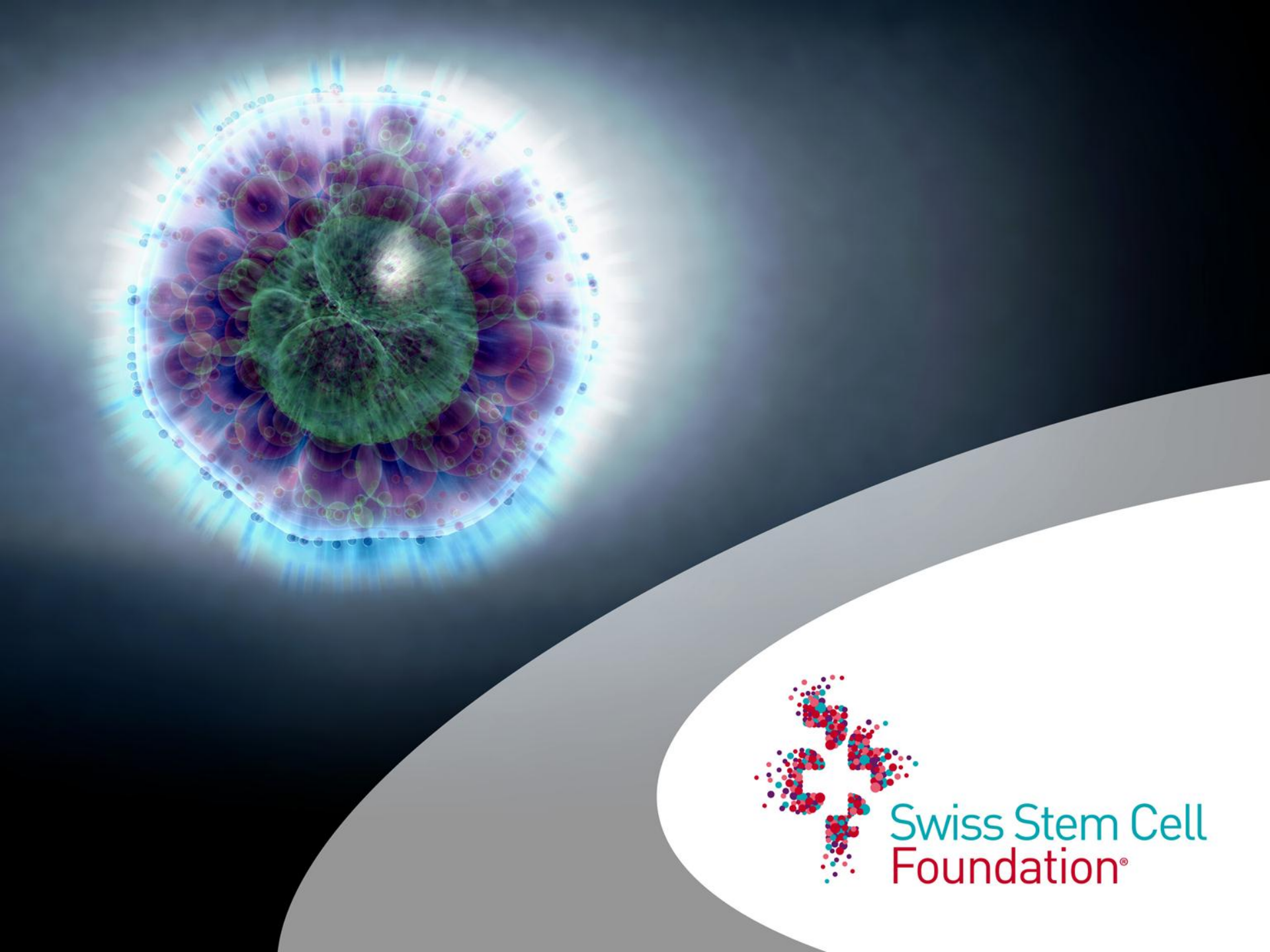
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Differentiating ASCs in tendon

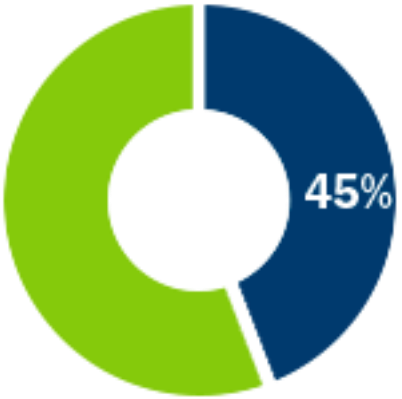
iCAST2017

Deborah Stanco

Introduction

Introduction

- Tendon disorders are common and lead to:
  - significant disability and pain
  - healthcare cost and lost productivity



- 45% of musculoskeletal lesions worldwide
- 2000 new cases/year only in Switzerland
- mean insurance cost of 23,843 CHF

Sharma P, Maffulli N. 2006
Service de centralization des statistiques de l'assurance accident LAA

Introduction

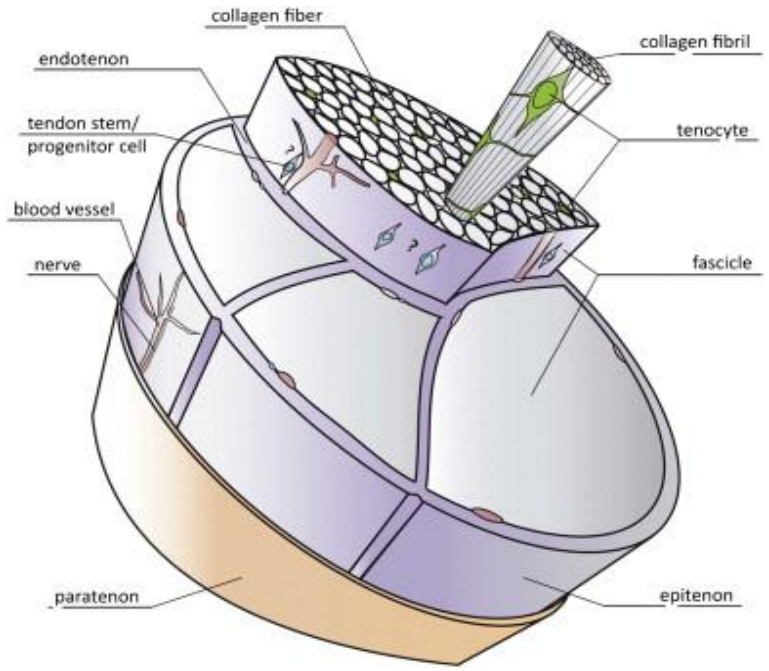
- A wide range of injury mechanisms exist leading to tendinopathy or tendon rupture
    - Tears in healthy tendons acutely overloaded (high impact event) or lacerated (knife injury)
    - Tendinitis or tendinosis in overuse conditions (elite athletes) or intrinsic tissue degeneration (age-related degeneration)
- Tendon biology and related injury mechanisms are still poorly understood

Maffulli N, et al. Clin Sports Med 2003
Sharma P, Maffulli N. Musculoskelet Neuronal Interact 2006

Introduction

Tendon tissue composition:

- Extracellular matrix (ECM) 95%:
  - Collagen type I, III
    Proteoglycans (decorin)
    Glycoproteins (tenascin, COMP)
- Cells 5%:
  - Tenocytes
    TSCPs
- Blood vessels and nerve low%



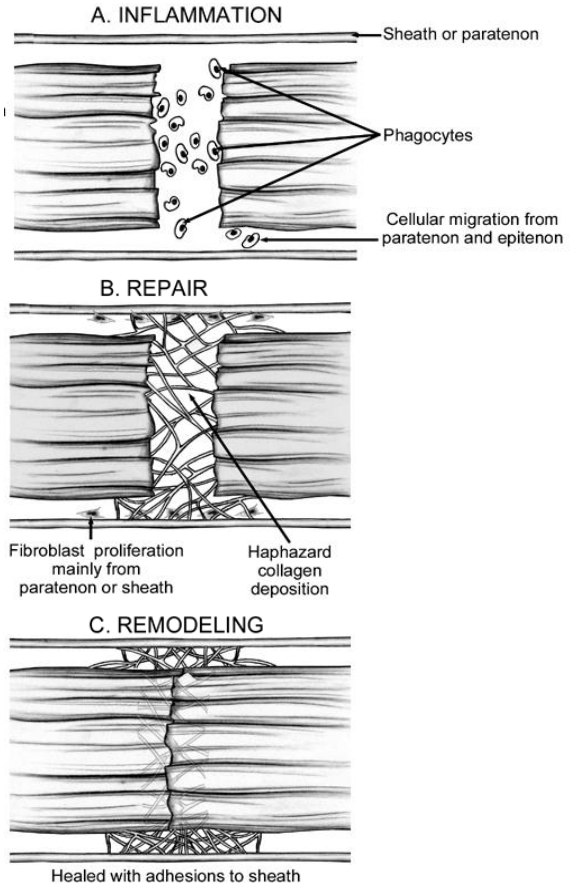
From Docheva D et al. Adv Drug Deliv Rev 2015; 84:222-39

