



University of
Natural Resources
and Life Sciences

Department of Biotechnology



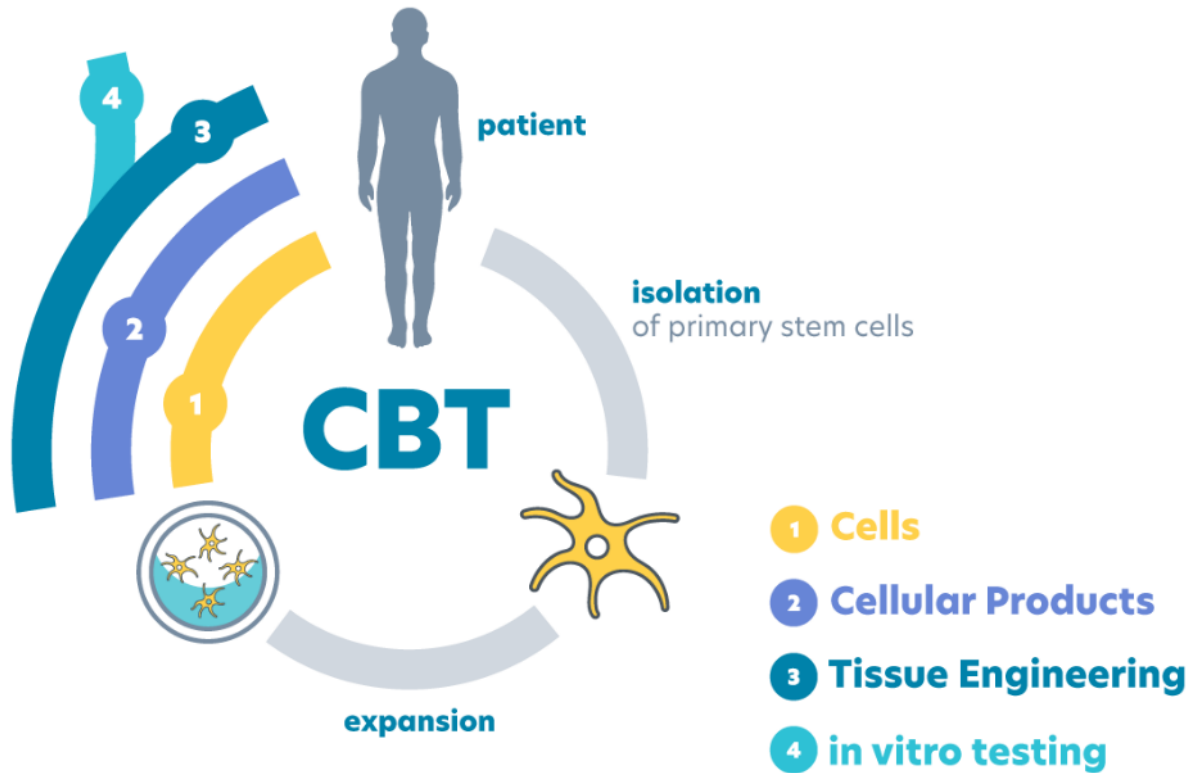
Institute of
Cell & Tissue
Culture Technologies



Mimicry of physiological aspects using hydrogels

Online-Fokusevent: Towards replacement of animal-derived components in complex human *in vitro* models

Farhad Chariyev-Prinz, PhD



Physiologic culture conditions

Culture Techniques



Hypoxia



3D



Dynamic



Medium components

Members:

Prof. Dr. Cornelia Kasper



Post Docs

Dr. Dominik Egger

Dr. Farhad Chariyev



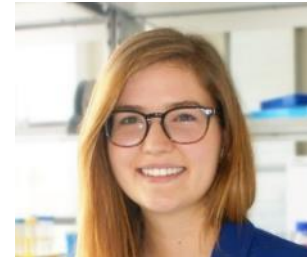
PhD students

Sabrina Nebel

Ilias Nikolits

Julia Moldaschl

Oscar Garcia



Technicians

Sarah Mühlbacher

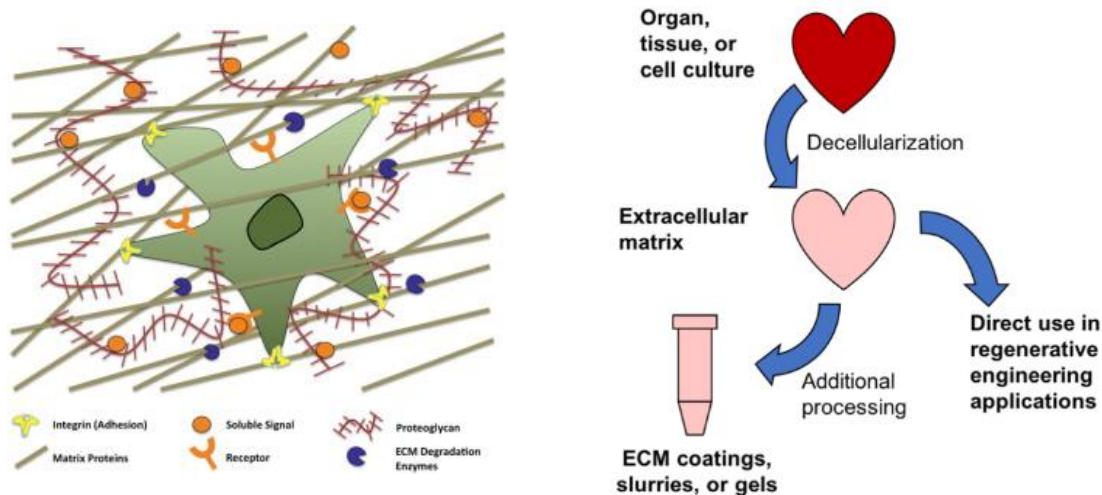
Tanja Wukovits



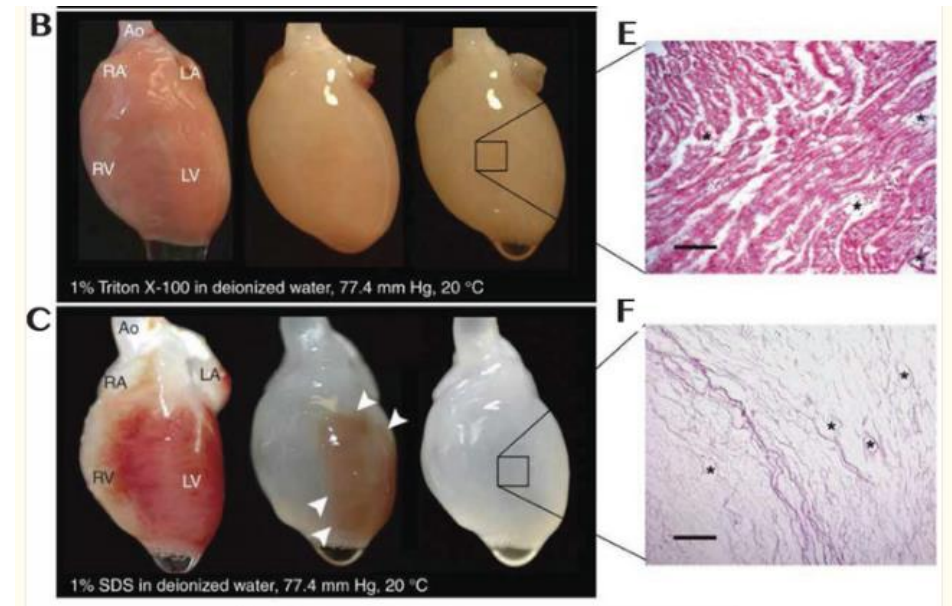
Natural 3D environment: decellularization of tissues



- Decellularized tissues and organs can **potentially mimic native physiology and can serve as scaffolds for cell laden transplants**
- Critical points for effective decellularization:
 - No cells left in the scaffold**
 - Tissue architecture is preserved**
 - ECM proteins are unaffected**



