Forum Inżynierii Materiałowej
Materials Engineering Forum
- The Materials Engineering and Metallurgy Committee of the Polish Academy of Sciences
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3D printing technologies and bio-ink materials in medical applications

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MATERIALS
DESIGN
DIVISION

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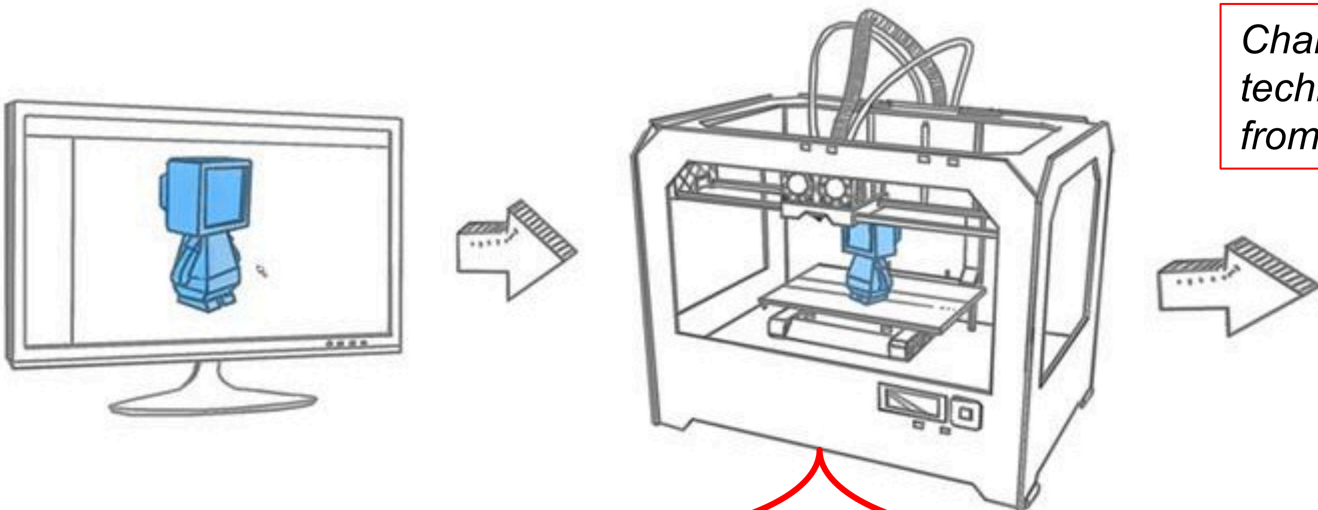
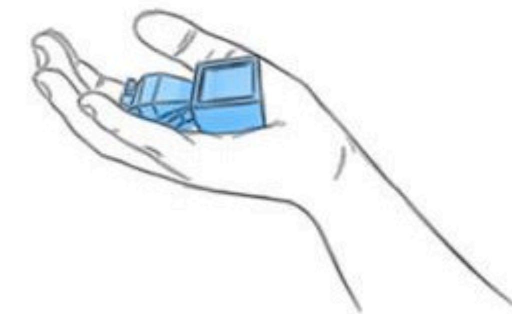
3D printing

- **3D printing**, also known as additive manufacturing (AM), refers to various processes used **to create a three-dimensional objects** of almost any shape or geometry **based on a 3D computer model**.

Charles Hull developed the technology for printing 3D objects from digital data in 1984.





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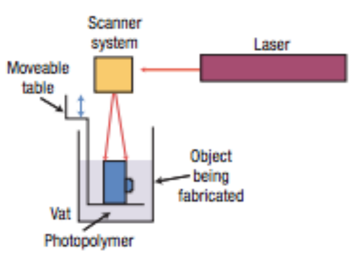
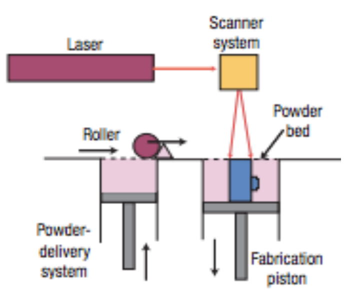
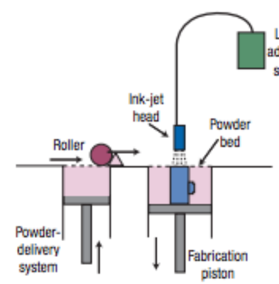


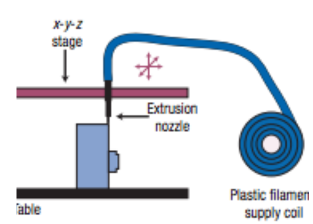
Stereolithography



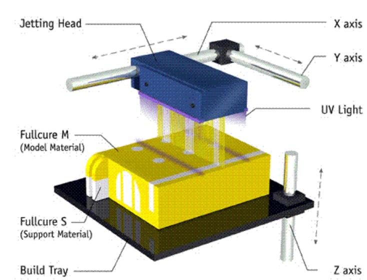
Selective laser sintering



3D printing



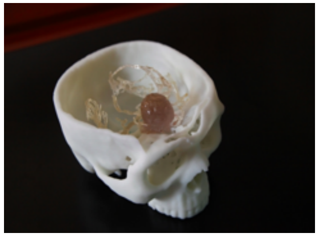
Fused deposition modelling



The Objet PolyJet Process

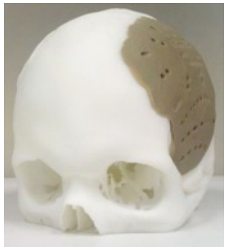
3D printing in medicine

3D Printing surgical models and templates

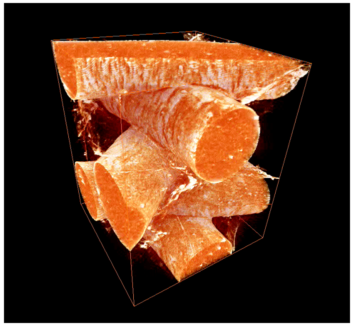




3D Printing prosthesis and implants

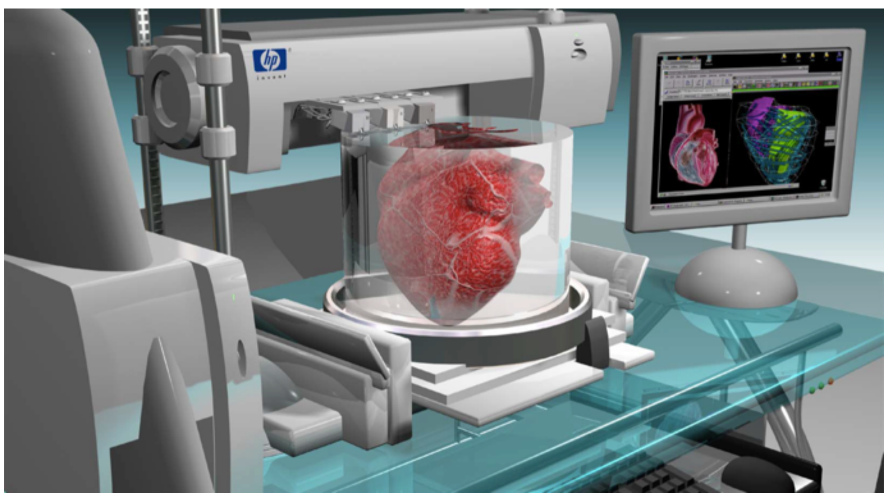
3D Printing of drugs and drug delivery systems

