



STIMULI-RESPONSIVE BIOENGINEERED BIOMATERIALS: 3D *IN VITRO* MODELS & REGENERATIVE MEDICINE THERAPIES



Consiglio Nazionale
delle Ricerche



**CNR
ISSMC**

Istituto di Scienza, Tecnologia
e Sostenibilità per lo Sviluppo
dei Materiali Ceramici

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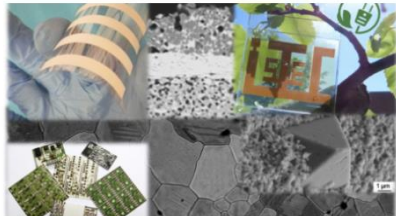
PHILADELPHIA, PA
JULY 13-18, 2025

**NEXT-GENERATION
DELIVERY INNOVATIONS**

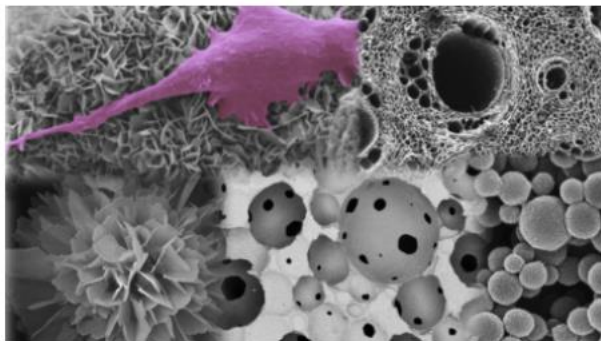
NATIONAL RESEARCH COUNCIL of ITALY - CNR



Nanotechnologies and nanosafety



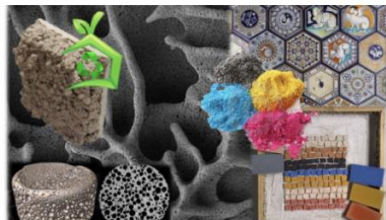
Energy and mechatronics



Health and wellbeing



Materials for extreme applications



Environment building and cultural heritage



LA
RICERCA
VENUTA
DAL
FUTURO



Istituto di Scienza, Tecnologia
e Sostenibilità per lo Sviluppo
dei Materiali Ceramici

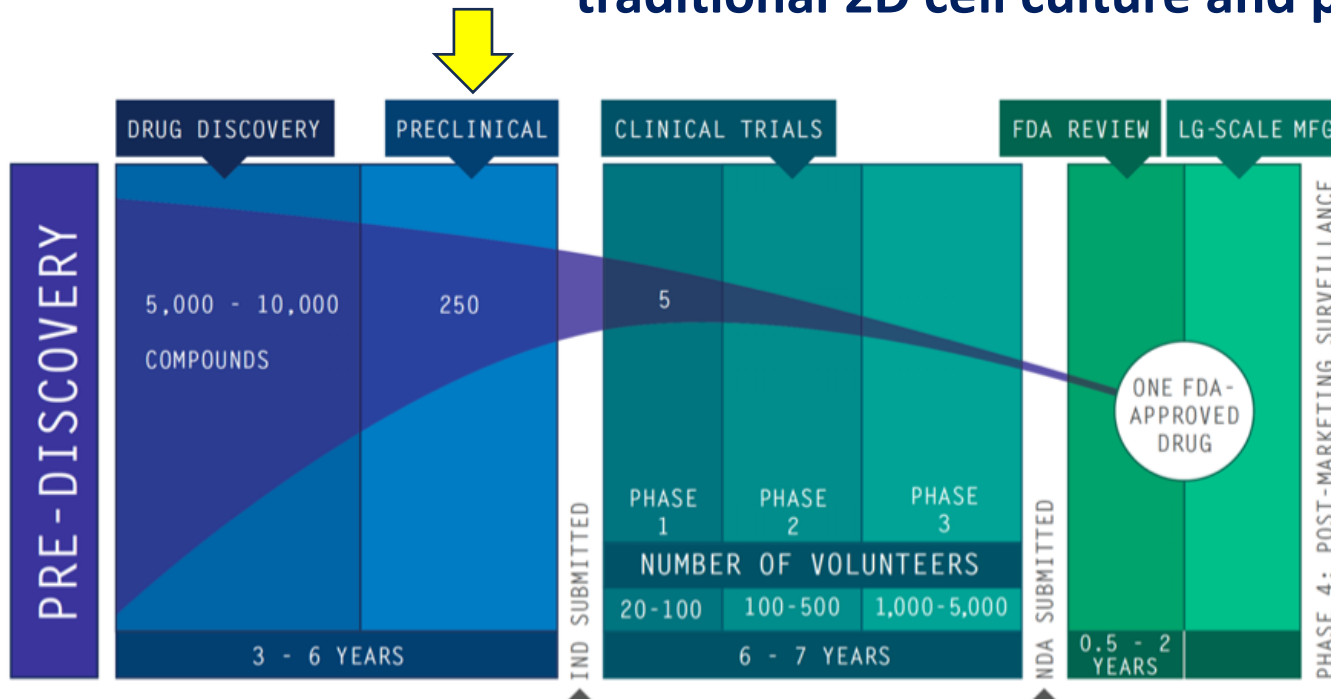
www.issmc.cnr.it

ALTERNATIVE METHODS TO ANIMAL TESTING



- *In Silico* Approaches
- *Ex Vivo* and Human-Based Methods
- *In Vitro* Models → Use of human cells, tissues, spheroids, organoids, organ-on-chip systems and **SCAFFOLDS**
- Integrated Testing Strategies

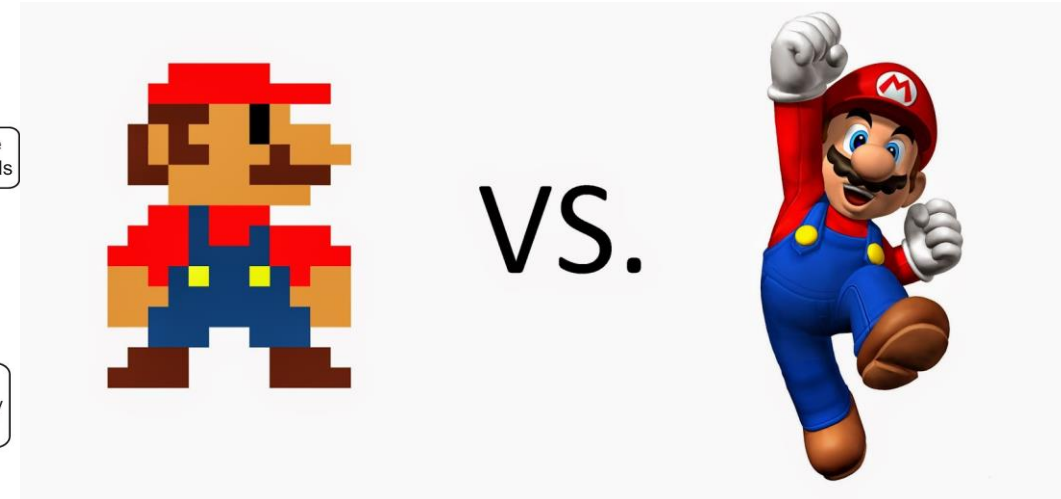
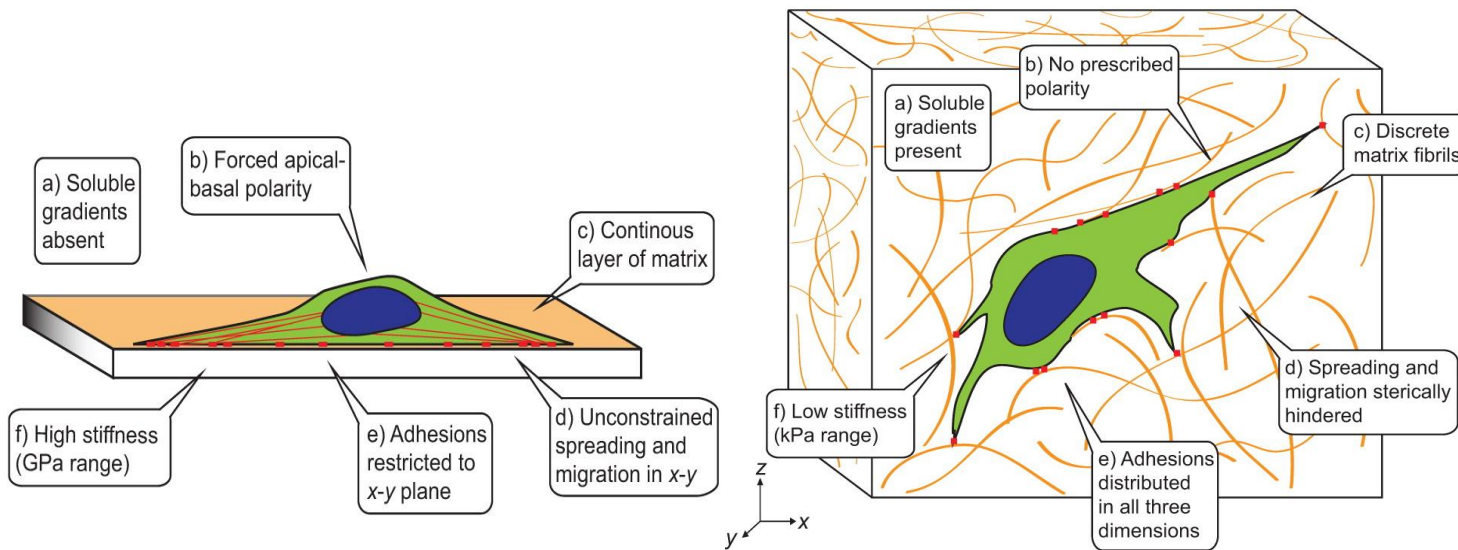
Novel *in vitro* cell culture models offer promising approaches that help bridge the gap between traditional 2D cell culture and preclinical/clinical studies

