

Kevin BOURGE

# Contribution of epitranscriptomics in the characterization of chondrogenic differentiation of synovial fluid MSCs



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UMR 7365 CNRS – Université de Lorraine

Director: Jean-Yves JOUZEAU

***Team 5 : Bio-ingénierie, Médecine Régénérative et Caractérisation Tissulaire***

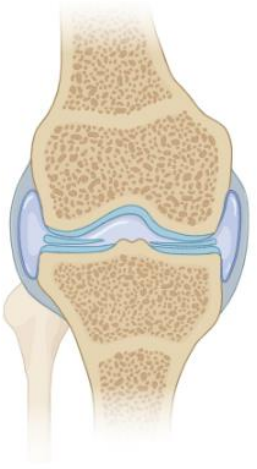
Supervised by:

Pierre GILLET (PU-PH1, HDR)

Astrid PINZANO (DR2 CNRS, HDR)



# Introduction

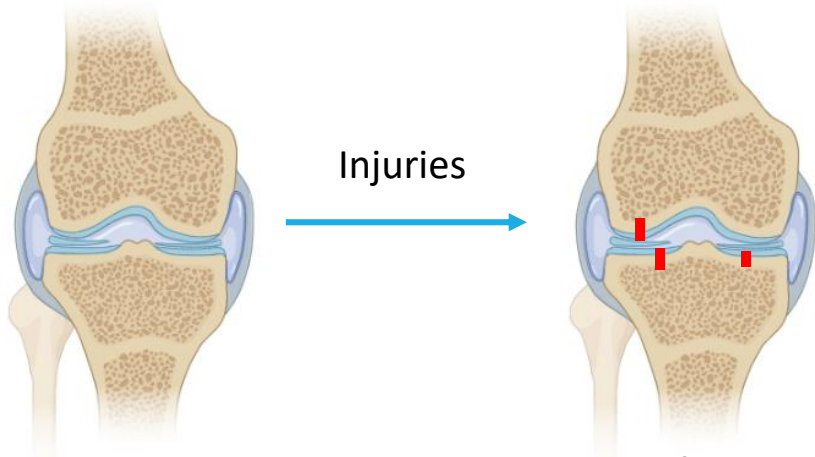


### Articular cartilage

- Aneural
- Avascular



*Low self  
reparation  
capacity*

**Articular cartilage**

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- Avascular



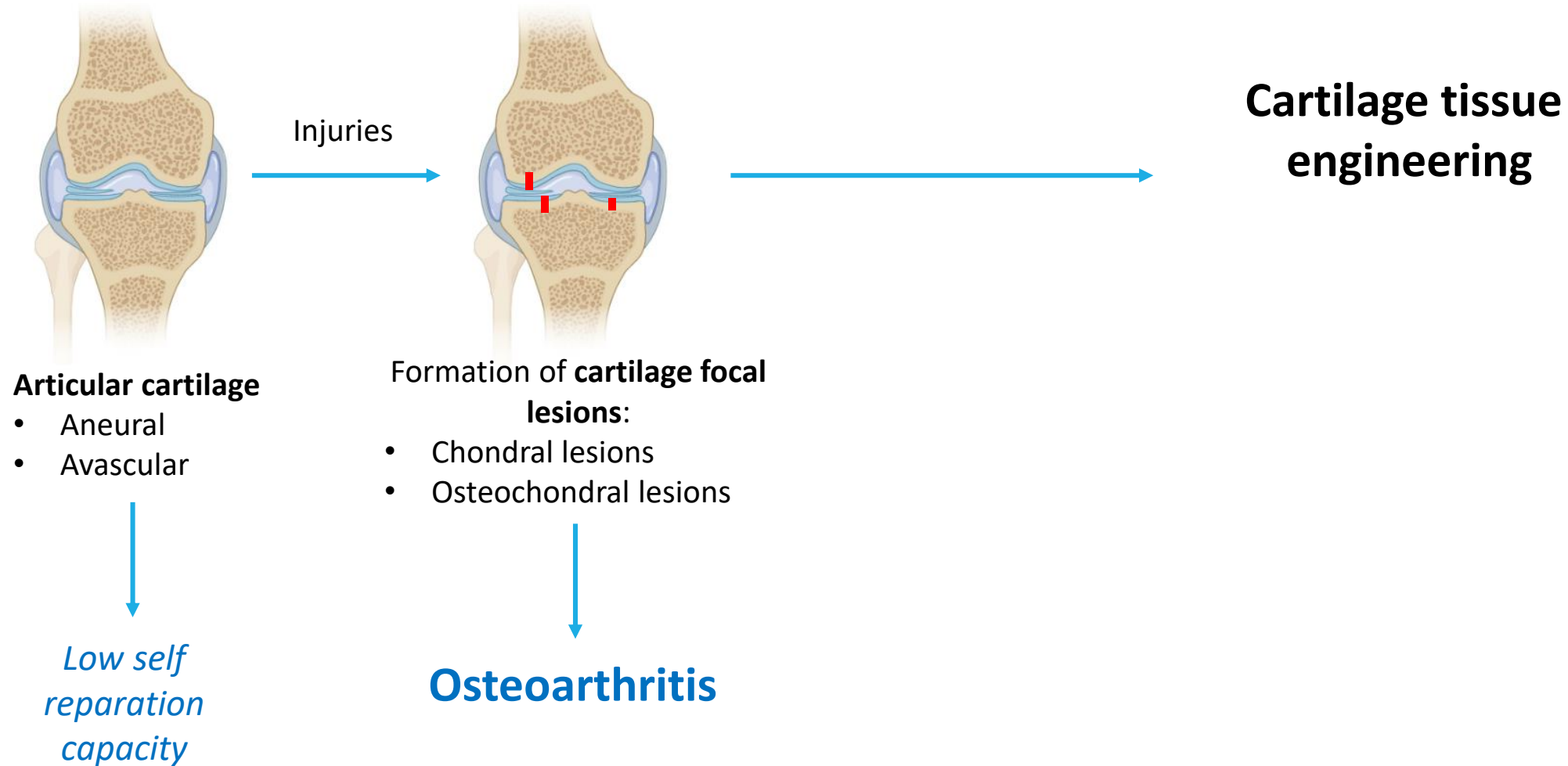
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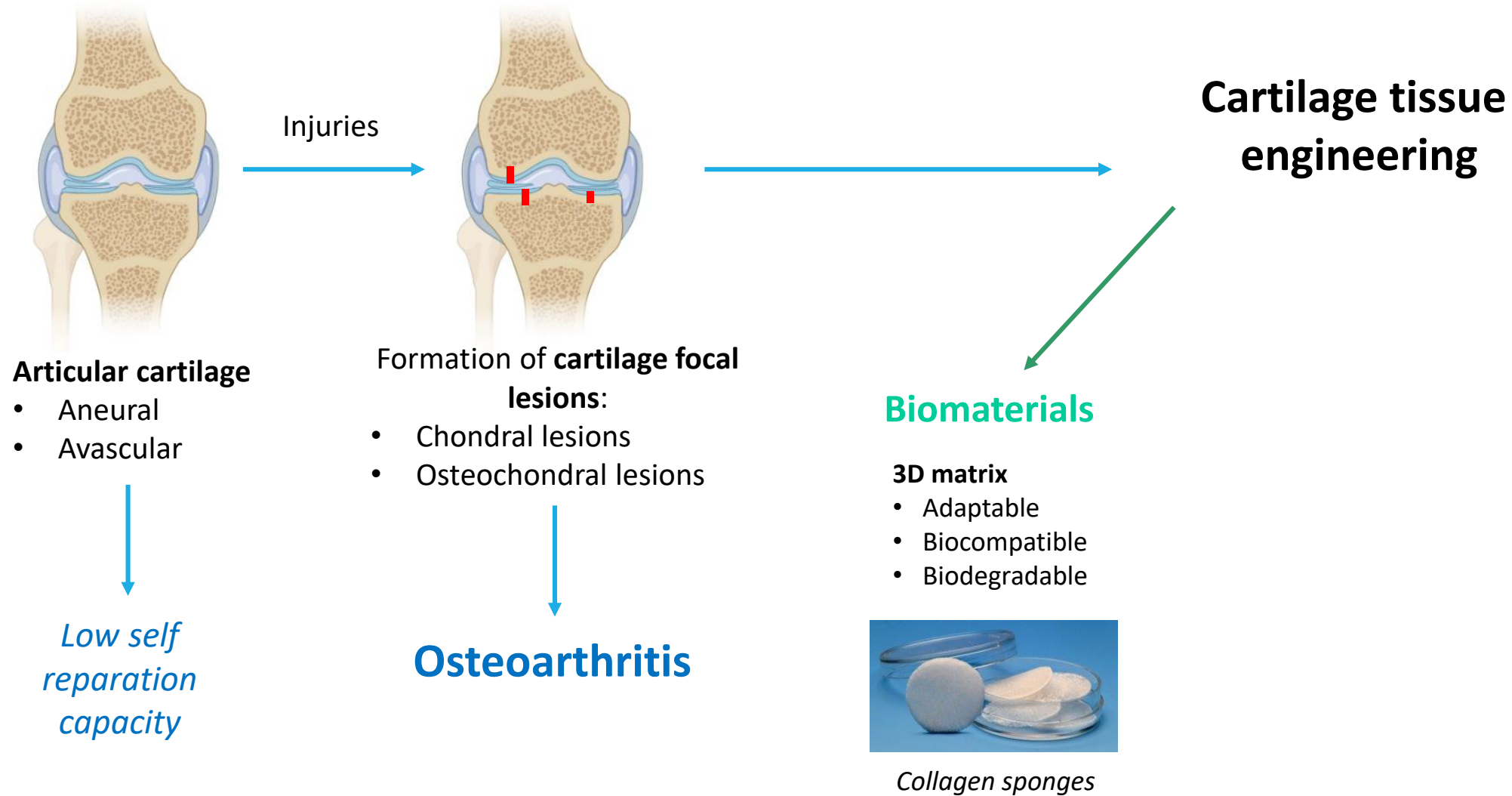
**Formation of cartilage focal  
lesions:**

