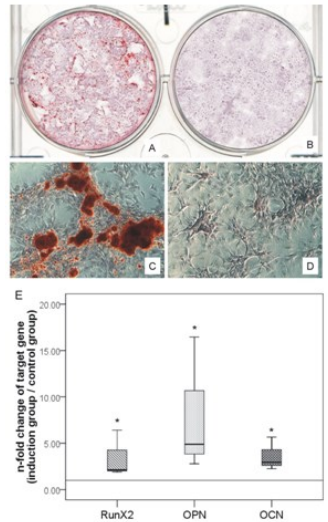
Normal tendon contains multi-potent stem cells

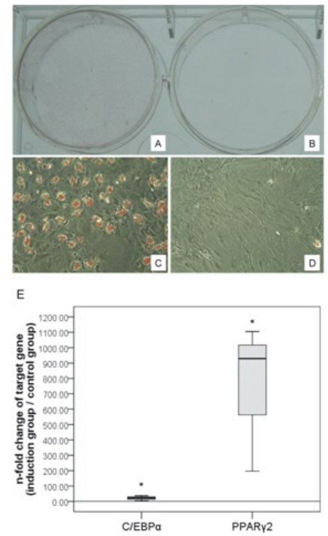


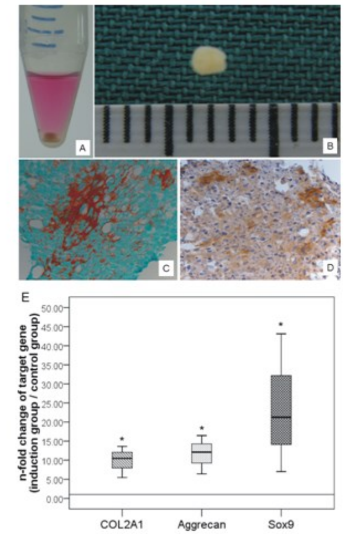
Bi, et al. Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche. Nat Med. 2007 Oct;13(10):1219-27.

Tendon-derived Stem Cells (TDSCs) in Normal Rat Tendon Tissue









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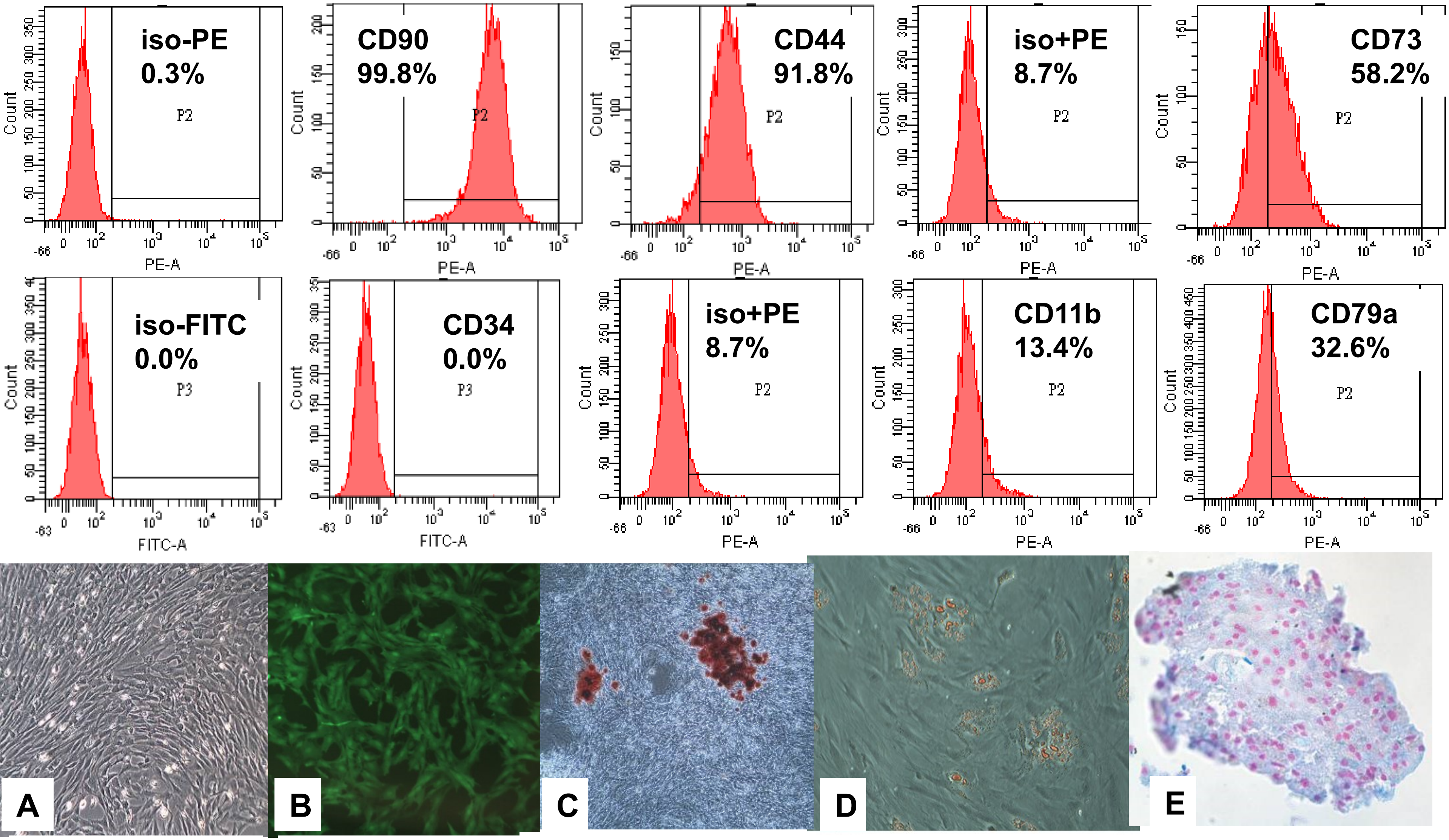
Original Article

Isolation and Characterization of Multipotent Rat Tendon-Derived Stem Cells

Yun-Feng Rui, M.Phil.,[1,2] Pauline Po Yee Lui, Ph.D.,[1,2] Gang Li, Ph.D.,[1,2] Sai Chuen Fu, M.Phil.,[1,2] Yuk Wa Lee, M.Phil.,[1,2] and Kai Ming Chan, M.D.[1,2]

Isolation and Characterization of TDSCs



GFP-Rat BMSCs

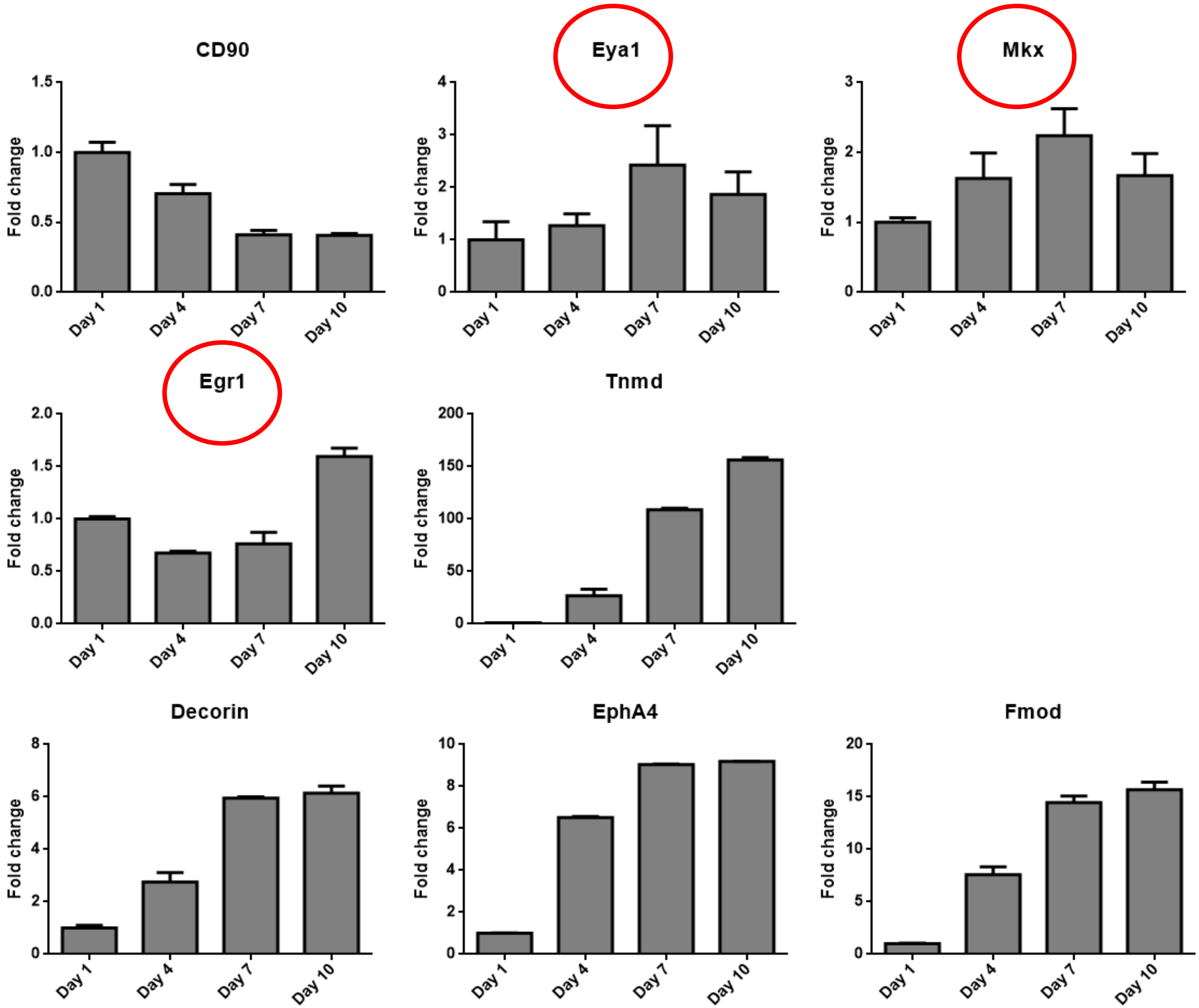
Fluorescent Image

Calcium Nodule Staining
---- Osteogenisis

Oil O Red Staining
----Adipogenisis

Alcian Blue Staining
----Chondrogenisis

TDSCs have the potential of spontaneous tenogenic differentiation



Tenogenic Differentiation Markers

Cell Tissue Res (2014) 356:287–298
DOI 10.1007/s00441-014-1834-8

REVIEW

Crucial transcription factors in tendon development and differentiation: their potential for tendon regeneration

Huanhuan Liu · Shouan Zhu · Can Zhang · Ping Lu · Jiajie Hu · Zi Yin · Yue Ma · Xiao Chen · Hongwei OuYang

—Original Article—

Regulation of Tenomodulin Expression Via Wnt/β-catenin Signaling in Equine Bone Marrow-derived Mesenchymal Stem Cells

Shihori MIYABARA[1], Yohei YUDA[1], Yoshinori KASASHIMA[2], Atsutoshi KUWANO[2] and Katsuhiko ARAI[1*]
[1] Department of Tissue Physiology, Tokyo University of Agriculture and Technology, Tokyo 183-8509, Japan

Table 2. Comparison of mRNA level between the tenodon and monolayer BMSC by qRT-PCR analysis

Gene	Tenodon	Monolayer BMSC
Tenomodulin	0.00057 ± 0.00012 *	0.00006 ± 0.00001
Col1a2	0.11810 ± 0.03612	0.15139 ± 0.02522
Col3a1	0.00880 ± 0.00251	0.13373 ± 0.02121
Col12a1	0.07856 ± 0.01431	0.24827 ± 0.03142
Col14a1	0.01458 ± 0.00373 *	0.00003 ± 0.00001
Decorin	29.65080 ± 2.85643 *	0.70031 ± 0.14381
Fibromodulin	0.11311 ± 0.02413 *	0.00599 ± 0.00143
Lumican	1.16473 ± 0.28143	0.94606 ± 0.14877
Tenascin-C	0.01858 ± 0.00143	0.01010 ± 0.00131

- ■Scleraxis (Scx)
- ✓Mohawk (Mkx)
- ✓Egr1 and Egr2
- ■Sox9
- ■Six1/2
- ■Eya1/2

- ■Tenomodulin (Tnd)
- ■Decorin
- ■Fibromodulin
- ■Col4a1

Tenogenic Markers (Tenocytes Vs. TDSCs)