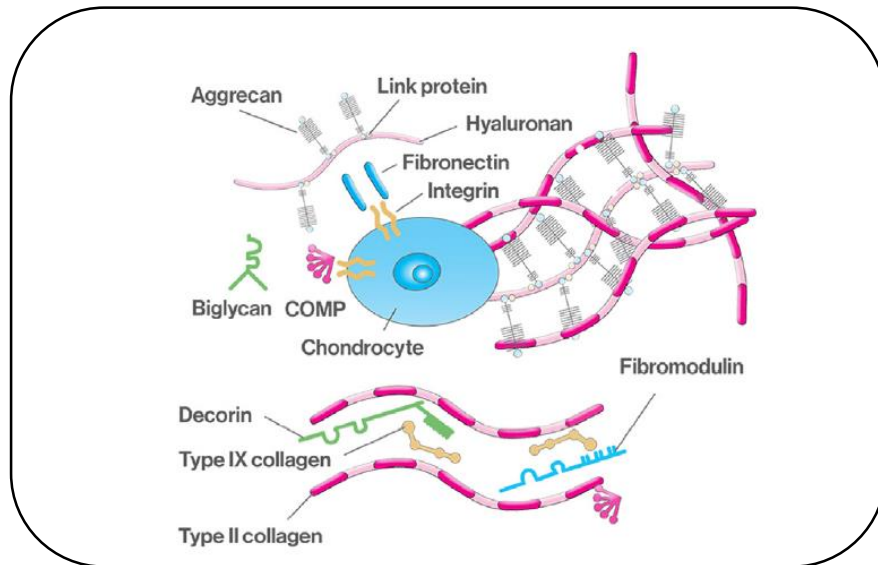
Solid (e.g. patches)



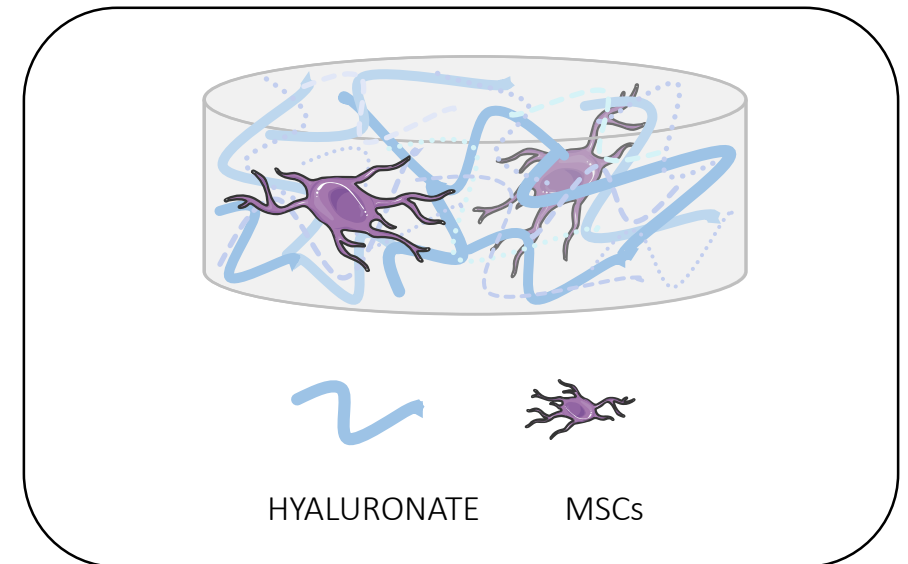
ECM mimicry with hydrogels



Extracellular Matrix



Hydrogel





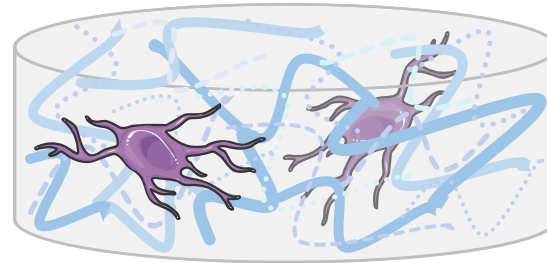
Examples of natural polymers

Material	Origin	Bioactive	Biodegradable	Common 3D structures	Common TE applications
<u>ECM proteins</u>					
Collagen	Tissue	Yes	Yes	Scaffolds, coatings, hydrogels	Varied
Fibronectin	Tissue	Yes	Yes	Patterning, surface coatings	Bone
Fibrin	Tissue	Yes	Yes	Scaffolds, gels, glues	Cardiovascular, neuronal
Laminin	Tissue	Yes	Yes	Scaffolds, gels (as a conjugate)	Neuronal
Elastin	Tissue	Yes	Yes	Scaffolds, conjugates	Vascular, skin
Other proteins					
Gelatin	Collagen/ECM	Yes	Yes	Scaffolds, hydrogels	Bone, cartilage
Silk fibroin (SF)	Silk	No	Yes	Fibers, scaffolds	Cartilage, bone
<u>ECM glycosaminoglycans (GAGs)</u>					
Hyaluronic acid (HA)	ECM	Yes	Yes	Hydrogels, scaffolds (conjugates)	Cartilage, bone
Chondroitin sulfate (CS)	ECM	Yes	Yes	Scaffolds, hydrogels	Cartilage
Aggrecan	ECM	Yes	Yes	Hydrogels (conjugates)	Cartilage
Other polysaccharides					
Chitosan	Crustaceans	No	Yes	Scaffolds, hydrogels, sponges	Varied
Starch	Plants	No	Yes	Scaffolds (conjugated)	Bone
Alginate	Algae	No	No	Hydrogels	Varied
Agarose	Algae	No	No	Hydrogels	Cartilage
Carrageenan	Algae	No	Yes	Hydrogels	Cartilage, bone
Gellan gum (GG)	Bacteria	No	No	Hydrogels	Cartilage, bone, skin

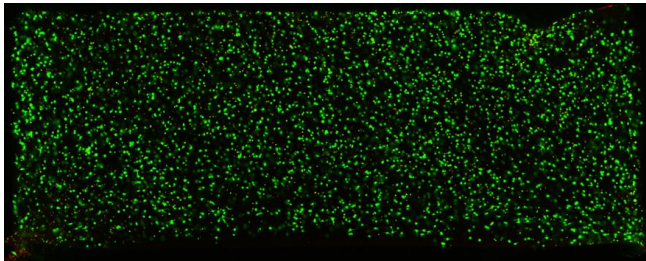
Hydrogel systems for various applications