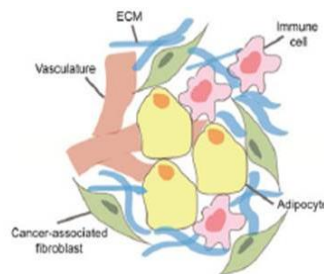


IF YOU AREN'T USING **ADVANCED CELL CULTURE, WHAT DISCOVERIES COULD YOU BE MISSING?**

ABSENCE OF PATHO-PHYSIOLOGICALLY RELEVANT *IN VITRO* MODELS

(understanding the organs' behavior or diseases, their mechanisms and progression, effective screening of potential therapy)





INTERDISCIPLINARITY

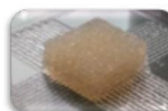
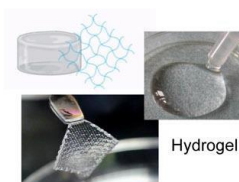
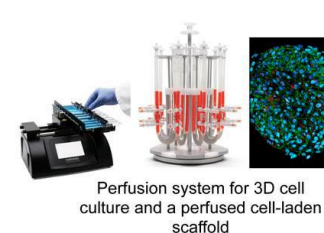
Heterogeneous cell population:

- Differentiated cells
- Stem Cells
- Endothelial cells
- Immune cells

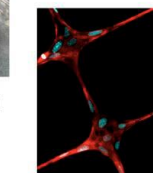
Dynamic extracellular matrix



3D model mimicking the complex tissue ecosystem



Bioprinted scaffold

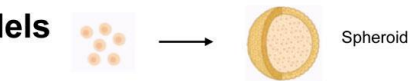
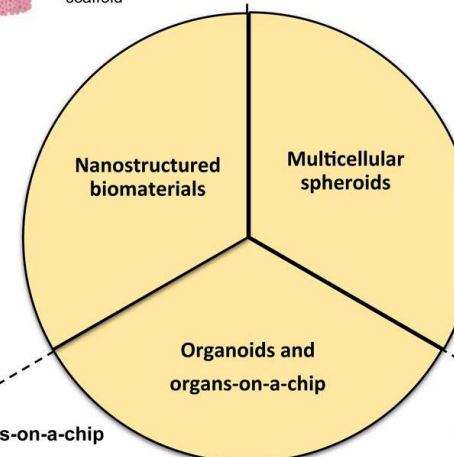


Bioprinted microfibers imaging

3D technologies for *in vitro* models



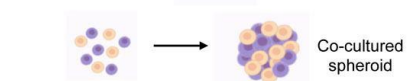
Macroporous scaffold



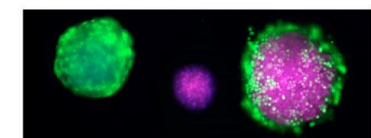
Spheroid



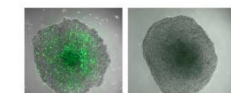
Nanofibers incorporation during spheroid formation



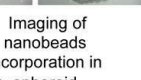
Co-cultured spheroid



Spheroid imaging

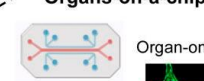


Organoids

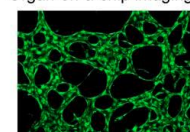


Imaging of nanobeads incorporation in spheroid

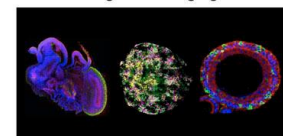
Organs-on-a-chip



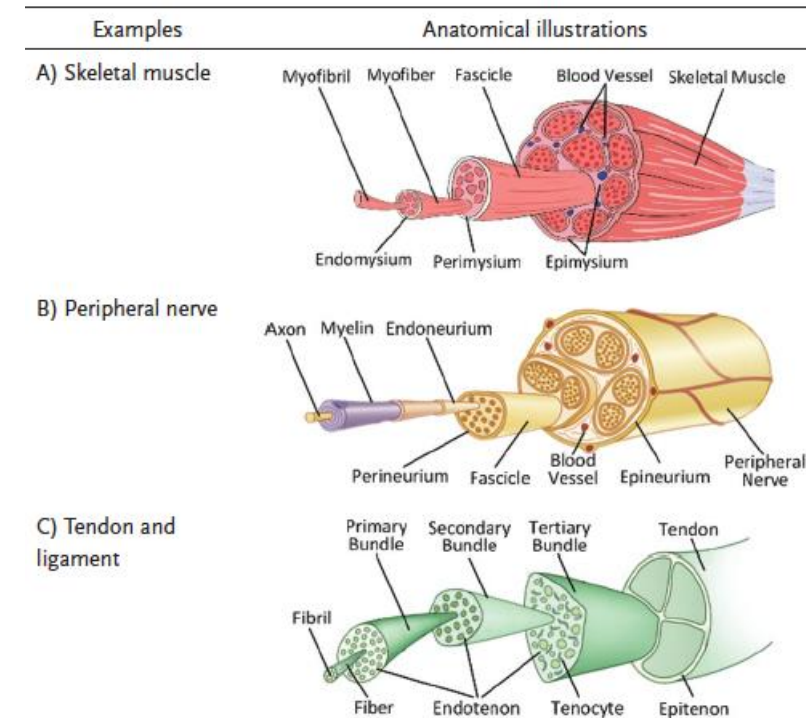
Organ-on-a-chip imaging



Organoid imaging

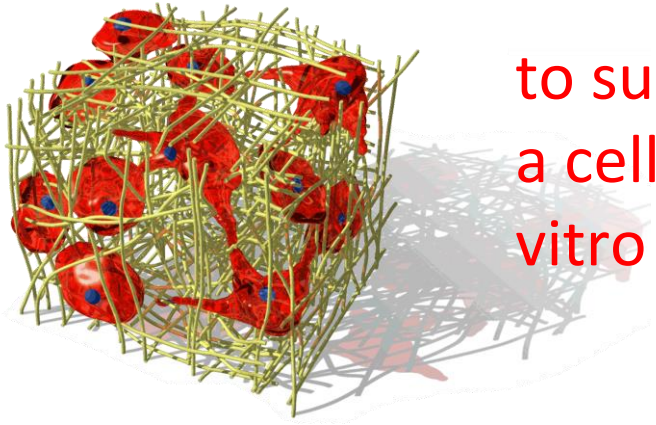


**CONTROLLED CELLULAR ALIGNMENT AND
BIOELECTRICAL STIMULATION
PLAY A CRUCIAL ROLE IN THE
NANO/MICROARCHITECTURE OF MANY HUMAN
TISSUES DICTATING THEIR BIOLOGICAL AND
MECHANICAL FUNCTION**

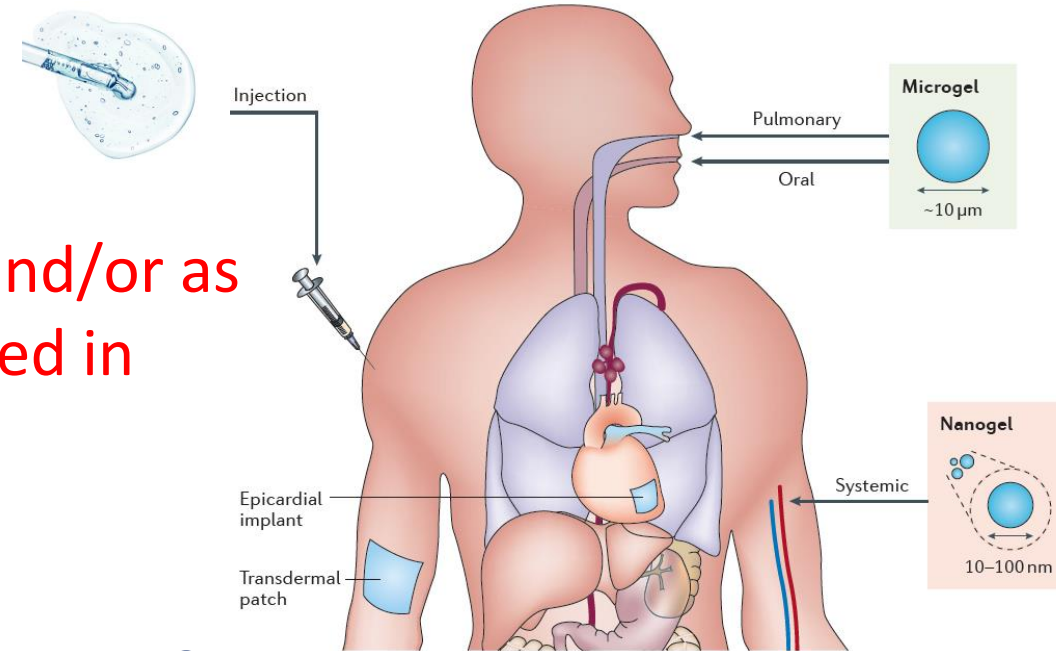


WHAT HAPPENS IN CASE OF TRAUMA, TUMOR OR OTHER DISEASE?