	A Tendon	A TDSC	P Tendon	P TDSC
Scx	1	1.52	1	0.11
pMkx	1	0.17	1	0.05
Egr1	1	0.12	1	0.53
Egr2	1	0.98	1	1.69
Eya1	1	0.00011	1	***
TNMD	1	0.002	1	0.01
Col1a1	1	0.56	1	0.60
Col3a1	1	5.08	1	4.81
Fomd	1	0.07	1	0.02
Decorin	1	0.00	1	0.01
Lox	1	4.62	1	9.54
EphA4	1	0.95	1	1.78
TenC	1	2.19	1	1.03
Six	1	0.92	1	6.73
CD90	1	34.42	1	276.00
CD73	1	1.36	1	4.11
NS	1	1.66	1	1.58

miR-124 is involved in regulation of tenogenic differentiation by targeting tenogenic transcription factors

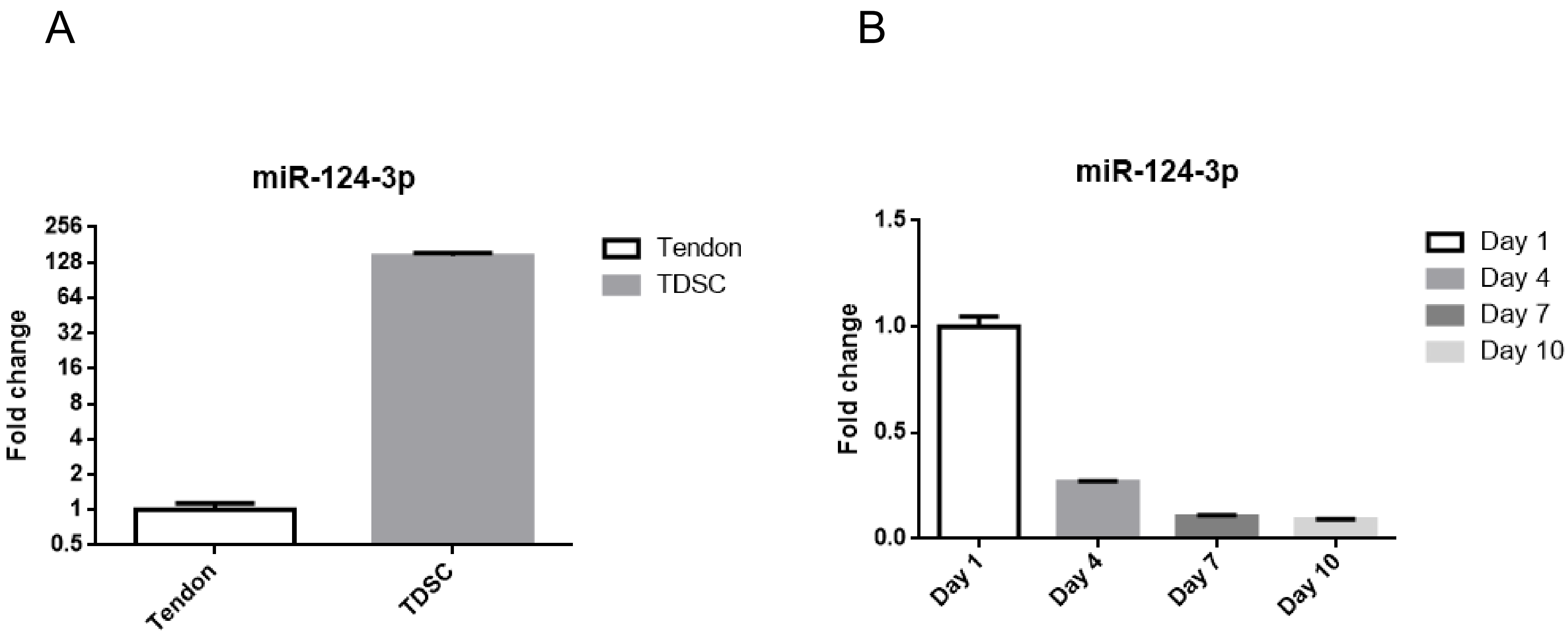
miR-124 →

- ✓Mkx
- ✓Egr1
- ✓Eya1

Bioinformatics tools prediction

<table>
<tr><th></th><th>predicted consequential pairing of target region (top) and miRNA (bottom)</th></tr>
<tr><td>Position 262-268 of MKX 3' UTR
rno-miR-124</td><td>5' ...UUGCCAUUACAGUAAGUGCCUUG...
|||| |||||||
3' CCGUAAGUGGCG-CACGGAAU</td></tr>
<tr><td>Position 774-780 of EGR1 3' UTR
rno-miR-124</td><td>5' ...AAGUUUCACGUCUUGGUGCCUUU...
||||| |||||||
3' CCGUAAGUGGCG---CACGGAAU</td></tr>
<tr><td>Position 843-849 of EYA1 3' UTR
rno-miR-124</td><td>5' ...GCACAAACUCCUGCAGUGCCUUA...
|||||||
3' CCGUAAGUGGCGCACGGAAU</td></tr>
</table>

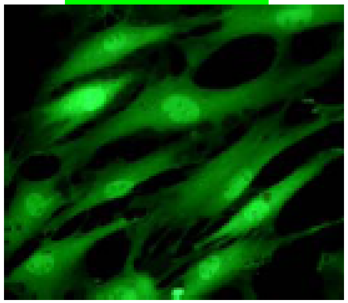
miR-124 is up-regulated in TDSCs and reduced duirng spontaneous tenogenic differentiation ofTDSCs



The mRNA expression level of miR-124-3p was much higher in TDSC compared with that in tendon (**A**), and down-regulated during the process of spontaneous tenogenic differentiation (**B**).

Tenogenic Differentiation of BM-MSCs

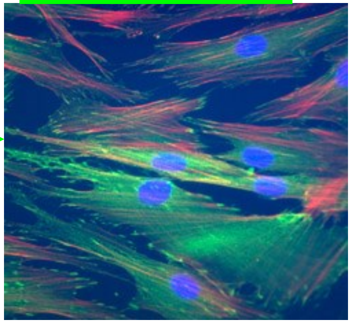
BMSCs

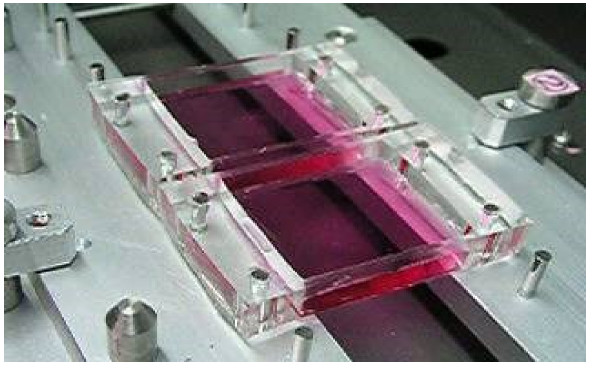


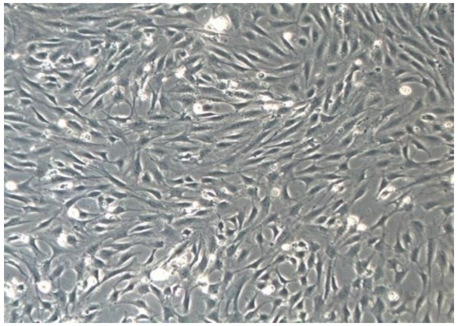
Tenogenic Differentiation

Growth factors; mechanical stimuli; and local environment.

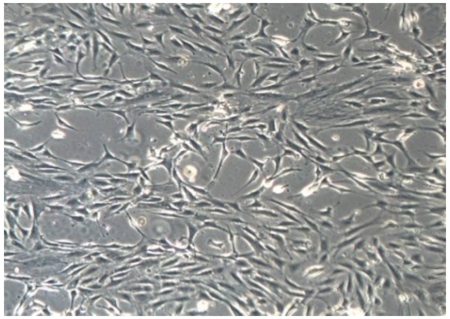
Tendon fibroblasts







No stretching



Stretch at 8% Strain,
30 circles / min, 4h

In vitro tensile loading to promote tendogenic differentiation (unpublished data)

The use of growth factors to control tenogenic differentiation of BM-MSCs

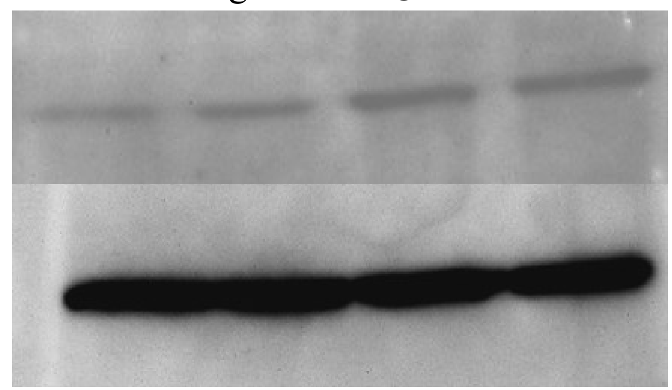
Tendon Related Markers:

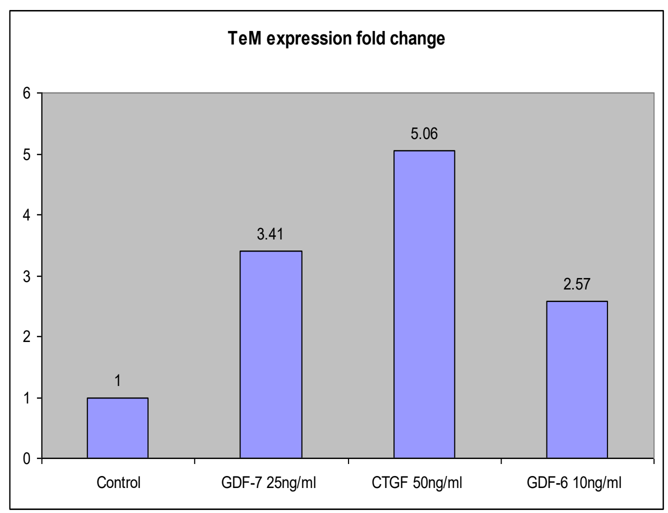
- Scleraxis (Scx)
- Tenomodulin (TeM)
- Tenascin C (TnC)
- Collagen Type I
- Decorin
- Biglycan
- Smad8
- Epha4

Control | GDF-7 25ng/ml | CTGF 50ng/ml | GDF-6 10ng/ml

TeM 37KD

b-actin 40KD





GDF-6 GDF-7 and CTGF can increase TeM expression

CTGF & Ascorbic Acid

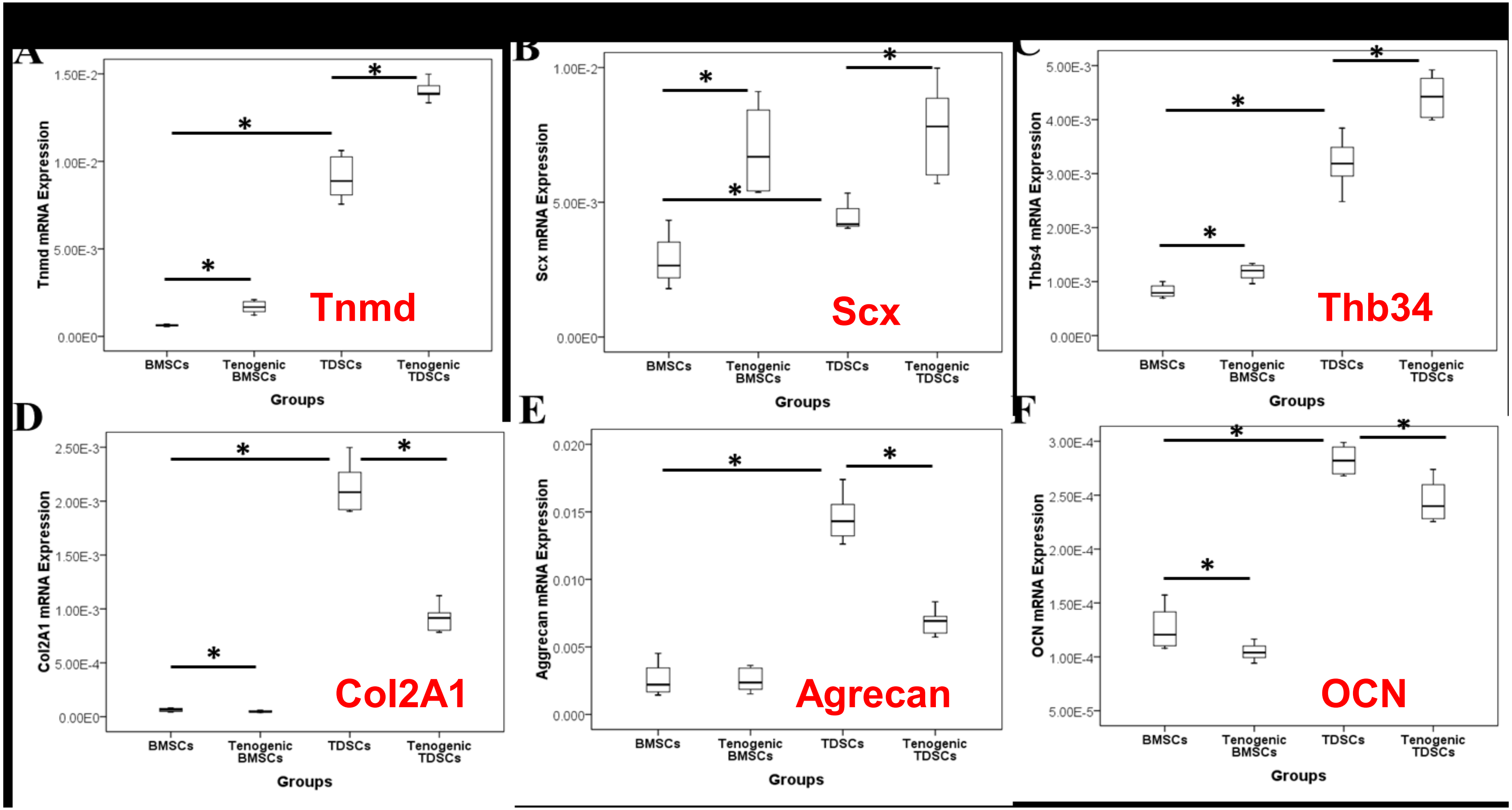
CTGF *(Lee CH, 2010)*

- **In vivo CTGF promoted postnatal connective tissue to undergo fibrogenesis rather than ectopic mineralization.**
- **CTGF promoted fibroblastic differentiation of MSCs.**