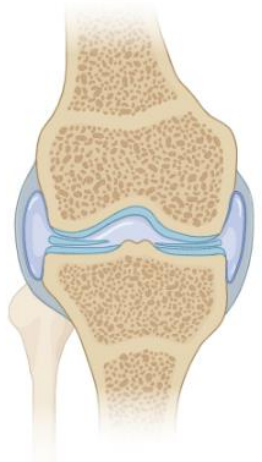
- Chondral lesions
- Osteochondral lesions



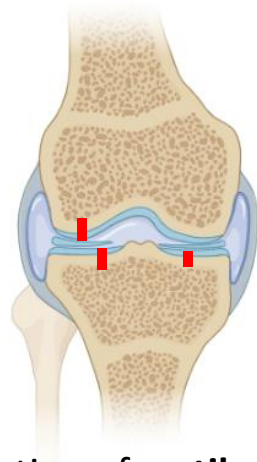
**Osteoarthritis**







Injuries



**Articular cartilage**

- Aneural
- Avascular



*Low self repair capacity*

Formation of **cartilage focal lesions:**

- Chondral lesions
- Osteochondral lesions



**Osteoarthritis**

**Cartilage tissue engineering**

**Biomaterials**

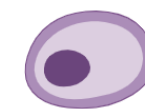
**3D matrix**

- Adaptable
- Biocompatible
- Biodegradable



*Collagen sponges*

**Cells**

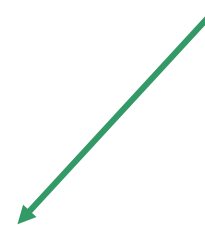


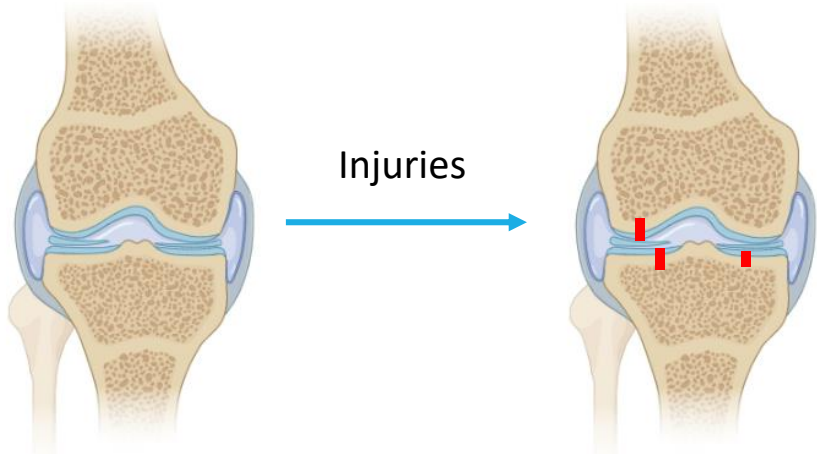
**Chondrocytes**

- Limited accessibility
- Dedifferentiation in monolayer culture



**Mesenchymal Stem Cells (MSCs)**





Injuries

**Articular cartilage**

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**Mesenchymal Stem Cells (MSCs)**