BIOMATERIAL-BASED STRATEGIES



to support new tissue ingrowth and/or as a cell delivery vehicle, --> advanced in vitro models



Nat Rev Mater 1, 16071 (2016)

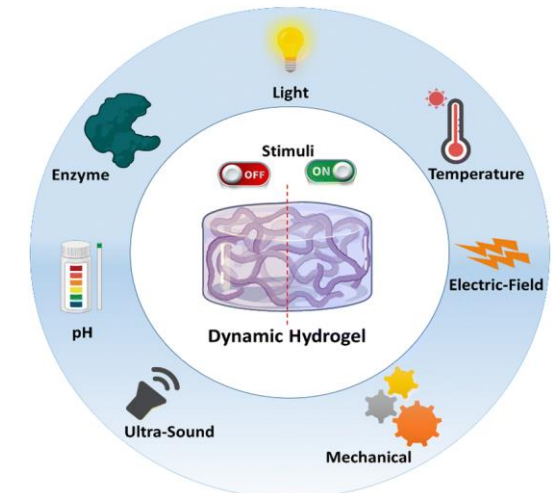
WHAT ARE STIMULI-RESPONSIVE MATERIALS?

SMART MATERIALS WITH THE ABILITY TO CHANGE THEIR PHYSICAL OR CHEMICAL PROPERTIES IN RESPONSE TO STIMULI

(temperature, pH, light, electric or magnetic fields, biochemical signals, etc.)

WHY ARE THEY RELEVANT?

Dynamically respond to the body's environment or external triggers



BIOINSPIRED STRATEGIES FOR 3D *IN VITRO* MODELS & REGENERATIVE MEDICINE THERAPIES

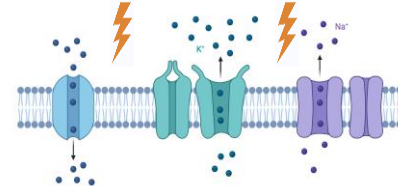
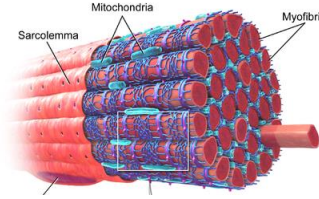


These 3D systems will evolve into 4D systems when electromagnetic field or ultrasound are applied:

○ **ALIGNED EXTRACELLULAR MATRIX**



SKELETAL MUSCLE



○ **BIOELECTRICITY**



NERVOUS SYSTEM



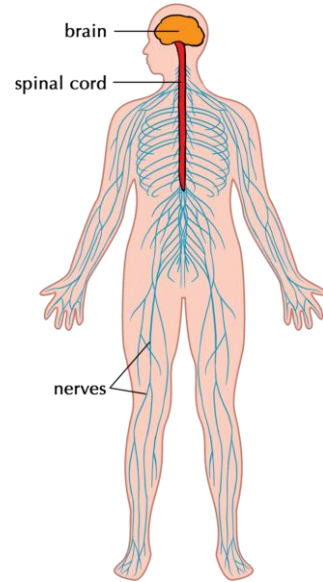
Volumetric Muscle Loss (VML) involves > 20% muscle loss with functional impairment and it is associated with neuromusculoskeletal damage

INJECTABLE MAGNETIC HYDROGEL

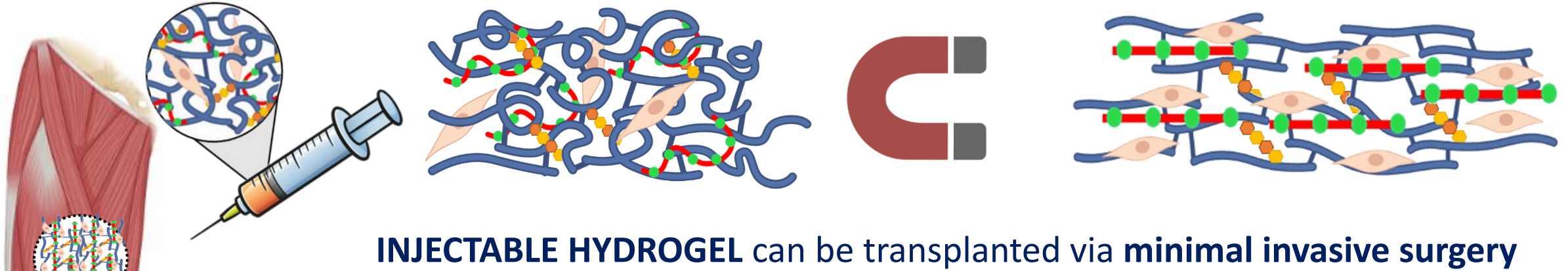
Damage to the Nervous System (CNS), resulting from trauma or neurodegenerative conditions, involves substantial neural tissue loss and functional deficits, with minimal potential for regeneration

PIEZOELECTRIC SCAFFOLD

NO EFFECTIVE THERAPIES OR ADVANCED *IN VITRO* MODELS CAPABLE OF SUPPORTING SIGNIFICANT FUNCTIONAL REGENERATION OR DRIVING MAJOR THERAPEUTIC ADVANCEMENTS



INJECTABLE HYDROGEL WITH ANISOTROPIC ARCHITECTURE BY APPLYING A STATIC MAGNETIC FIELD



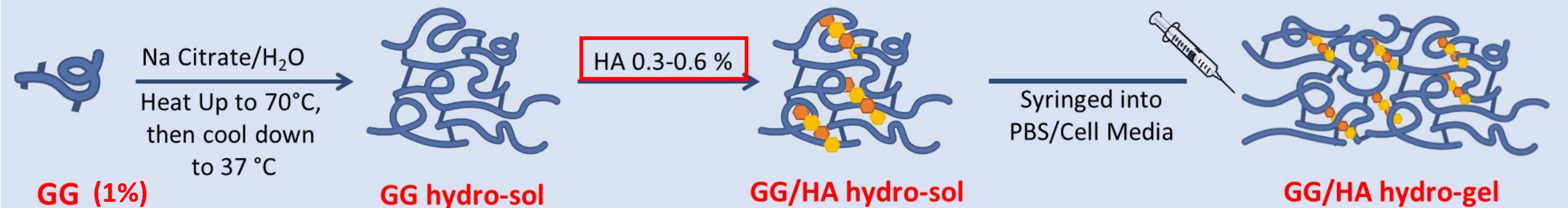
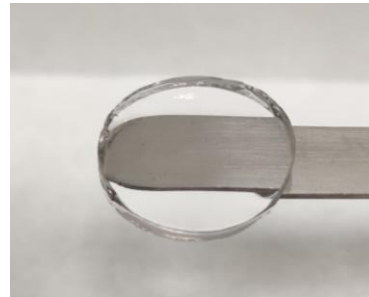
GELLAN GUM

bacterial polysaccharide known to be **biocompatible** and **biodegradable**

It can easily form a **hydrogel** by lowering the temperature or exposing it to cations

HYALURONIC ACID

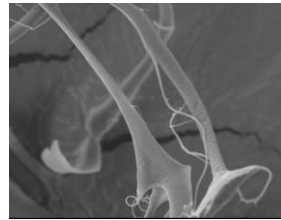
an important component of the extracellular matrix



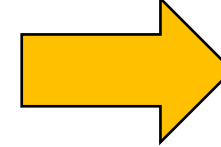
OUR STRATEGIES: MAGNETIC COLLAGEN FIBERS (MagFib)



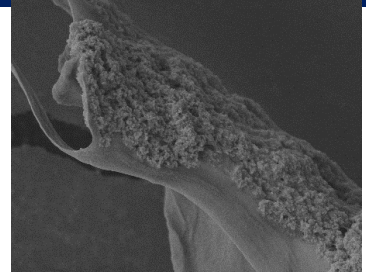
COLLAGEN



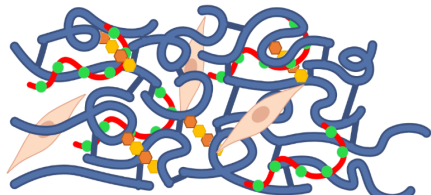
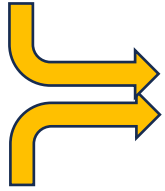
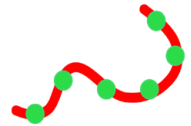
MAGNETIC NANOPARTICLES