Ascorbic Acid *(Omeroğlu S, 2009)*

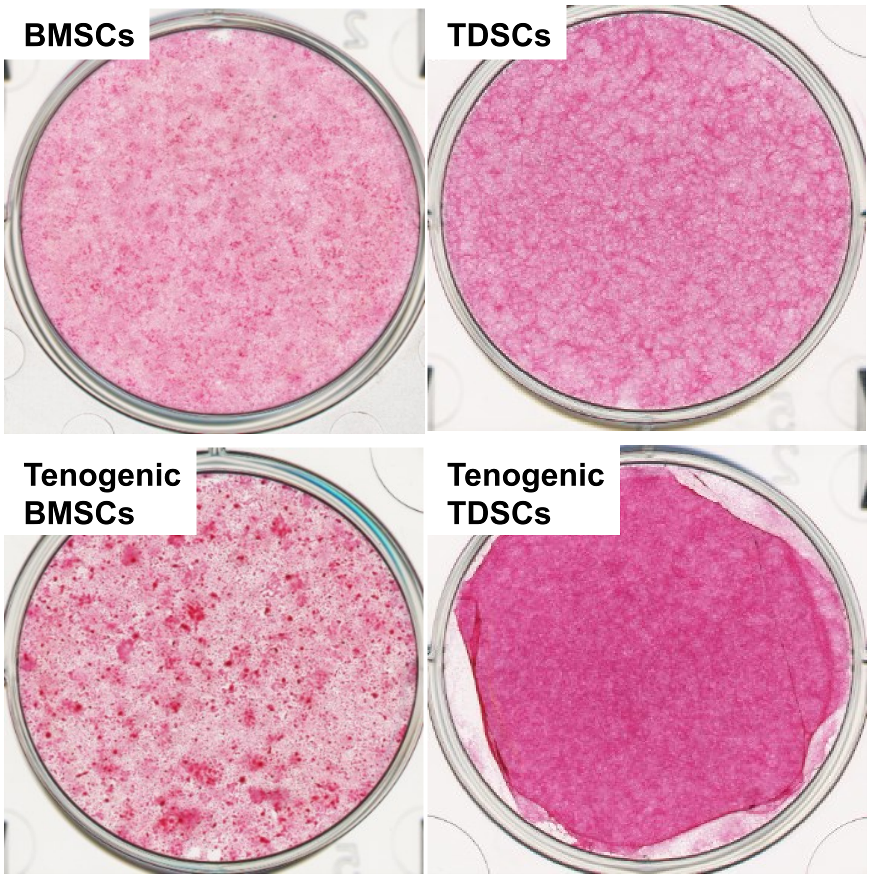
- **Vitamin C could stimulate the Achilles tendon healing because of early angiogenesis and increased collagen synthesis in a healthy rat model.**

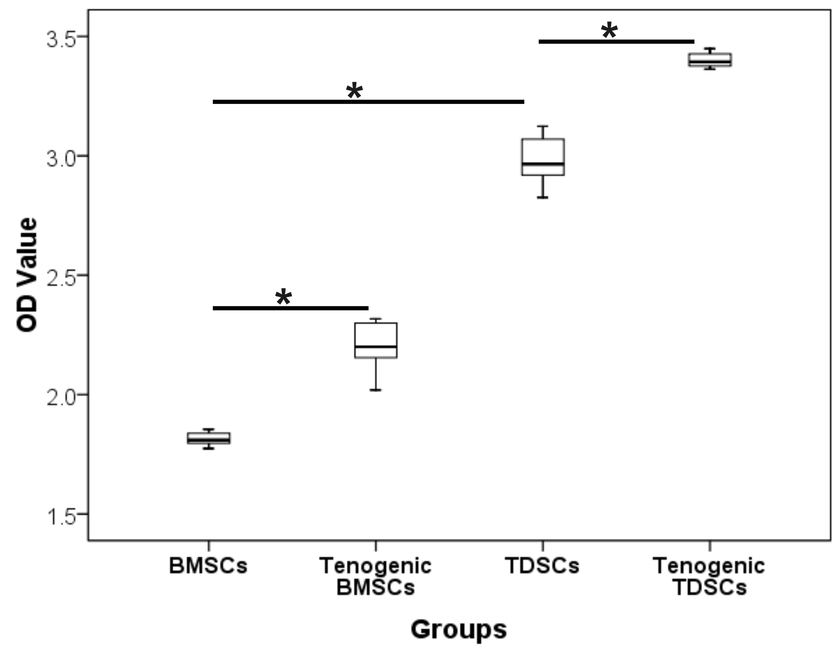
CTGF and Vit C Promoted Tendon-specific Markers Expression in TDSCs *in vitro*



1. Higher Tenogenic Differentiation Potential of TDSCs compared to BMSCs
2. CTGF (25ng/ml) did not promote Osteogenesis and Chondrogenesis of TDSCs and BMSCs

CTGF and Vit C Promoted ECM Production in TDSCs





Sirius red staining, 2 weeks, *$p<0.05$, N=6, Non-Parameter Tests, Mann-Whitney Test

Limitations & Challenges of Tendon Regeneration

Limitations:

Tendon healing is poor:

1. The tendon healed with poor tissue quality.
2. The regenerated fibrotic scar tissue could not regain its original mechanical strength.

(Miyashita et al., 1997)

Challenges:

How to improve tendon healing outcome ?

Tissue Engineering – can we use BM-MSCs or TDSCs to promote tendon healing or regeneration?

Tendon-derived stem cells (TDSCs), may be used to produce tendon tissues through tenogenic differentiation *in vitro*; and form neo-tendon and promote tendon healing *in vivo*.

Tendon-Derived Stem Cells (TDSCs) Promote Tendon Repair in a Rat Patellar Tendon Window Defect Model

Ming Ni,[1,2] Pauline Po Yee Lui,[1,2,3] Yun Feng Rui,[1,2] Yuk Wa Lee,[1,2] Yuk Wai Lee,[1,2] Qi Tan,[1,2] Yin Mei Wong,[1,2] Siu Kai Kong,[4] Pui Man Lau,[4] Gang Li,[1,2,3] Kai Ming Chan[1,2]

[1]Department of Orthopaedics and Traumatology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China, [2]The Hong Kong Jockey Club Sports Medicine and Health Sciences Centre, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China, [3]Program of Stem Cell and Regeneration, School of Biomedical Science, The Chinese University of Hong Kong, Hong Kong SAR, China, [4]Programme of Biochemistry, School of Life Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China

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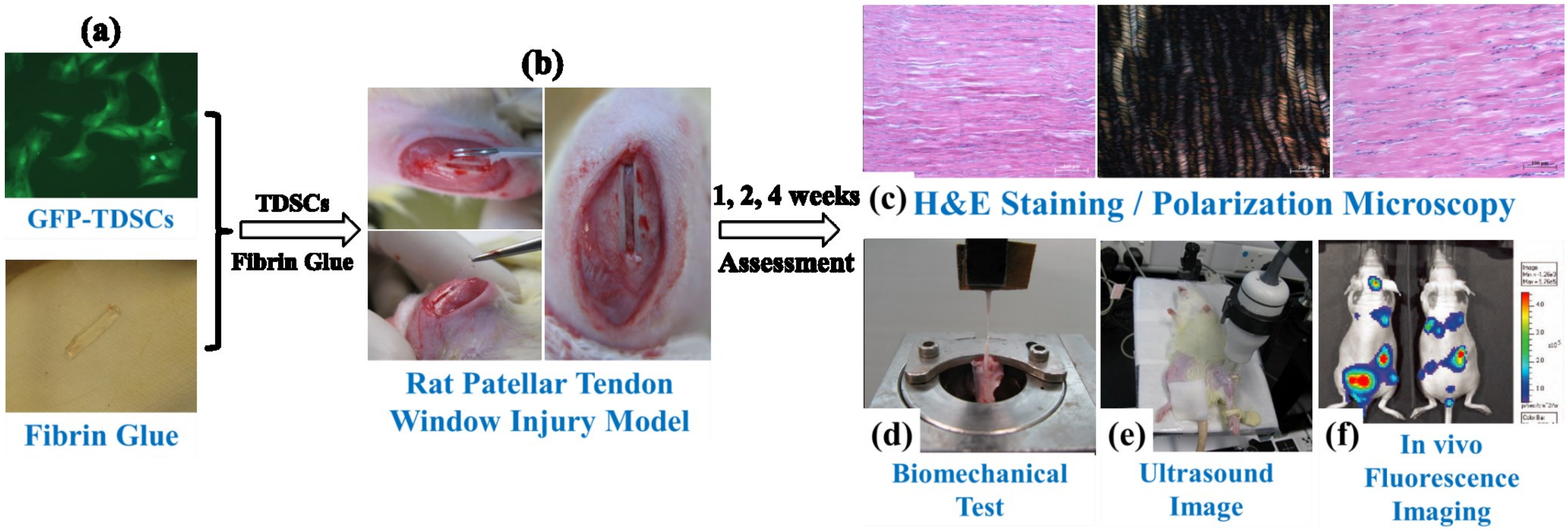
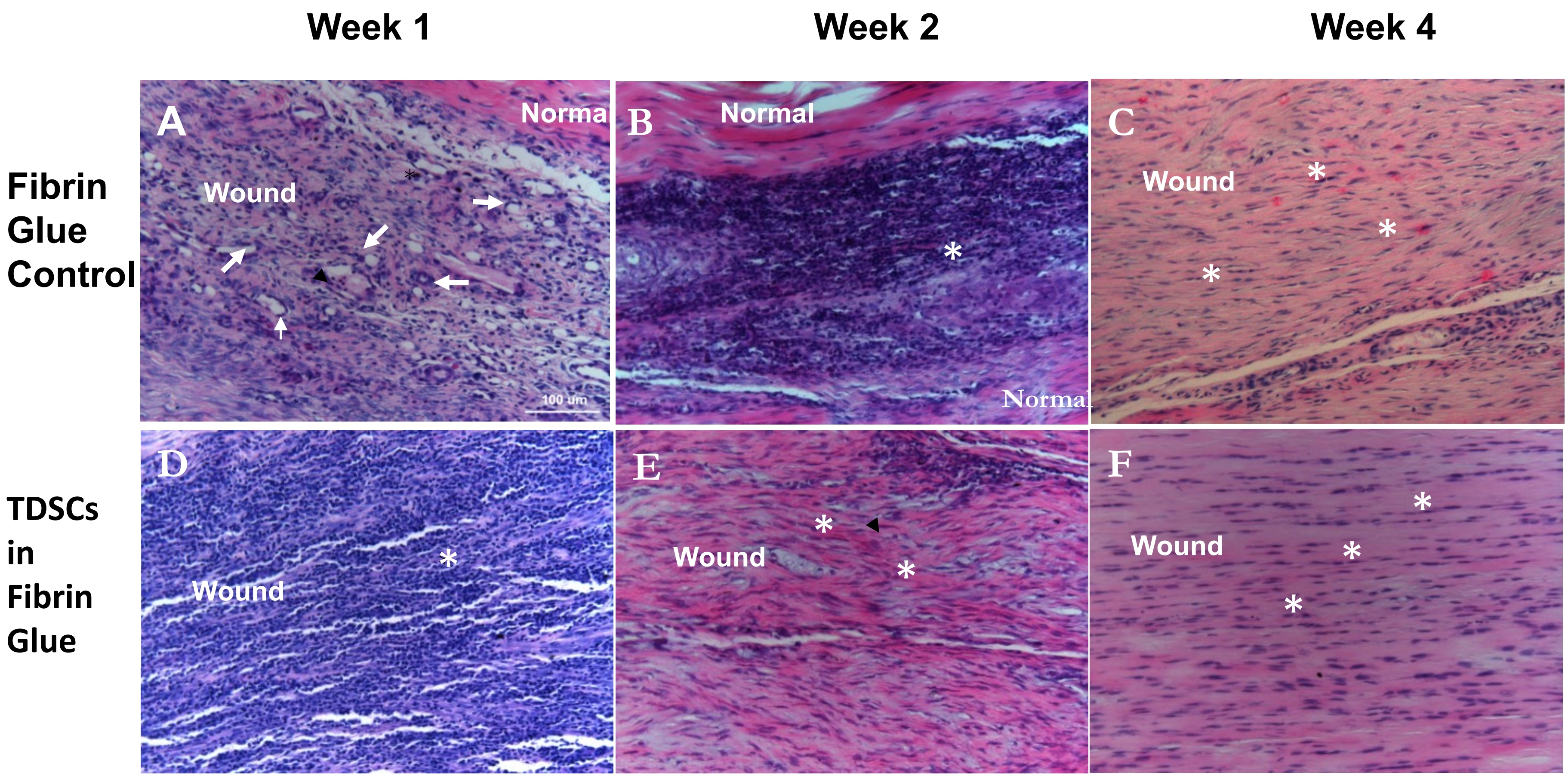
Journal of Orthopaedic Research, 2011.