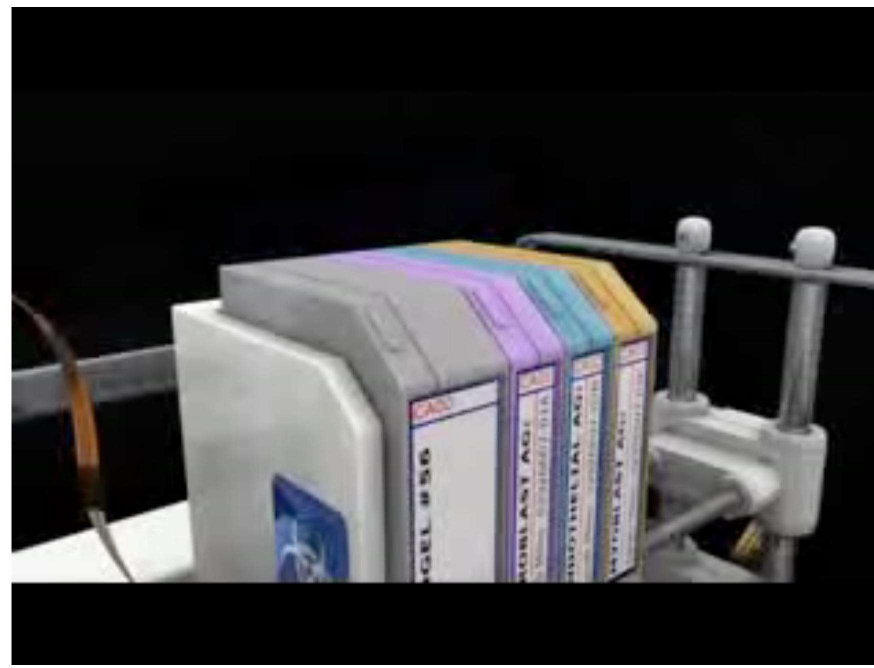


3D Printing in tissue and organ engineering

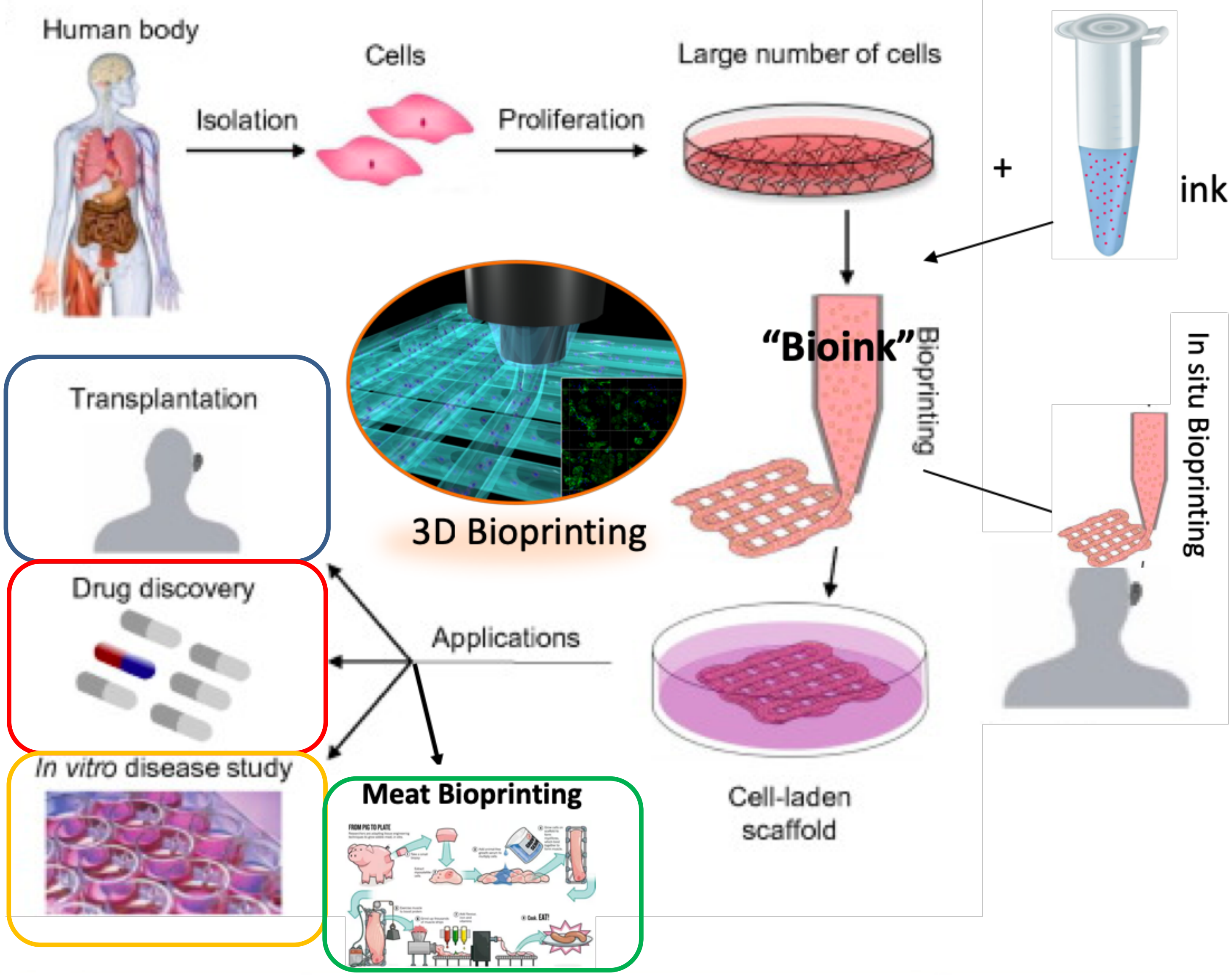
Bioprinting

- **Bioprinting** is an automated computer aided layer-by-layer deposition of **biological materials (bio-ink)** for manufacturing of functional tissues and organs.
- It allows, in a single step, for the generation of spatially controlled cell patterns through the deposition of a bioink.





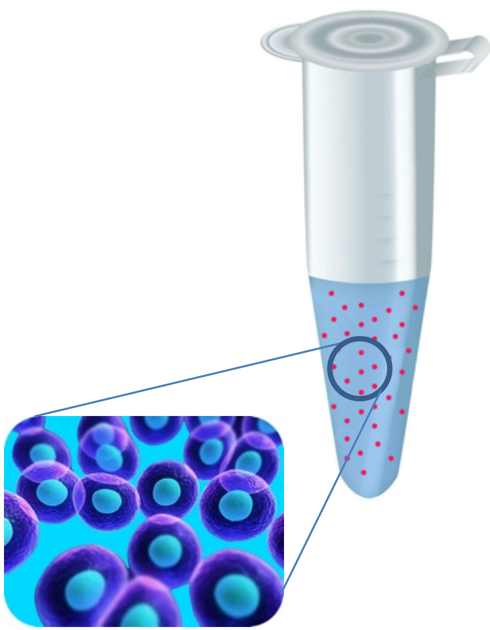
Biofabrication



Bioinks and Bioprinting process optimization

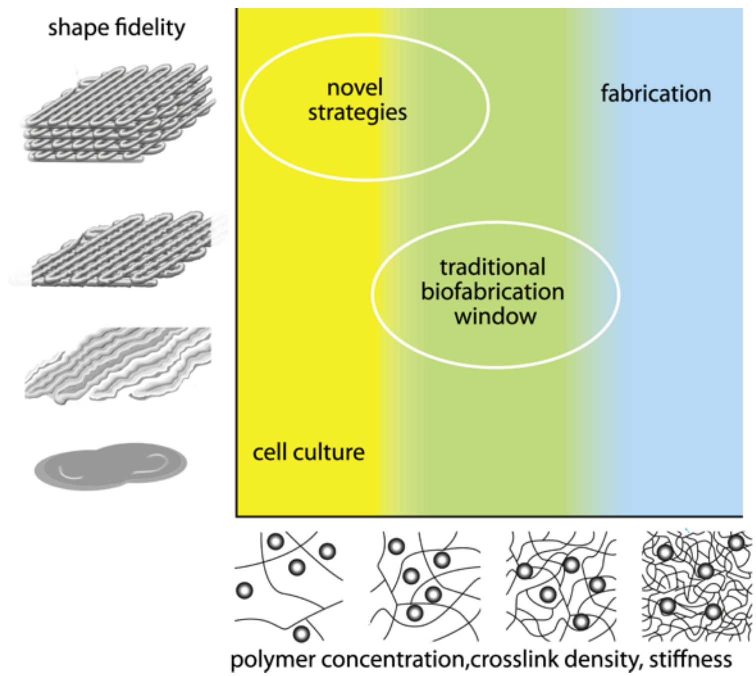
- ***Bioinks** are aqueous solutions of polymers in which cells are suspended and deposited in the form of hydrogel to fabricate a 3D construct.*

- Formulating a proper bioink is **challenging** as from one side it **has to provide an ideal environment for cells growth**, spreading and differentiation and from the other it deeply **influences the quality of printing**.