Introduction

Tendon healing process

	Inflammatory 0-2 days	Reparative 2-40 days	Remodeling 12 months
Cells and Matrix Changes	Platelets Neutrophilis Erytrhocytes Circulating-MSCs	Cellularity and Matrix production Collagen type III TSPCs activation	Cellularity and Matrix production Collagen type III Collagen type I
Molecular Changes	IL-6, IL1-β bFGF IGF-1 PDGF TGFβ VEGF	GDF-5, -6, -7 bFGF IGF-1 PDGF TGFβ VEGF	GDF-5, -6, -7 IGF-1 TGFβ

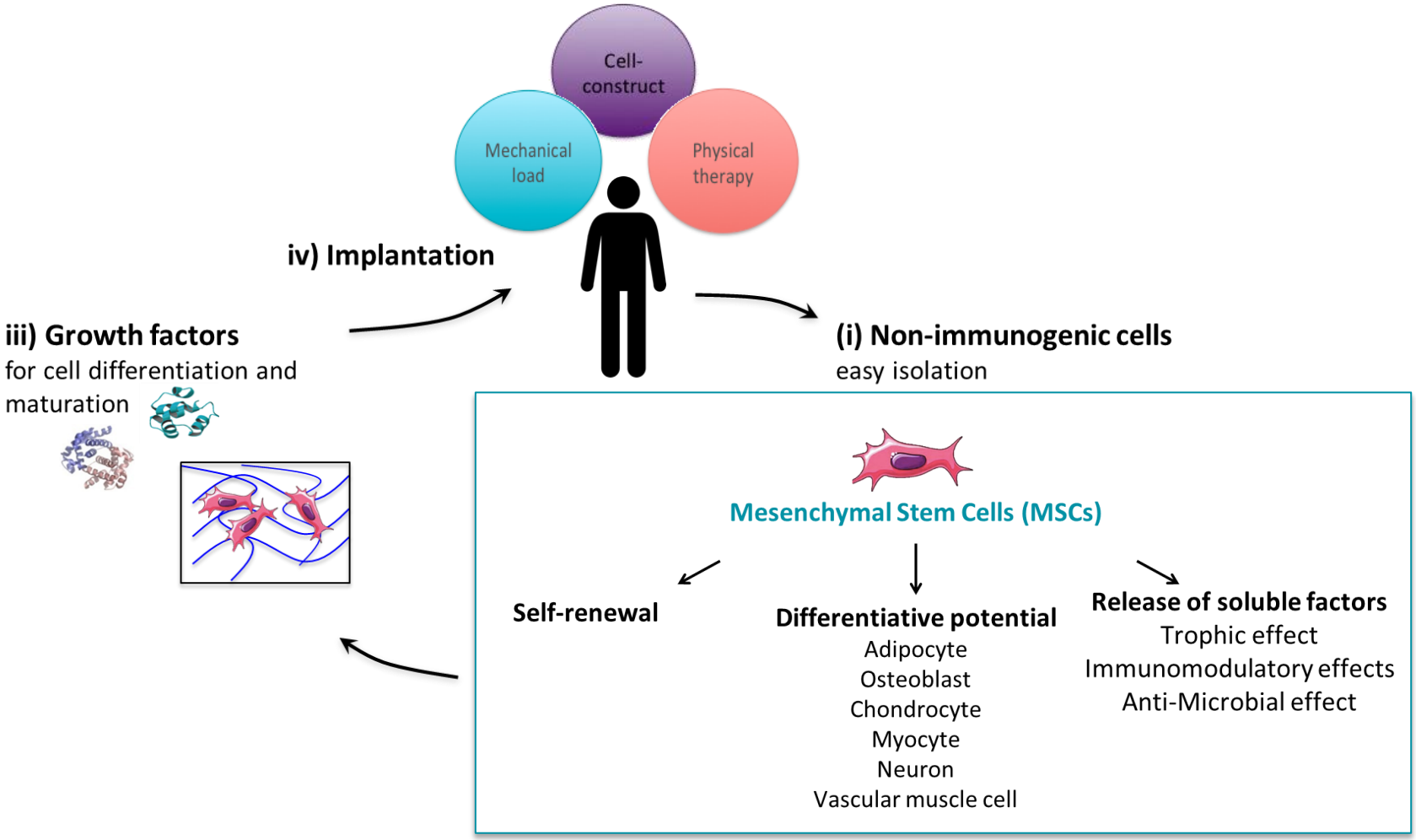
Modified from Docheva D et al 2015



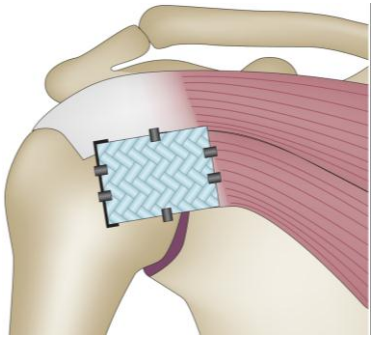
Clinical problem

- Tendon healing often results in scar tissue mechanically inferior, less able to perform the functions of a normal tendon and with high risk of reinjure
- Autografting and allografting are still considered to be the golden standards but they have limited availability, require an additional surgical procedure with potential complications, and are susceptible to immunorejection (Jakob M et al 2012)
- Stem cell transplantation and stem cell/scaffold-guided tissue engineering approaches are emerging as viable alternatives to grafting

Tendon Tissue engineering



Aim of the Study

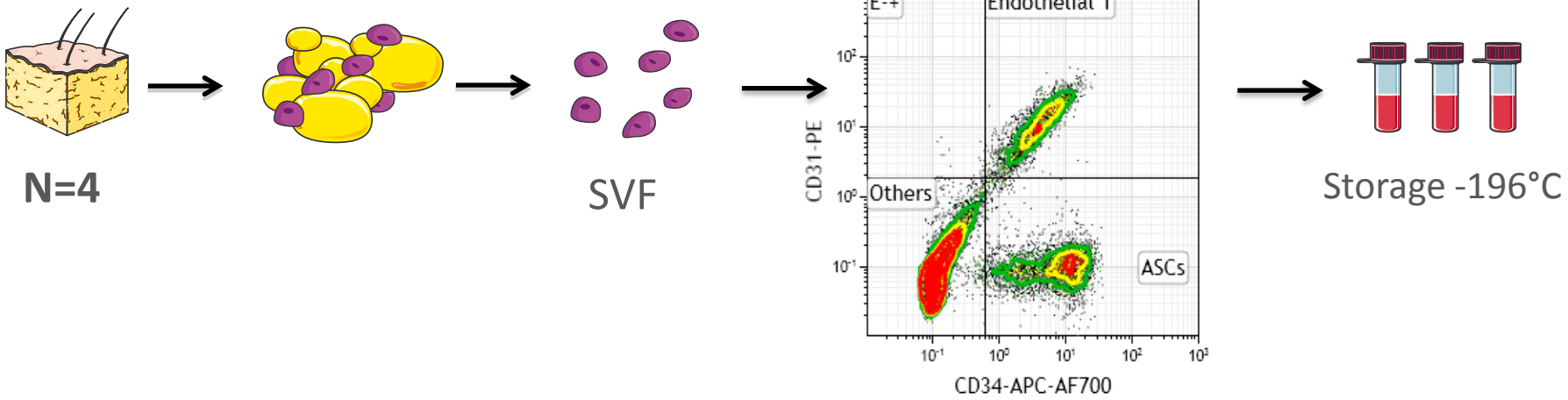


To develop of human ASC-rich tendon patch, GMP-compliant to deliver the regenerative and anti-inflammatory potential of ASCs directly in the site of injury

Q. Are ASCs able to differentiate into tenogenic lineage using a GMP-compliant inductive medium?

Methods

ASCs Isolation & Immunophenotyping



- Nucleated cells: Syto40
- Viability: 7-AAD
- ASCs: CD45(-) CD34(+) CD146 (-)
- Flow-Count Fluorospheres