TDSCs Promotes Tendon Healing



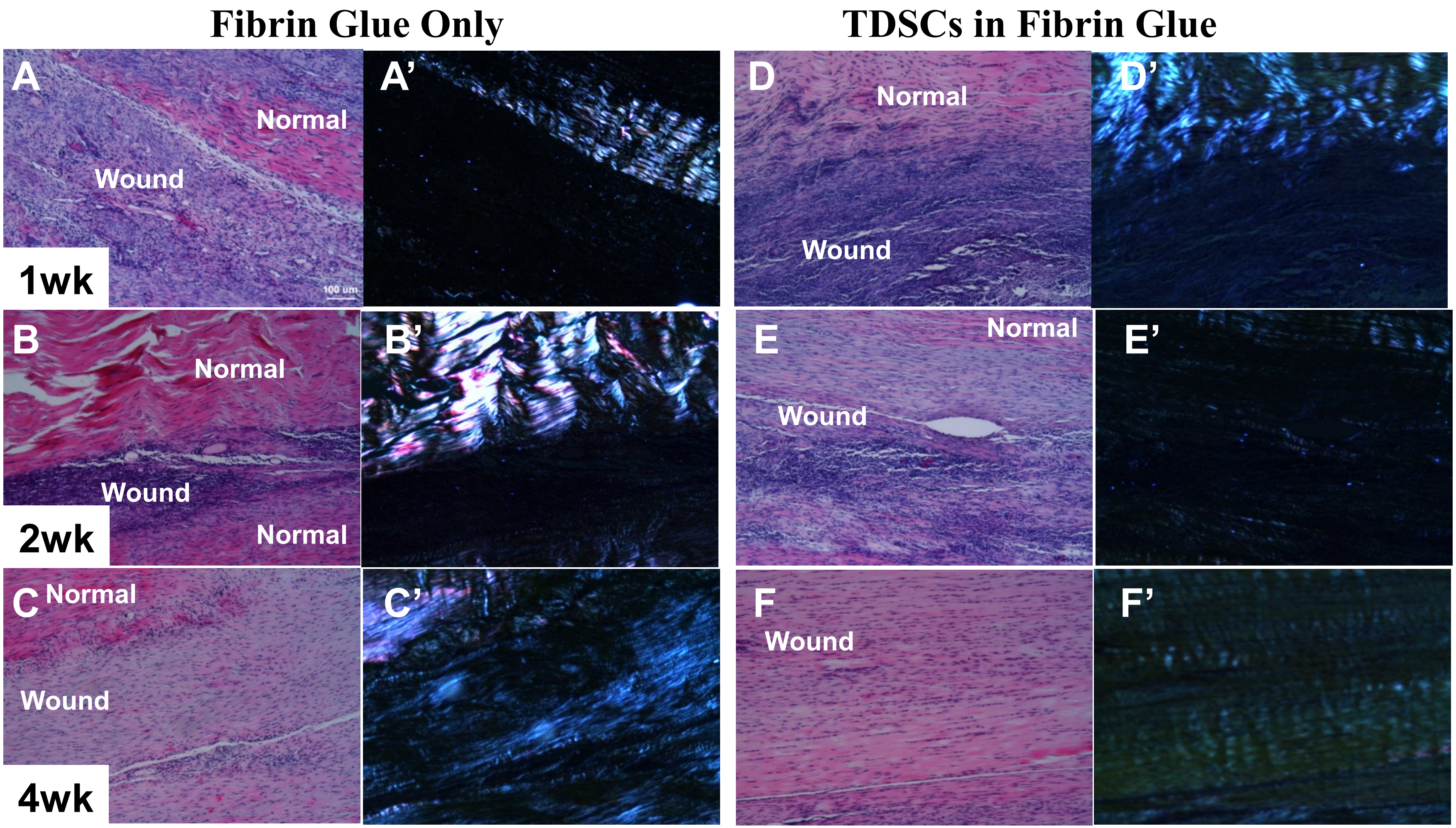
1. GFP-TDSCs were isolated from the intact patellar tendons of GFP rats and characterized following our previous study protocol.
2. Fibrin glue constructs with or without GFP-TDSCs was transplanted into the SD rat patellar tendon window defect.
3. The patellar tendons were harvested for ultrasound imaging, histology, ex vivo fluorescent imaging and biomechanical test at various time points.

TDSCs Promotes Tendon Healing- H&E staining



1. The cellularity was higher in the TDSCs group than control groups at week 1.
2. The healing cells became elongated at 2 and 4 weeks in the TDSCs group.
3. More extracellular matrices were produced in the TDSCs group than control groups at all time points.

TDSCs Promotes Tendon Healing- polarized microscopy

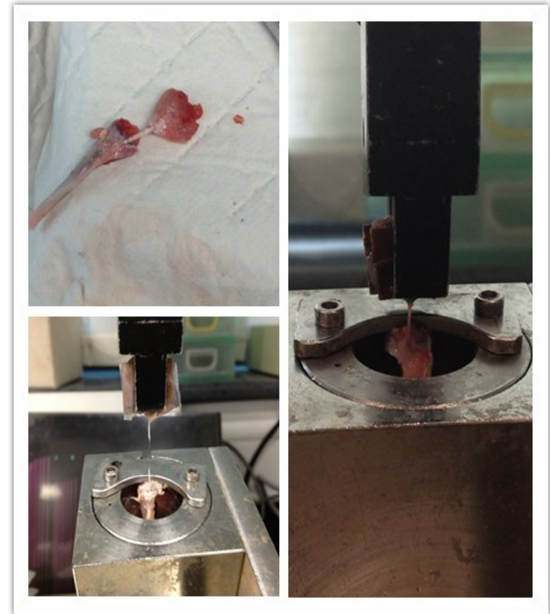


1. The collagen birefringence increased with healing in both groups.
2. Higher collagen birefringence was observed in the TDSCs group than control groups at all time points, suggesting better collagen fiber alignment.

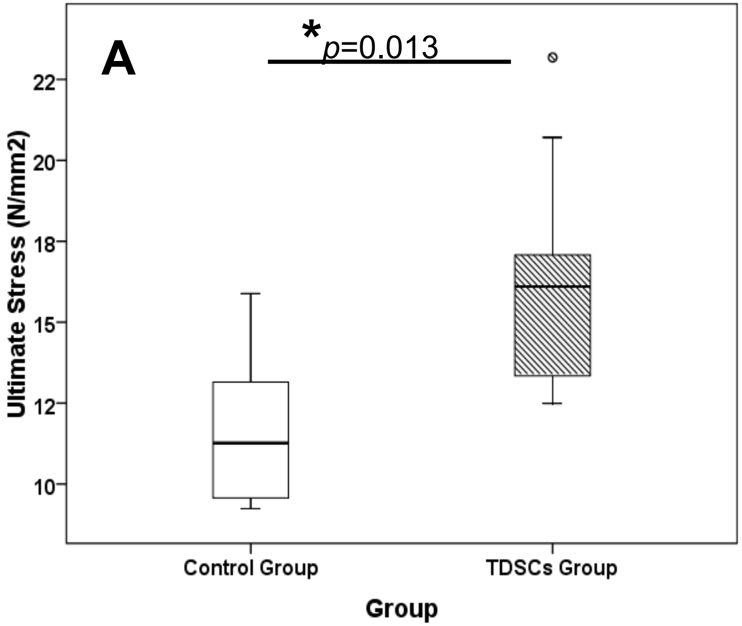
Biomechanical Test

Control VS TDSCs:

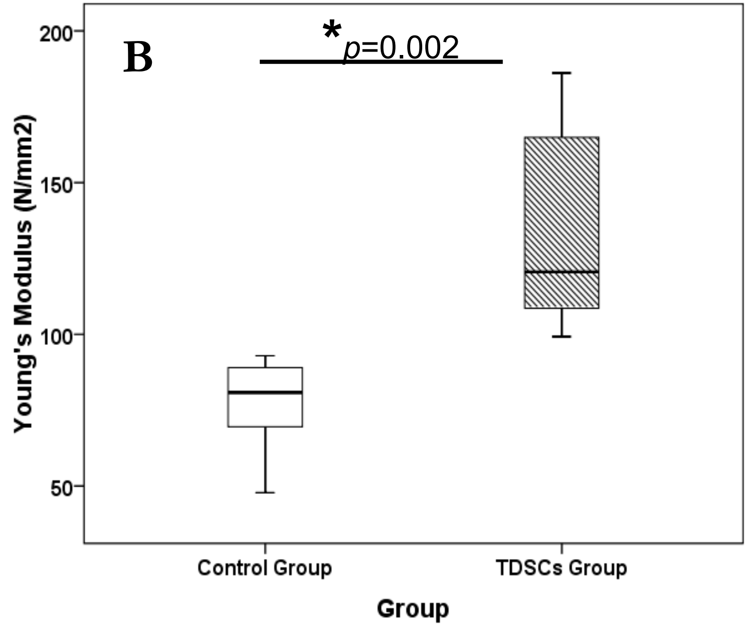
- At 4 weeks post-op, the Ultimate Stress and Young's Modulus in the TDSCs group was significantly higher than that of control group.