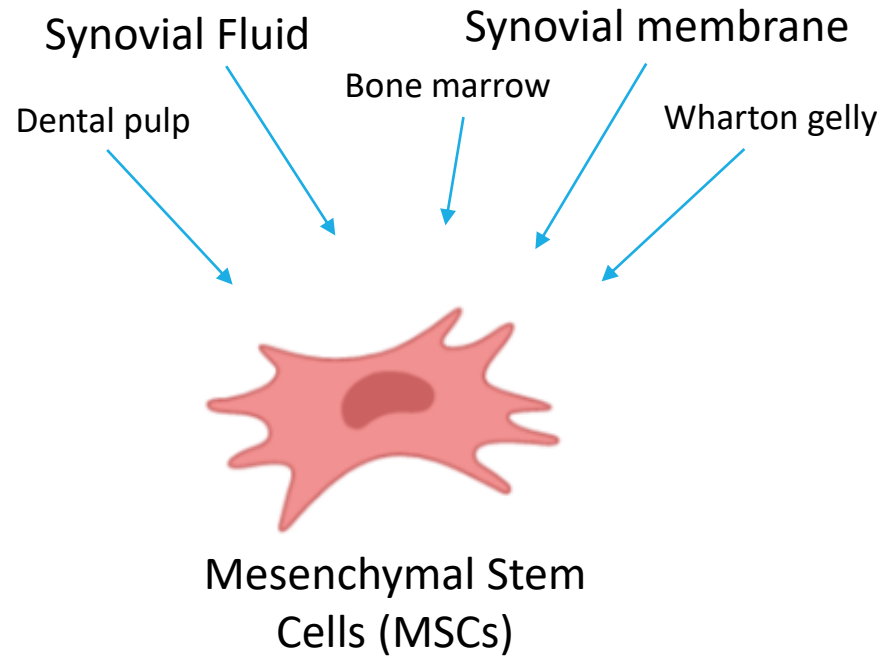
**Environment**

**Growth factors**  
(TGF-β1, BMP-2)

**Oxygen levels**

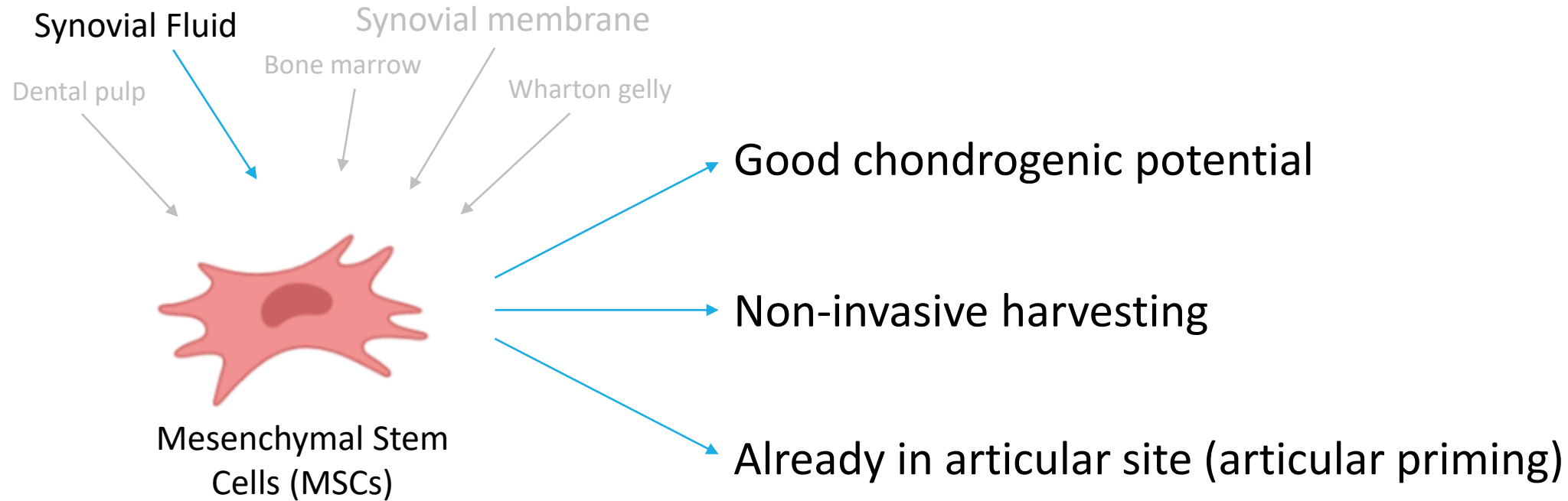


## MSCs sources

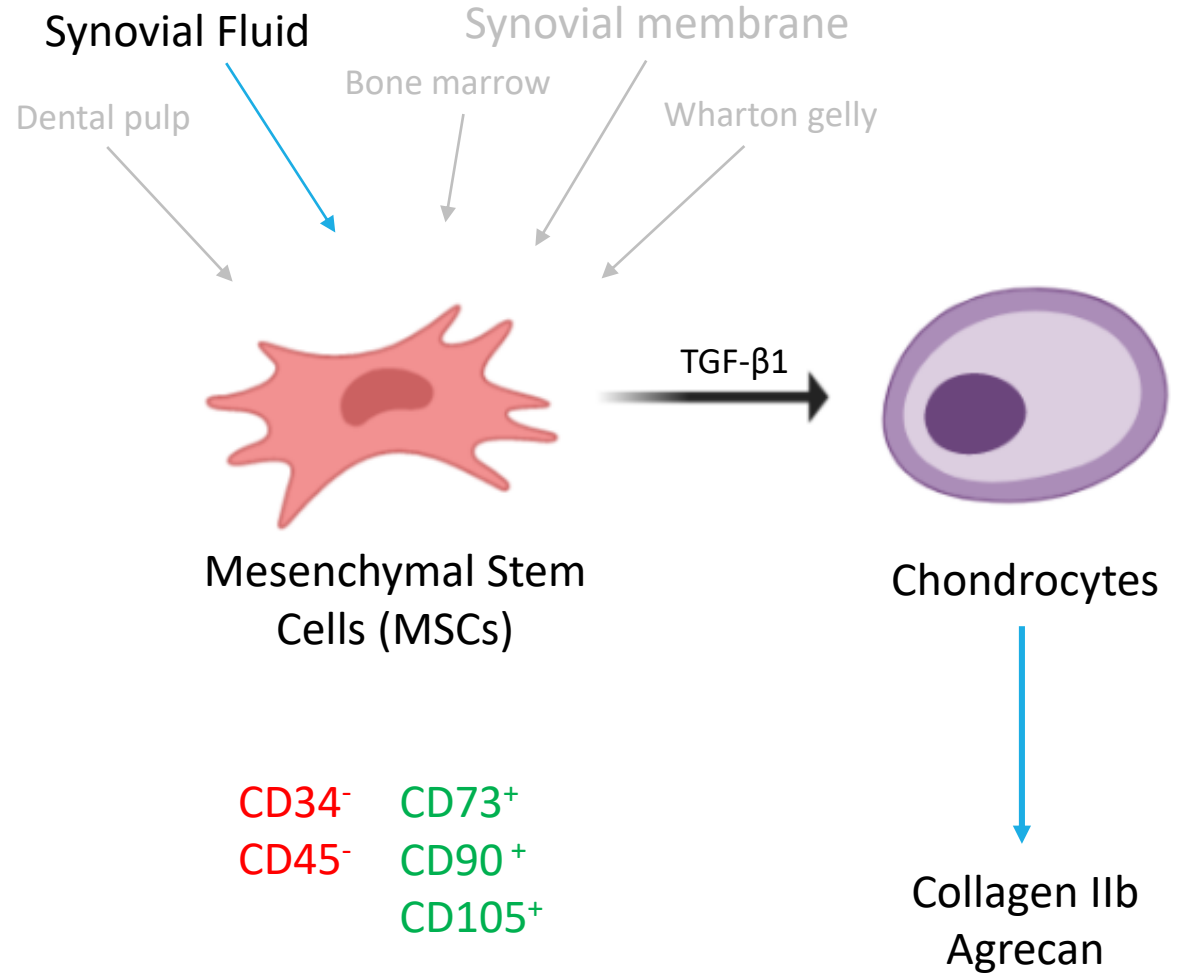


CD34<sup>-</sup>    CD73<sup>+</sup>  
CD45<sup>-</sup>    CD90<sup>+</sup>  
            CD105<sup>+</sup>

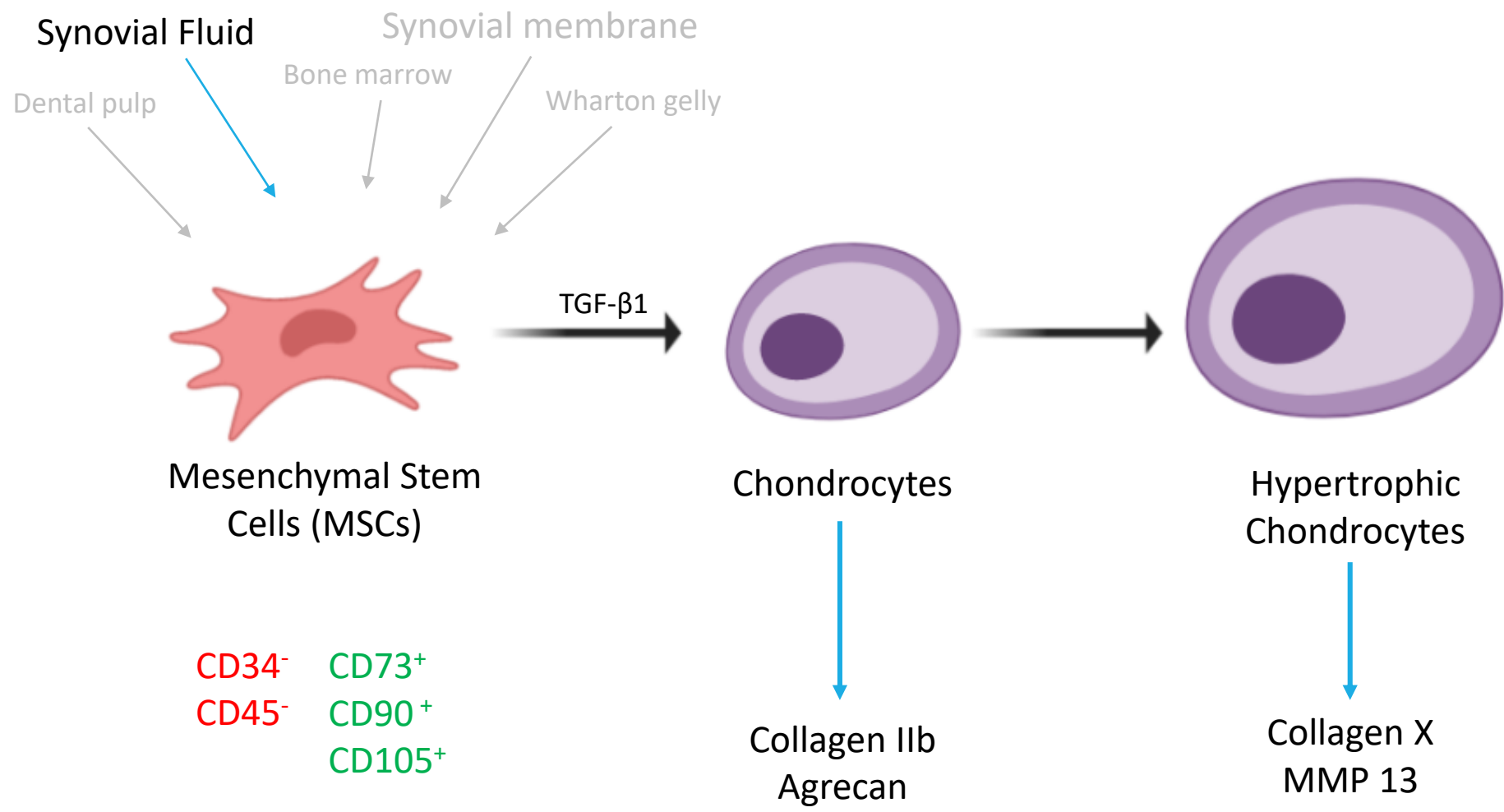
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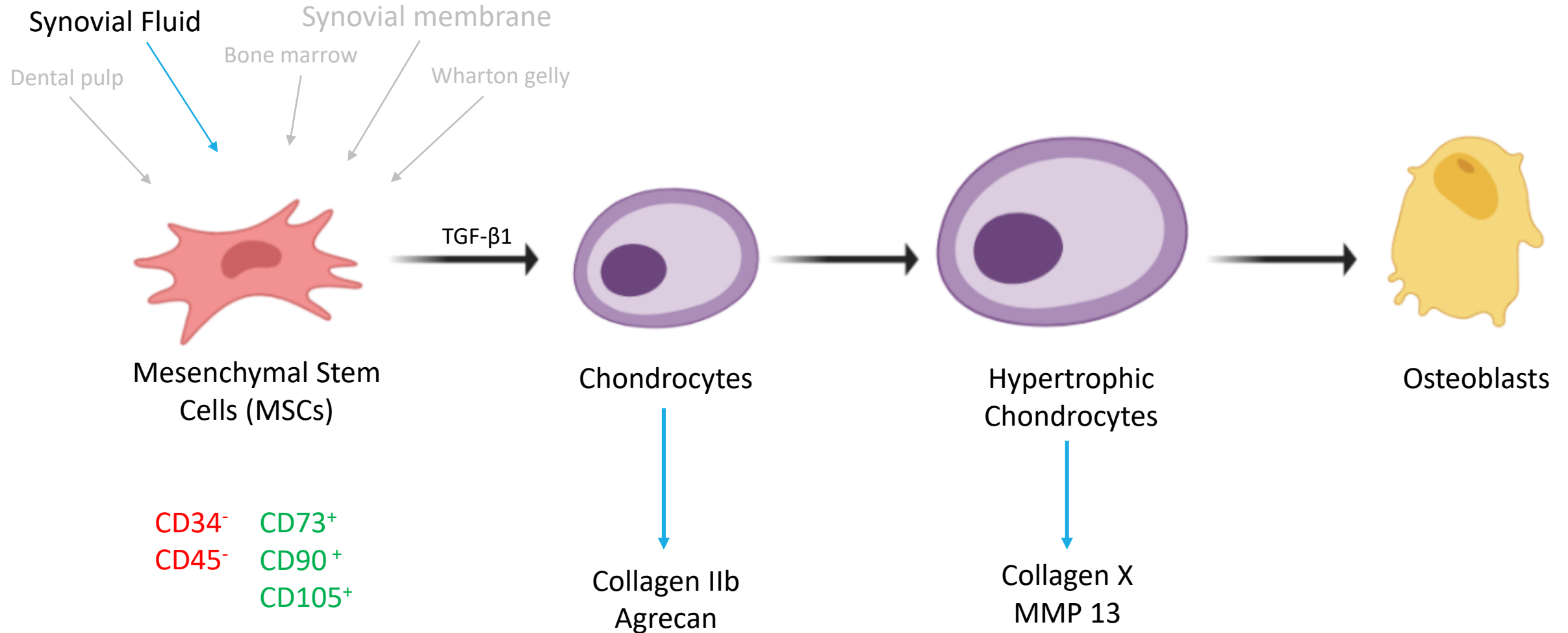
### MSCs sources