Minonzio *et al*, 2014

Methods: ASC culture

- **Culture on collagen-coated surfaces to mimic tendon environment**
- **Expansion in CTRL medium** according with the standard GMP-procedure:

  1) Stemulate® Pooled Human Platelet Lysate culture media supplement (**hPL**)

  2) Serum free medium (**SF**) developed in our laboratories constintg of ITS, human albumin, bFGF, TGFb1, PDGF-AB, PDGF-BB supplementation

- **Induction of cells at P4 in hPL or SF TENO medium for 14 days**:

  - CTRL hPL/SF supplemented with

    50 µg/ml Ascorbic acid, 50 ng/ml BMP-12, 100 ng/ml CTGF and 10ng/ml TGFb3

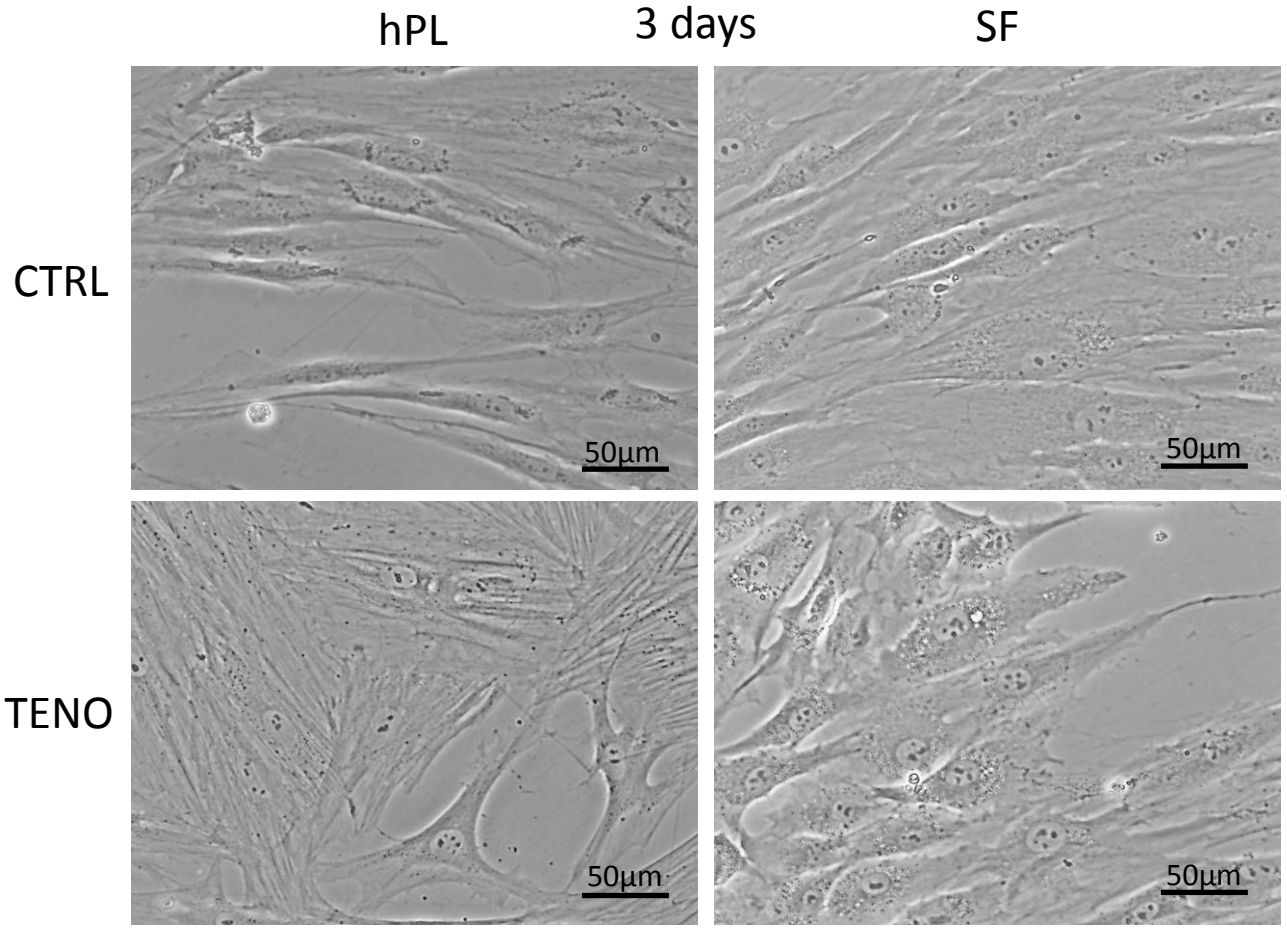
Methods: Evaluations

- Morphological appearance (optical microscopy)
- Expression of tendon related genes (RT-PCR): scleraxis (SCX), collagen type I (COL1A1) and type III (COL3A1), cartilage oligomeric matrix protein (COMP), metalloproteinase 3 and 13 (MMP3 and MMP13)
- Immunofluorescence to detect the transcription factor scleraxis that play a central role in promoting fibroblast proliferation and matrix synthesis in tendons
- Staining to evaluate collagen type 1 deposition

Results

Morphological Appeareance:

- Differentiated cells more rounded and with more cytoplasmatic content vs CTRL



Magnification 20X

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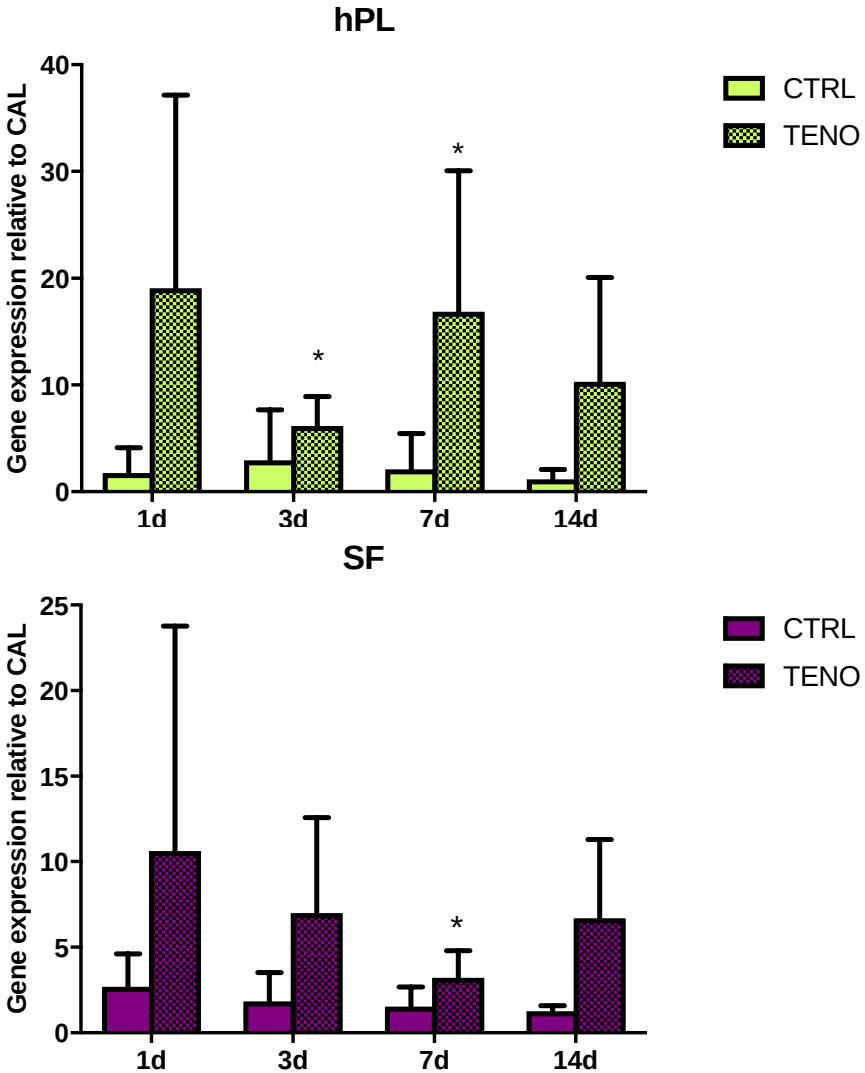
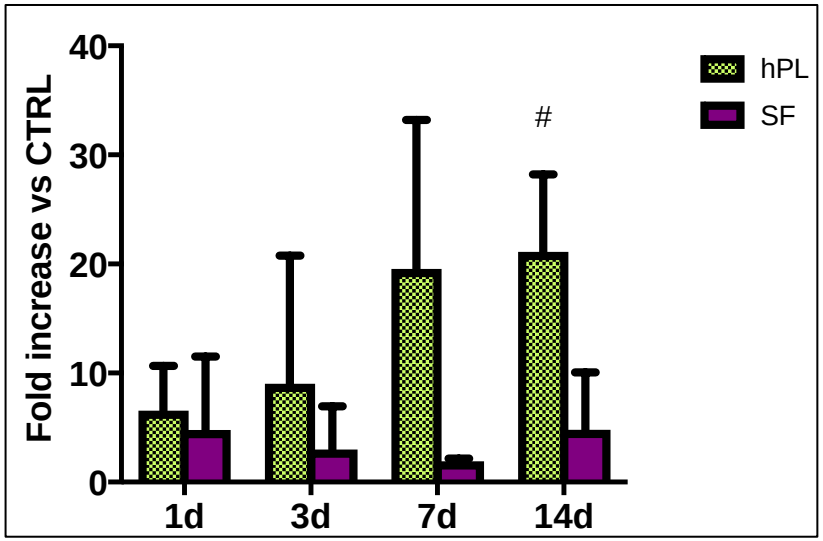
Upregulation of Scleraxis gene expression in both hPL and SF conditions