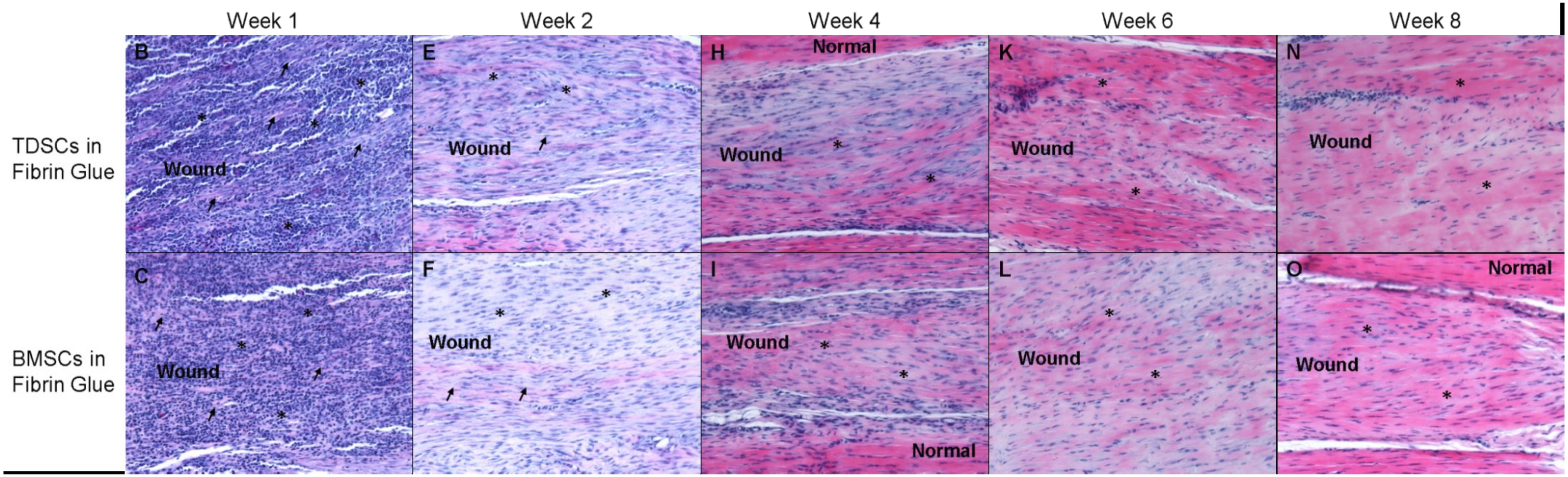
Ultimate Stress

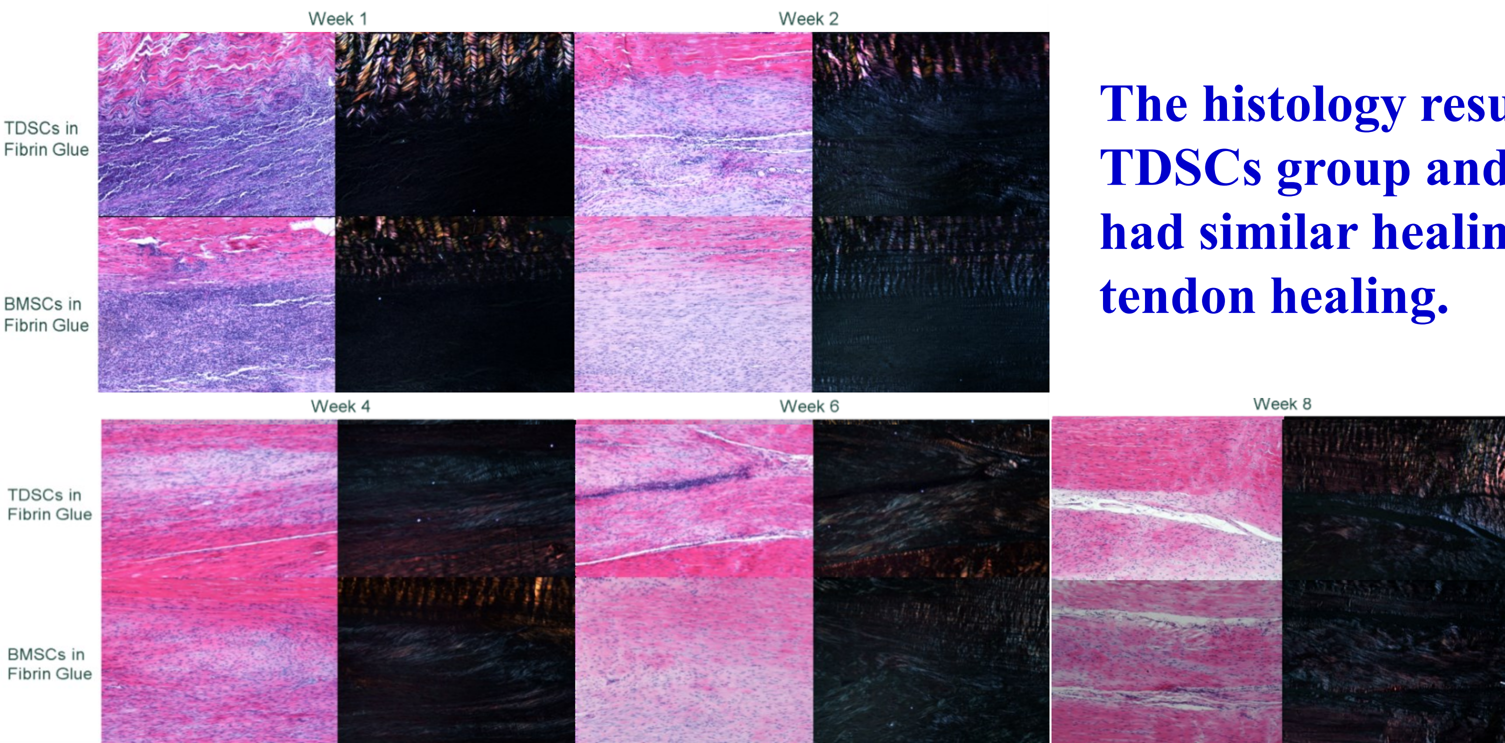


Young's Modulus



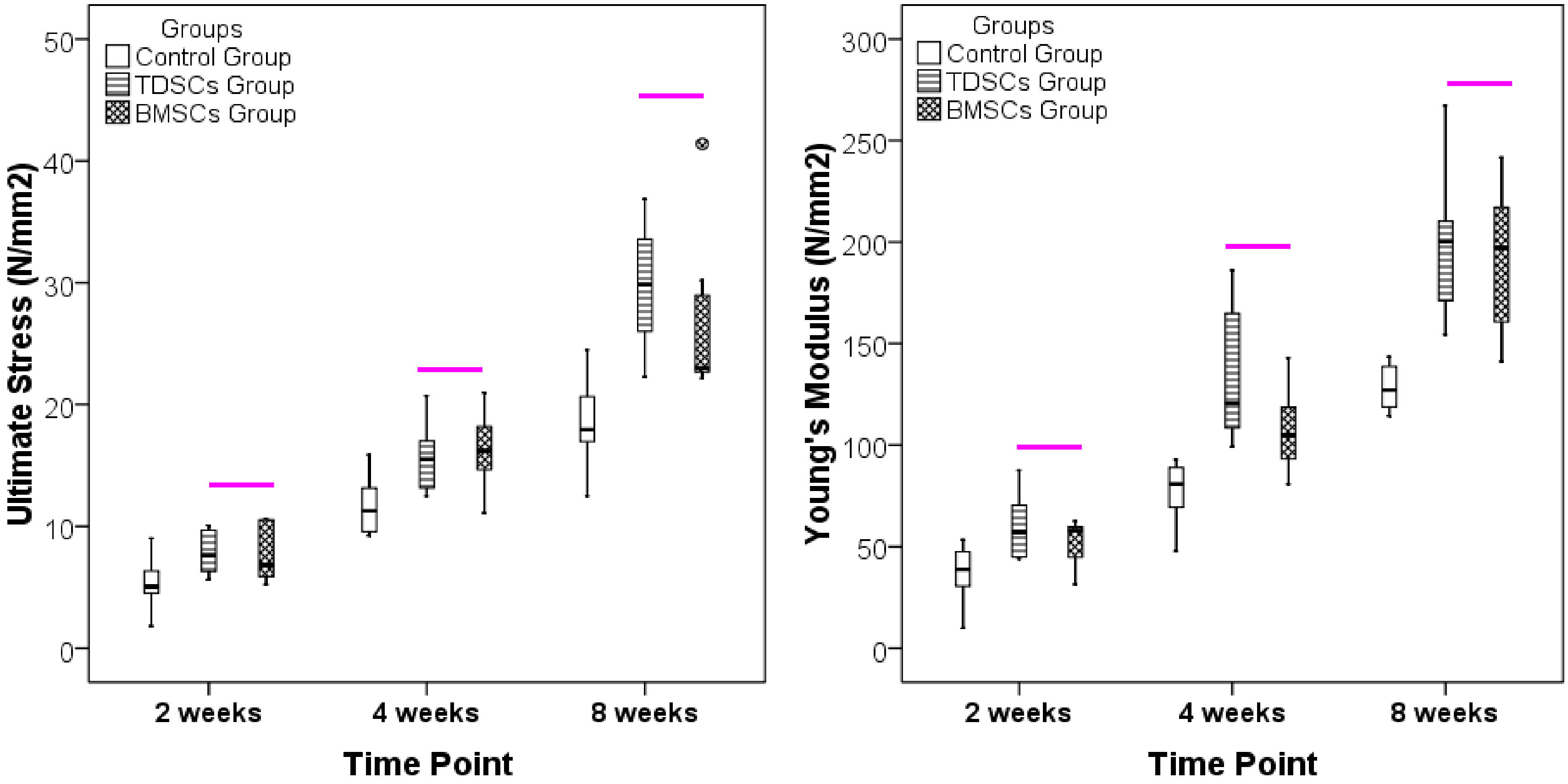
TDSCs *vs.* BM-MSCs in Tendon Repair





The histology results showed the TDSCs group and BMSCs group had similar healing quality in tendon healing.

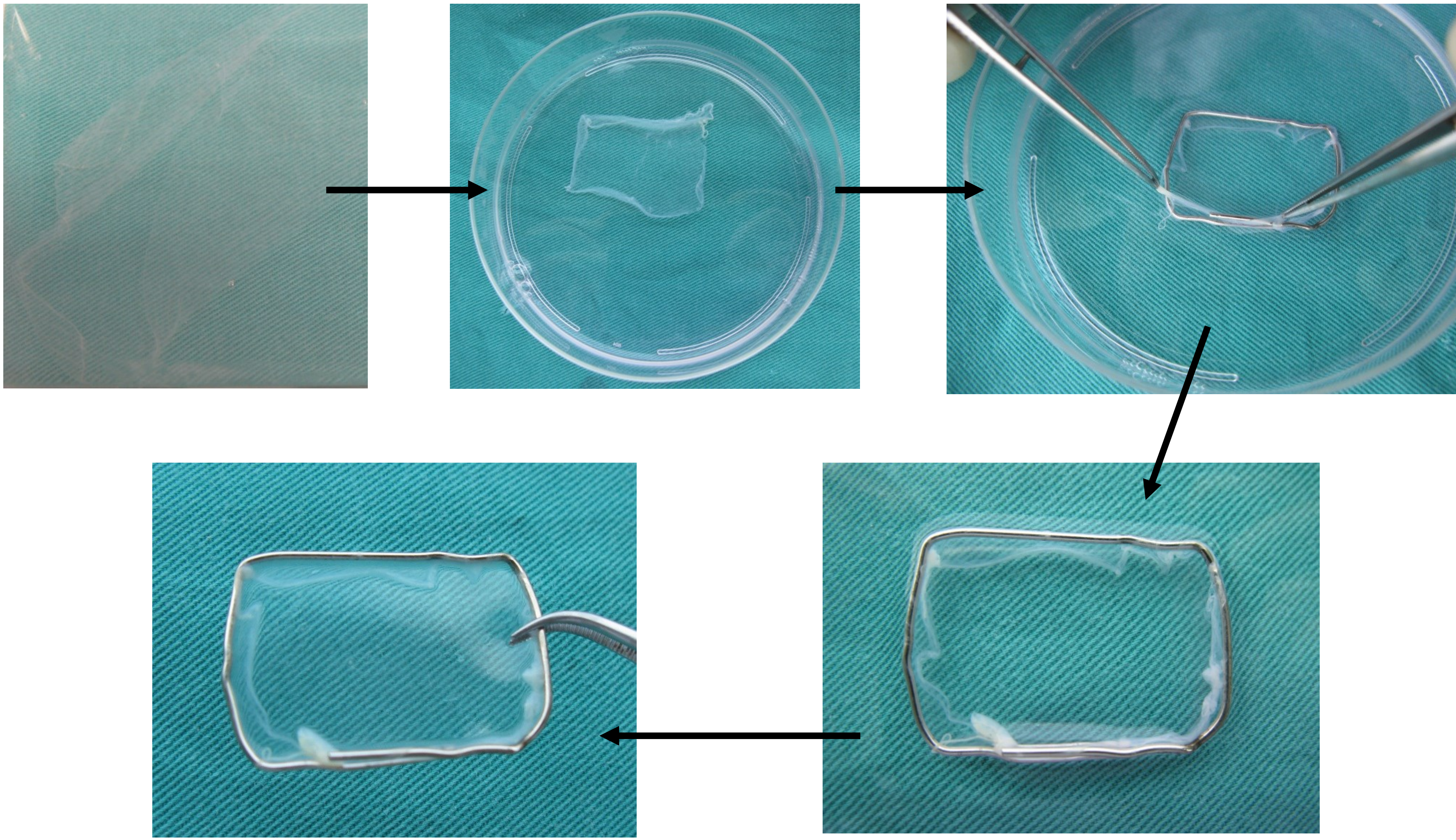
TDSCs *vs.* BMSCs in tendon healing



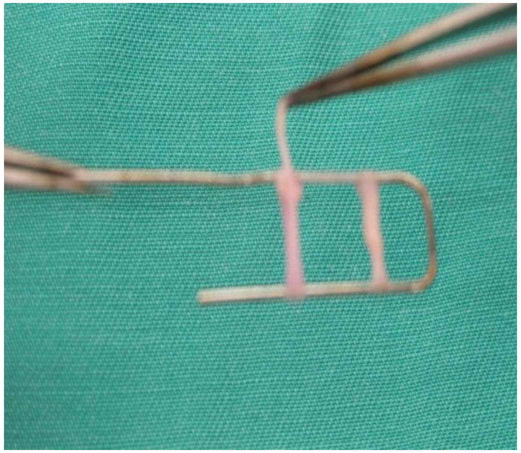
At all time points, the Ultimate Stress and Young's Modulus in the TDSCs and BM-MSCs groups had no significant difference.