Main features of an **ideal** bioink:

- ➢ Printable
- ➢ Biocompatible and Bioactive
- ➢ ECM Biomimetic
- ➢ Support cell proliferation, differentiation
- ➢ Allow proper tissue maturation
- ➢ Tailored degradation
- ➢ Dynamic mechanical behaviour (if needed)

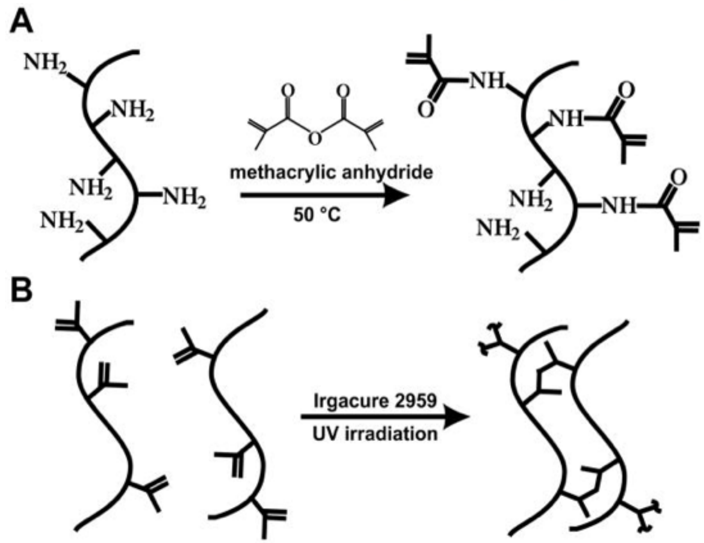


Moda J et al. Biofabrication, 2016,

Bio-ink materials

Materials	Synthetic or natural	Gelation mechanisms	Gelation speed
Acrylated PEG or multi-arm PEG	Synthetic	Photopolymerization	Seconds–minutes
Collagen	Natural	pH and temperature	Hours
Thiolated hyaluronic acid	Natural	pH-mediated Michael addition	Minutes–hours
Thiolated hyaluronic acid	Natural	Photopolymerization thiol–ene	Seconds
Methacrylated hyaluronic acid	Natural	Photopolymerization	Minutes
Tyramine hyaluronic acid	Natural	Chemical cross-linking (oxidative coupling, tyramine–H_2O_2)	Seconds
Gelatin	Natural	Temperature	Minutes–hours
Methacrylated gelatin	Natural	Photopolymerization	Minutes
Alginate	Natural	Chemical cross-linking (Ca^{2+})	Seconds
Fibrin	Natural	Enzymatic reaction (thrombin–fibrinogen)	Seconds

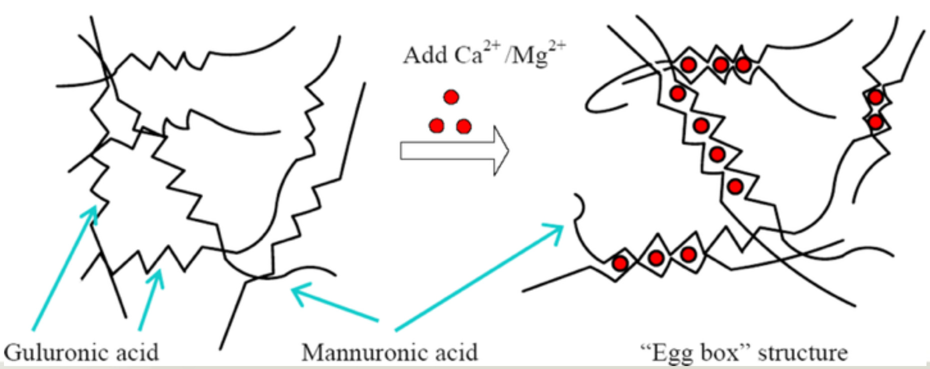
GelMA



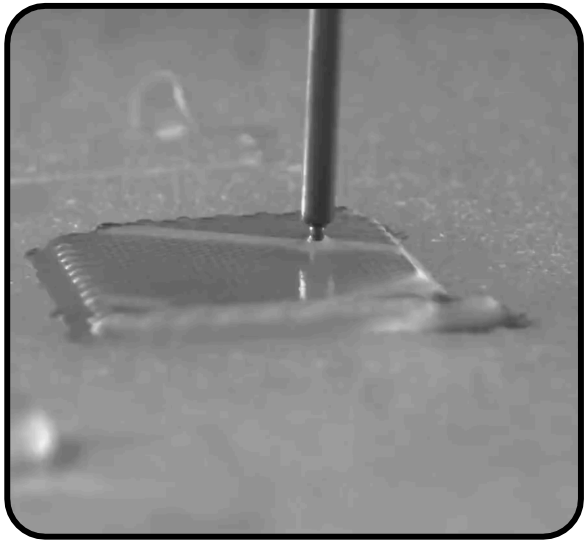
Photocurable biopolymers:
GelMA – Gelatin methacrylamide,
CS-AEMA – Chondroitin sulfate 2-aminoethyl methacrylate
HAMA – Methacrylated hyaluronic acid
PEGMA-fibrinogen)

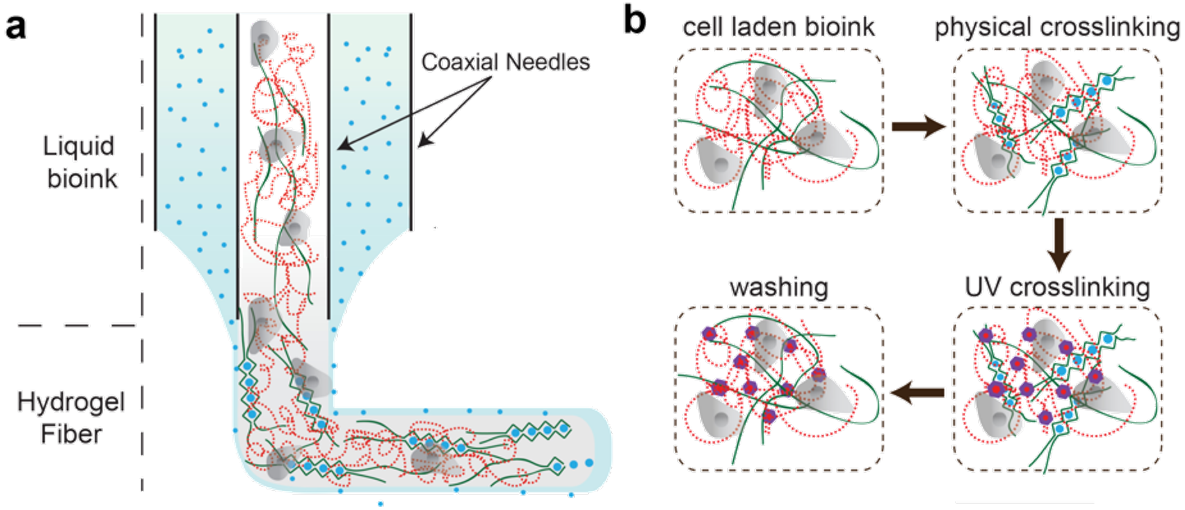
(UV or visible light crosslinking)