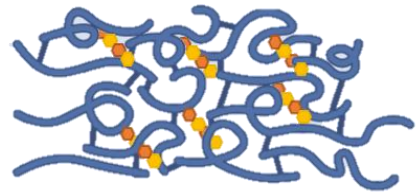
MagFib



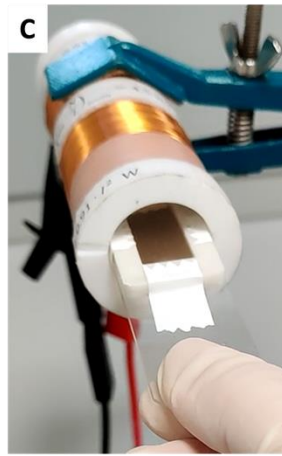
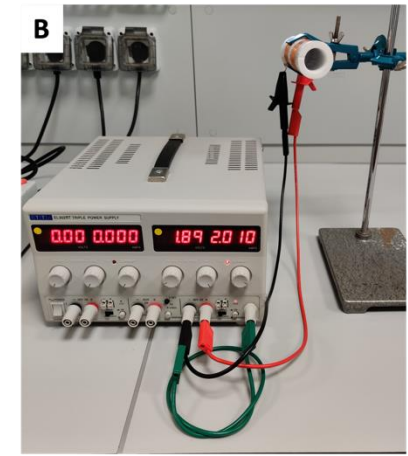
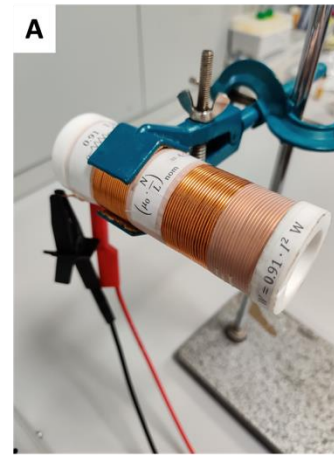
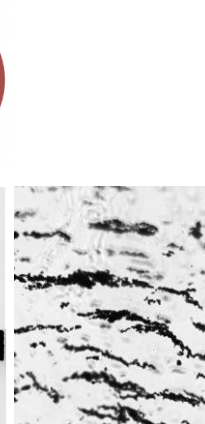
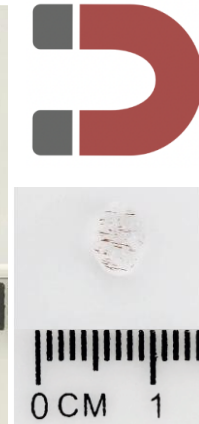
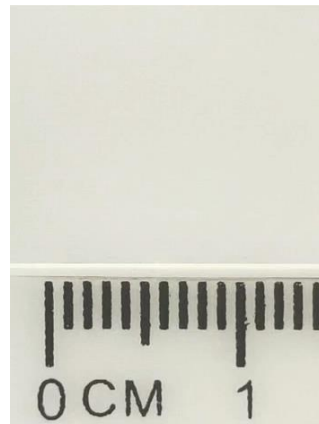
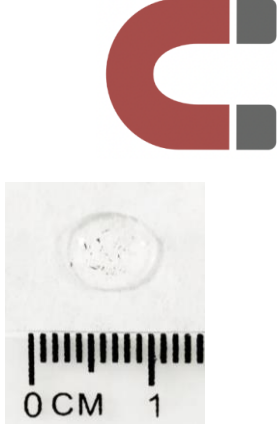
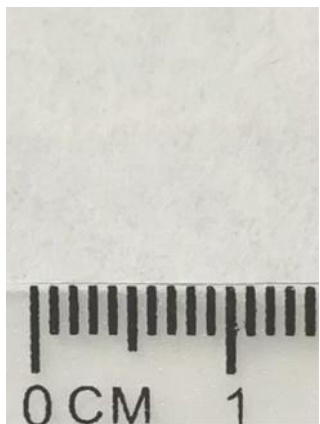
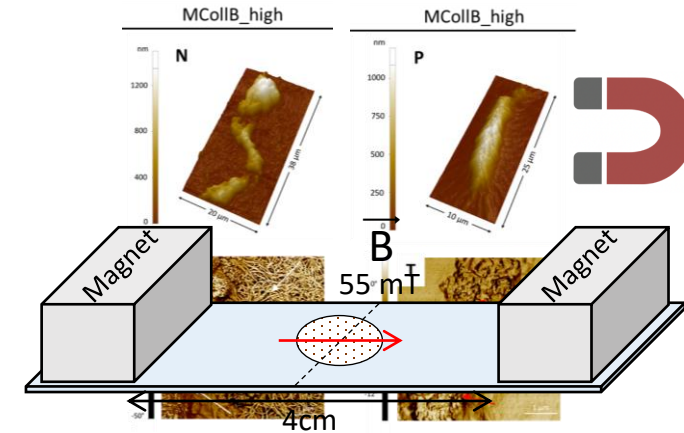
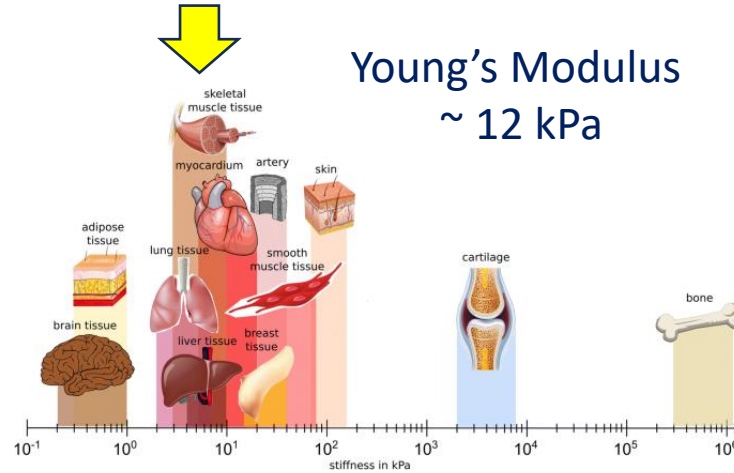
Type I, most abundant collagen of the human body