Cell encapsulation in 3D/hydrogel



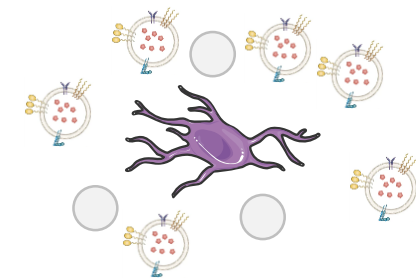
EXPANSION



DIFFERENTIATION



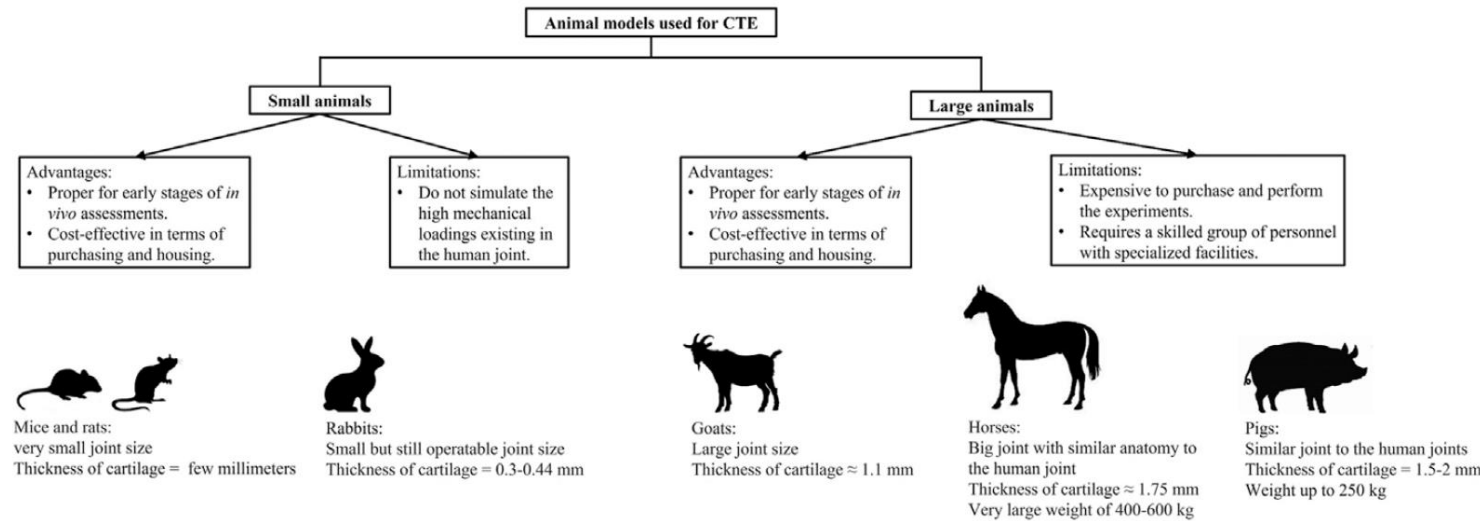
EV SECRETION



What can affect these processes?

- availability of binding sites and mechanical properties

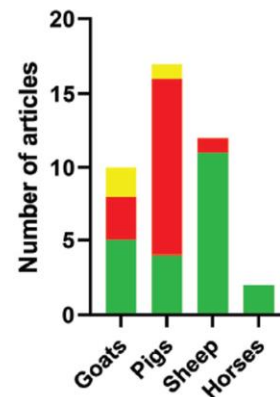
Animal testing in Articular Cartilage Tissue Engineering (AC TE)



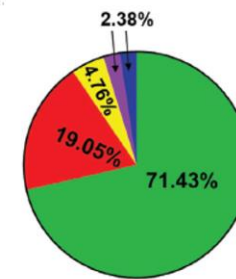
- Factors to consider:**
- size, weight and age
 - joint size
 - cartilage thickness
 - defect location and size
 - load distribution
 - appropriate controls
 - cost and lastly convenience of the operation and animal handling

No considerable benefits of MSC-seeded constructs compared to cell-free counterparts *in vivo*

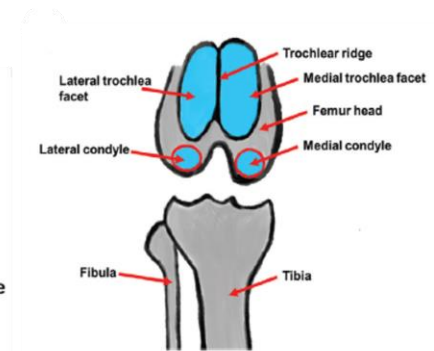
- Biomaterial
- Cell density
- Maturation
- Mechanical loading