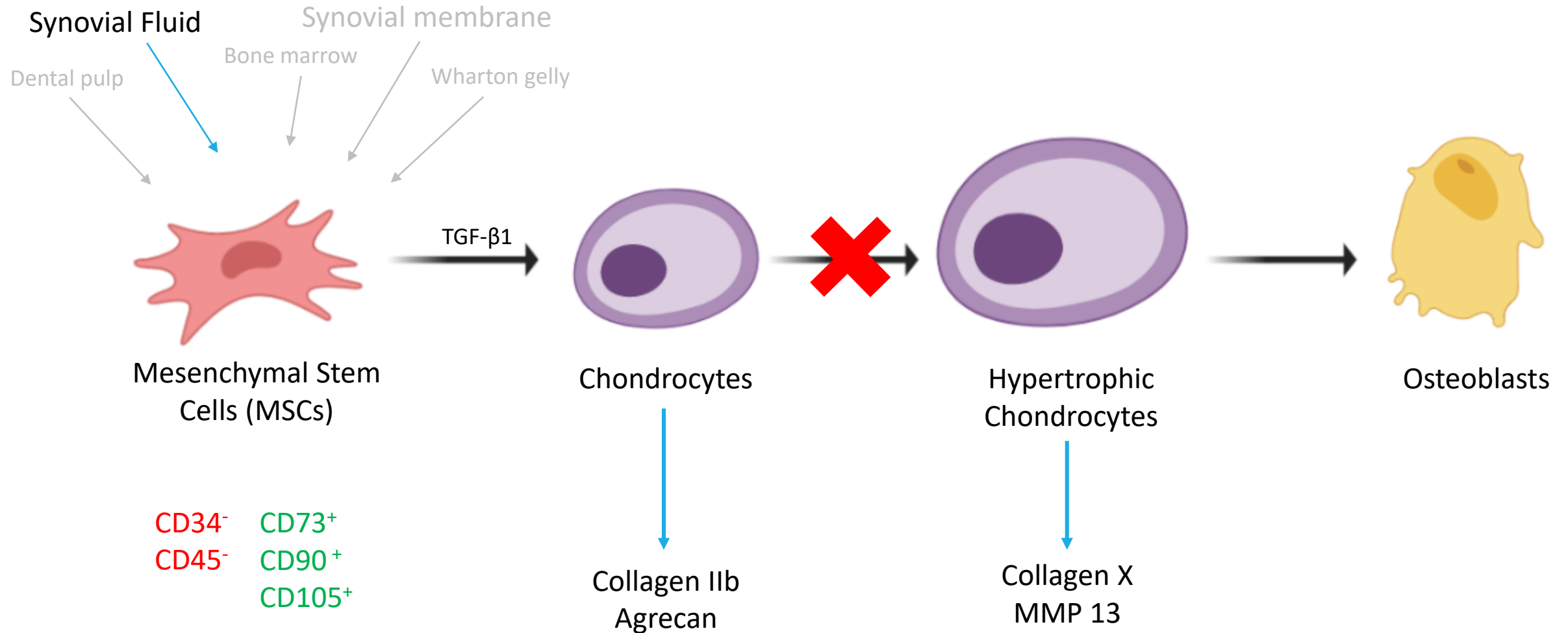
### MSCs sources



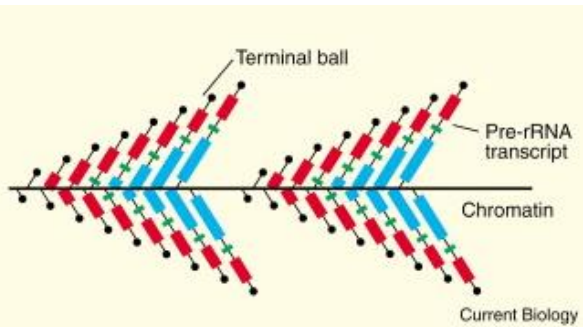
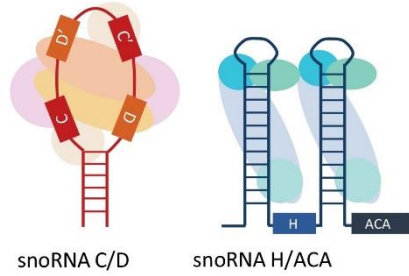
## MSCs sources



## MSCs sources

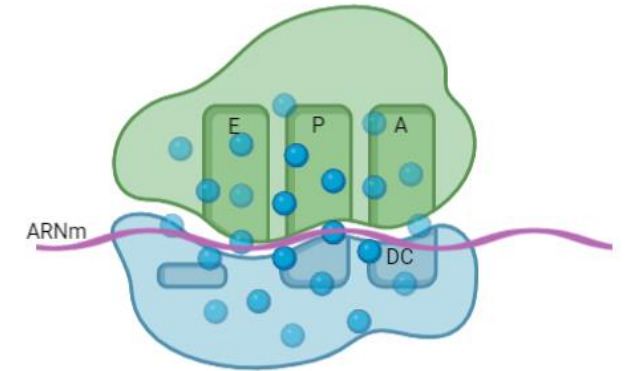
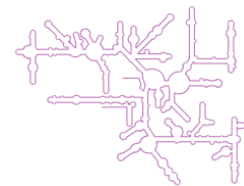
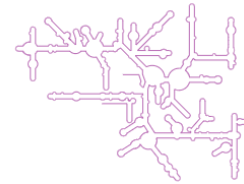


### 2'O methylations and pseudouridynilations

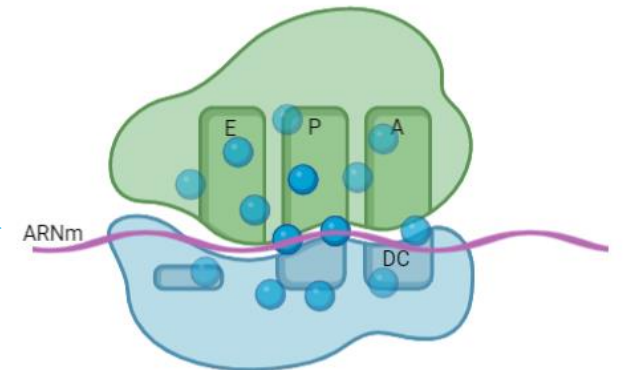


rRNA maturation and chemical modifications

Differential epitranscriptomic modifications



Specialized ribosome



→ Methylations: influences rRNA structure and interactions

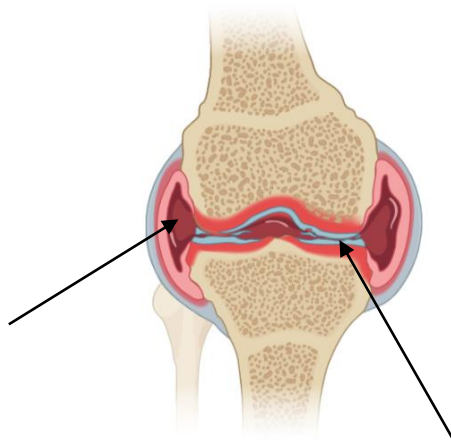


# Objectives



## Cells sources

Synovial fluid  
MSCs



Native cartilage cells  
Chondrocytes

### Innovative source of MSCs

- Little studied yet
- Easy to harvest
- On articular site



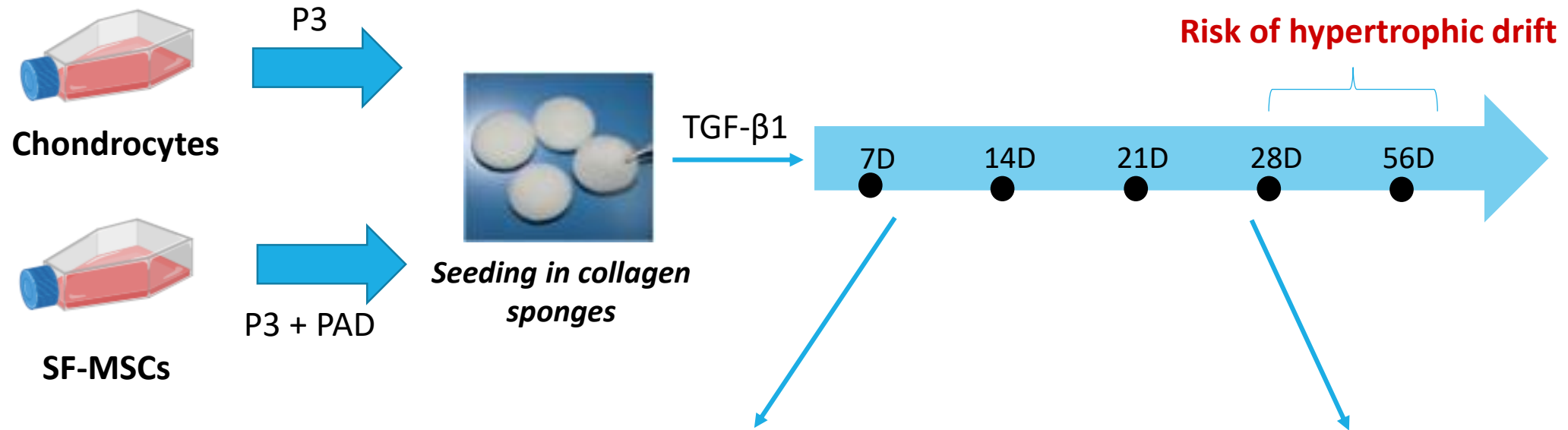
## Objectifs



We aimed to characterize SF-MSCs VS chondrocytes using a classic chondrogenic index completed by an epitranscriptomic mapping along their chondrogenic differentiation to identify new markers.