INJECTABLE MAGNETIC HYDROGEL



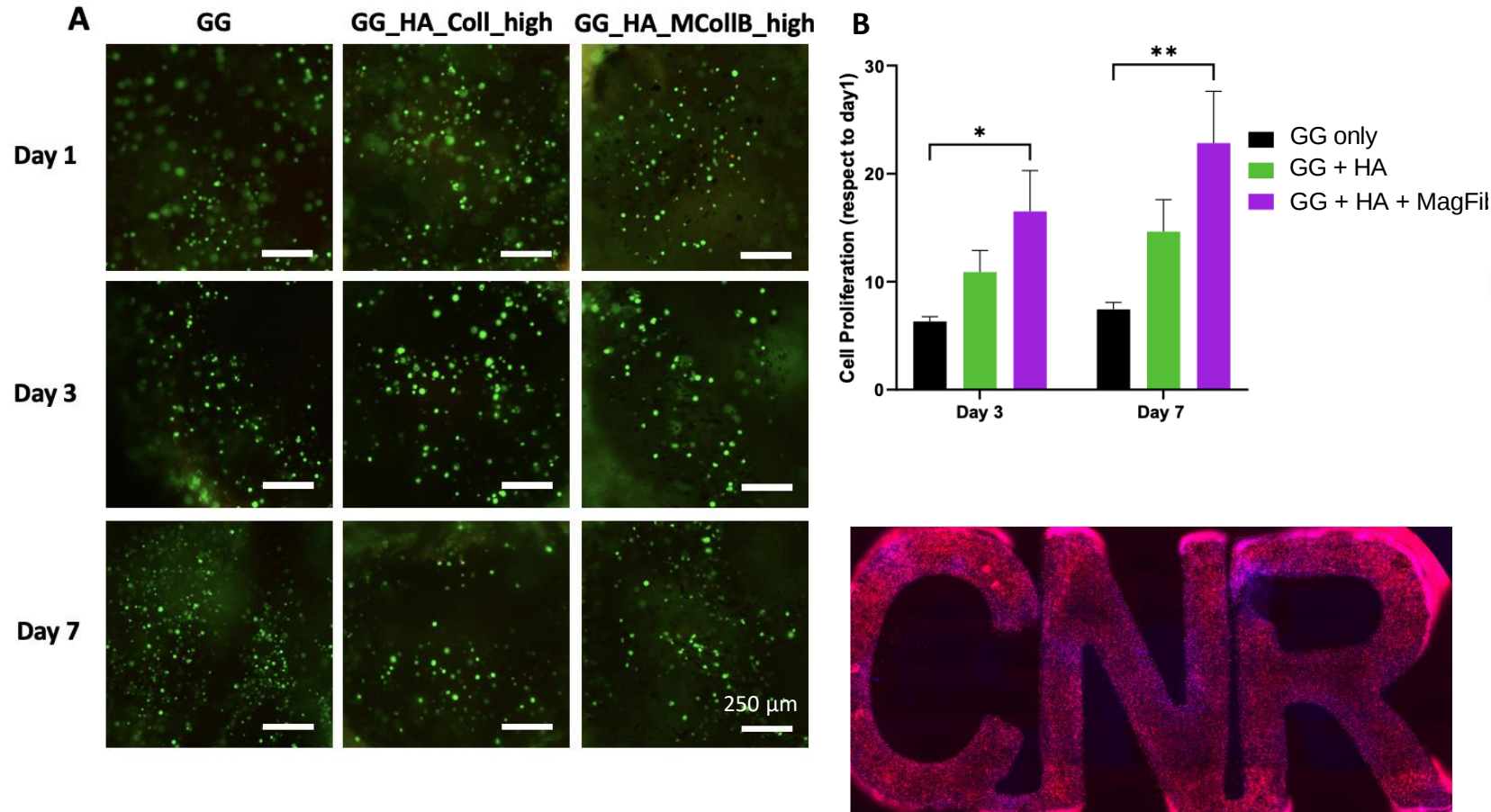
Young's Modulus
~ 12 kPa



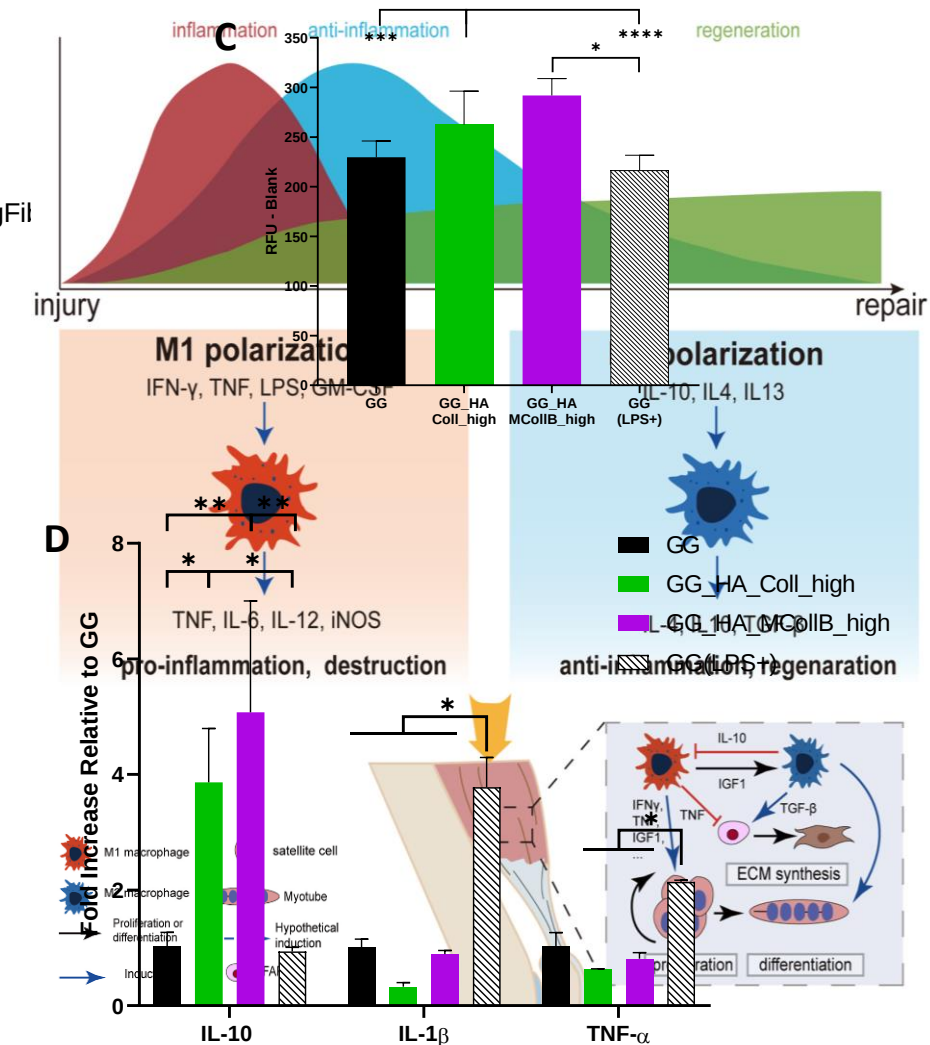
BIOLOGICAL BEHAVIOUR – *in vitro* study



Preliminary evaluation of hydrogel cytotoxicity (BALB3T3 cells)



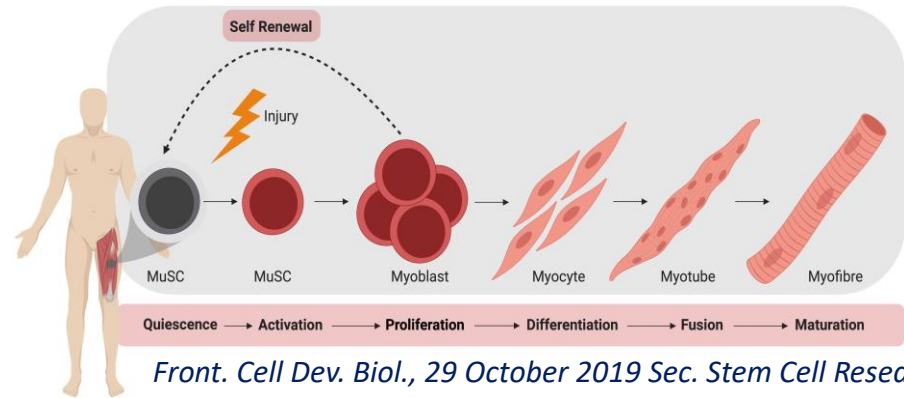
Immunomodulation (Raw 264.7 cells)



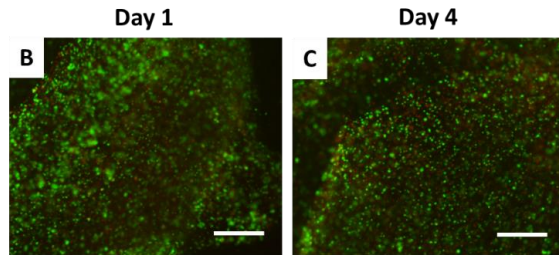
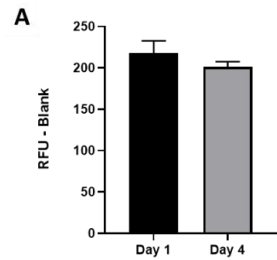
All the formulations tested showed good cytocompatibility
(cells embedded into the hydrogel) + M2 activation

BIOLOGICAL BEHAVIOUR – *Muscle Cells*

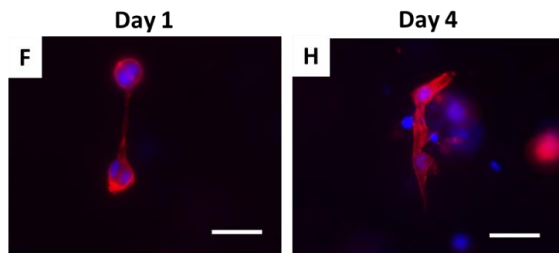
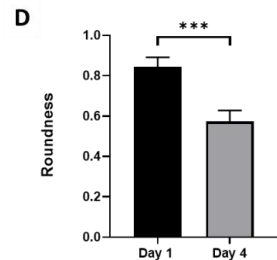
Muscle cells (C2C12 cell line)



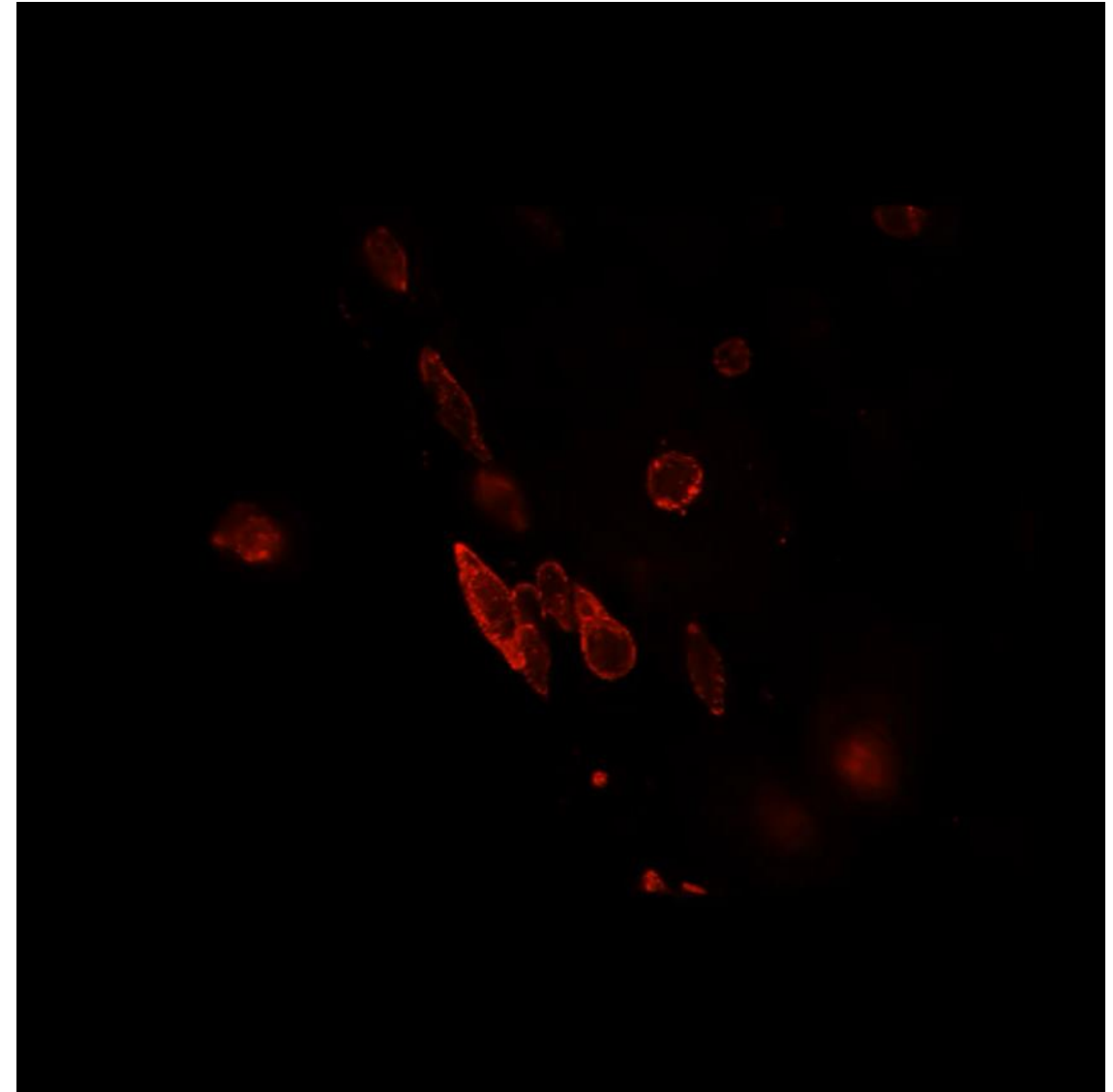
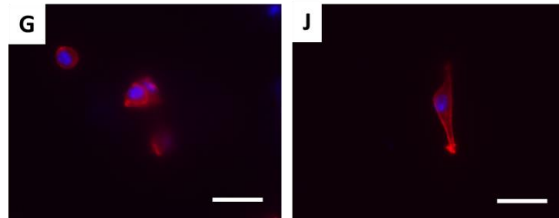
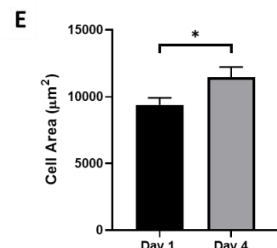
Front. Cell Dev. Biol., 29 October 2019 Sec. Stem Cell Research