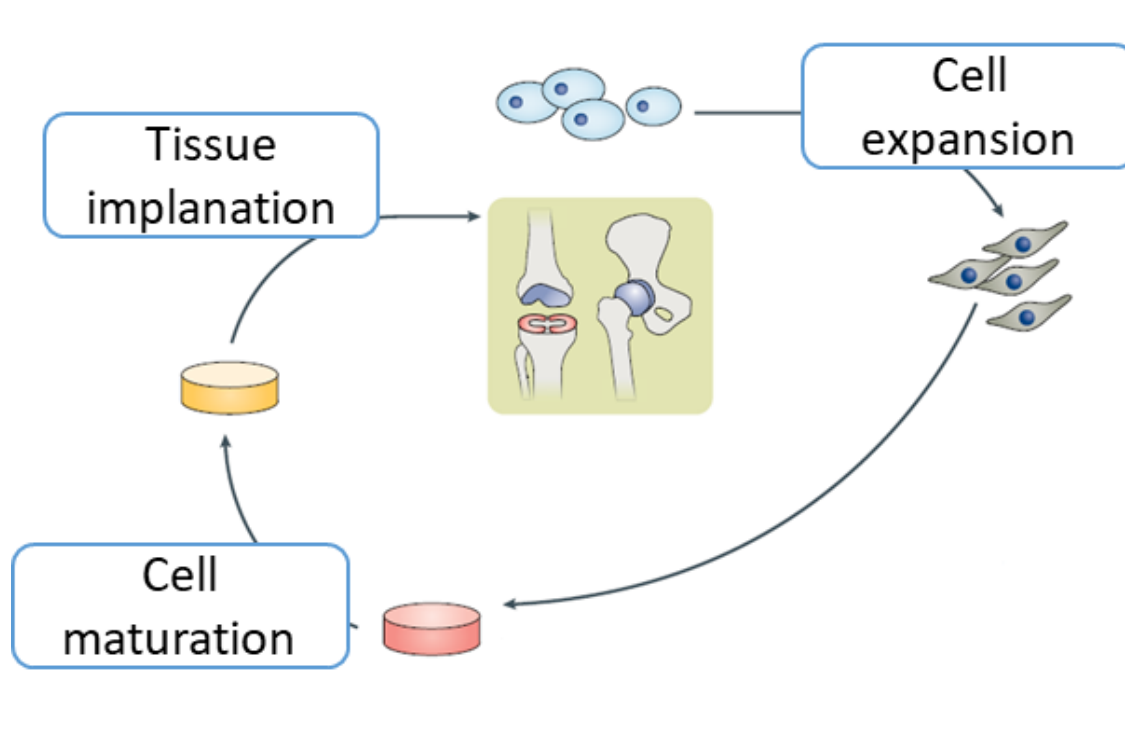
Does not say
Immature
Mature



Condyle
Trochlea
Condyle and trochlea
Femur head
Condyle and patellar groove

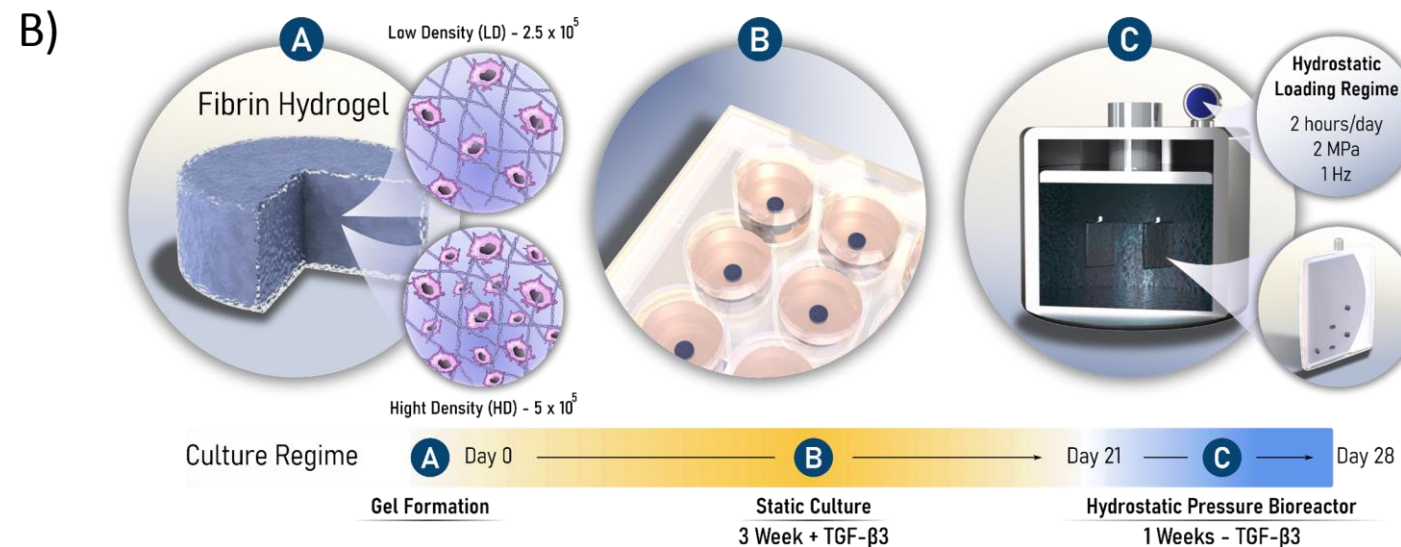
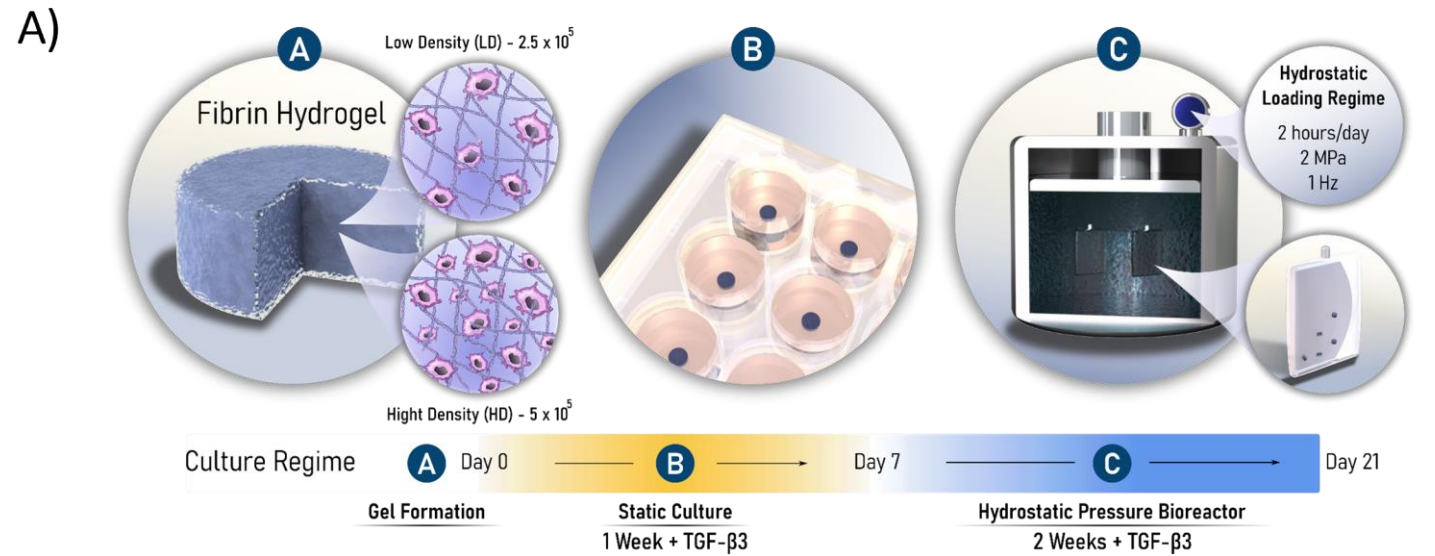


Mimicry of physiological aspects *in vitro*: *Hydrogels and bioreactors*

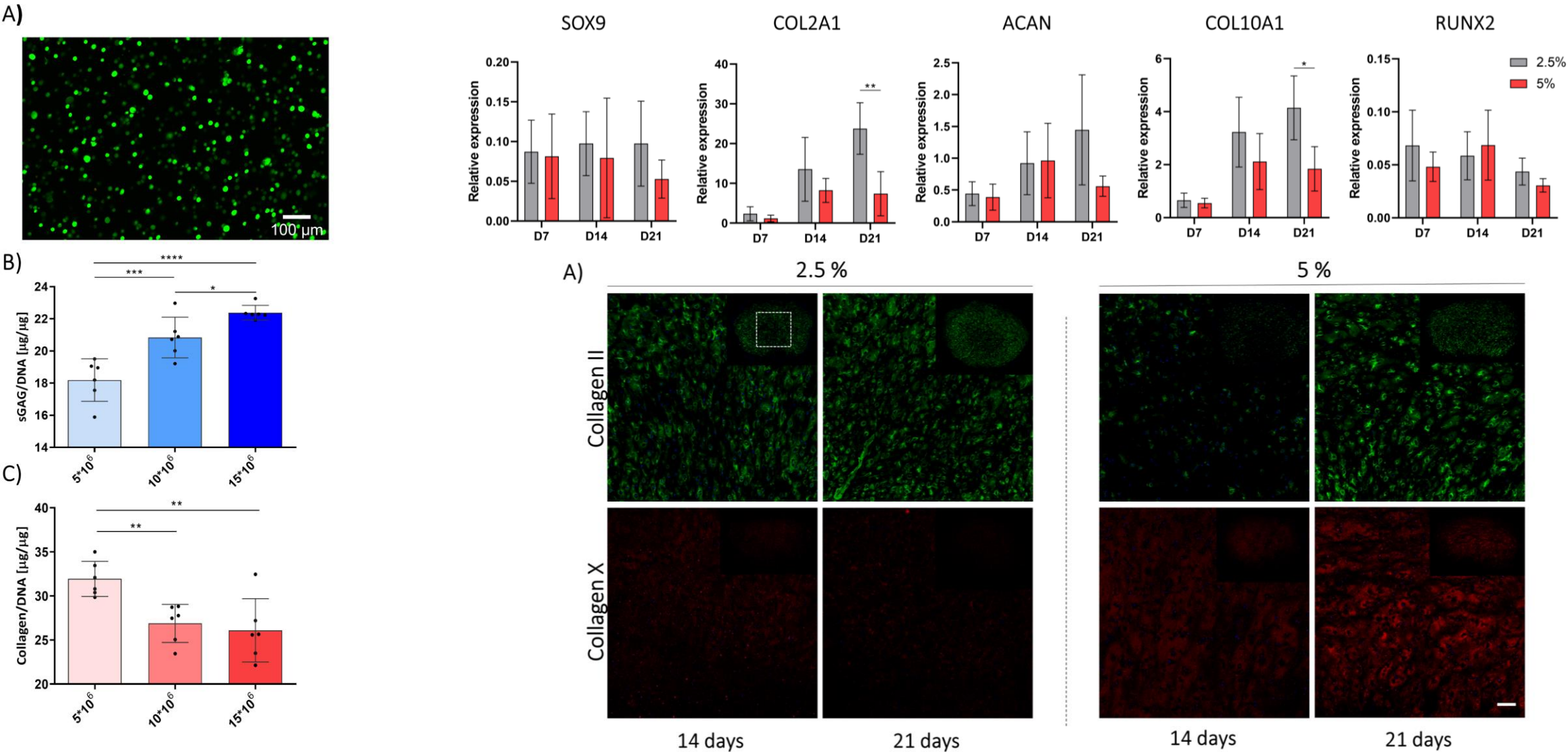


- Physioxia
 - 5% O₂
- 3D environment
 - Fibrin hydrogels
- Mechanical stimulation
 - *Hydrostatic pressure*

Mimicry of physiological aspects *in vitro*



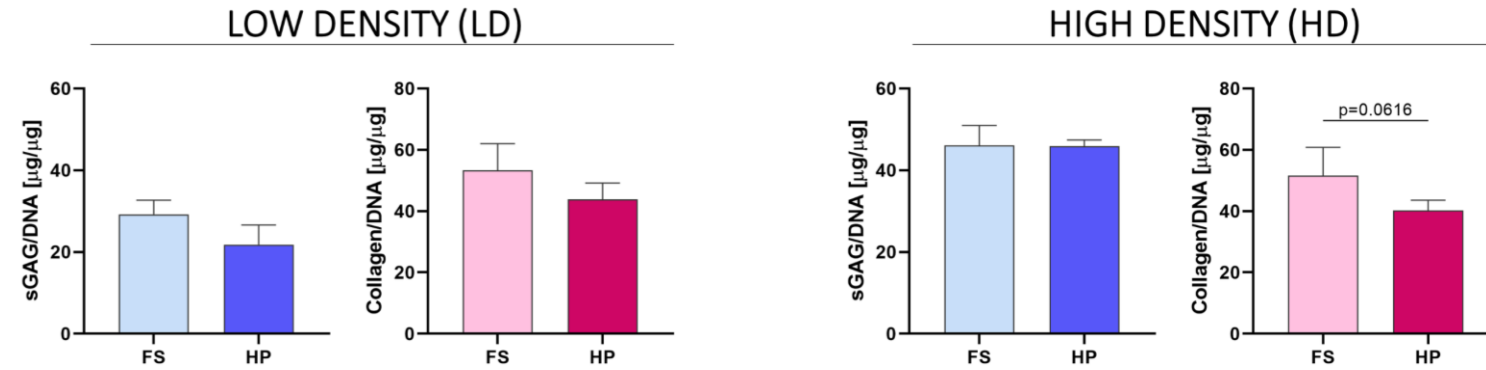
Effect of cell density and biomaterial concentration on ECM deposition