# Material and methods



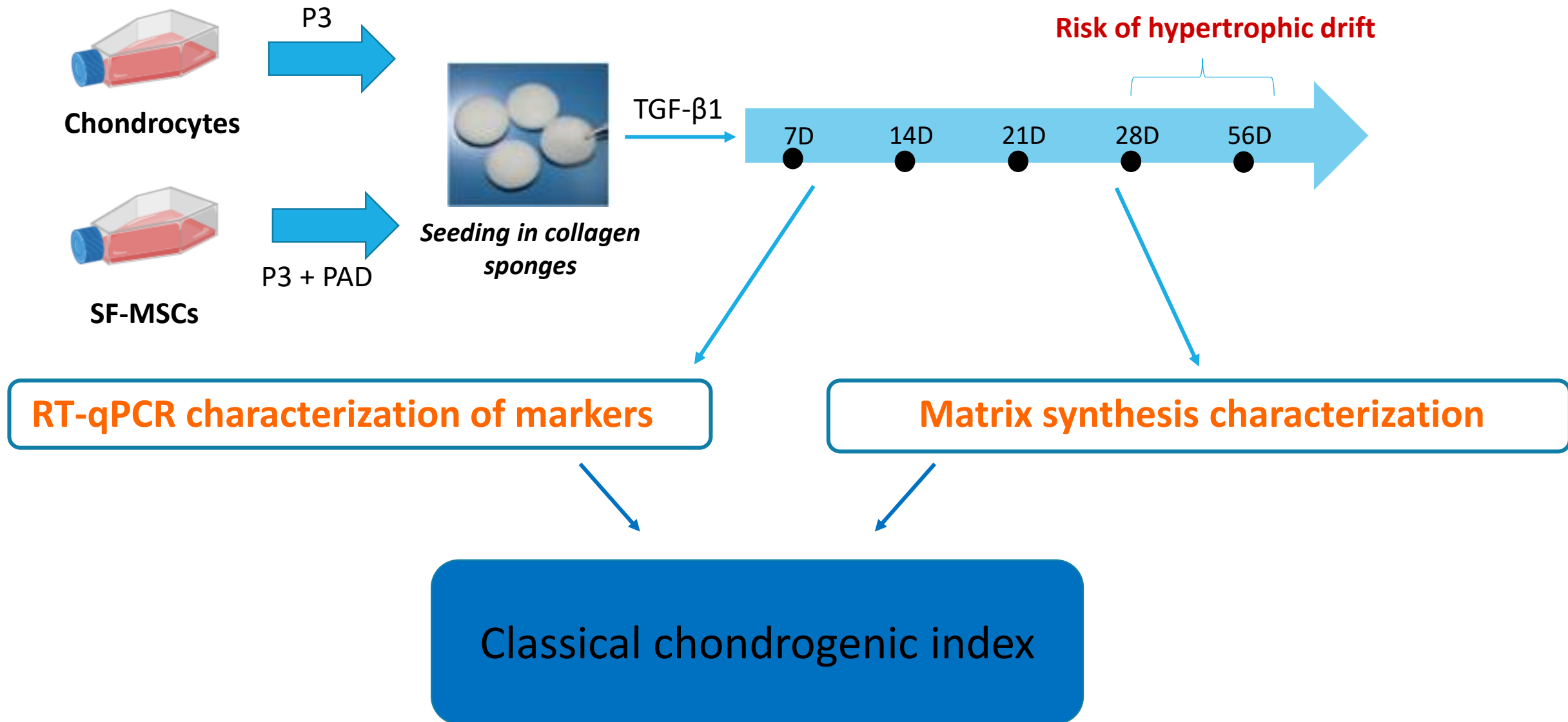
### RT-qPCR characterization of markers

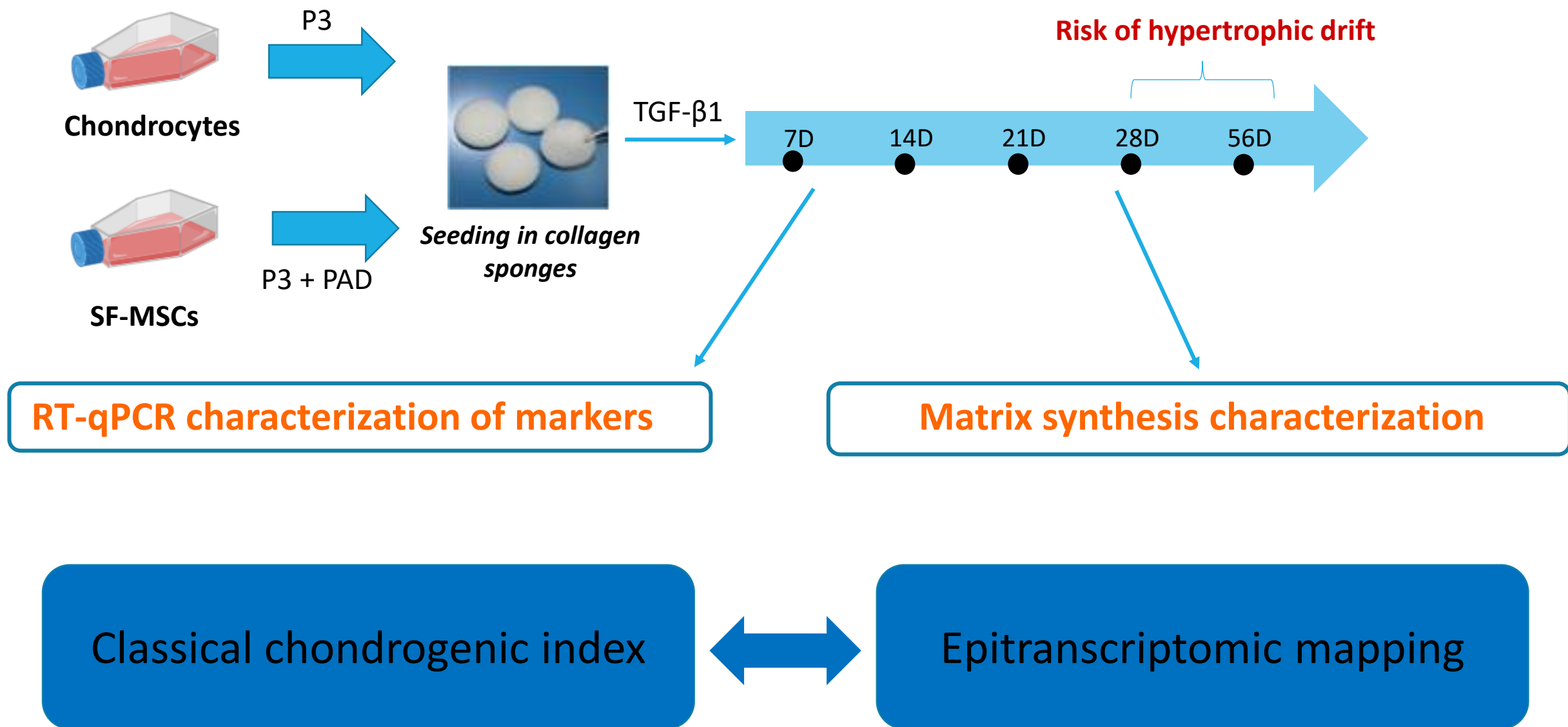
- **Chondrogenic** (*COL2*, *ACAN*, *SOX9*)
- **Fibrotic** (*COL1*)
- **Hypertrophic** (*COL10*)
- **Ostéogenic** (*RUNX2*)

→ Comparison with P0 chondrocyte

### Matrix synthesis characterization

- **Alcian blue** staining (*GAGs*)
- **Sirius red** staining (*Fibrillar Collagens*)
- **Immunohistochemistry** (Type II Collagen and Agrecan)