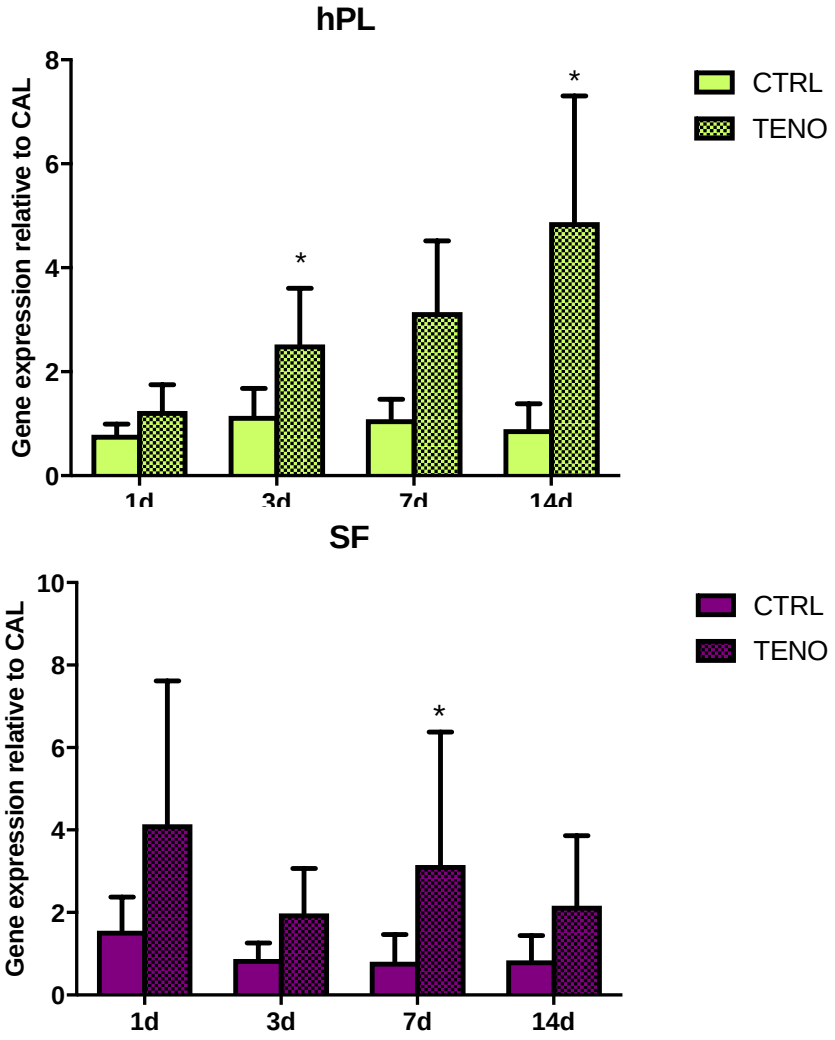


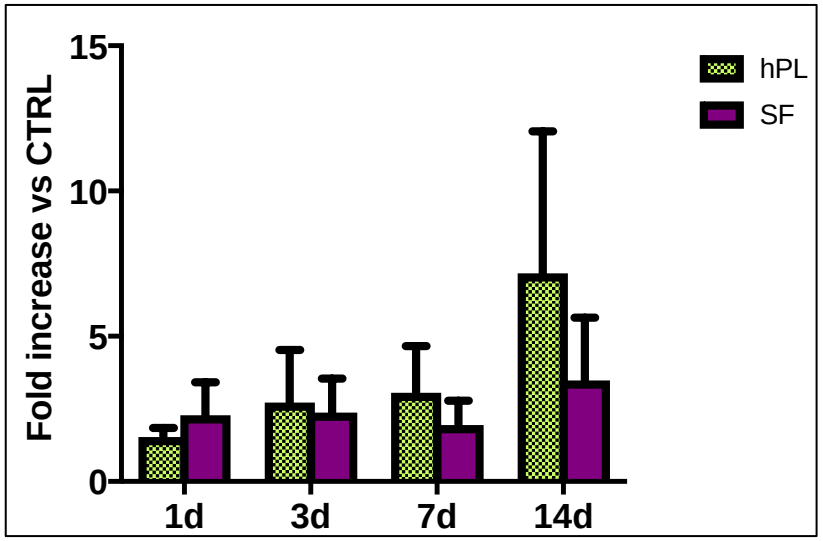


Significance vs CTRL * $p<0.05$; vs SF # $p<0.05$

Swiss Stem Cell Foundation

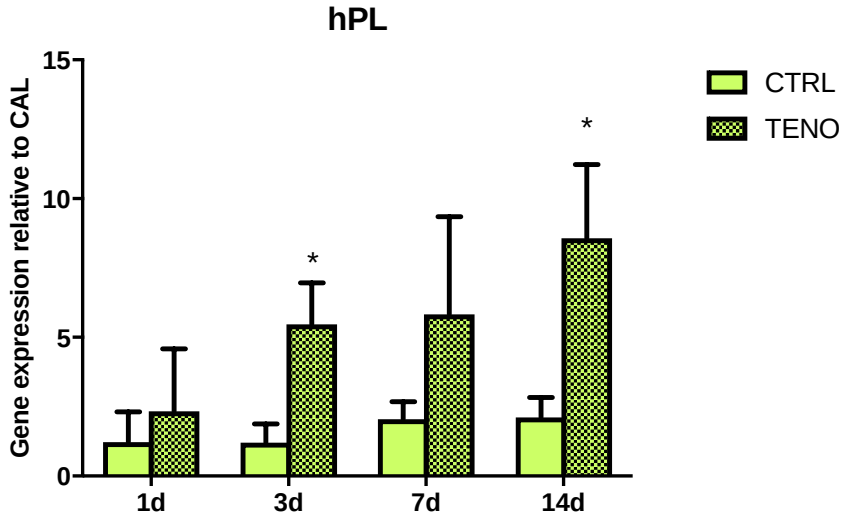
Upregulation of COL1A1 expression in both hPL and SF conditions