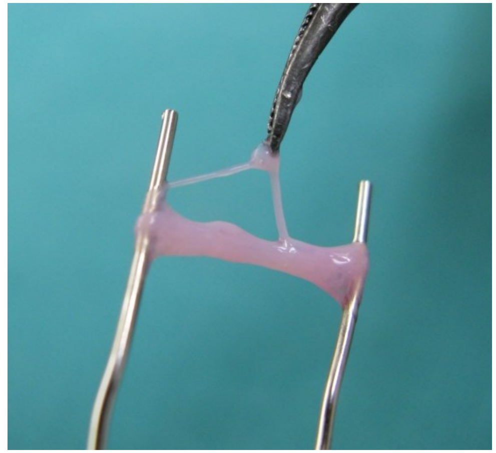
Engineered TDSCs Cell Sheets *in vitro*

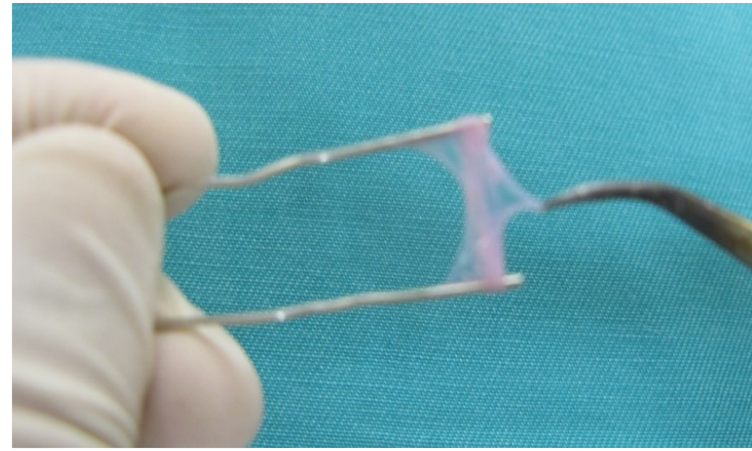
TDSCs were treated by CTGF and Vit C for 2 weeks