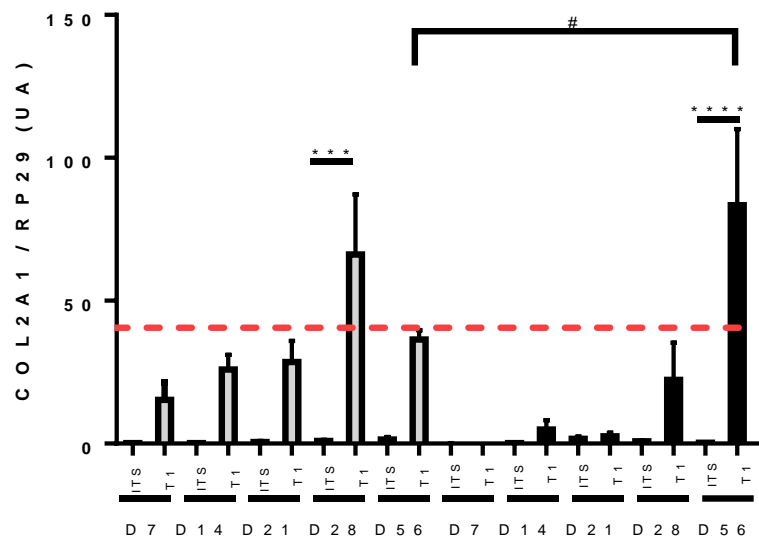




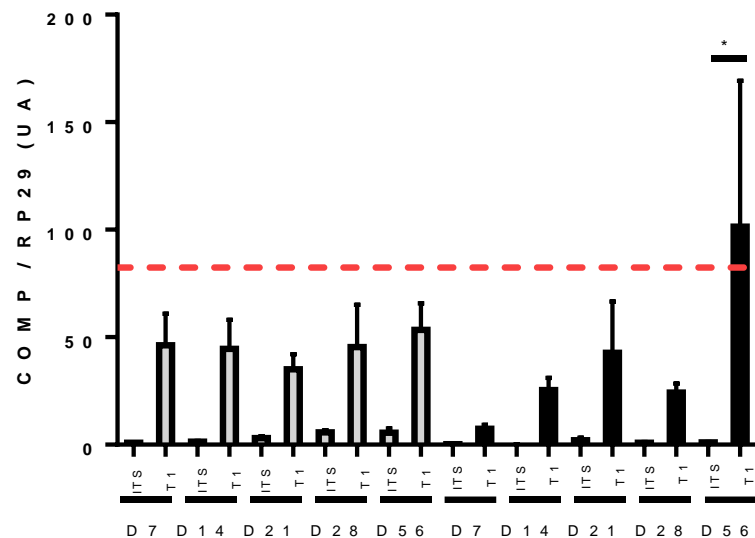


**Results**

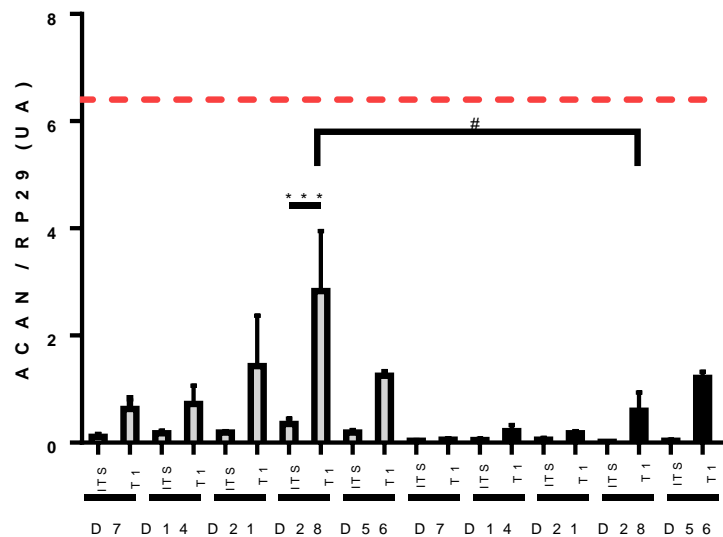
COL2A1



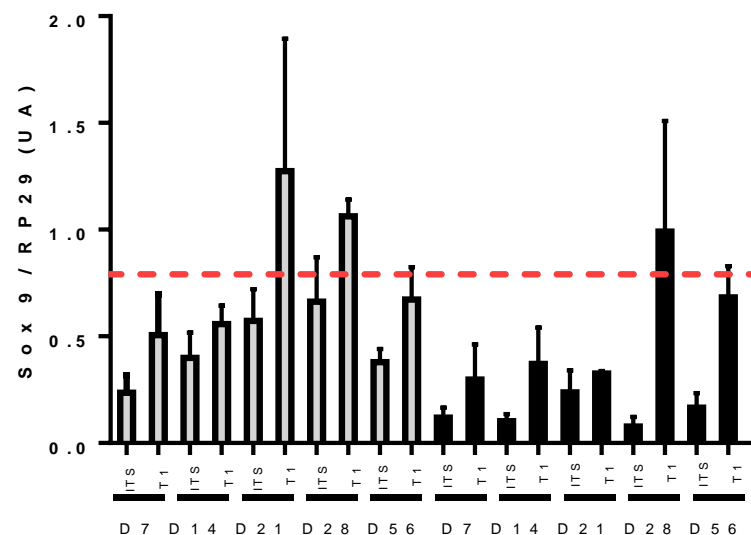
COMP



ACAN

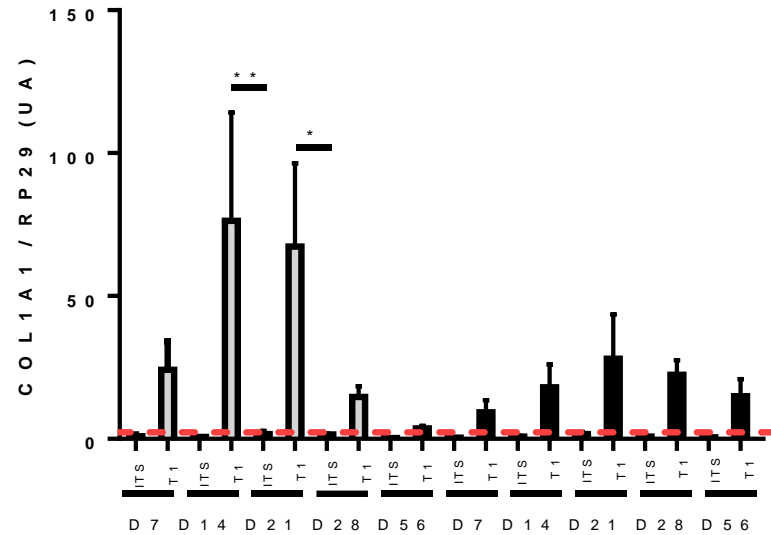
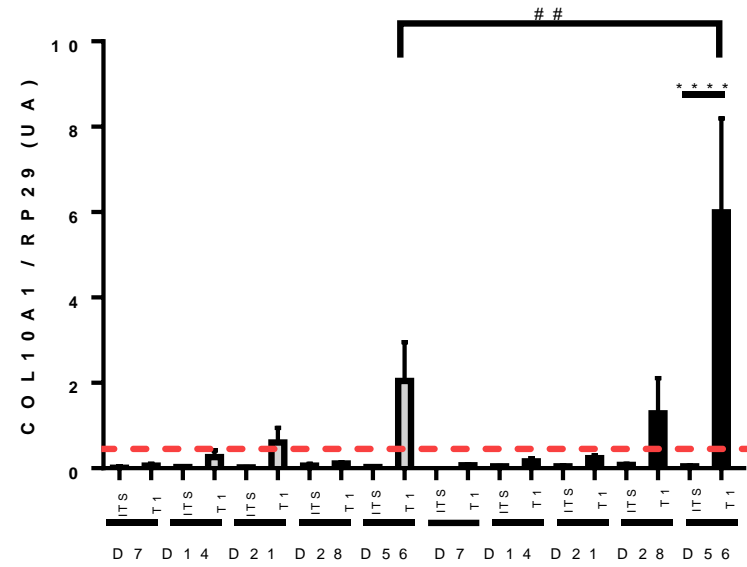
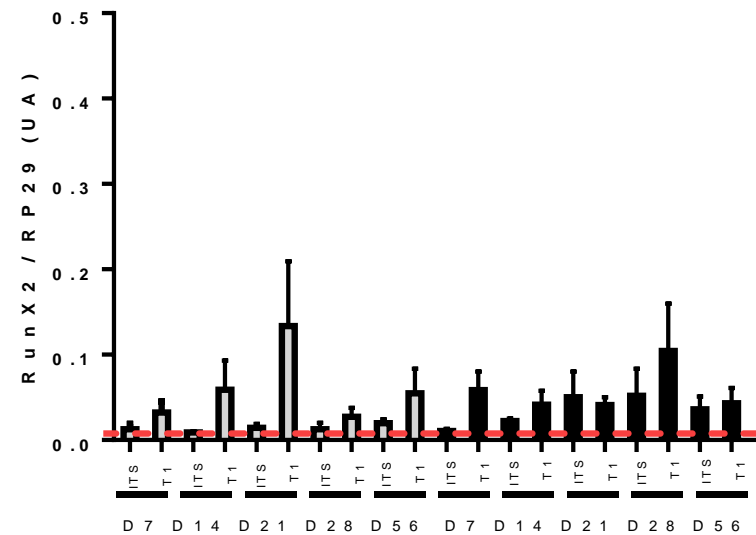
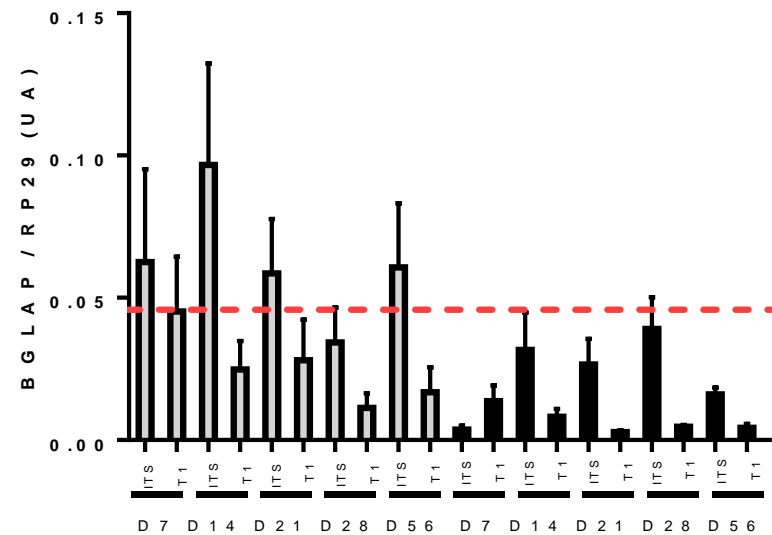


SOX9



Chondrocytes
  SF-MSCs  
 PO Chondrocytes

Mean +/- SEM,  
 two way ANOVA ITS VS T1 \* p<0,05 ; \*\* p<0,01 ; \*\*\* p<0,001; \*\*\*\* p<0,0001,  
 SF-MSCs VS chondrocytes T1 # p<0,05 ; ## p< 0,01