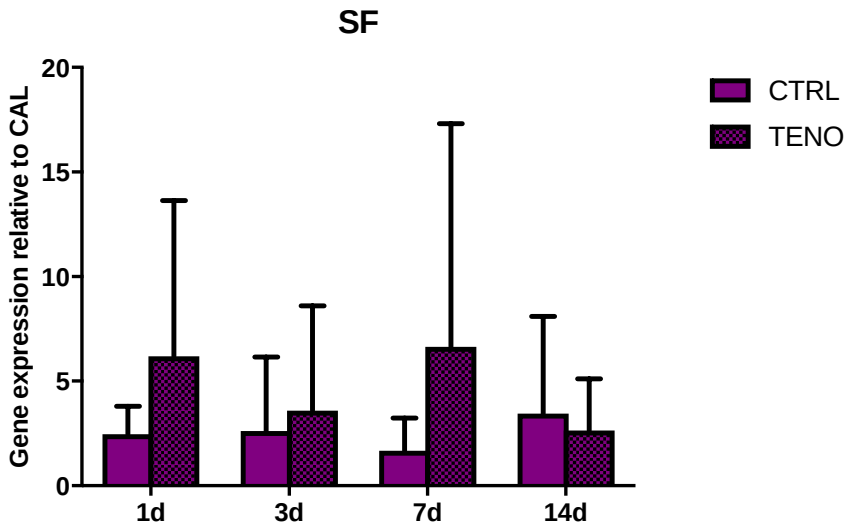


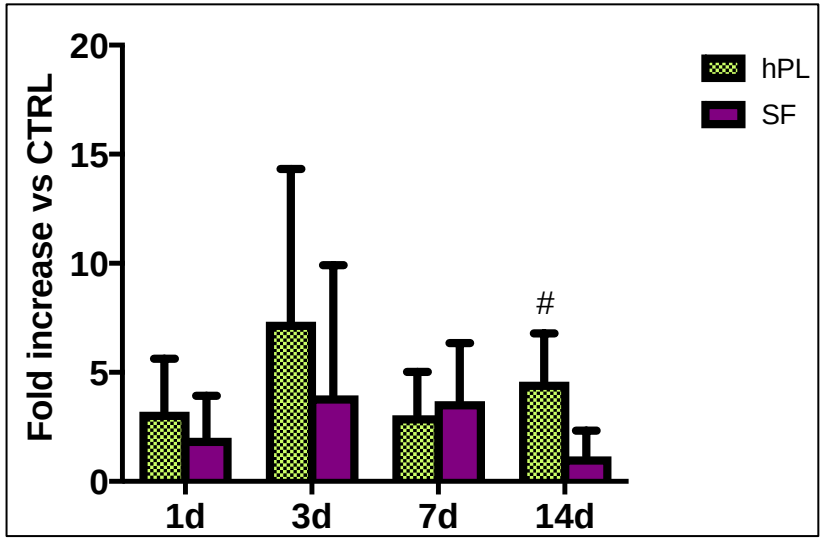


Significance vs CTRL * p<0.05; vs SF # p<0.05

Upregulation of COL3A1 expression in both hPL and SF conditions

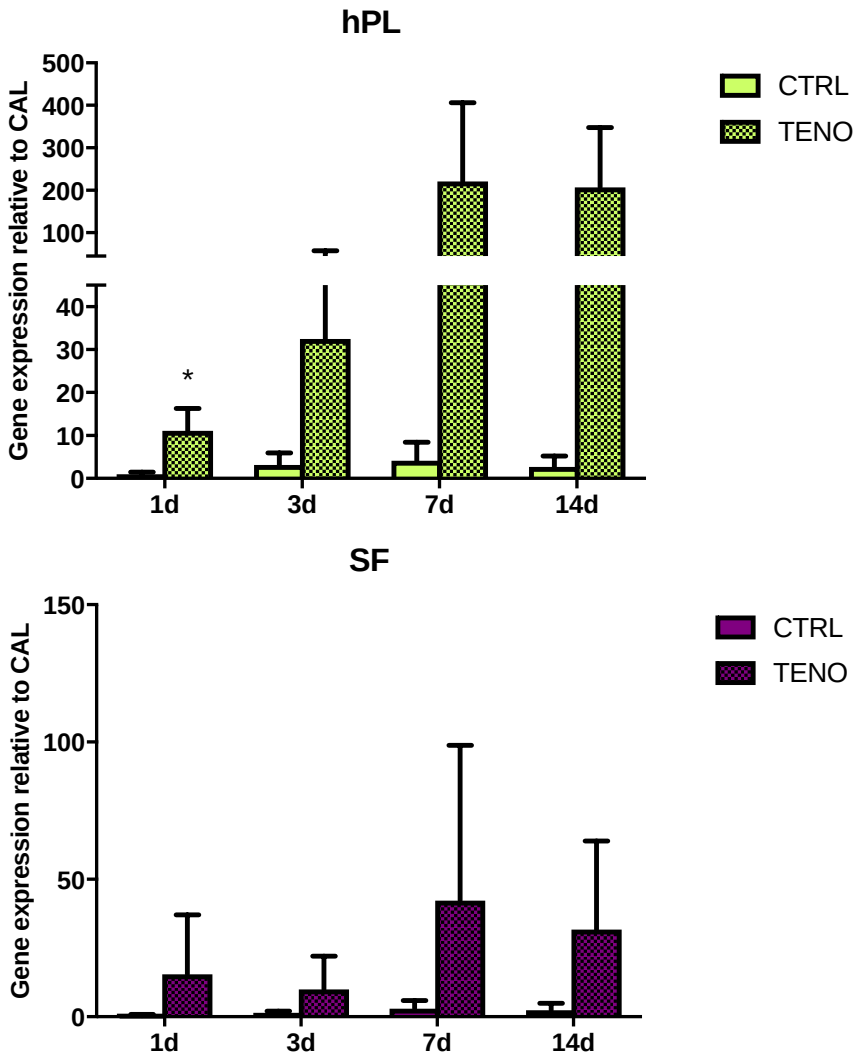


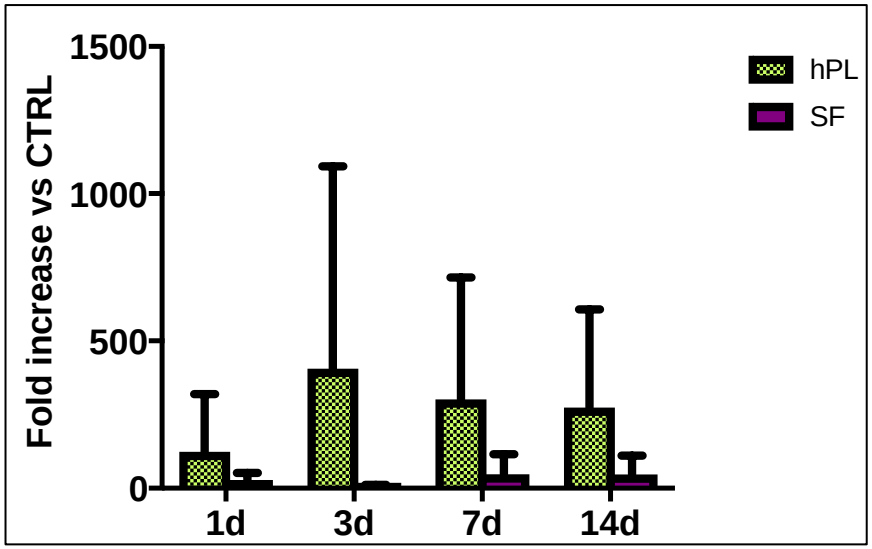




Significance vs CTRL * $p<0.05$; vs SF # $p<0.05$

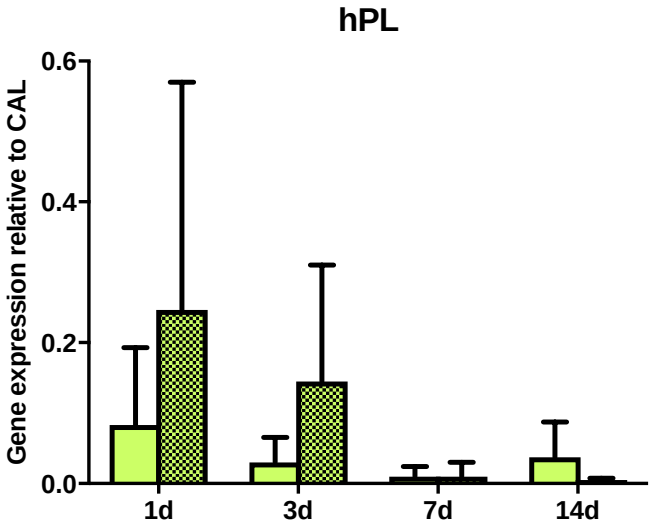
Upregulation of COMP expression in both hPL and SF conditions