HIGH RATIO OF **LIVE CELLS** compared to **dead cells**



Roundness → cells start spreading and elongating within the hydrogels over time

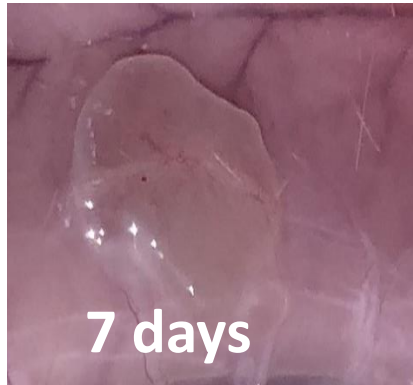


BIOLOGICAL BEHAVIOUR – *in vivo* study

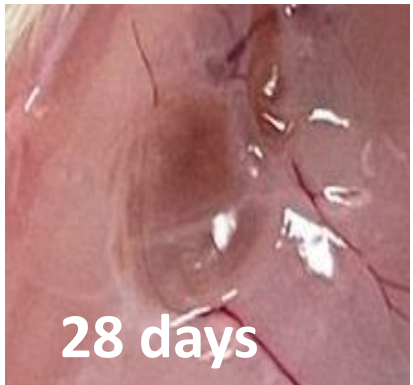
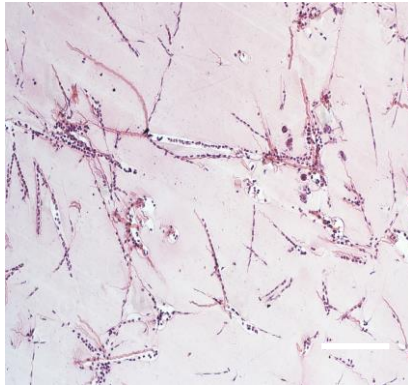


The hydrogel gelled rapidly (< 2 minutes)

No local inflammation was detected up to 28 days

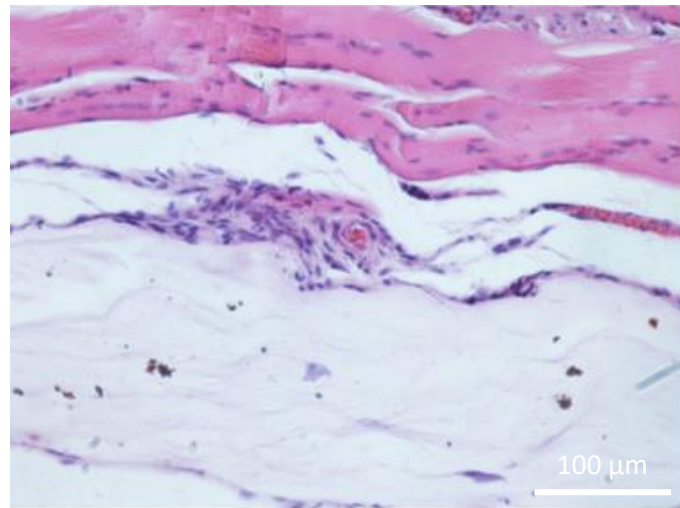
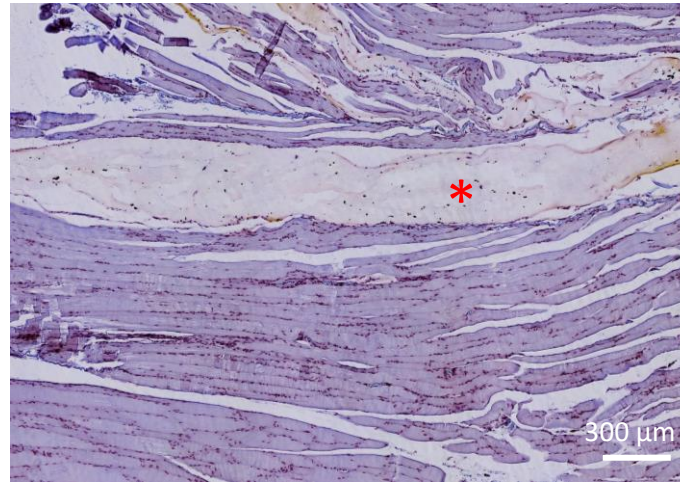


7 days

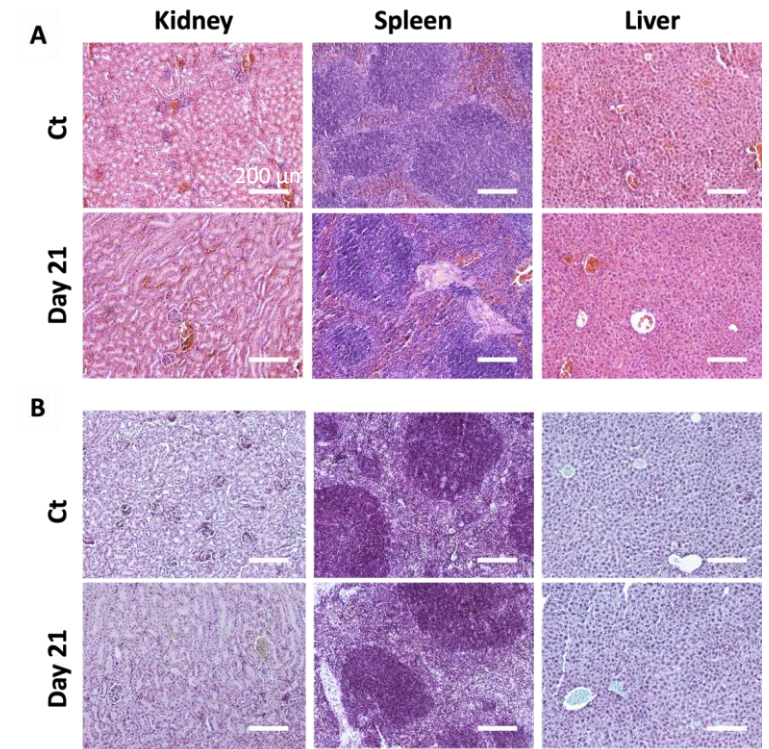


28 days

SUBCUTANEOUS INJECTION



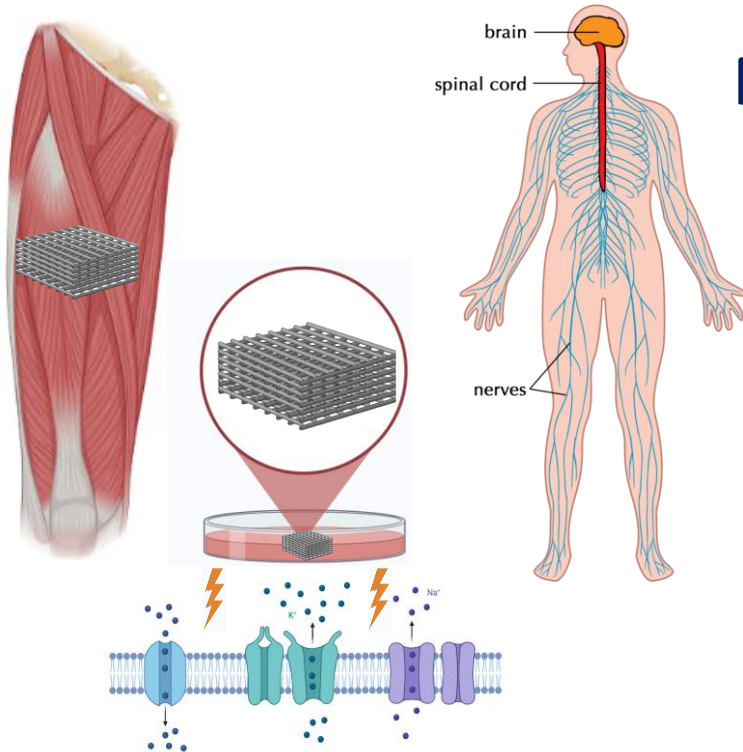
INTRAMUSCULAR INJECTION (TA)