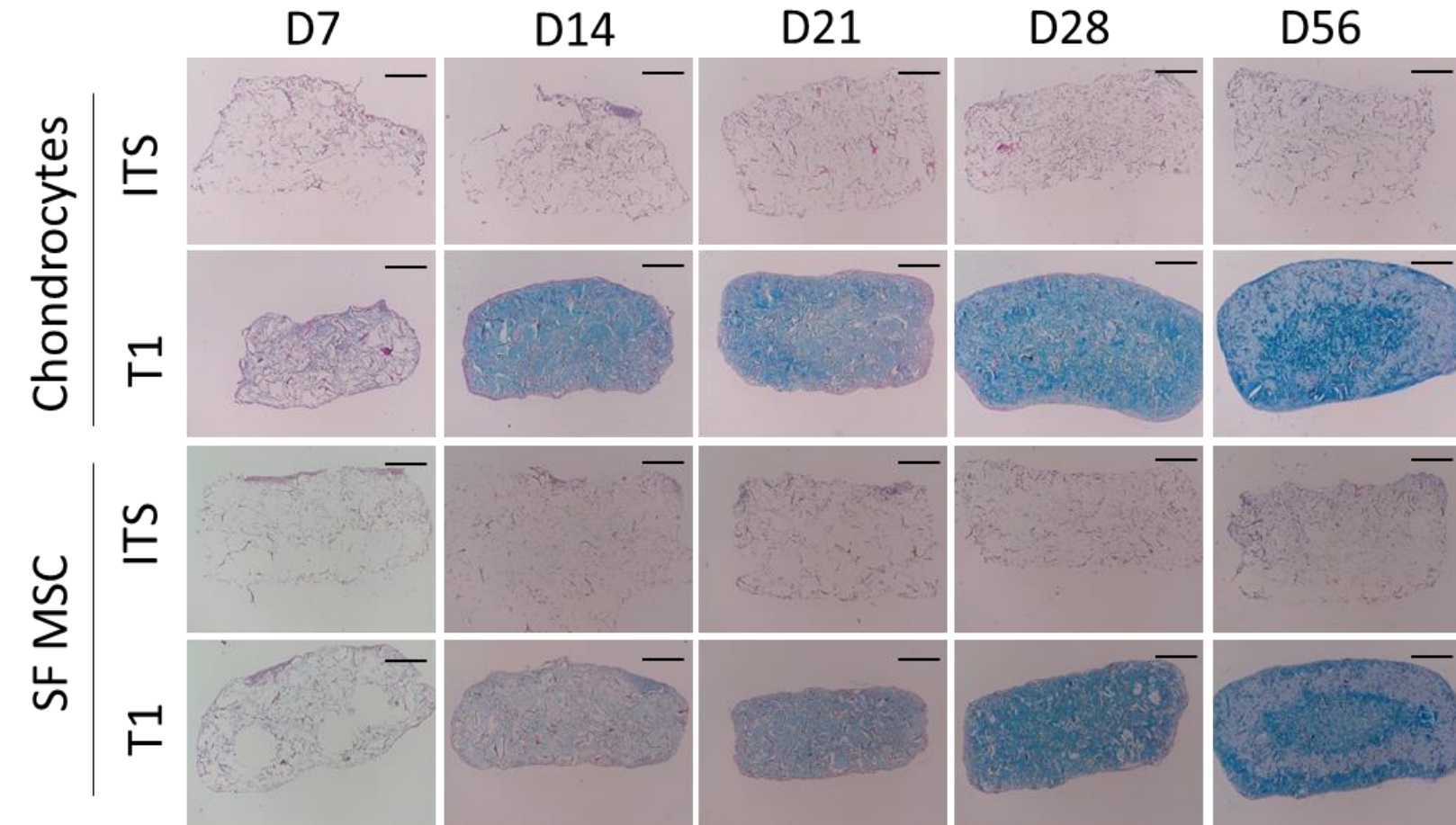
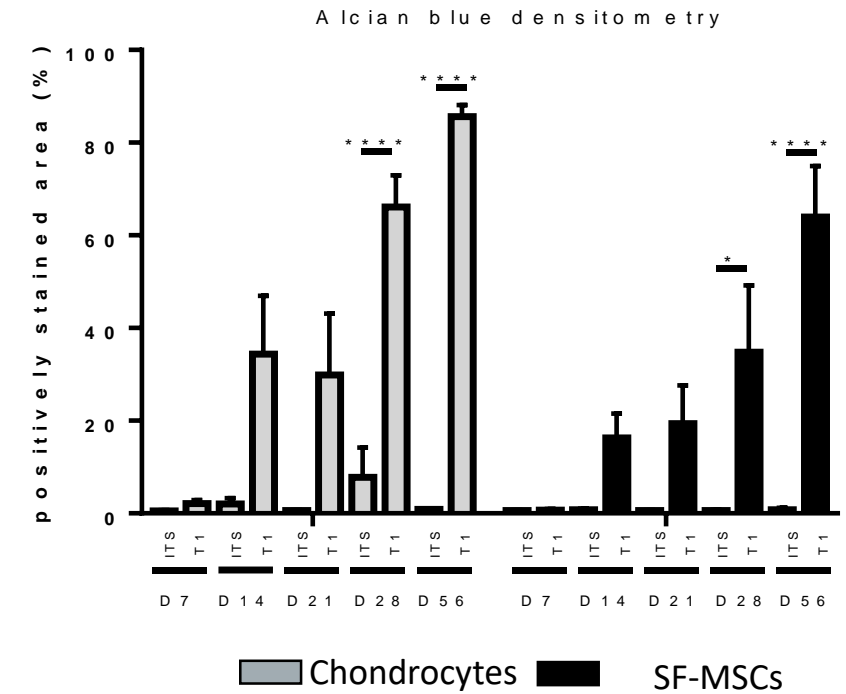
*COL1A1**COL10A1**RUNX2**BGLAP*

Chondrocytes
  SF-MSCs  
 PO Chondrocytes

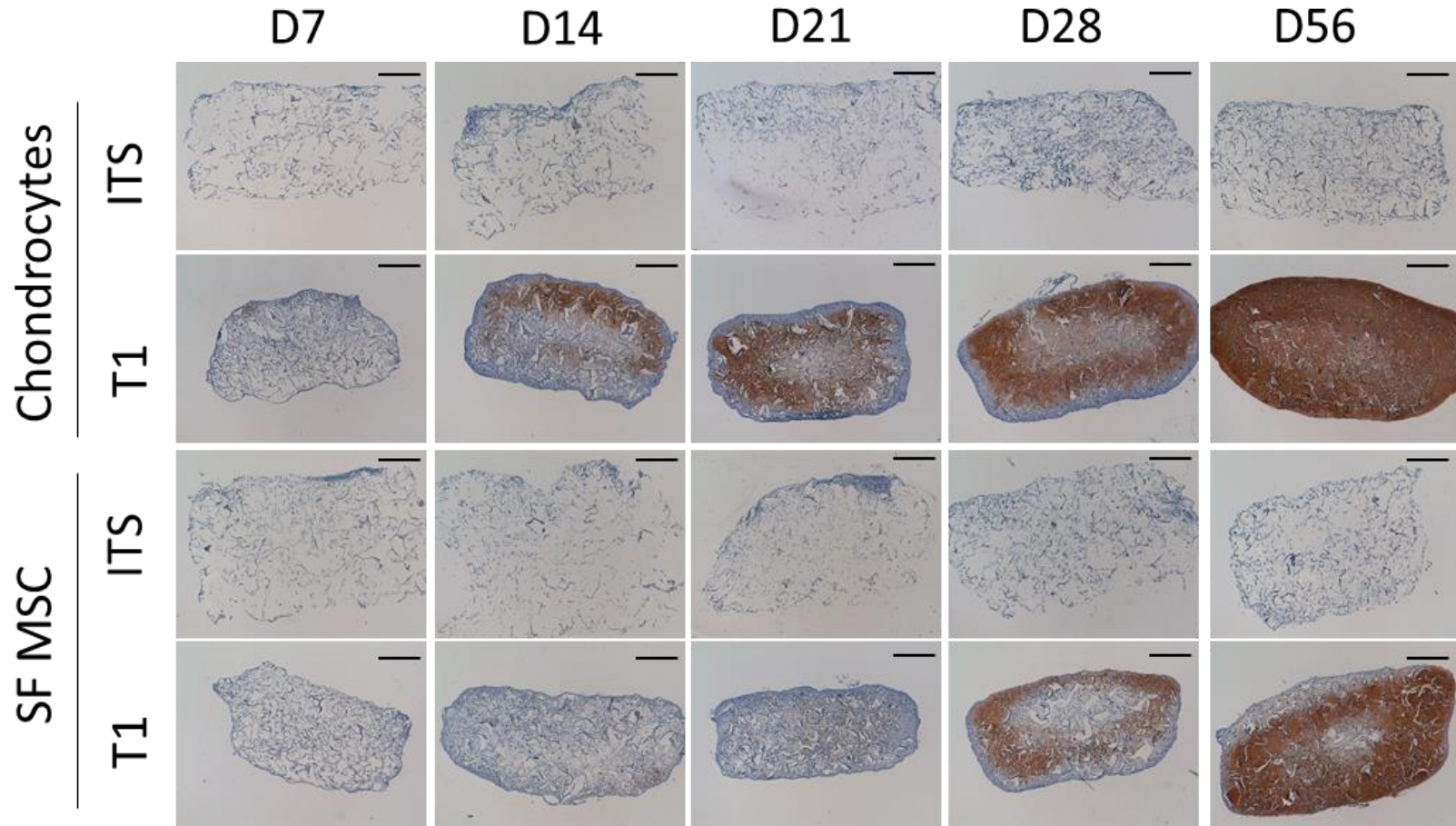
Mean +/- SEM,  
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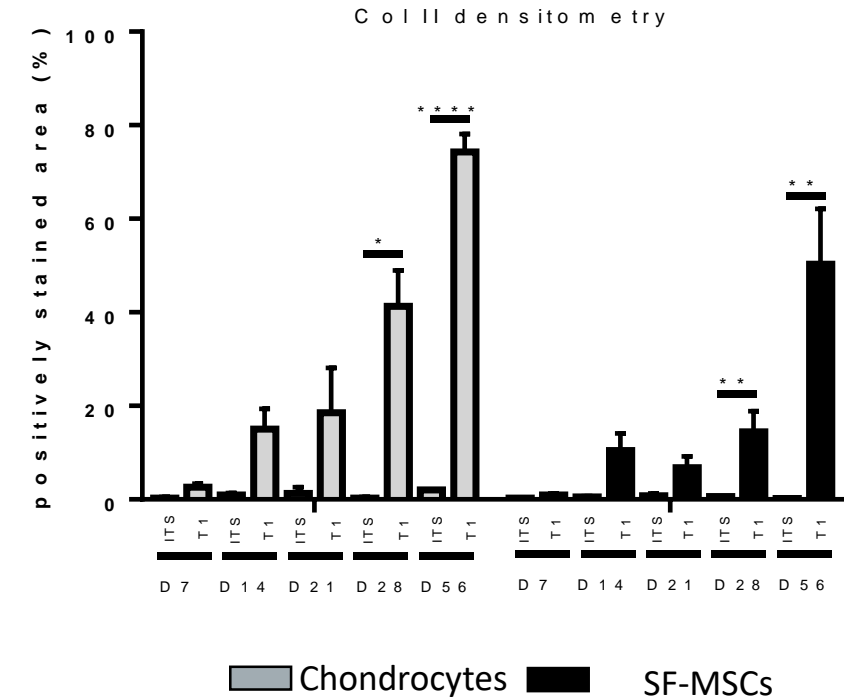
## Alcian blue staining