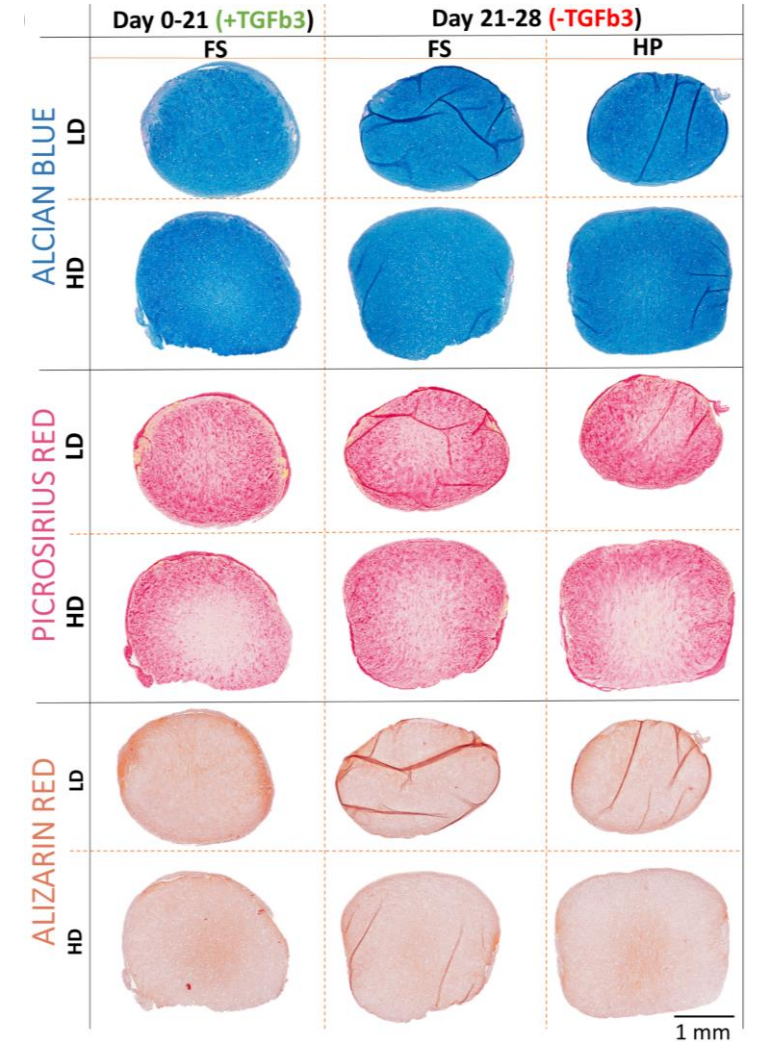
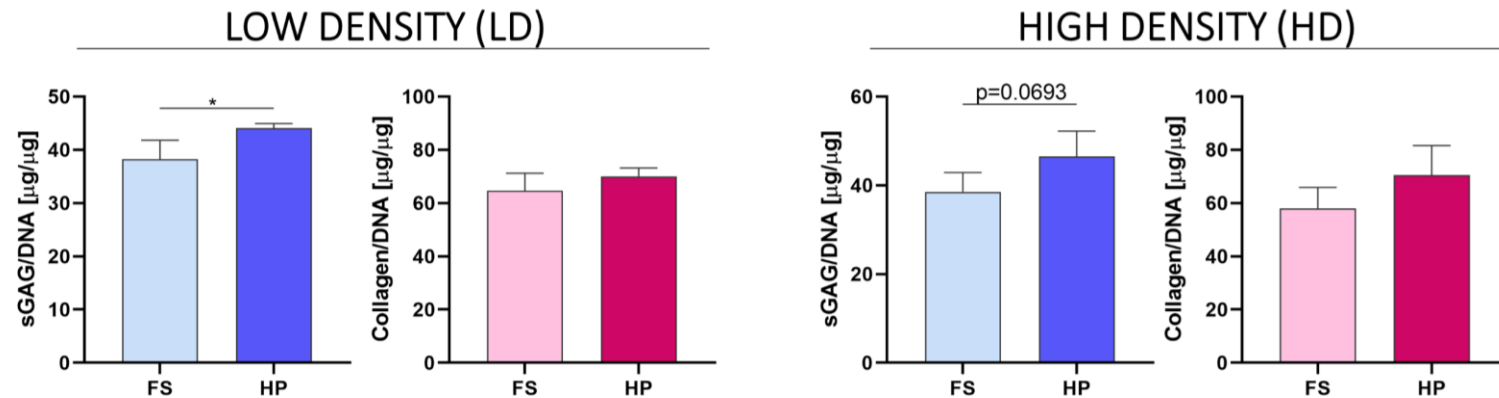


No effect of HP during chondrogenesis; subtle benefits after priming

During differentiation



After priming





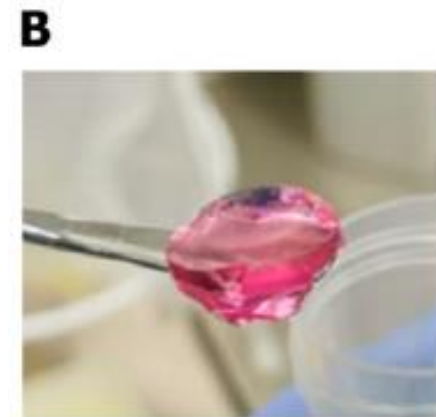
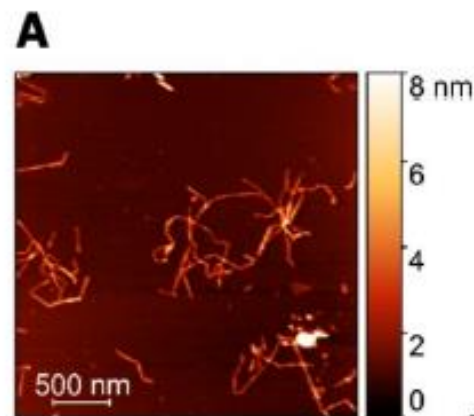
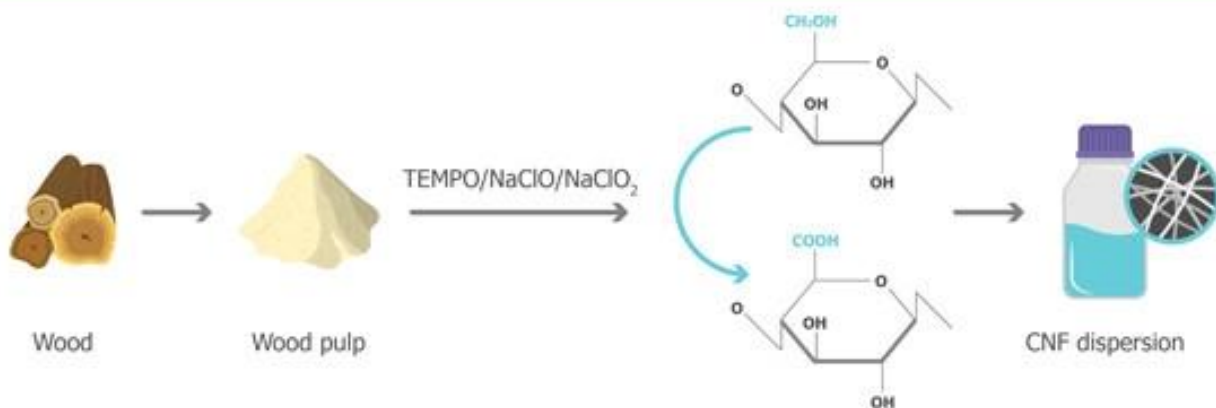
- Employment of hydrogels and bioreactors for *in vitro* modelling of cartilage maturation
 - Development of appropriate rehabilitation therapies after surgeries (microfracture)
 - Testing platform for drugs and compounds
 - More effective use of animal studies (3R, greater probability of success; Reduction)
- Limitations:
 - FBS employment: animal-derived, variation, differential effect on differentiation
 - Fibrin: expensive and complicated handling