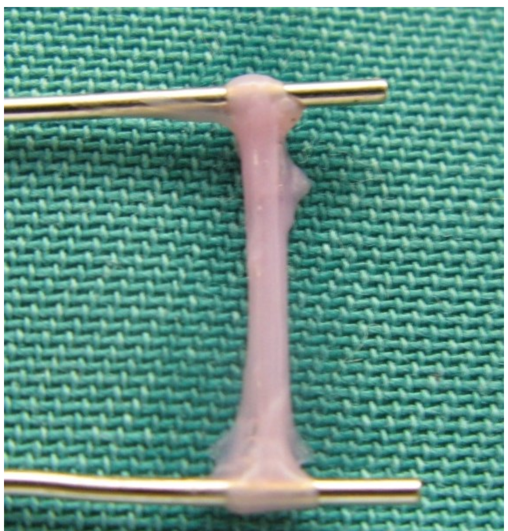


Engineering Tendon Using TDSCs Cell Sheets



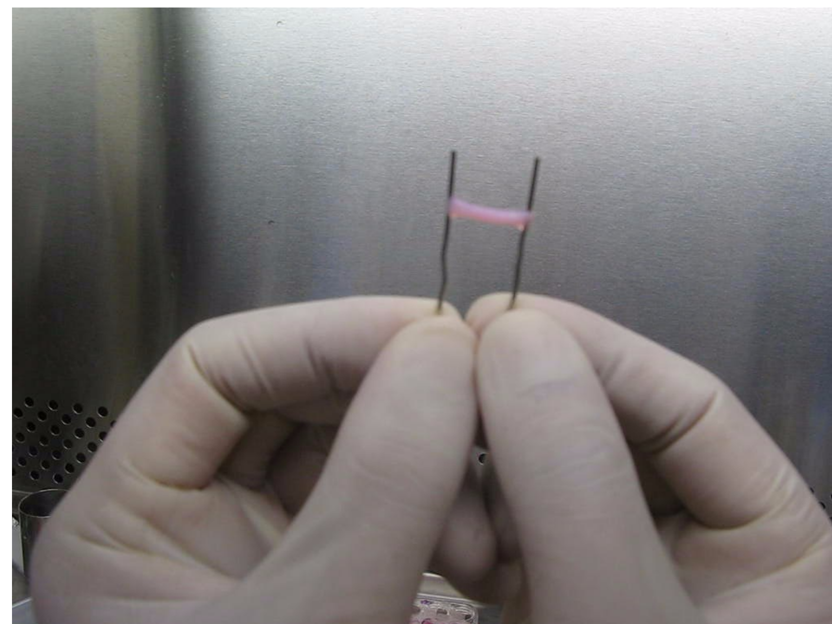


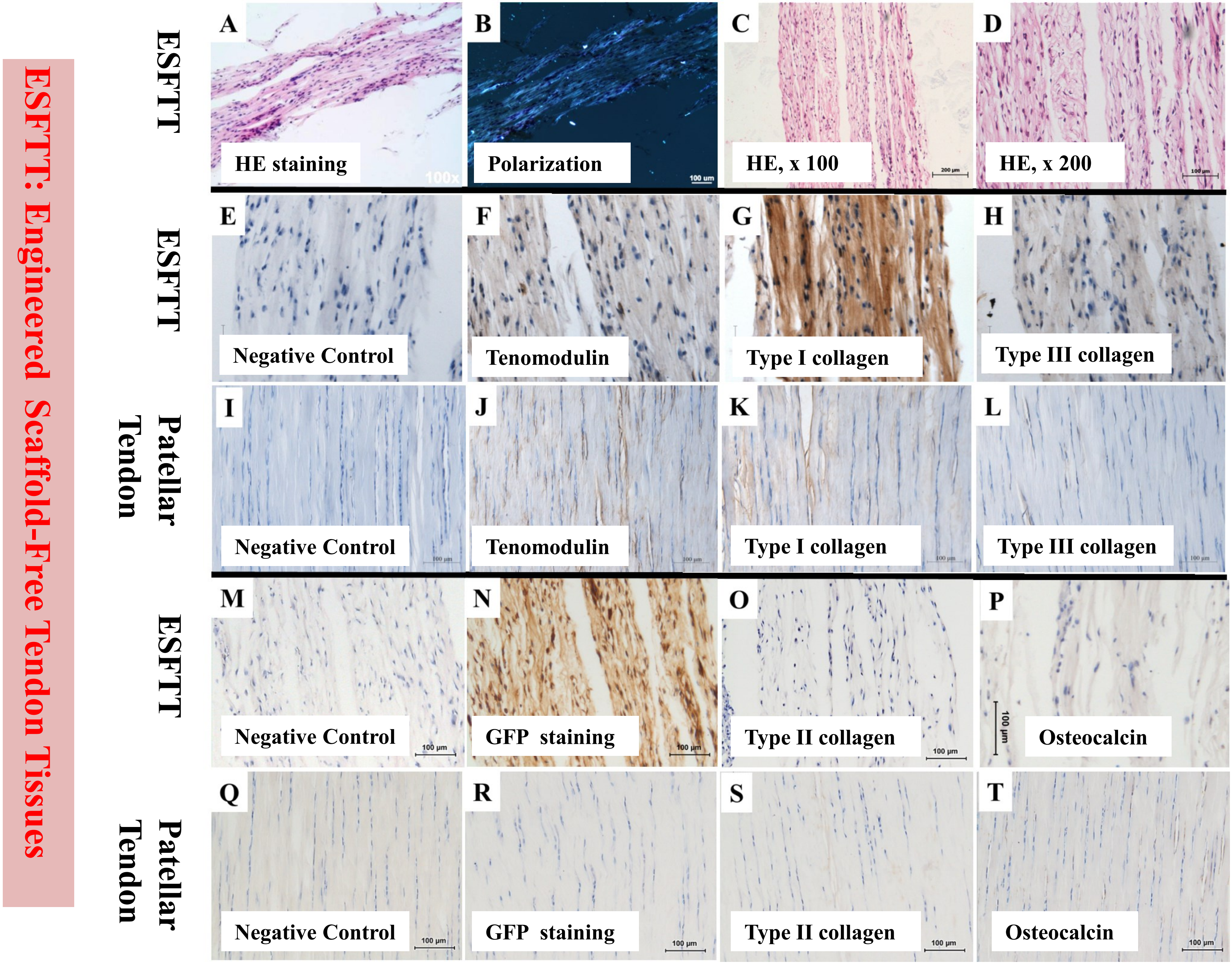




Engineered Tendon

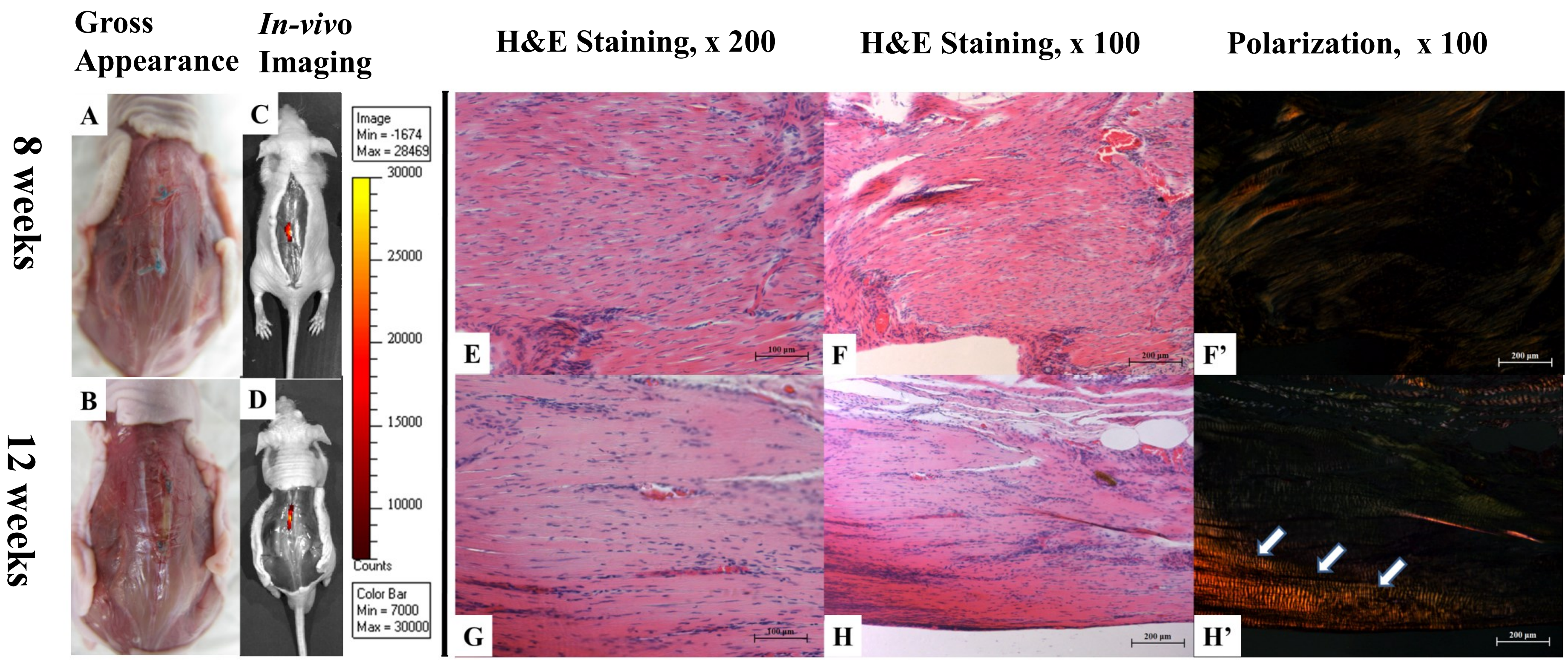
Intact Patellar Tendon





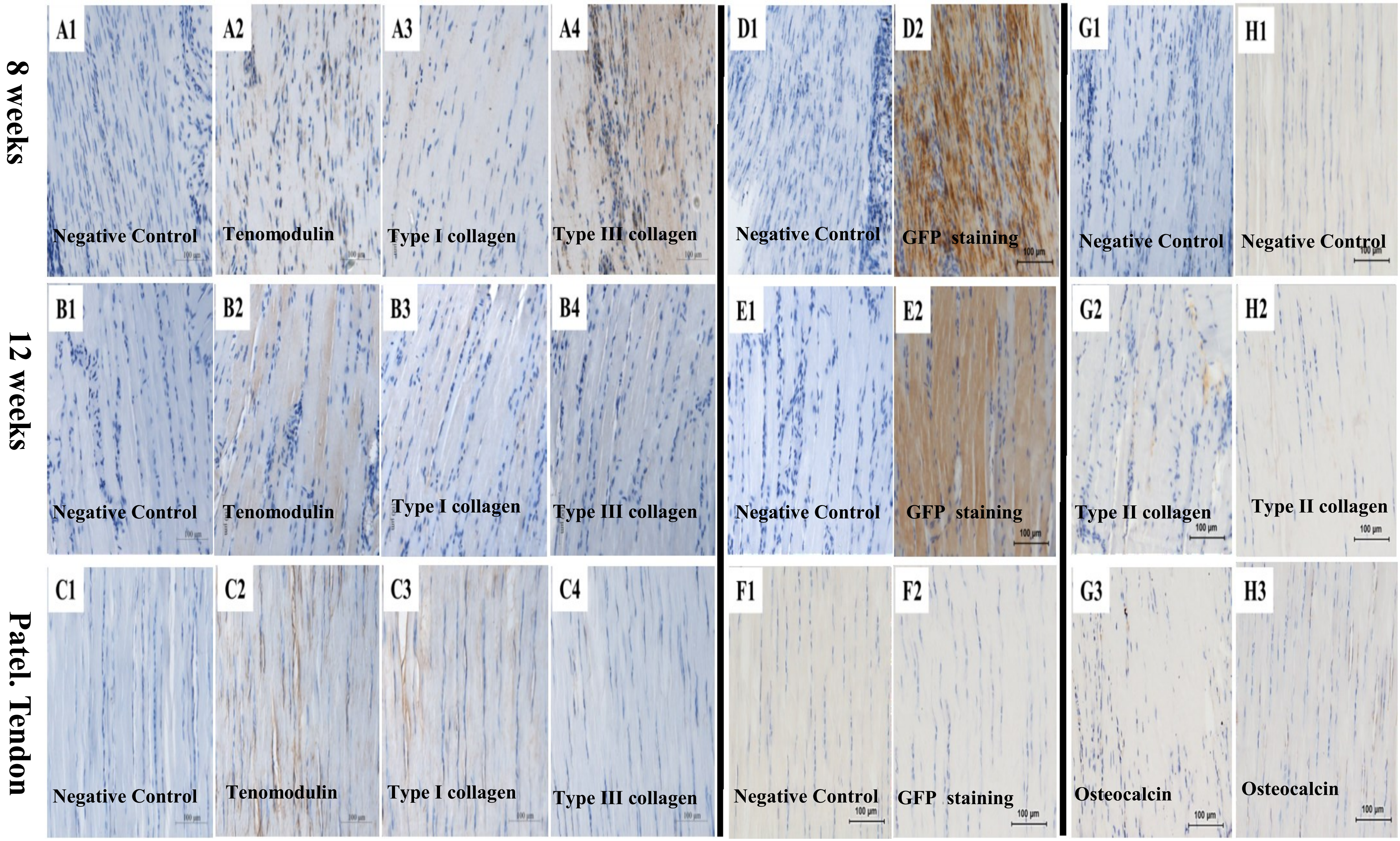
ESFTT: Engineered Scaffold-Free Tendon Tissues
ESFTT
ESFTT
Patellar Tendon
ESFTT
Patellar Tendon
A
HE staining
100x
B
Polarization
100 um
C
HE, x 100
D
HE, x 200
E
Negative Control
F
Tenomodulin
G
Type I collagen
H
Type III collagen
I
Negative Control
J
Tenomodulin
K
Type I collagen
L
Type III collagen
M
Negative Control
100 μm
N
GFP staining
100 μm
O
Type II collagen
100 μm
P
100 μm
Osteocalcin
Q
Negative Control
100 μm
R
GFP staining
100 μm
S
Type II collagen
100 μm
T
Osteocalcin
100 μm

ESFTT formed neo-tendon tissues in nude mice

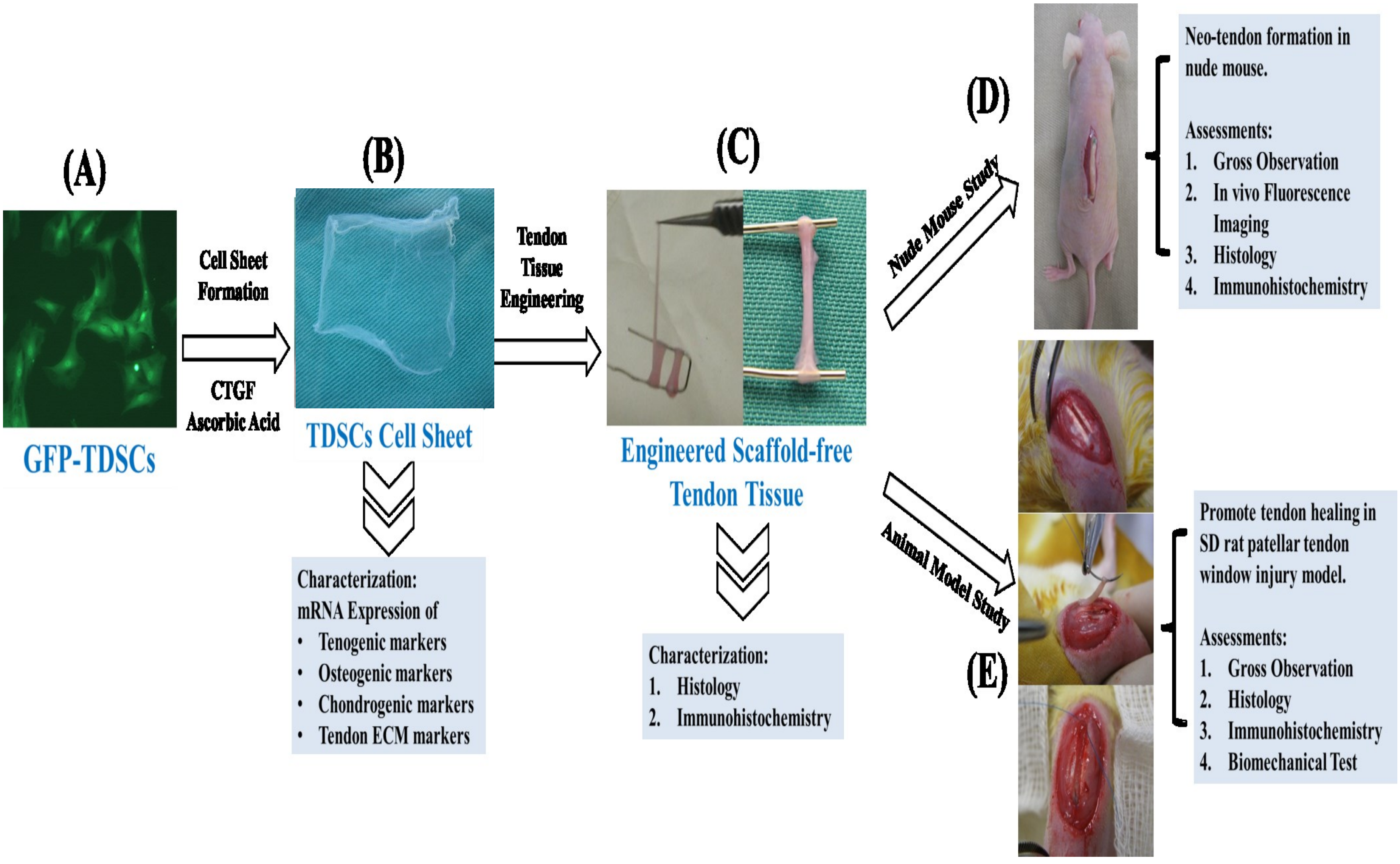


Engineered tendon sheets by TDSCs formed neo-tendon tissues in nude mice.