Scale bars= 400  $\mu$ m

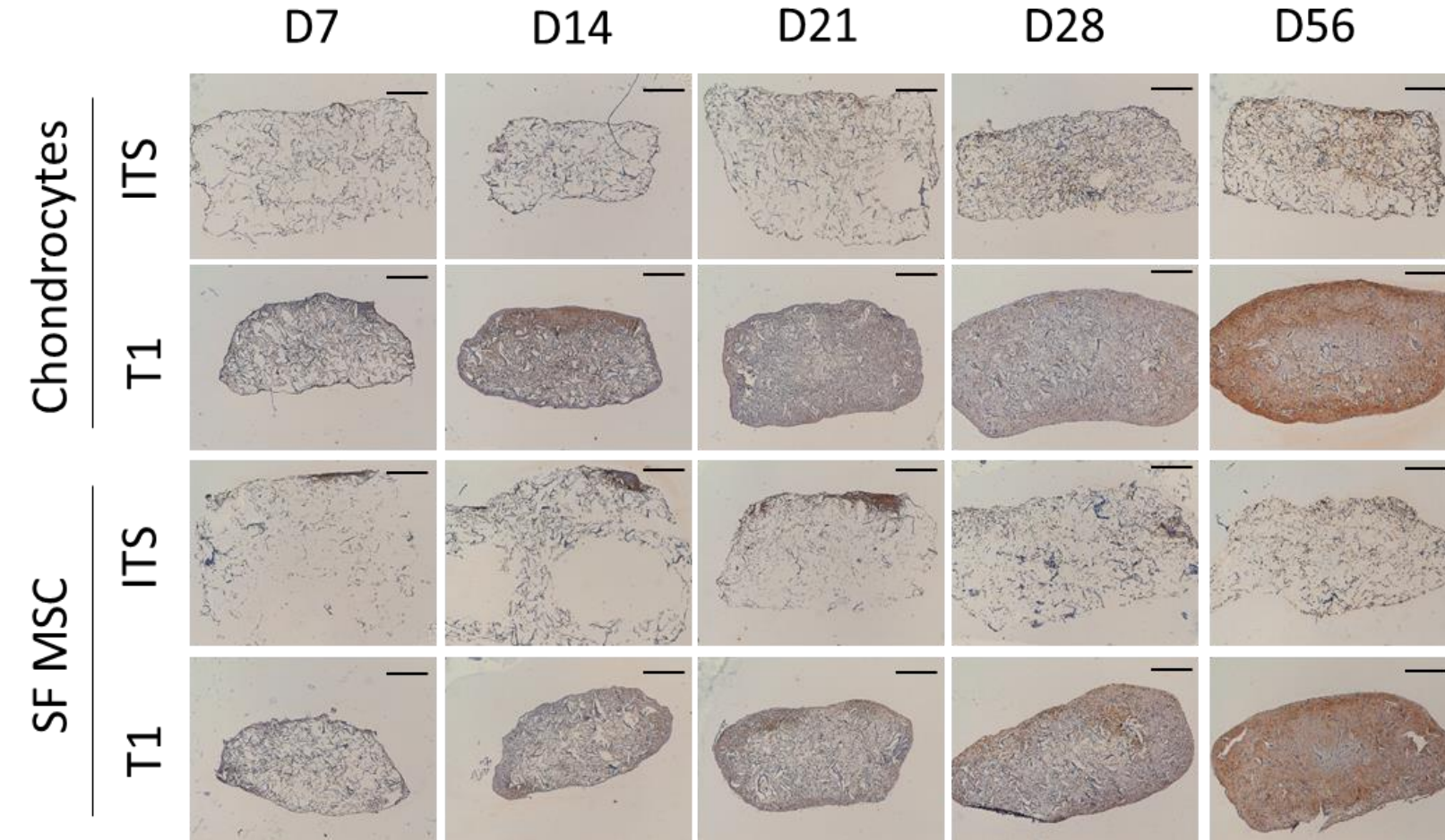
## Type II Collagen immunohistochemistry



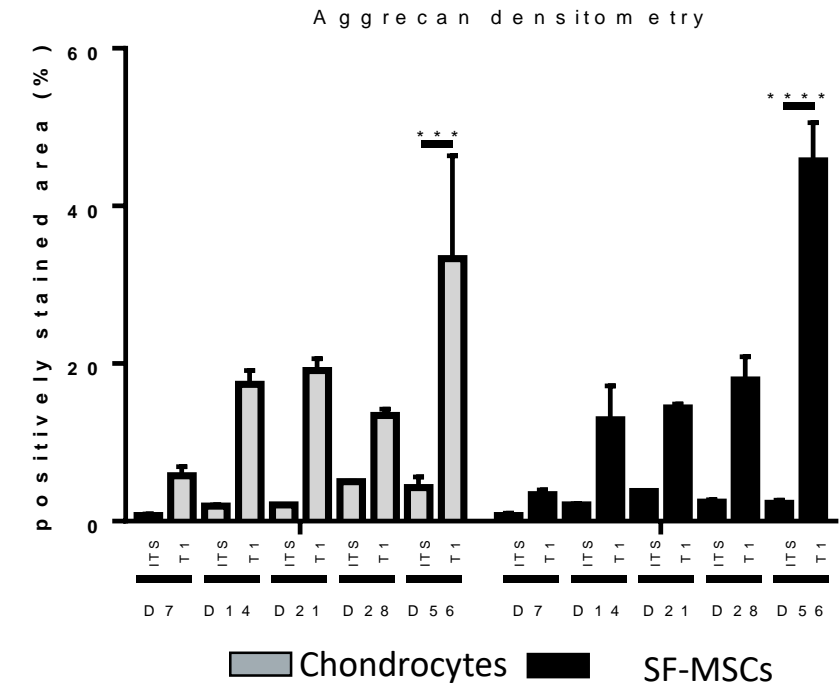
Scale bars= 400  $\mu$ m



## Agrecan immunohistochemistry



Scale bars= 400  $\mu$ m



## Epitranscriptomic mapping

Chondrocytes showed heterogeneity in their rRNA methylation profile at P0, depending on the age of the patients

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For both chondrocytes and SF-MSCs, TGF- $\beta$ 1 induced changes on their rRNA methylation levels



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Chondrocytes showed heterogeneity in their rRNA methylation profile at P0, depending on the age of the patients

For both chondrocytes and SF-MSCs, TGF- $\beta$ 1 induced changes on their rRNA methylation levels

Five differentially methylated sites were identified between chondrocytes and SF-MSCs.



# Conclusions

SF-MSCs can undergo a good chondrogenic differentiation and are able to synthesize a dense matrix, rich in proteoglycans and in type II Collagen



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rRNA are modified along chondrocytes and SF-MSCs differentiation in biomaterials

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rRNA are modified along chondrocytes and SF-MSCs differentiation in biomaterials

Chondrocytes and SF-MSCs show differently methylated rRNA

# Thank you for your attention

## Team 5: BioReMaTCh:

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**Ghita SEKKAT**

**Magali DETZ**

**Laurent GALOIS**

**Didier MAINARD**

**Damien LOEUILLE**

**Elise PAPE**

**Nicolas GAMBIER**

**Julien SCALA-BERTOLA**



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 **SFBTM**

 **CHRU NANCY**  **SFR**

 **IBS** **INGÉNIERIE**  
 **Lor** **BIOLOGIE**  
**SANTÉ EN** **LORRAINE**