Alginate gel formation in the presence of calcium ions

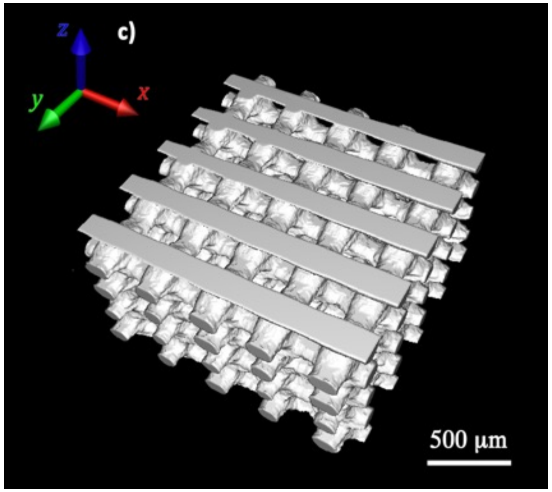


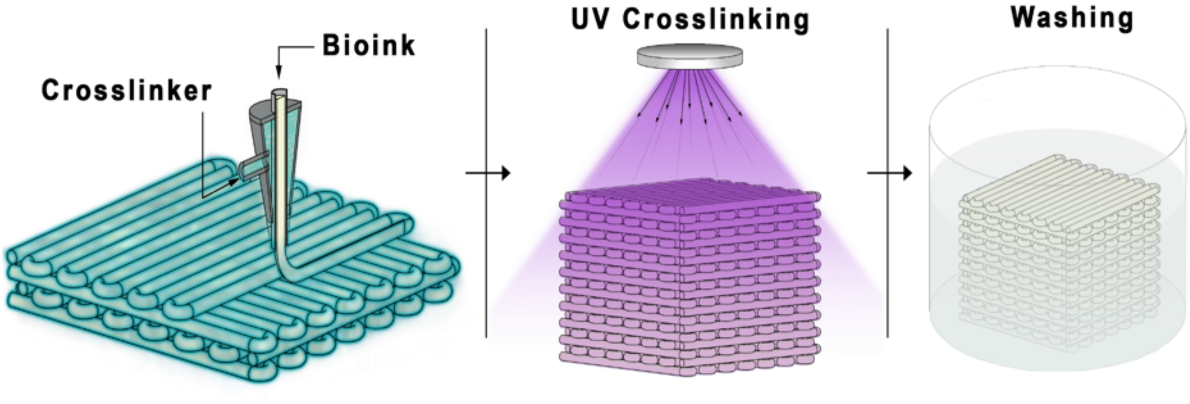
Co-axial extrusion-based bioprinting



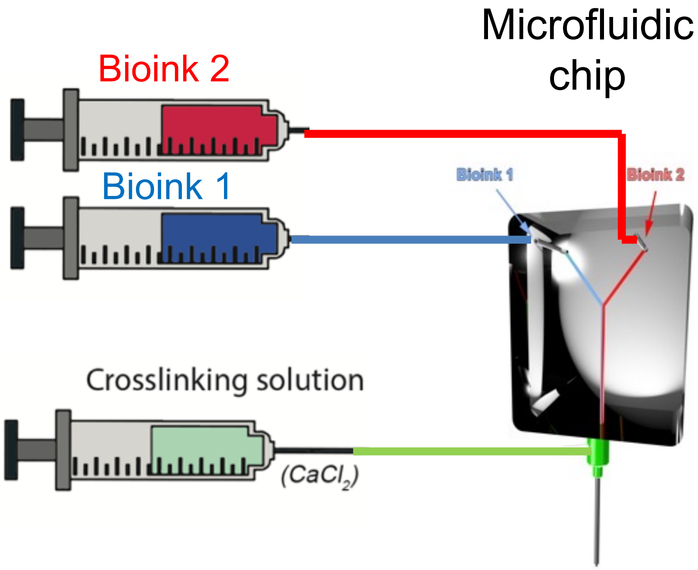


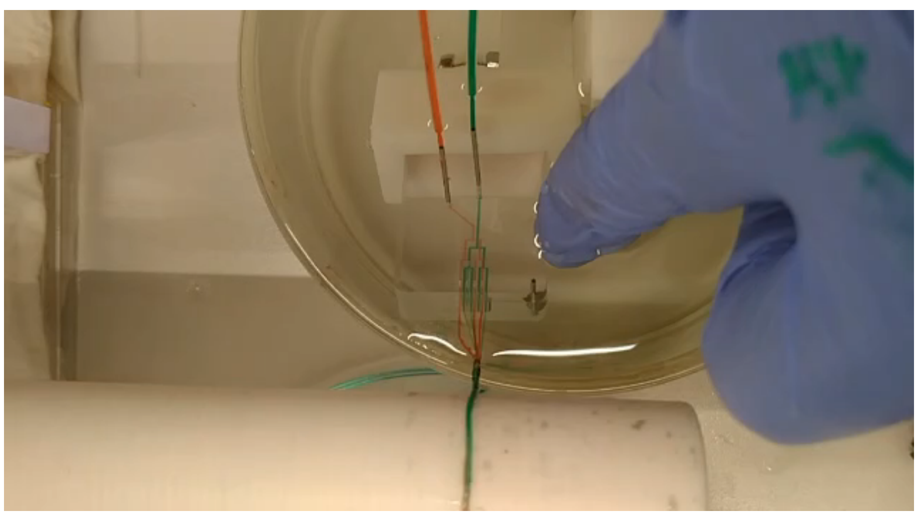
Dual cross-linking bioprinting

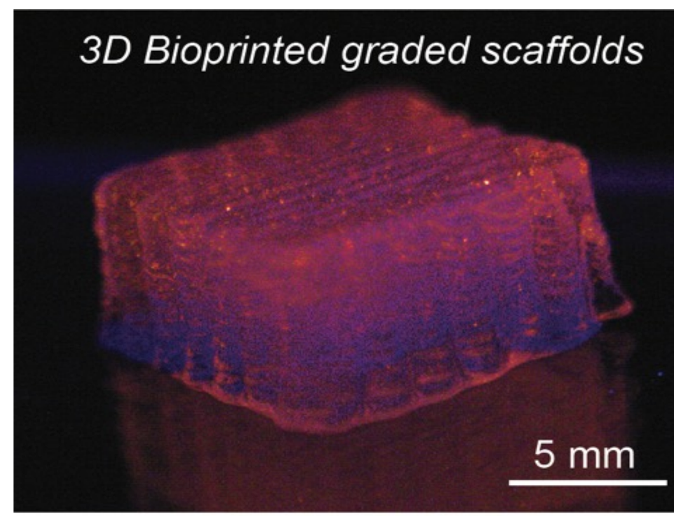




Microfluidic extrusion-based strategies

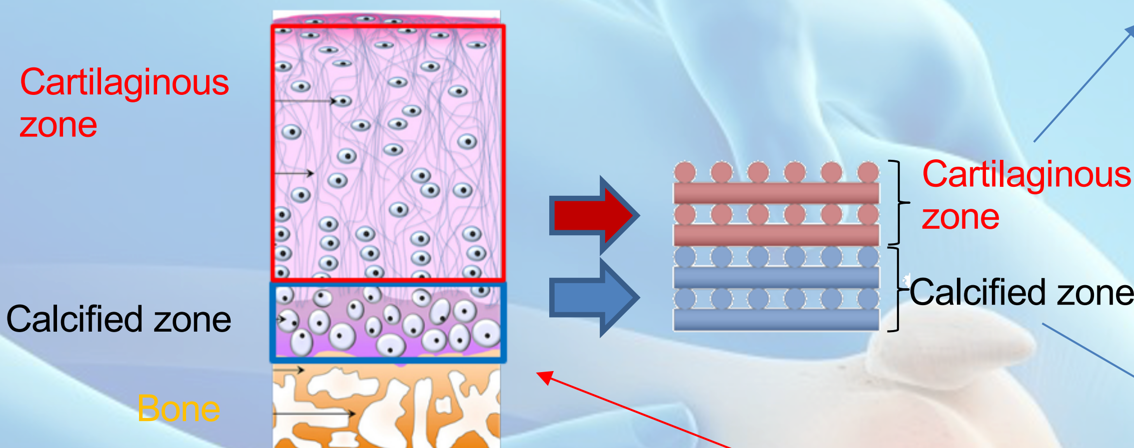






3D bioprinting of hydrogel constructs for zonal cartilage regeneration

Multi-material and multi-cellular deposition system



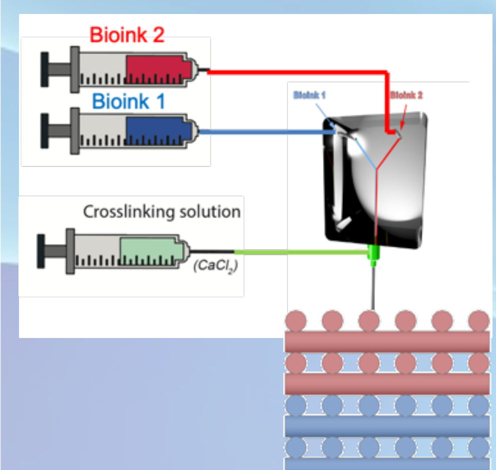
Bioink 2:

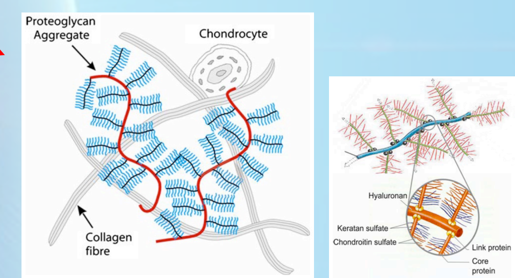
ALG: 4% w/w
GelMA: 6% w/w
CS-AEMA: 4% w/w
hAC: $2{,}5 \cdot 10^6$ cells/mL
hBM-MSCs: $7{,}5 \cdot 10^6$ cells/mL

Bioink 1:

ALG: 4% w/w
GelMA: 6% w/w
CS-AEMA: 4% w/w
HA-MA: 0.5% w/w
TCP NPs: 0.5% w/w
hBM-MSCs: 10^7 cells/mL

Microfluidic chip



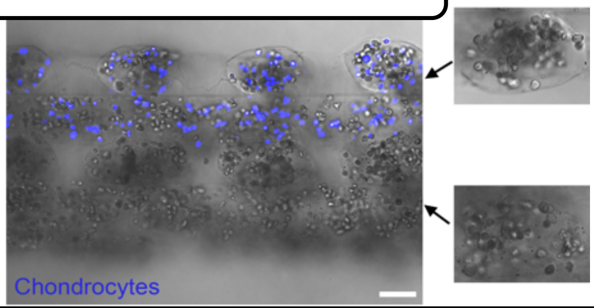


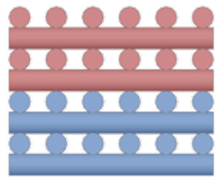
100 million people worldwide suffer from osteoarthritis