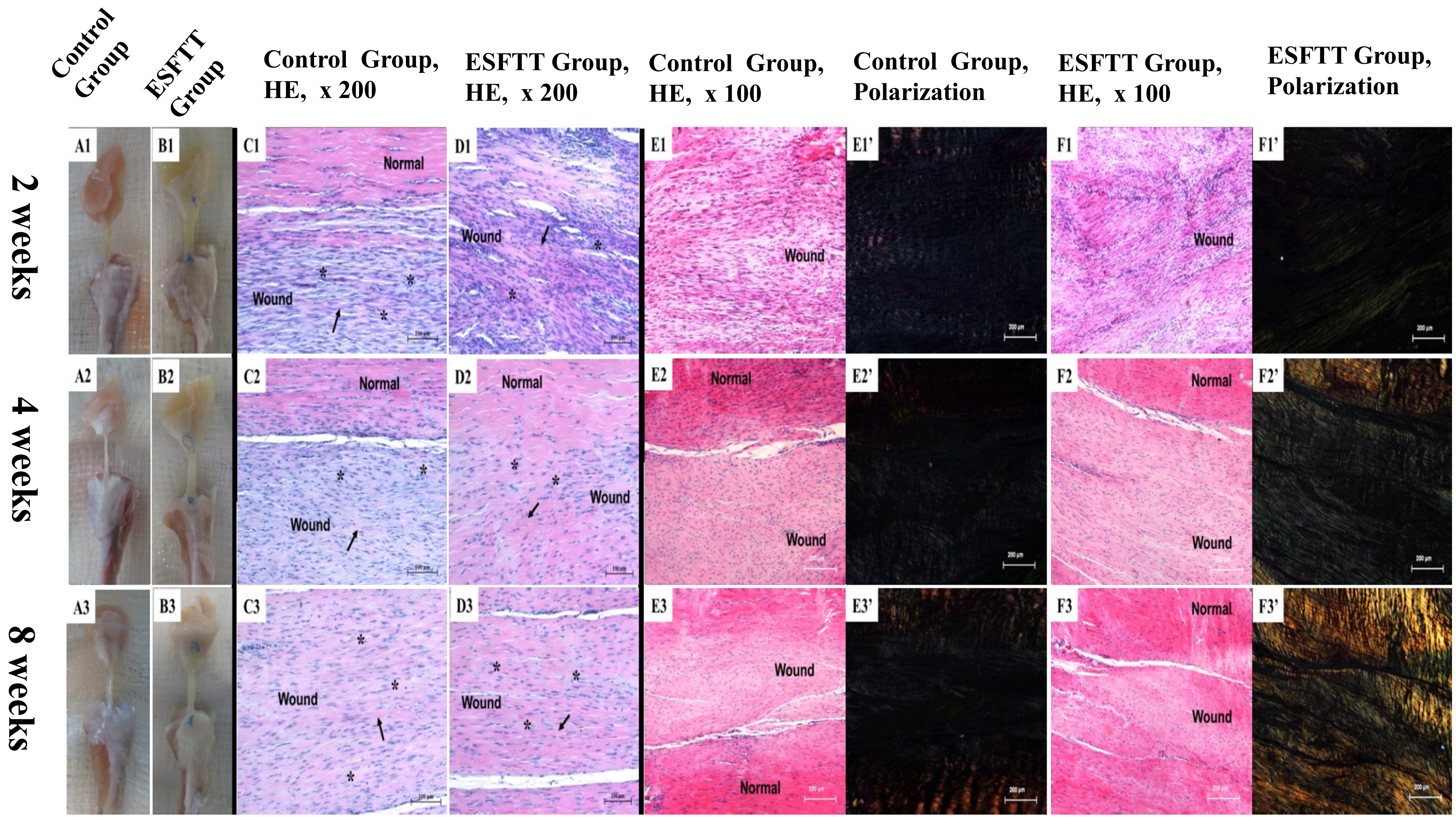
ESFTT Forms Neo-tendon in Nude mouse



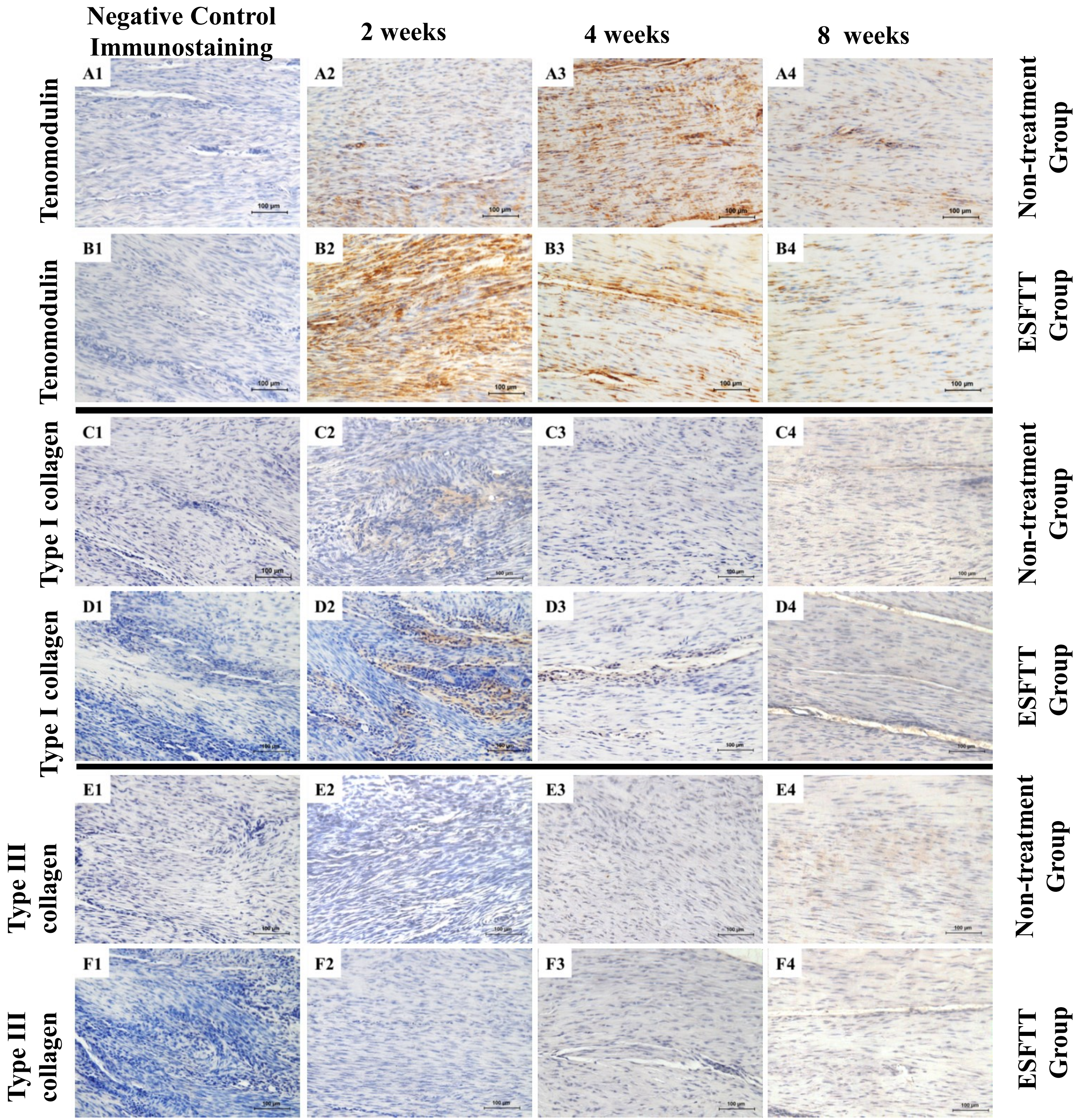
TDSCs Cell Sheet for Tendon Repair



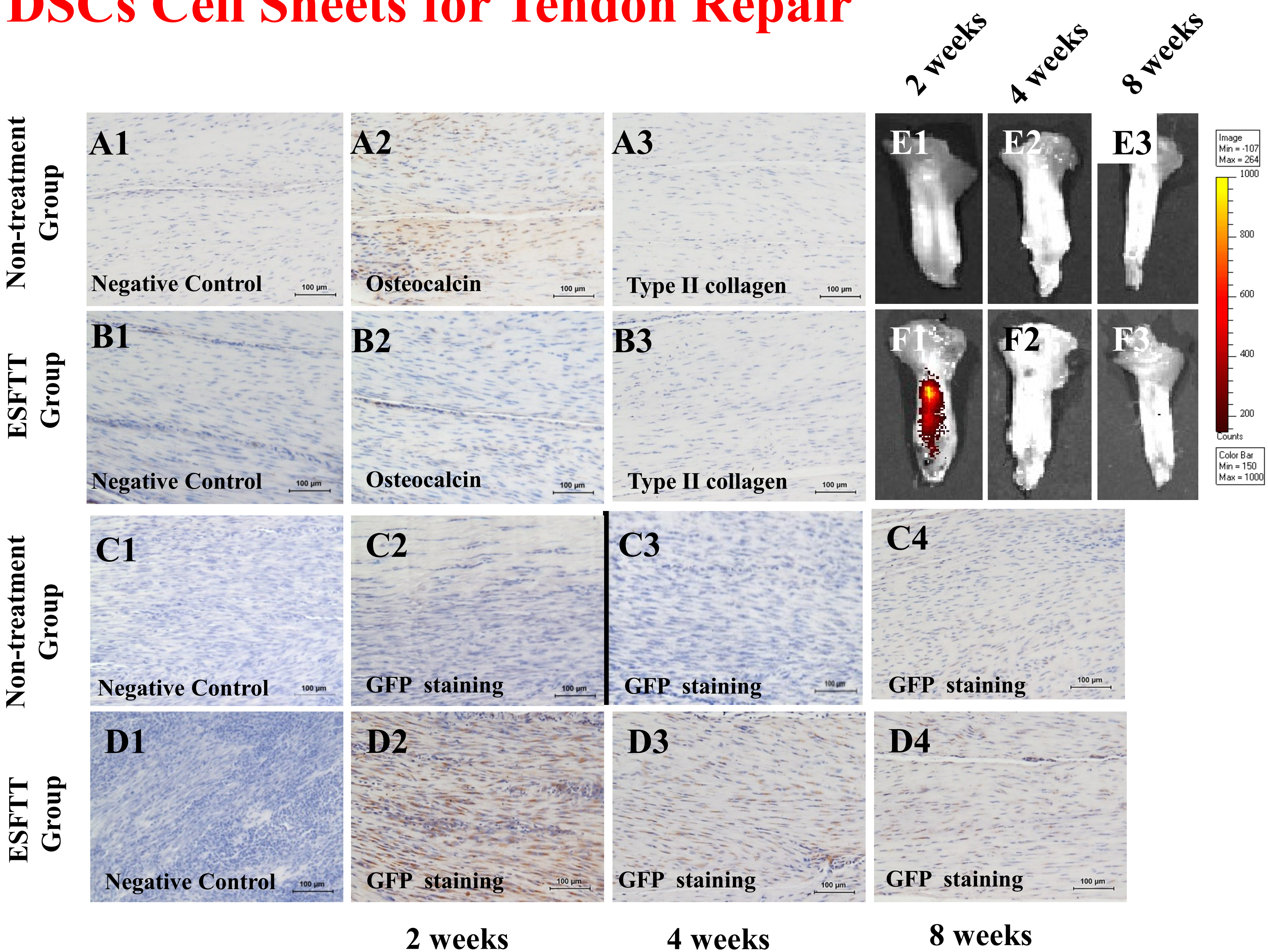
TDSCs Cell Sheet for Tendon Repair



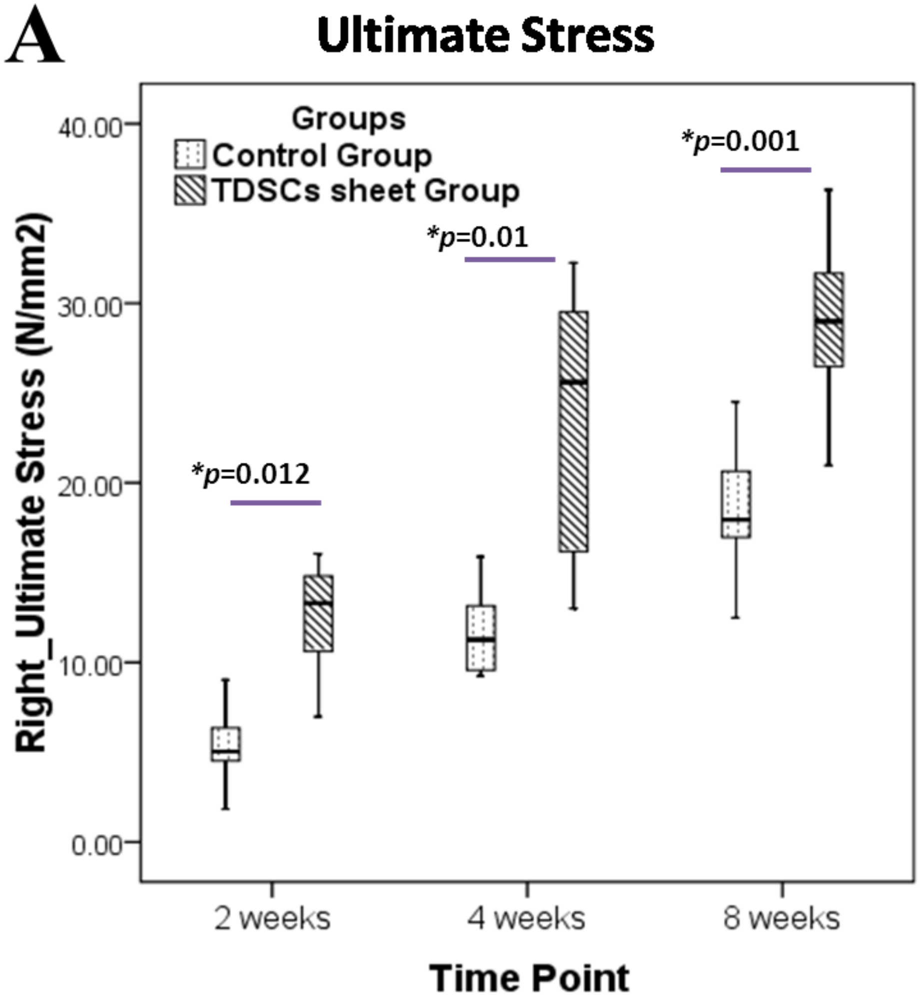
TDSCs Cell Sheet in Tendon Repair

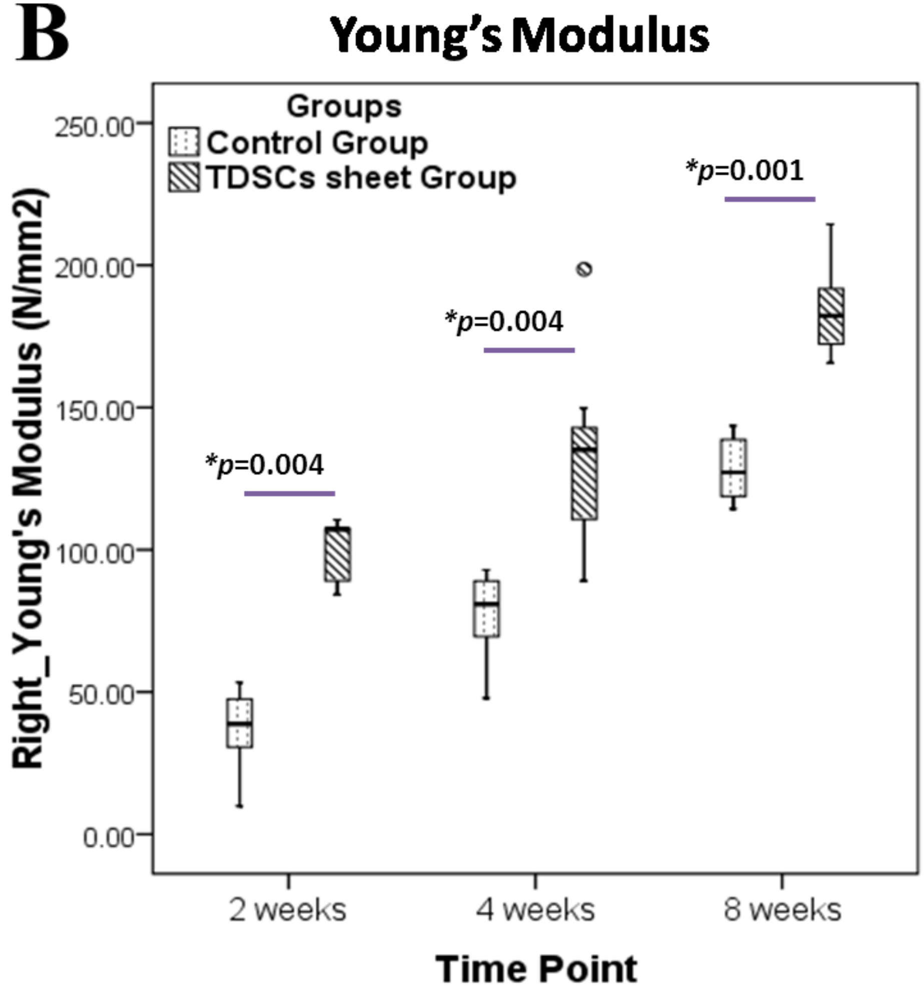


TDSCs Cell Sheets for Tendon Repair



TDSCs Cell Sheet for Tendon Repair





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Engineered scaffold-free tendon tissue produced by tendon-derived stem cells☆

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Conclusions

1. **TDSCs is a new cell source for tendon regeneration.**
2. **TDSCs showed its superiority in promoting tendon healing than that of BMSCs after stimulating with CTGF and ascorbic acid for 2 weeks.**
3. **The engineered TDSCs cell sheets formed neo-tendon tissues *in vivo*.**
4. **The engineered TDSCs cell sheets promoted tendon healing in a rat acute patellar tendon injury model, it may be a new strategy for tendon repair.**

Summary

1. More mechanistic studies of tenogenic differentiation are needed: epigenetic and genetic regulations; mechanical and environmental cues, etc.

2. The use of TDSCs or growth factors to guide or promote tendon regeneration in tendon disorders and injury.

3. Clinical studies: clinical trials and samples for further confirmation studies.

CUHK LiKS Stem Cell and Regeneration Lab Members

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