TEMPO-oxidized cellulose hydrogels for MSC culture



- Cellulose
 - Highly abundant and inexpensive
 - Suitable for *in vitro* culture of various cell types
 - TEMPO-oxidation
 - Formation of cellulose nanofibers (CNFs)

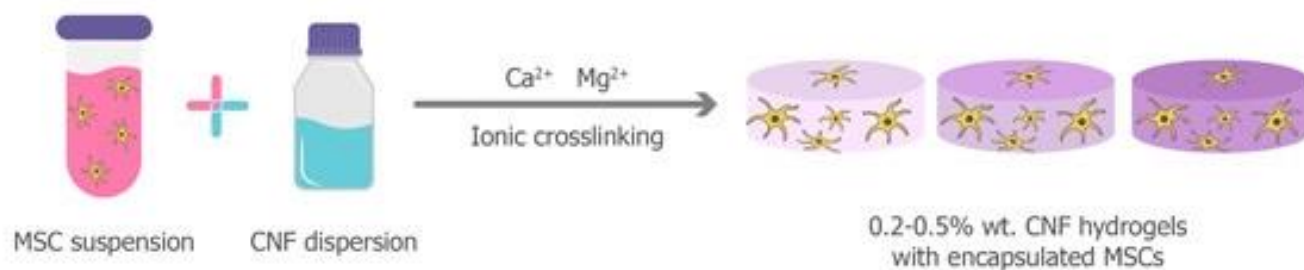
Preparation of TEMPO-oxidized CNFs



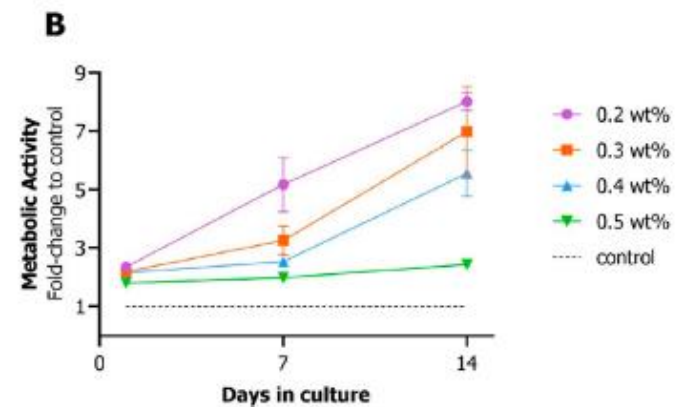
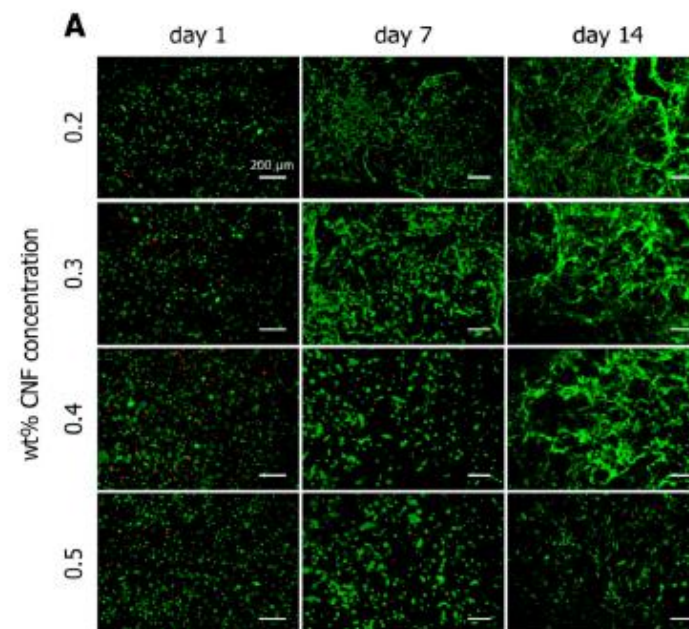
CNFs supports viability of hMSCs