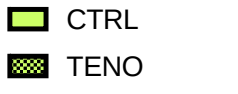


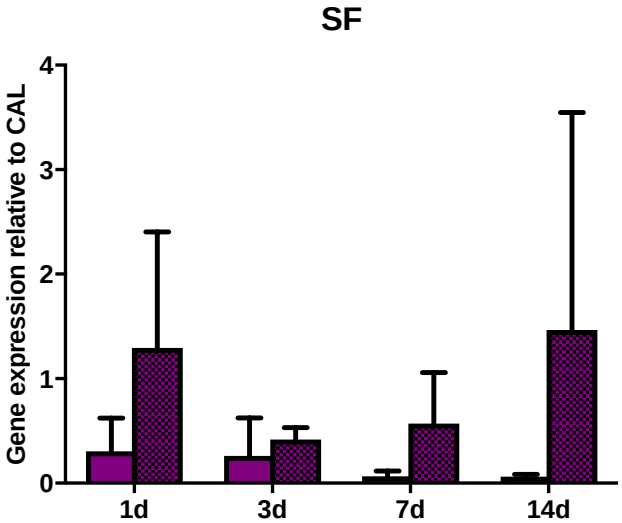


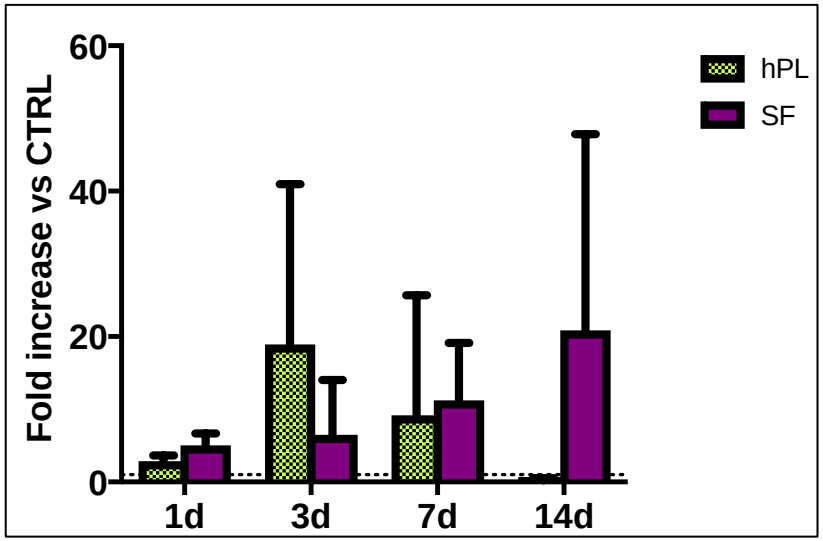
Significance vs CTRL * p<0.05; vs SF # p<0.05

Upregulation of MMP3 expression in both hPL and SF conditions

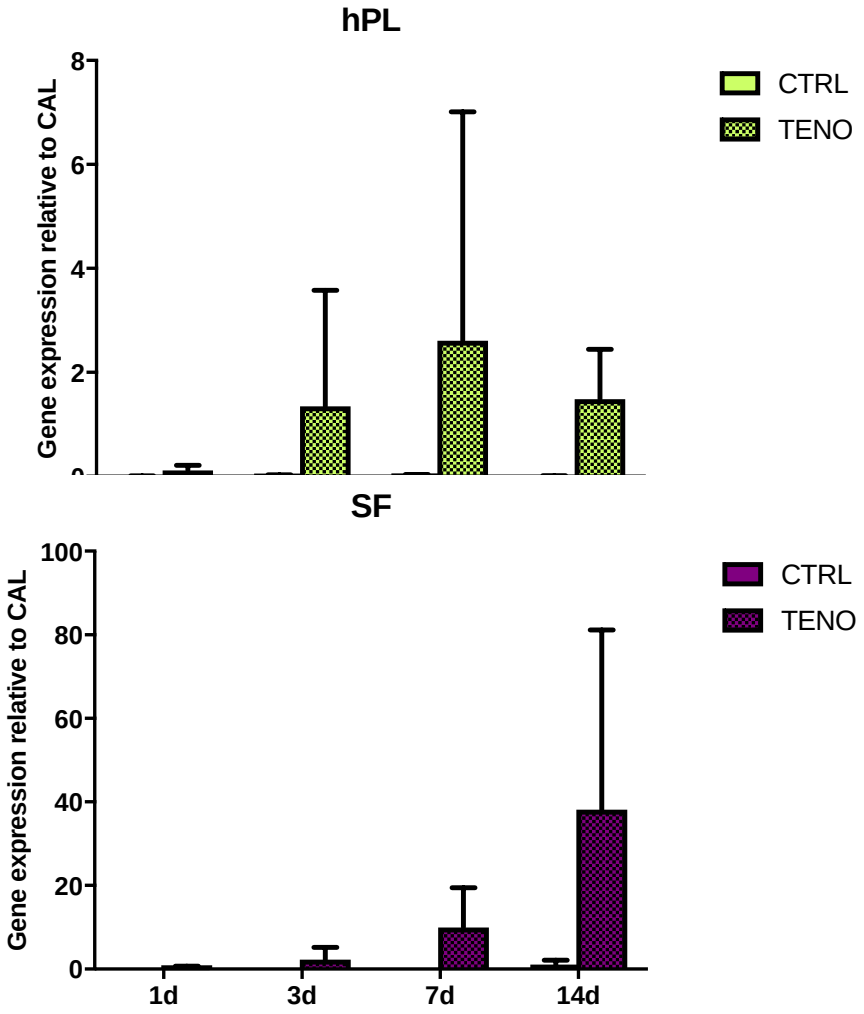


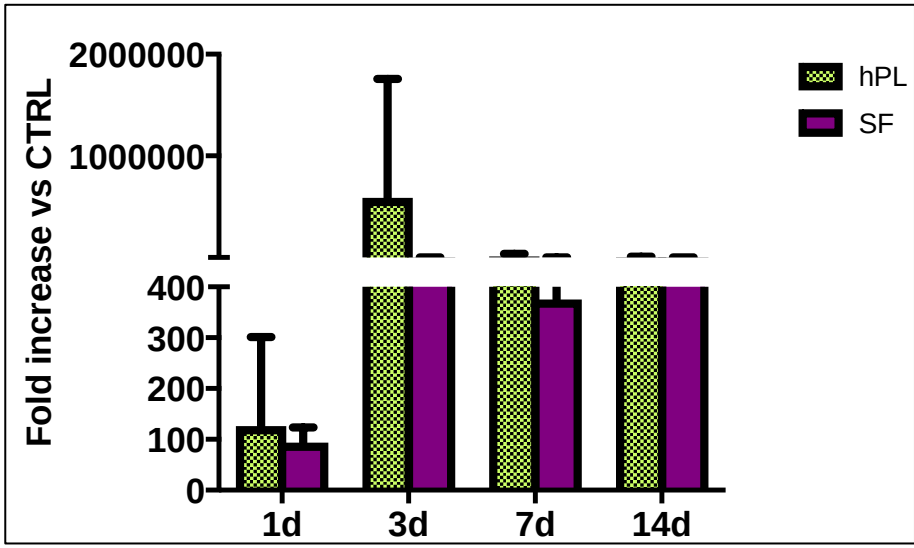






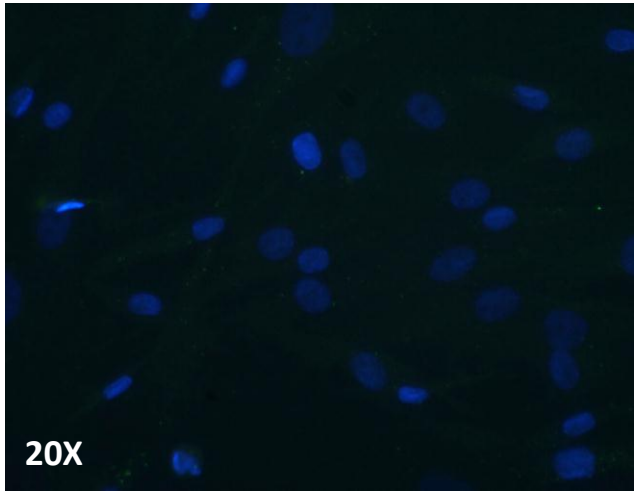
Upregulation of MMP13 expression in both hPL and SF conditions