ORGANS EXPLANTED

J Colloid Interface Sci. 2025. 678: 334–345 doi.org/10.1016/j.jcis.2024.09.121

Mater Today Bio. 2024. 27, 101110. doi.org/10.1016/j.mtbio.2024.101110



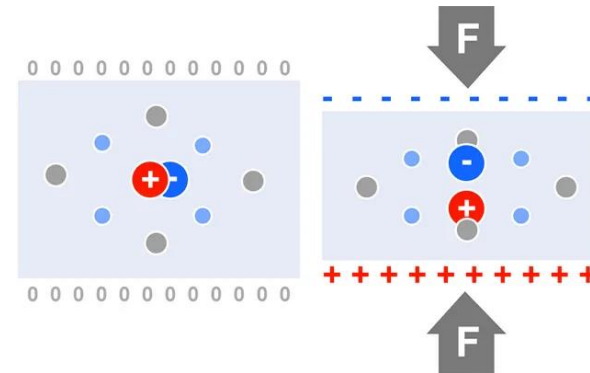
BIOELECTRICITY

- → Ultrasound

Using piezoelectric particles alone or combined with biopolymers

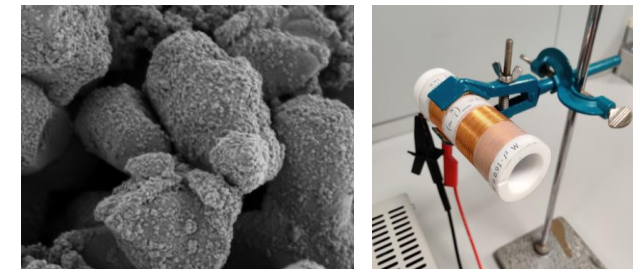


Piezoelectric scaffolds to provide electrical stimulation to the endogenous cells by the deformation of the piezo-particles



- → magnetic field (AMF)

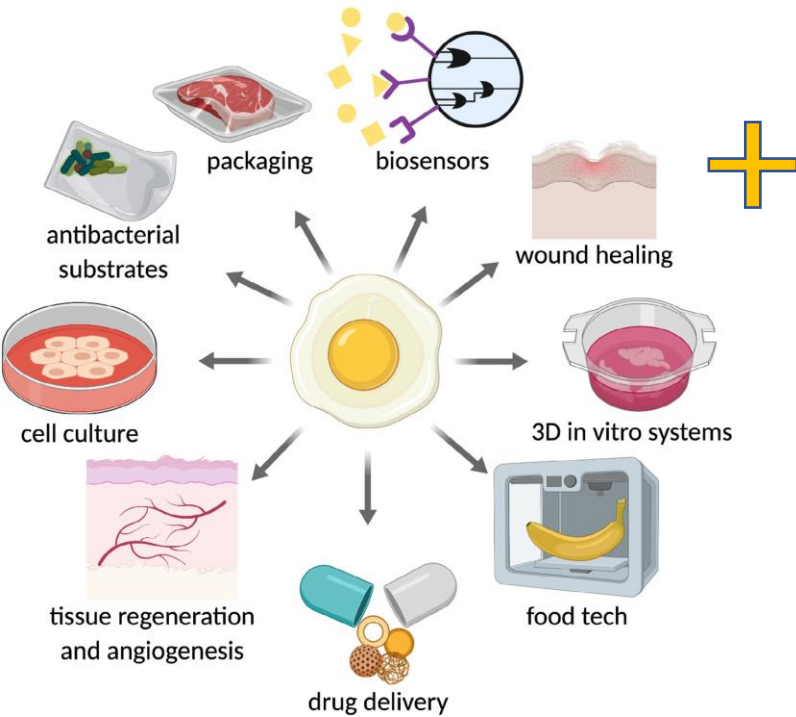
Coupling magnetic nanoparticles with biocompatible piezoelectric particles



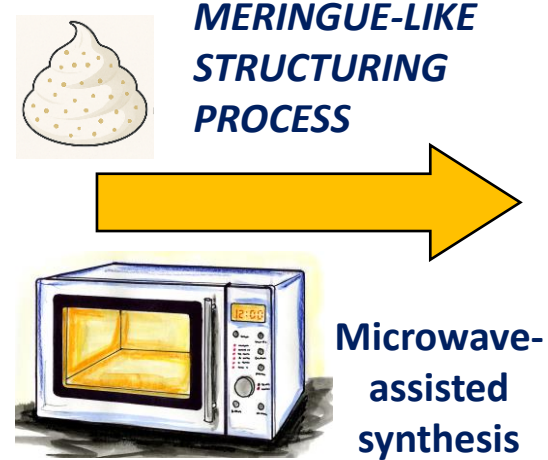
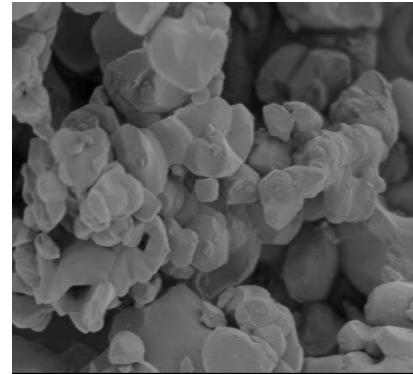
PIEZOELECTRIC EGG-WHITE SCAFFOLDS



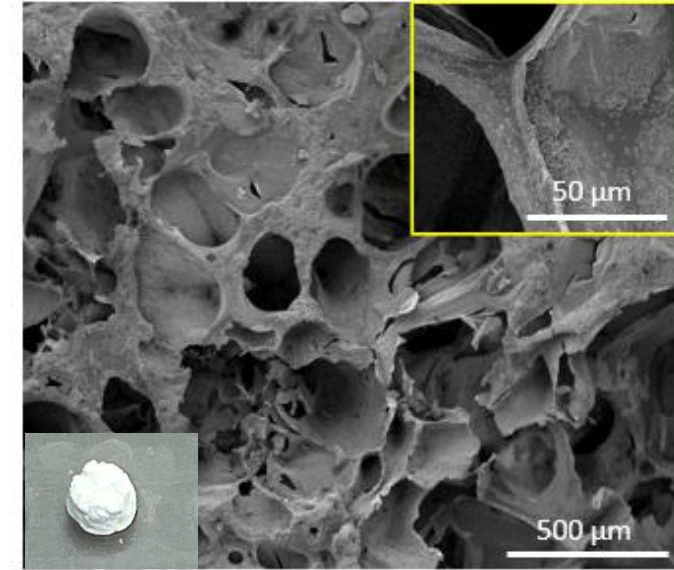
EGG-WHITE PROTEINS



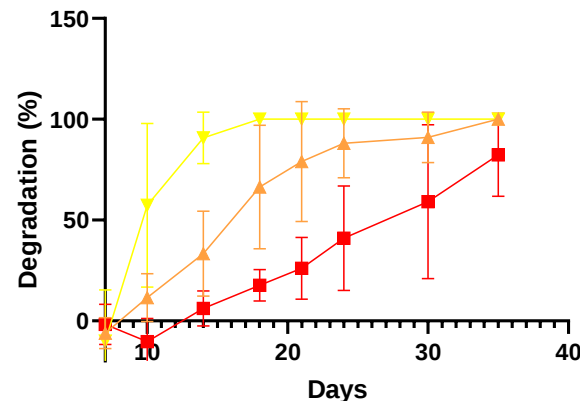
BaTiO₃



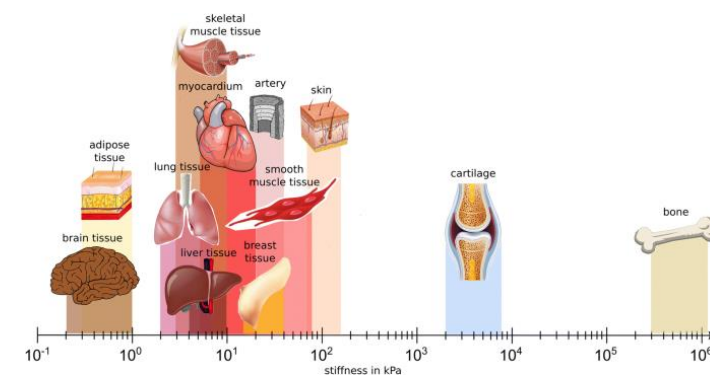
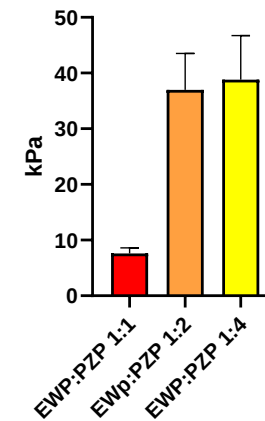
PIEZOELECTRIC SCAFFOLD