Idaszek J, et al. ***Biofabrication*** *2019*

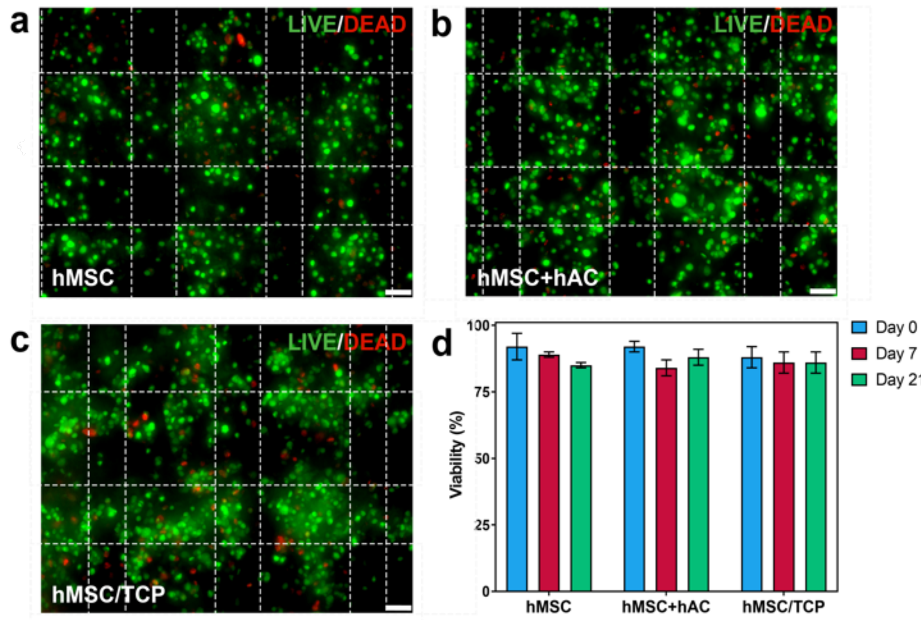
Biopriting od 3D bizonal articular cartilage

Confocal microscope

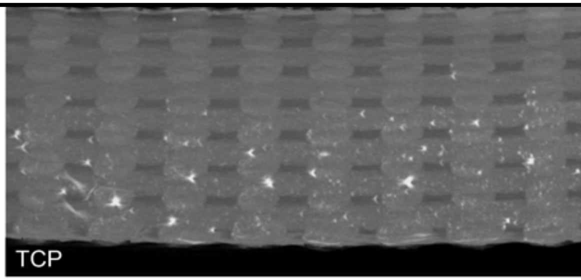




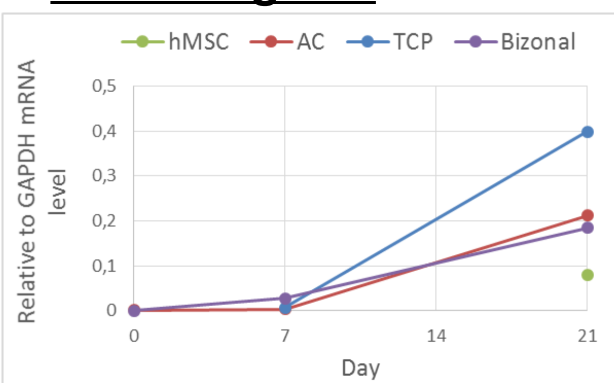
Live/dead fluorescent assay

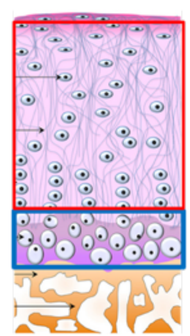


µCT 3D radiograph – TCP distribution

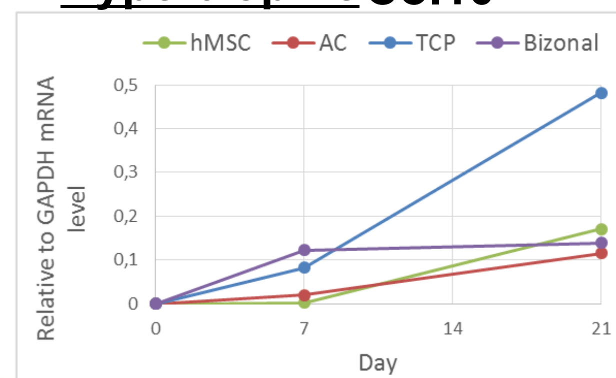


Chondrogenic Col2

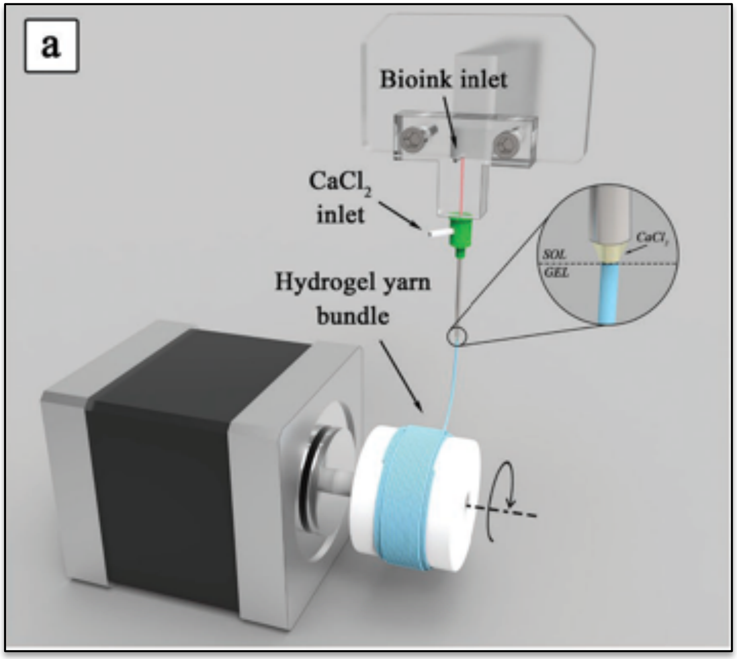


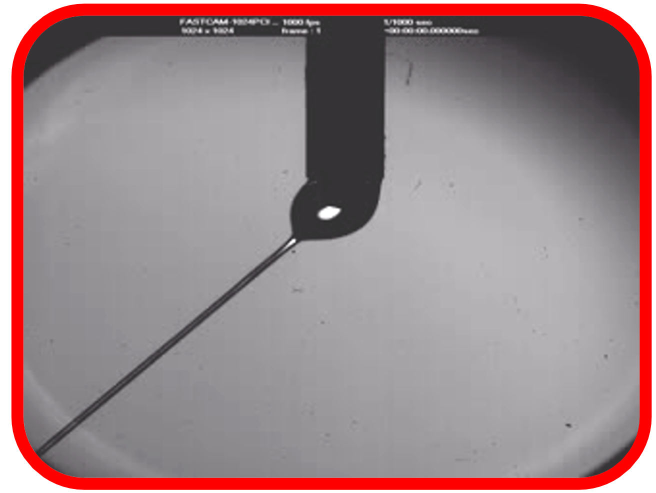


Hypertrophic Col10



Co-axial wet-spinning biofabrication





- ✓ The first crosslinking of the yarns during the spinning process
- ✓ Hydrogel fibers were then collected onto a rotating drum with 30 rpm motor speed, in order to obtain aligned fibrous structures
- ✓ A second crosslinking was performed by exposing the yarns to UV light for 30 s

Rinoldi C. et al. AHM, 2019,8 (7), 1970025

Highly aligned bio-fibers

✔ Highly aligned and compact fibers!

✔ Fiber diameter down to **100 μm**!!

- Highly aligned yarns recapitulate the structure and morphology of the **muscle** or **tendon**, mimicking the native tissue architecture

Skeletal muscles tissue engineering

Skeletal muscles are characterized by a precise and **hierarchical structure** that allows them to exert their main function: **contraction**.

This structure is composed of **unidirectionally aligned myotubes** surrounded by connective tissue, capillaries and nerves.