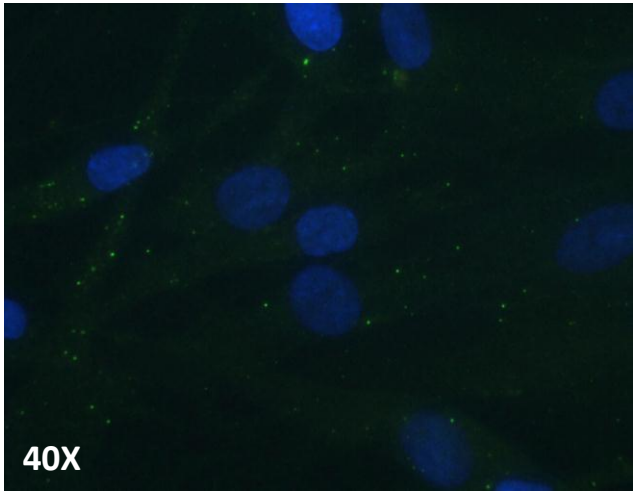




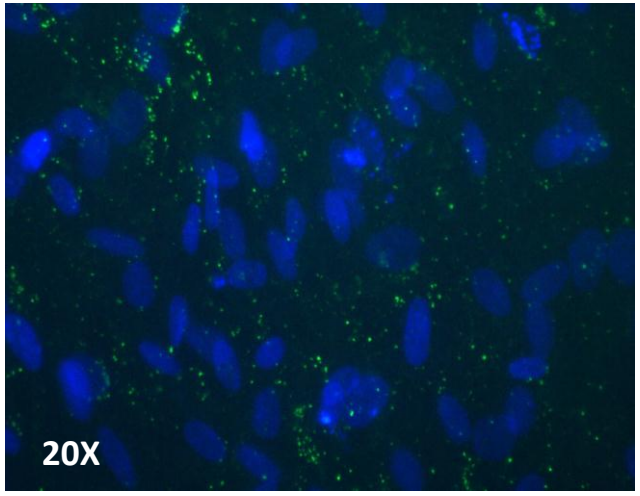
TENO hPL show SCX protein expression after 3 days of induction

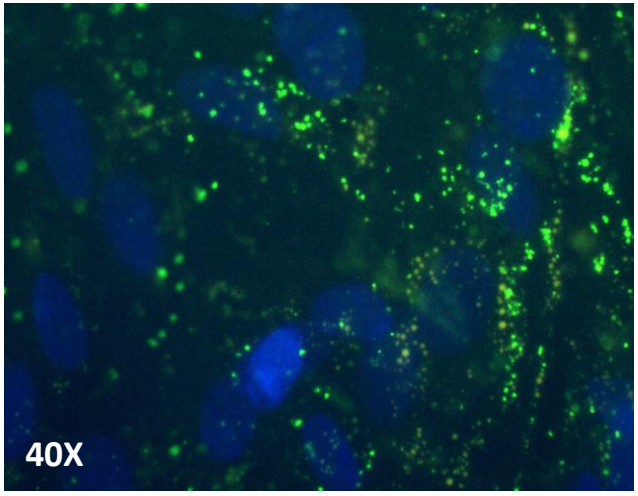
CTRL





TENO





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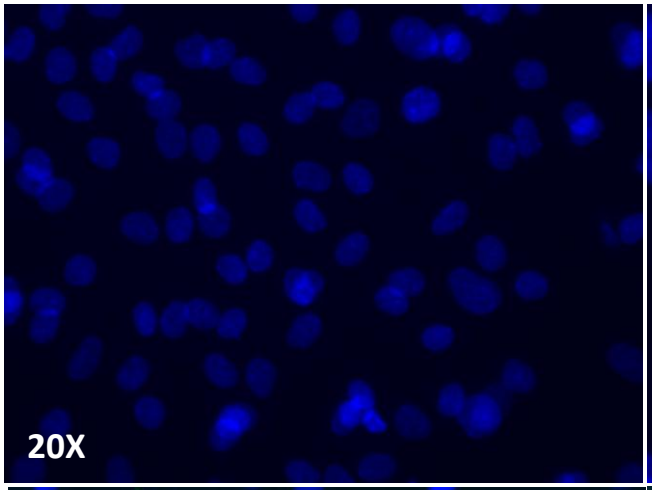
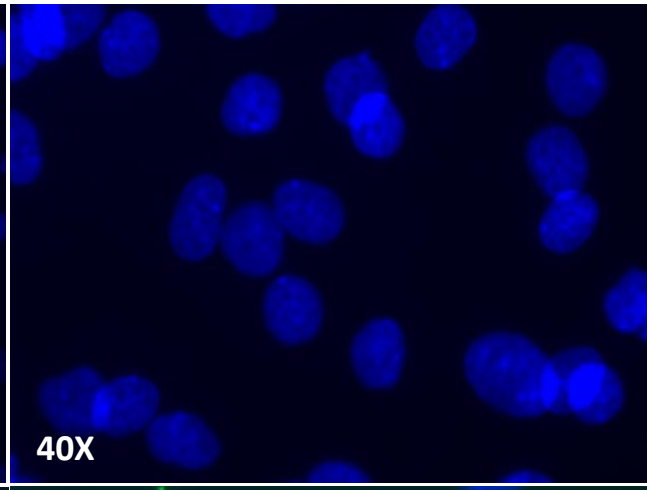
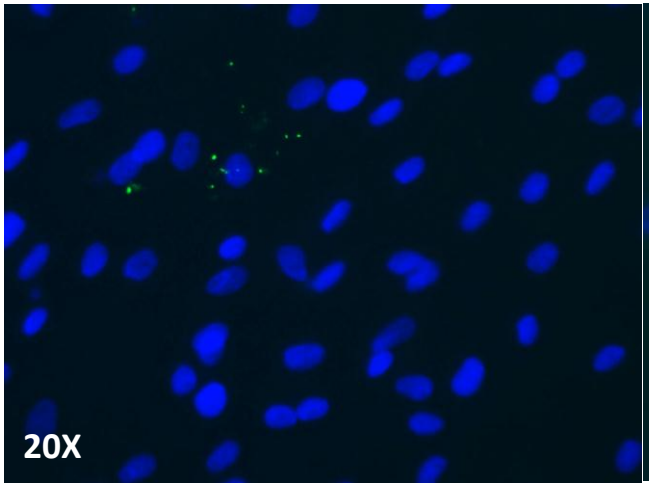
TENO SF show lower amount of SCX protein expression after 3 days of induction than TENO hPL