Preparation of CNF hydrogels with encapsulated MSCs

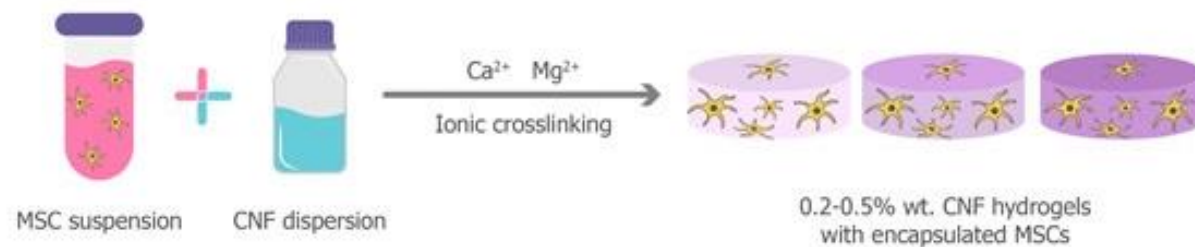


Evaluation of CNF hydrogels with encapsulated MSCs

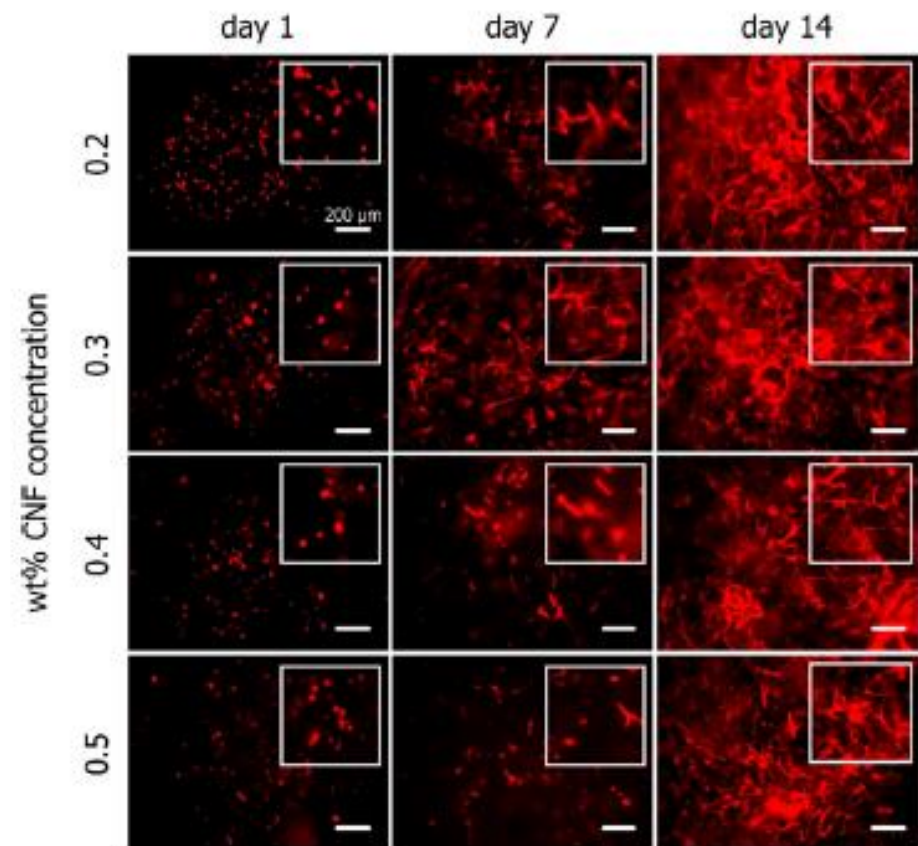


CNFs support spread morphology of hMSCs

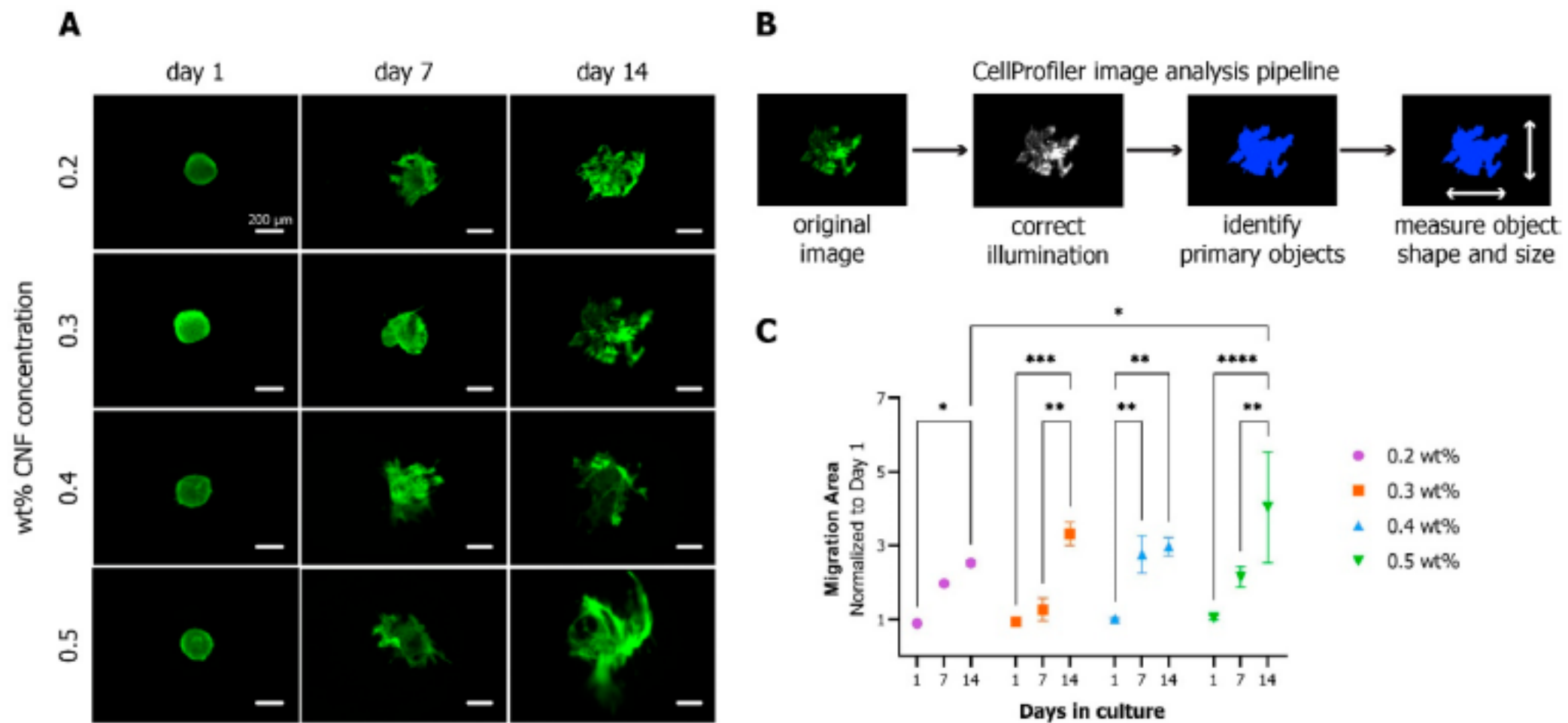
Preparation of CNF hydrogels with encapsulated MSCs



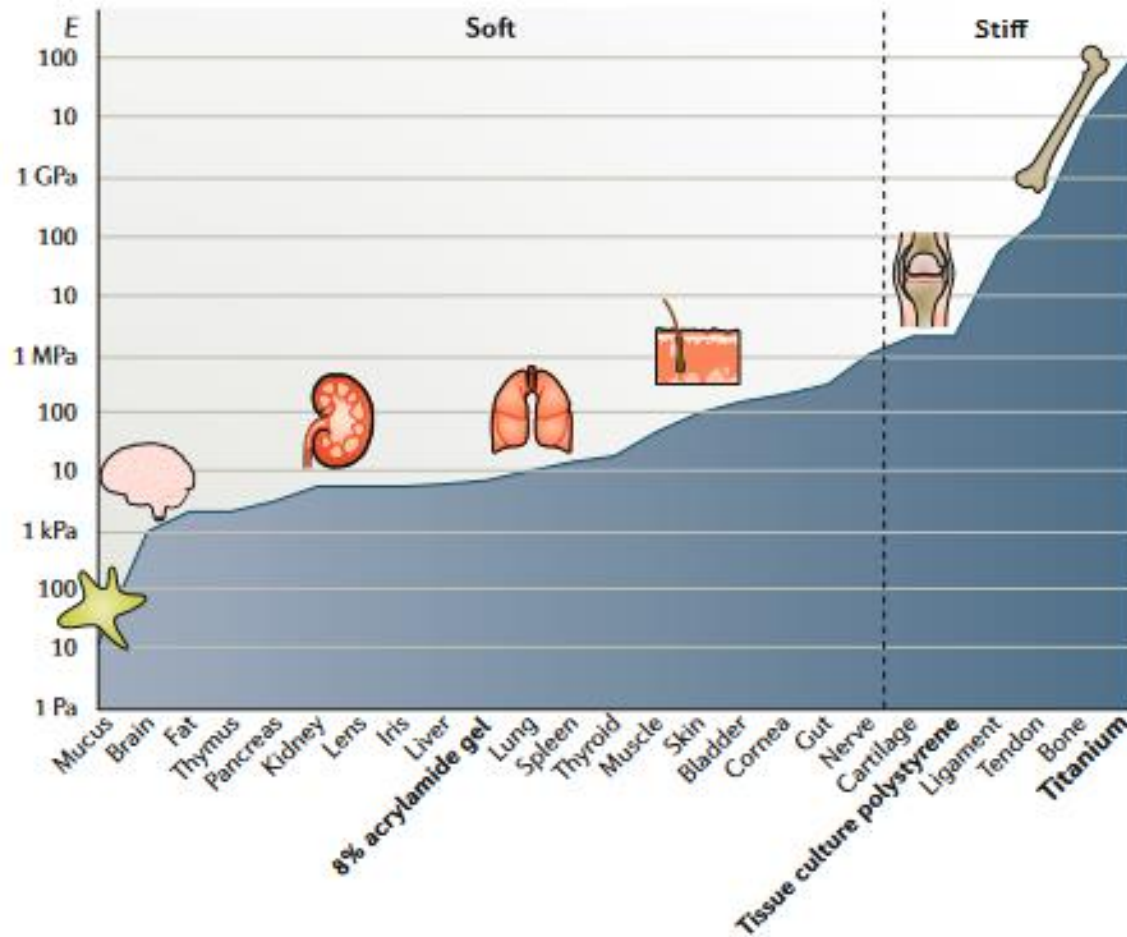
Evaluation of CNF hydrogels with encapsulated MSCs



CNFs allow migration of hMSCs



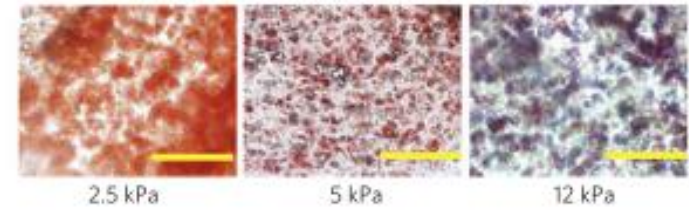
Challenges: Engineering lifelike tissue



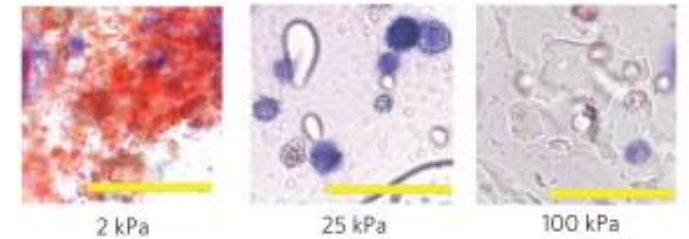
Mechanical properties of living tissues:

- Stiffness: Pascal to Gigapascal range
- Stress relaxation
- Stress stiffening

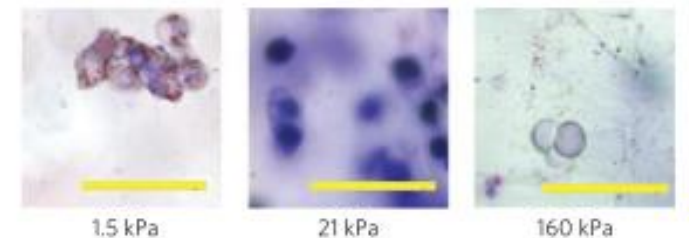
Alginate



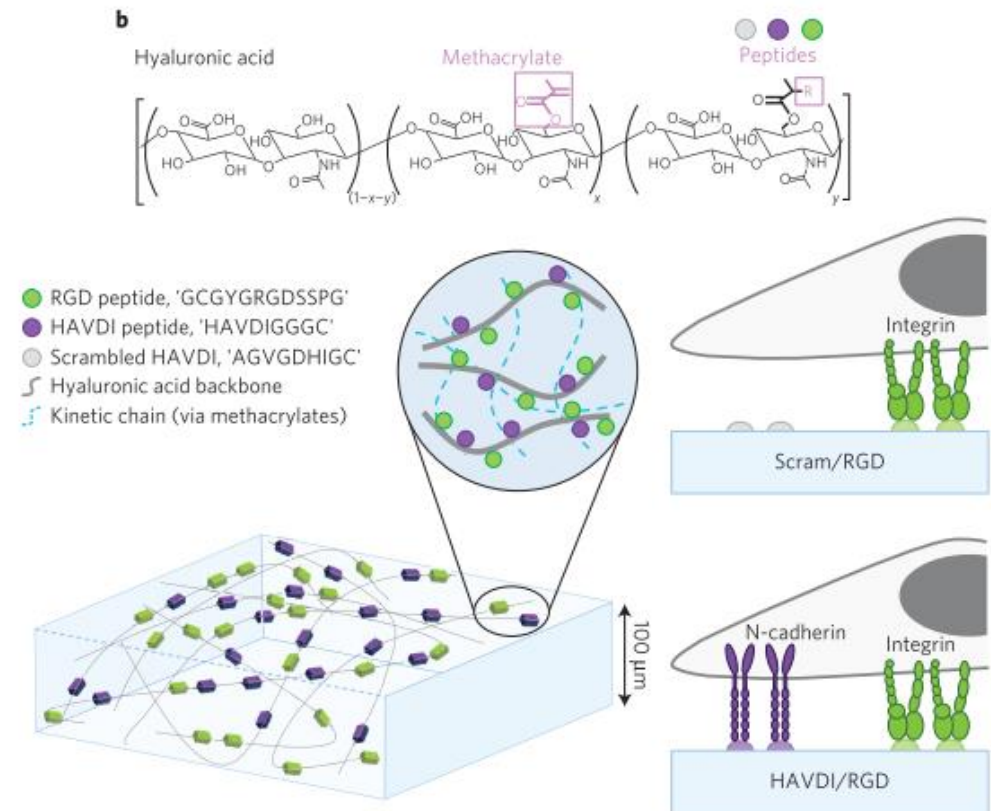
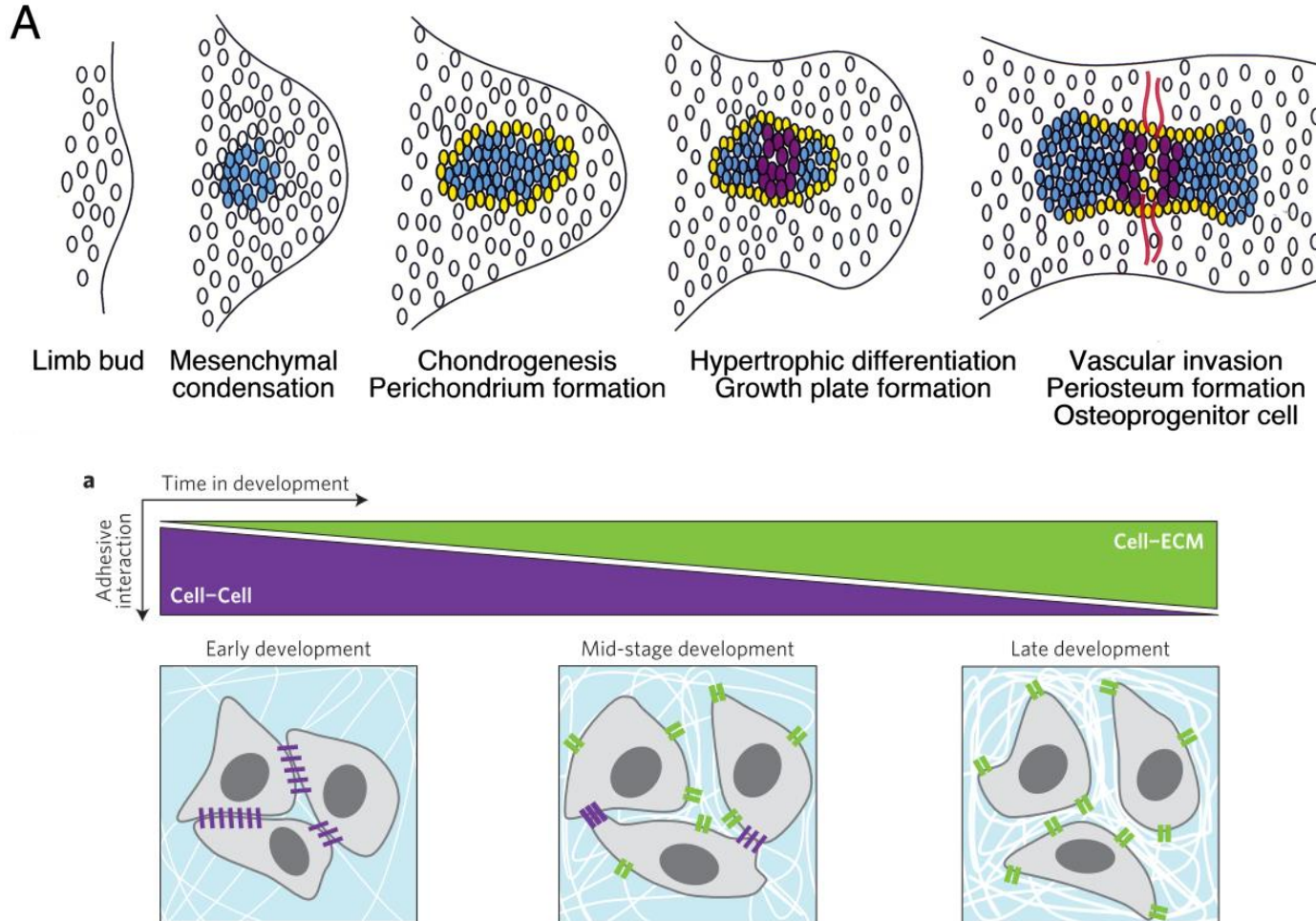
Agarose



PEG



Engineering lifelike tissues: *Ligand availability*



Acknowledgements

Current Lab members:

- Prof. Dr. Cornelia Kasper
- Dr. Dominik Egger
- Dr. Farhad Chariyev
- Sabrina Nebel
- Ilias Nikolits
- Julia Moldaschl
- Oscar Garcia
- Tanja Wukovits
- Sarah Mühlbacher
- Marta Serra
- Sylwia Kapanowska
- Carolia Rodrigues
- Luisa Baier

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