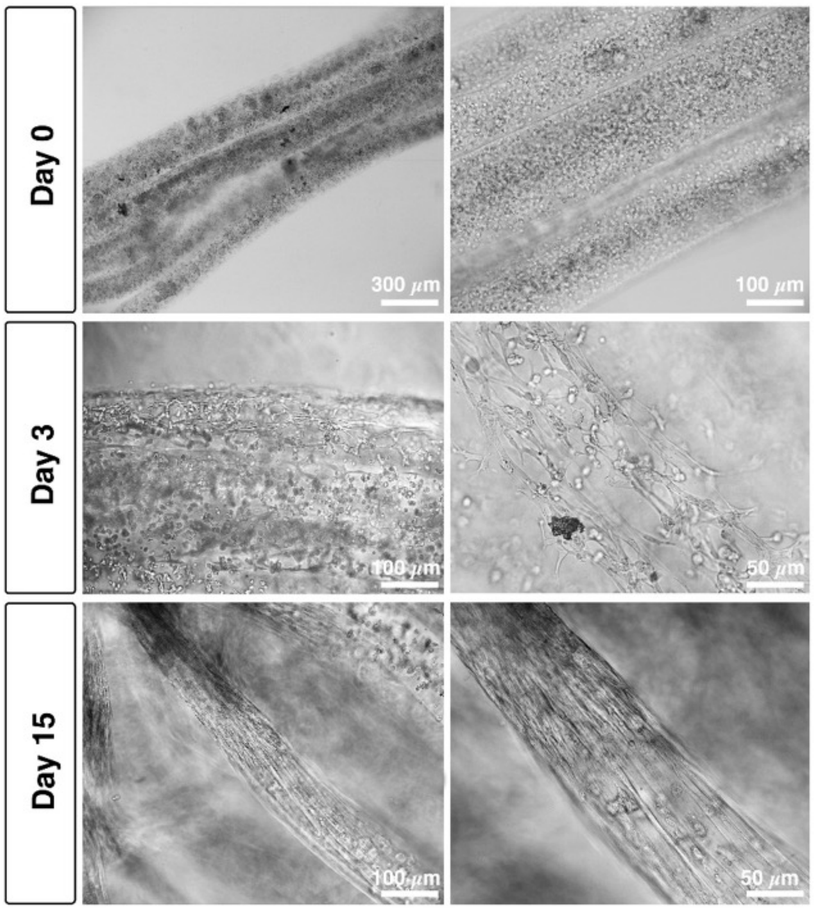
So far, several approaches have been developed, however **a functional model of skeletal muscle is still missing!**

3D bioprinting represents a suitable technology to engineer successfully artificial skeletal muscle!!

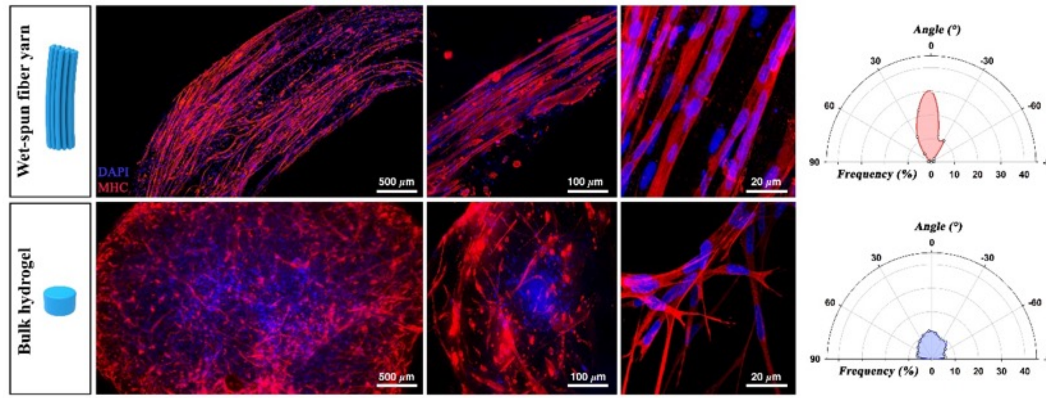
33 millions musculoskeletal injuries per year in United States alone !!!

Engineering muscle tissue

Cells: Skeletal muscle progenitors (murine mesoangioblasts) 2 x 10^7 cells/mL in hydrogel fibers



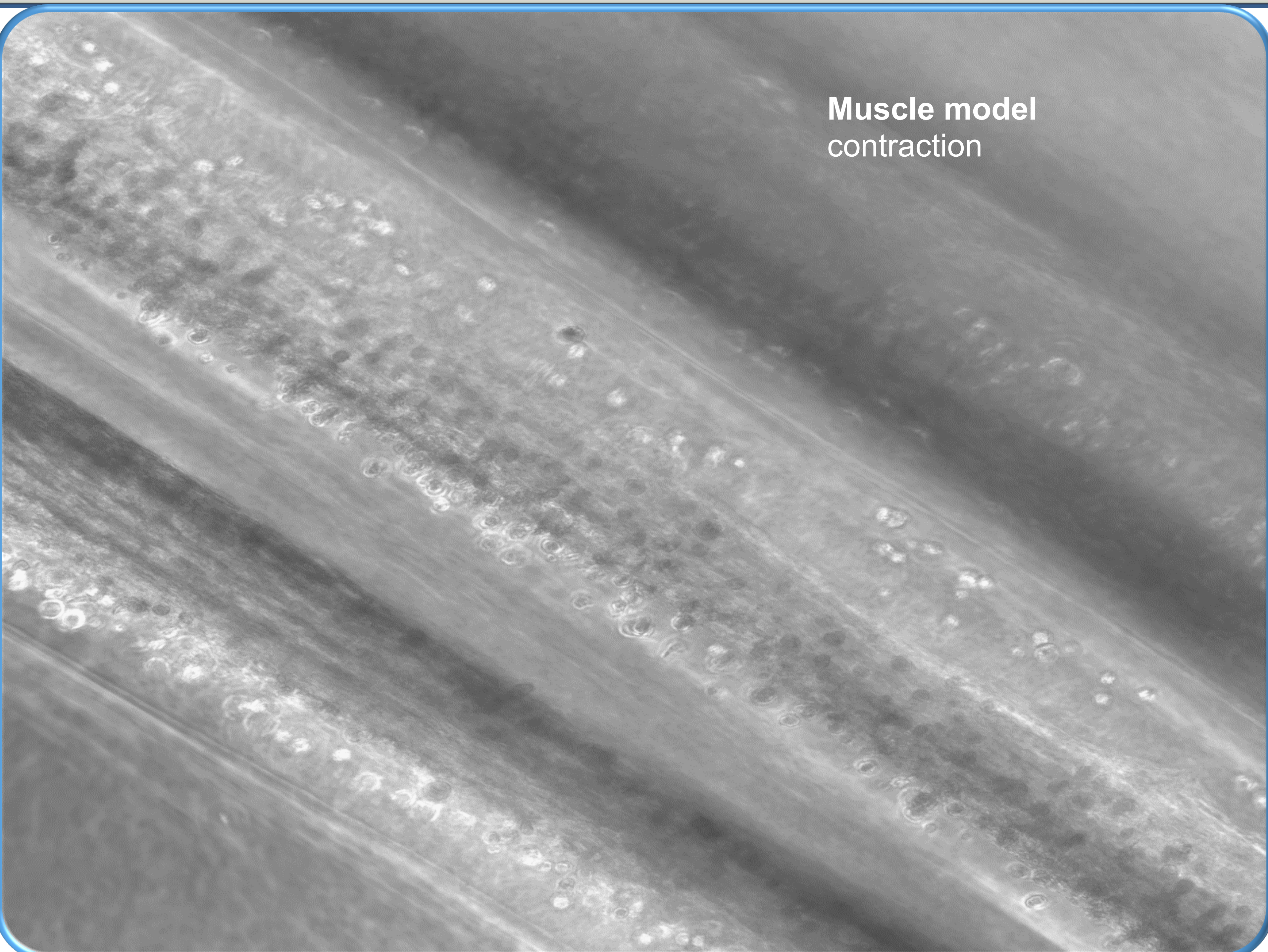
***In vitro* Mabs differentiation.**
MHC staining (red)



Cells rapidly started to elongate and fuse forming **highly aligned, long-range multinucleated myotubes**.

Day 15 revealing the progressive **formation of myofibers** and an outstanding **sarcomeric organization**.