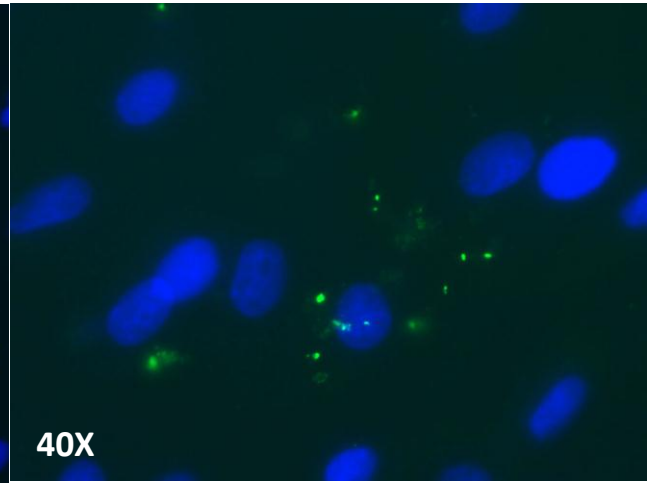
CTRL



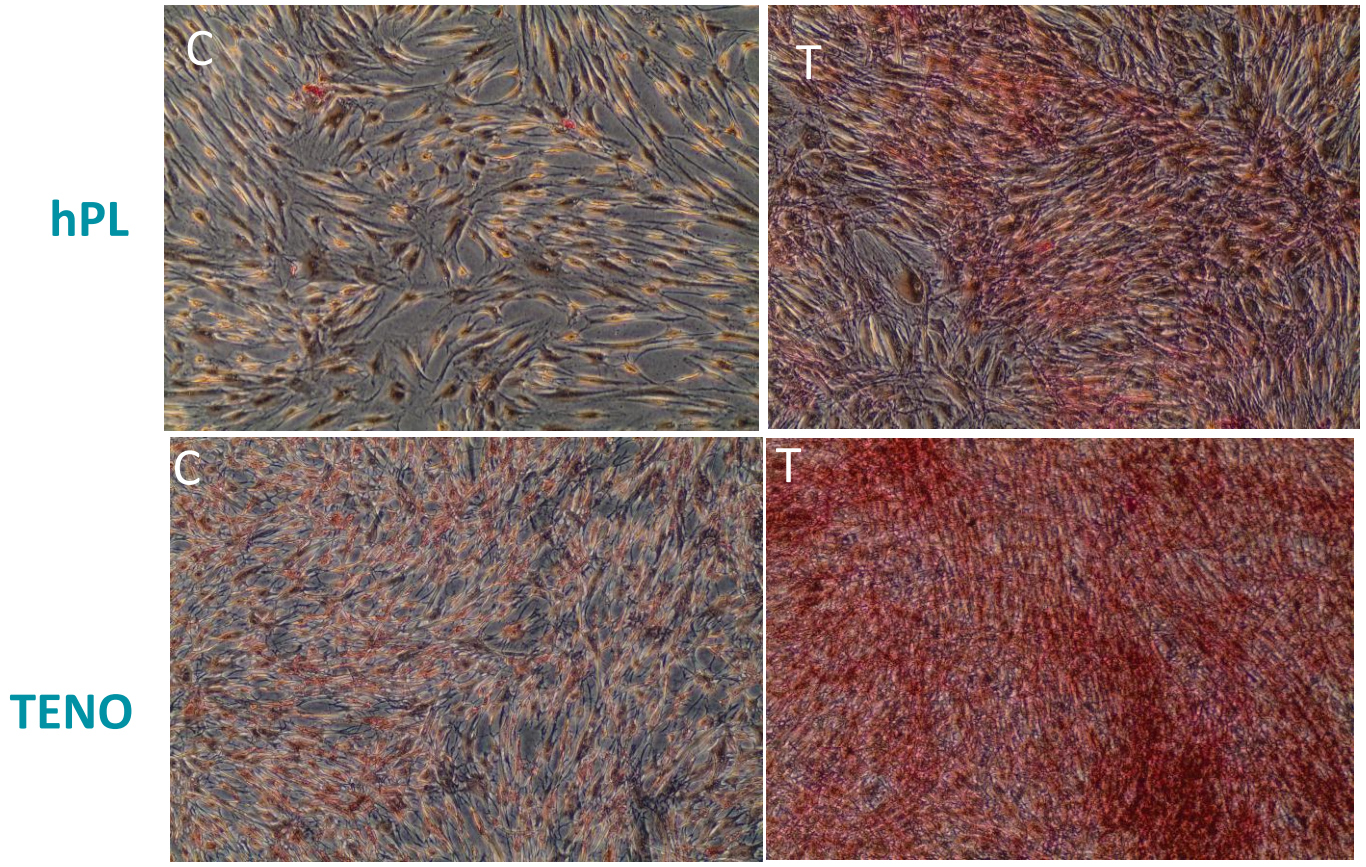


TENO





Collagene type I deposition at 7 days



Magnification 4X

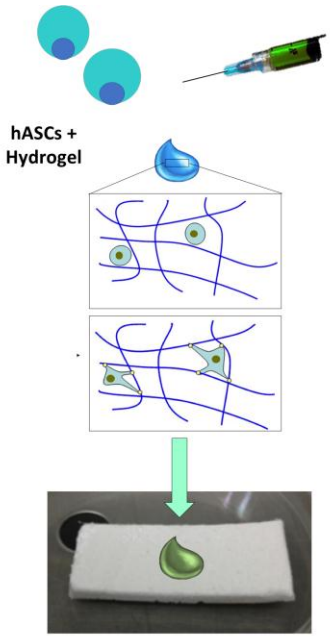
Conclusions

- ASCs expressed tendon related markers in both hPL and SF medium supplement with ascorbic acid and BMP-12, TGF-β3 and CTGF growth factors

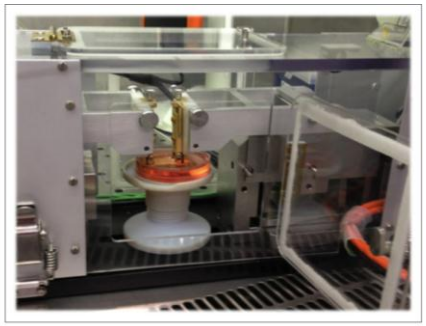
- High inter-donor variability: Sample size

Next steps

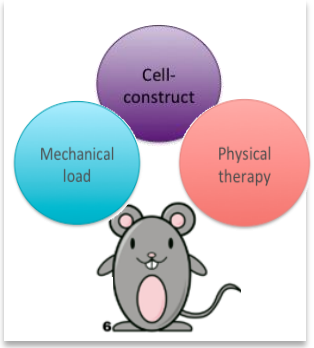
- Evaluation of tenogenic differentiation of ASCs in dynamic 3D condition using STIMIOTE bioreactor and physical therapy before to going to preclinical study



ASC-rich Tendon Patch



STIMIOTE

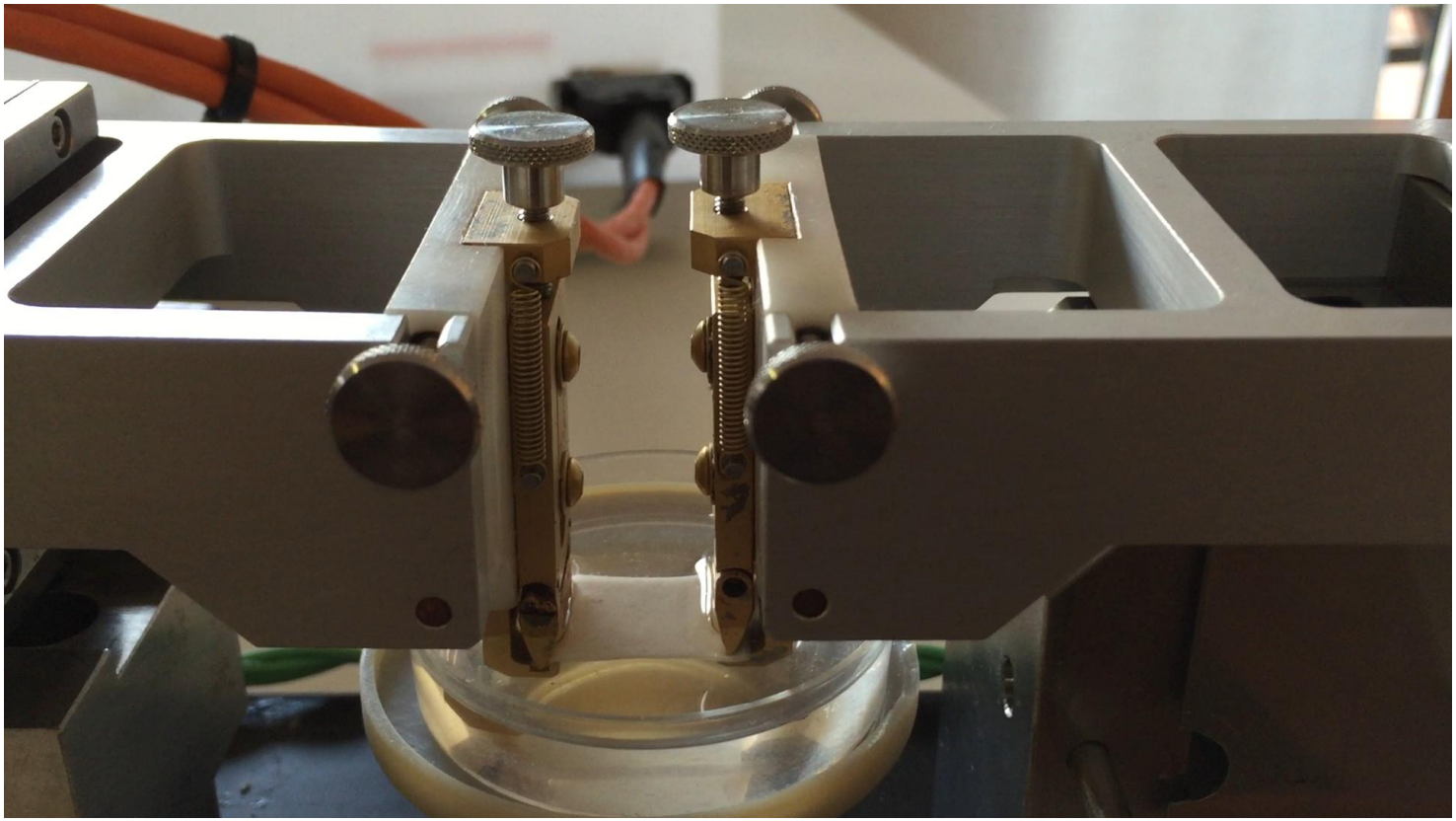


In vivo model

STIMIOTE

A medical device designed to generate a cyclical mechanical extension of a scaffold seeded with ASCs

STIMIOTE



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