Costantini et al., ***EMBO molecular medicine****, just accepted, doi: 10.15252/emmm.202012778*


Muscle model
contraction

Tendon injury, treatment and challenges

○ Tendon injury

✓Major tendon injuries are caused by physical activity (sport or professional activities), traumatic events or aging, degenerative process

✓The decreasing of musculoskeletal functions reduces people activity and quality of life

✓Affects an estimated number of **100 million people worldwide annually**

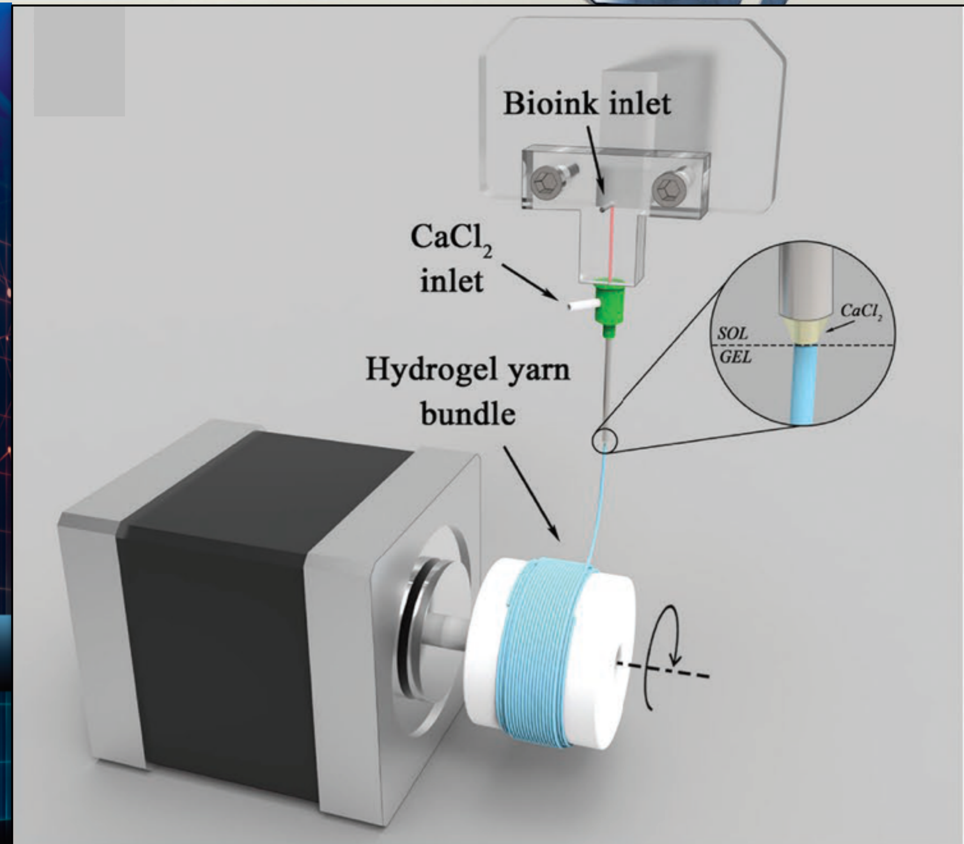
✓500,000 annual reconstructive surgeries of tendons in the US (more than 100 billion USD)

○ Challenges

✓**Due to its hypovascularity and hypocellularity, tendon has a weak intrinsic healing ability**

✓ Inability of the repaired tissue to regenerate the total functions of native tendon

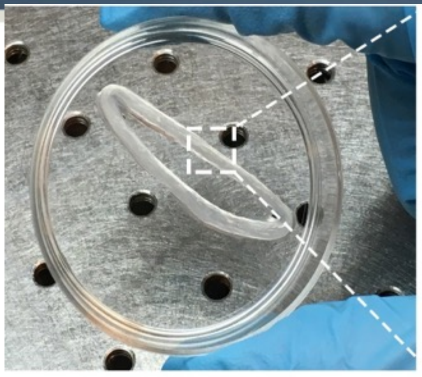
Co-axial wet-spinning biofabrication for tendon TE

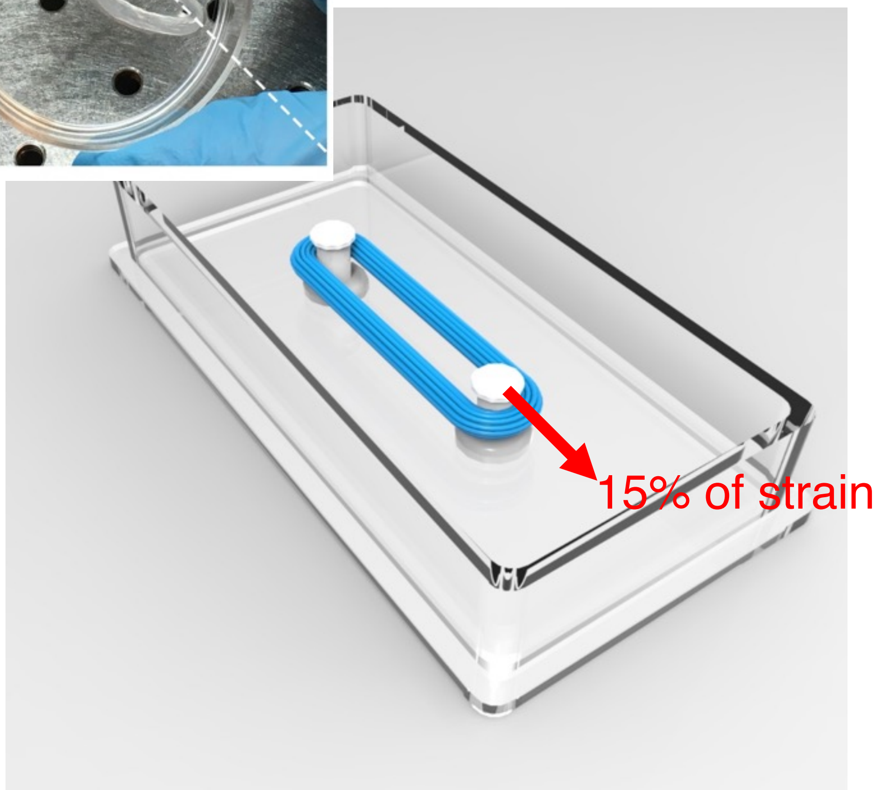


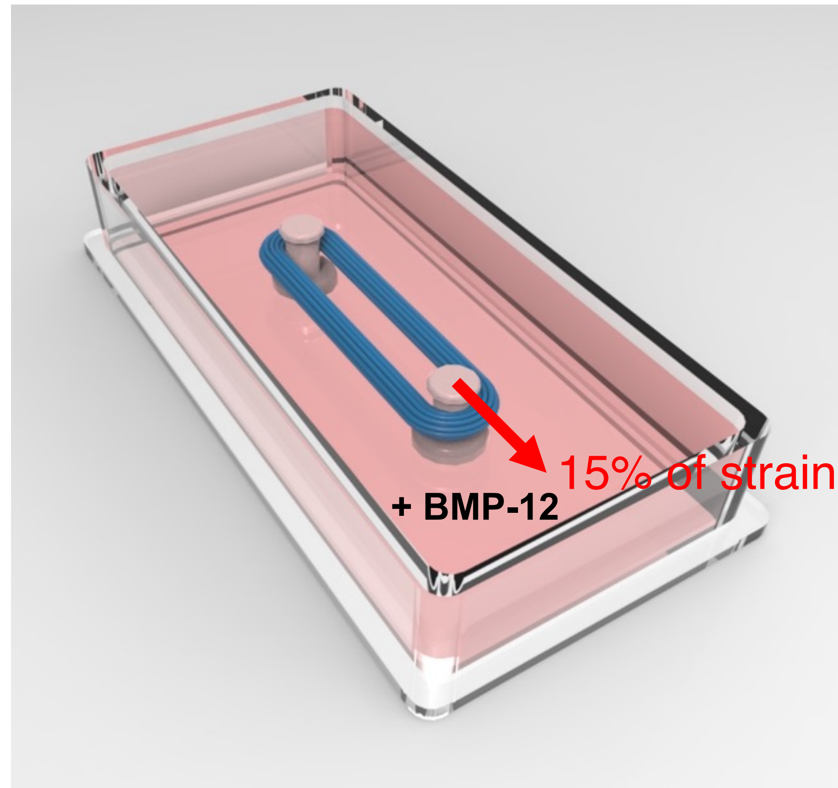
- ✓ Co-axial wet-spinning biofabrication
- ✓ **The bioink** was prepared by dissolving lyophilized GelMA 5% (w/v) and Alginate 4% (w/v) in HEPES containing 0.1% (w/v) of Irgacure 2959 as photoinitiator + hBM-MSCs

Rinoldi C. et al. AHM, 2019,8 (7), 1970025

Tissue construct stimulation







Mechanical stretching (MS)

Mechanical stretching

+ Biochemical stimulation (MBS)

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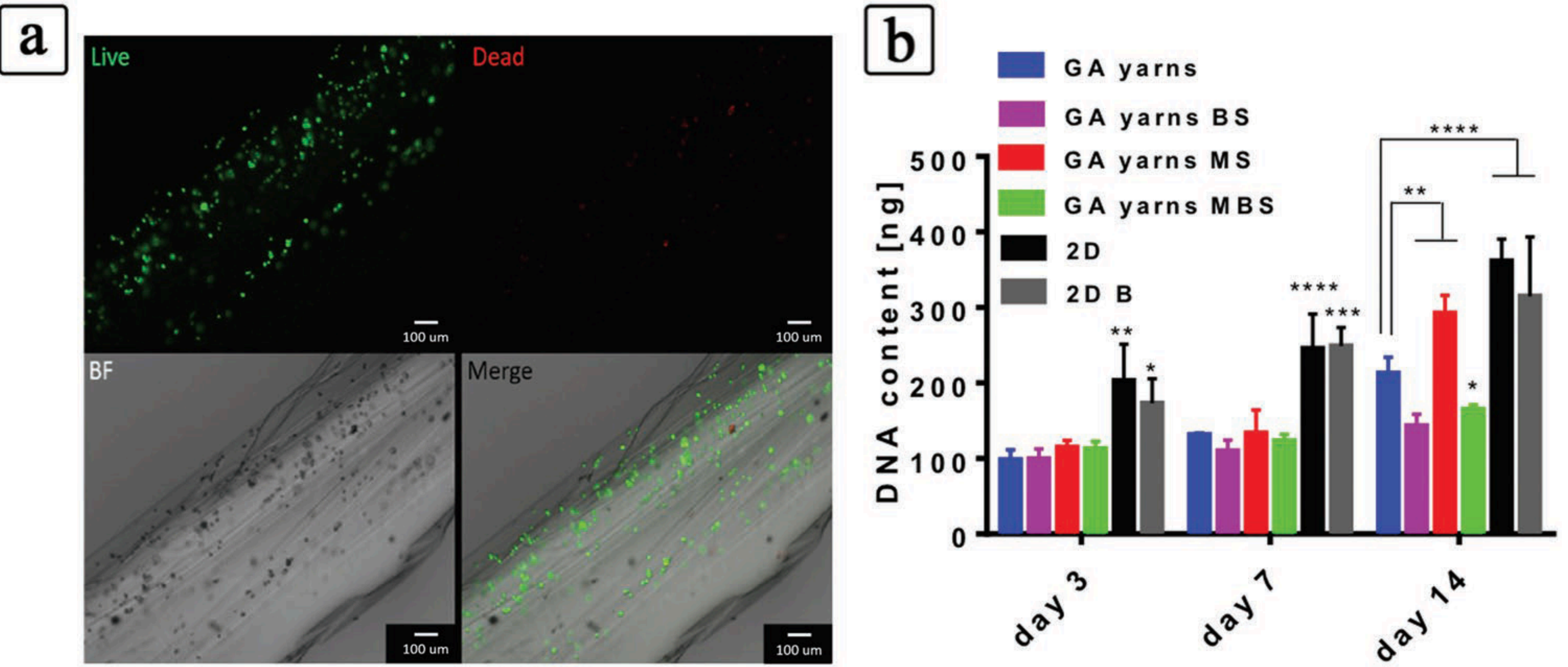
Biological response

Cell viability **>94%**



hBM-MSC viability, live/dead staining, performed 24 hours after fabrication process

- Mechano-transduction promotes cell growth
- BMP-12 led to a proliferation detriment

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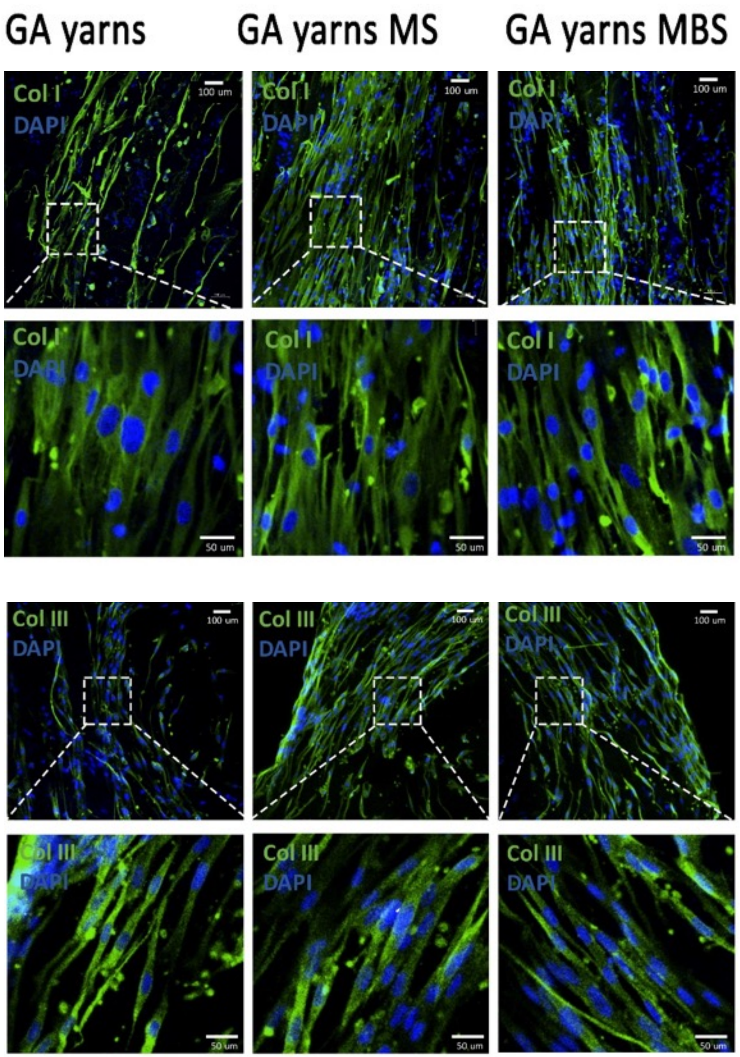
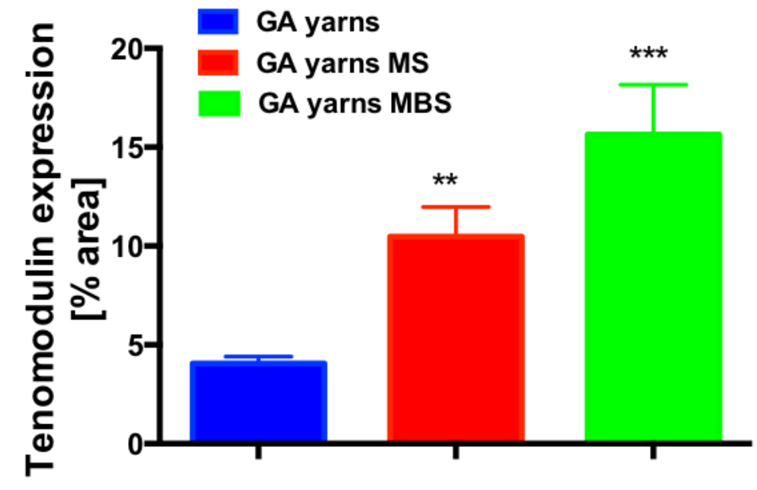
ECM proteins expression



- Aligned orientation and deposition of collagen type I and III leading to the formation of oriented matrix, which can mimic the native tendon ECM architecture



- Combination of mechanical and biochemical stimuli induces a synergistic effect on key tenogenic gene expression, leading to a more effective tenogenic differentiation of hBM-MSCs compared to non-stimulated condition.

Conclusions

- **Properly designed bio-ink and 3D biofabrication method are crucial for development of tissue engineered constructs and products**

- 3D microextrusion-based bioprinting allow for **high-resolution 3D biofabrication (<100 um) to reproduce natural tissue structures**.

- **Blending alginate with natural based polymers or dECM** allows to formulate **ECM biomimetic inks** that can be used for tissue engineering.

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Jan Brinchmann, OU, NO

iTE

START for TE

New Joint

HARVARD

MIT

NUS National University of Singapore

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BIO-IMPLANT

UNIA EUROPEJSKA EUROPEJSKI FUNDUSZ ROZWOJU REGIONALNEGO

NIMS National Institute for Materials Science

Bionic

CEPT Centrum Badań Przedklinicznych i Technologii

SEVENTH FRAMEWORK PROGRAMME

MATERA

norway grants

Centrum Studiów Zaawansowanych PW
Center for Advanced Studies WUT

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COST

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