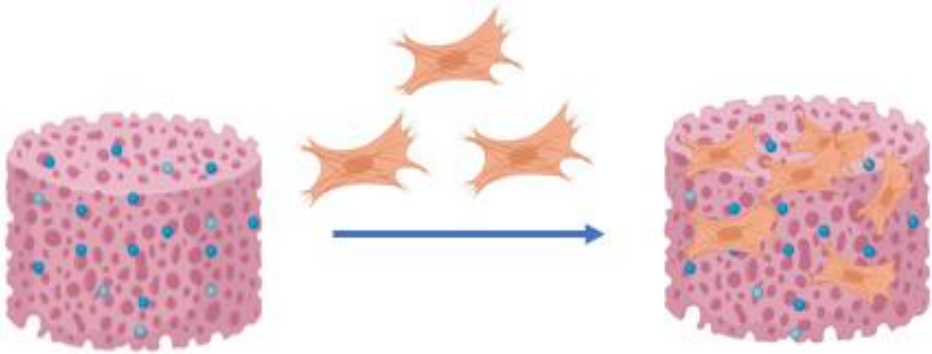
Stability evaluation – Mechanical Properties



Young's modulus

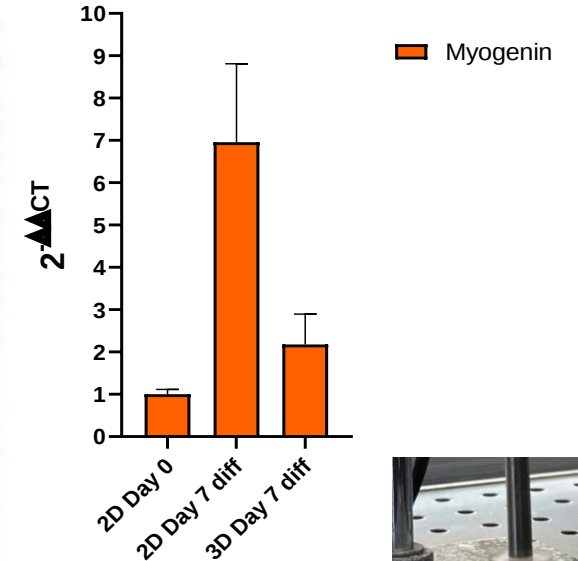
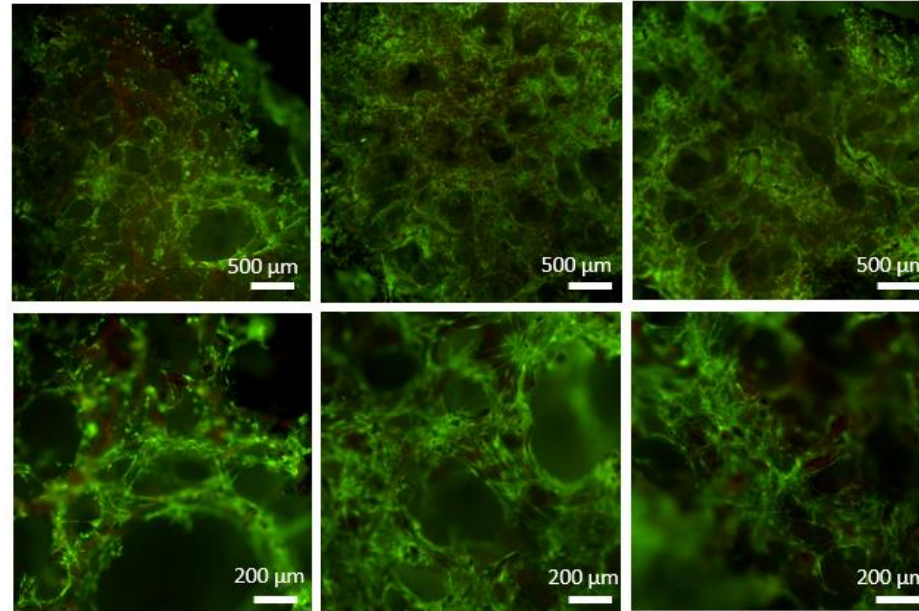


PIEZOELECTRIC EGG-WHITE SCAFFOLDS

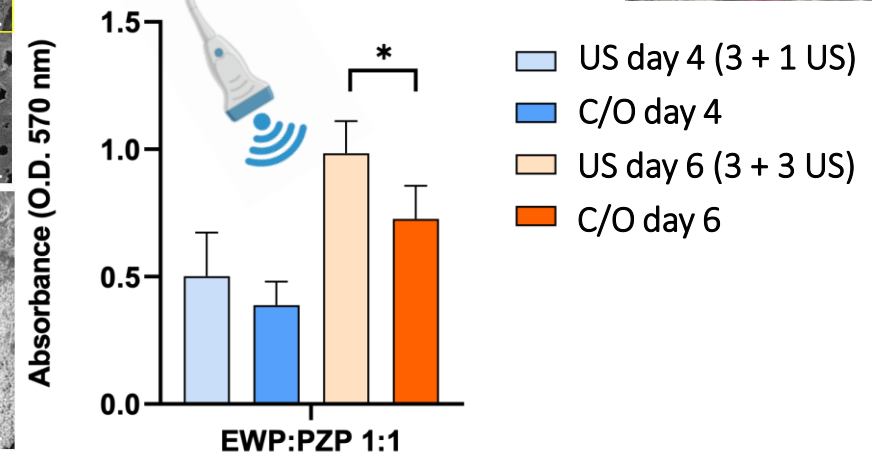
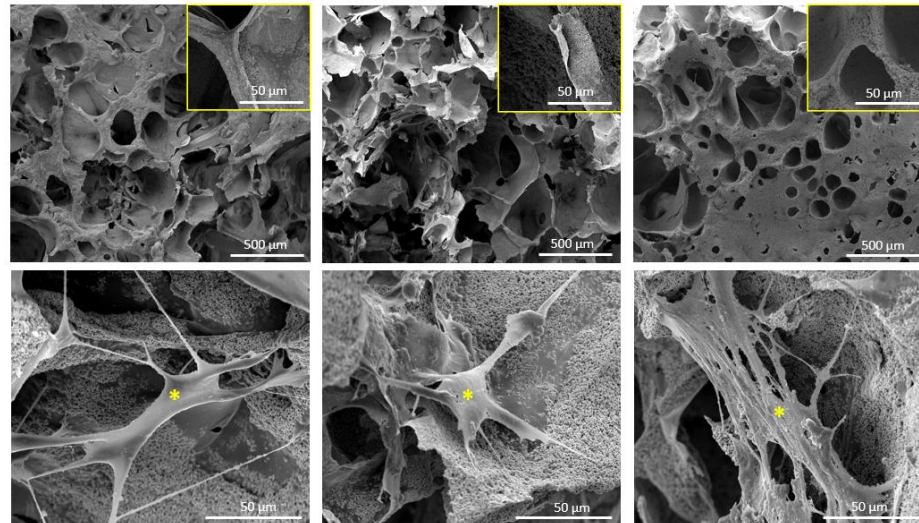
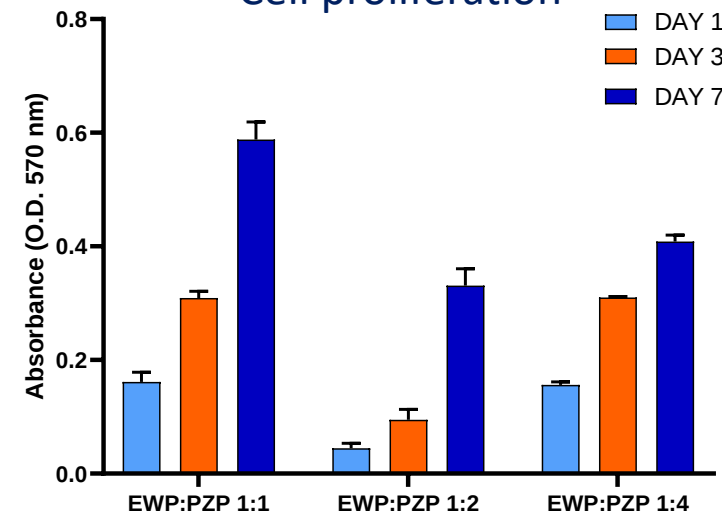


- **MUSCLE CELLS** (C2C12 cell line)
- **NEURONAL CELLS** (SH-SY5Y cell line)

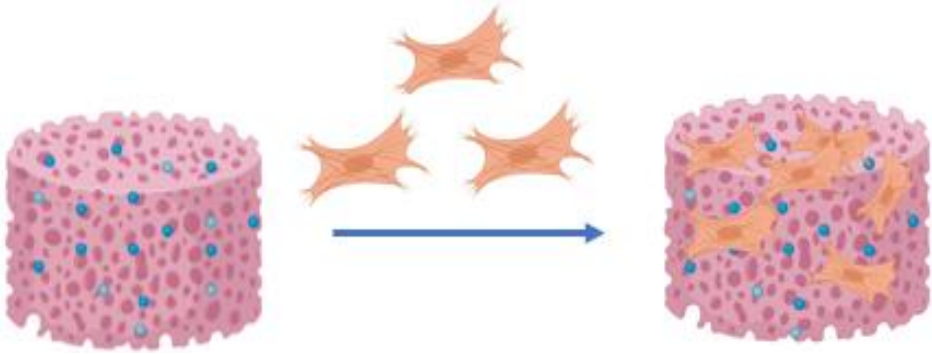
Cell Viability (day 7)



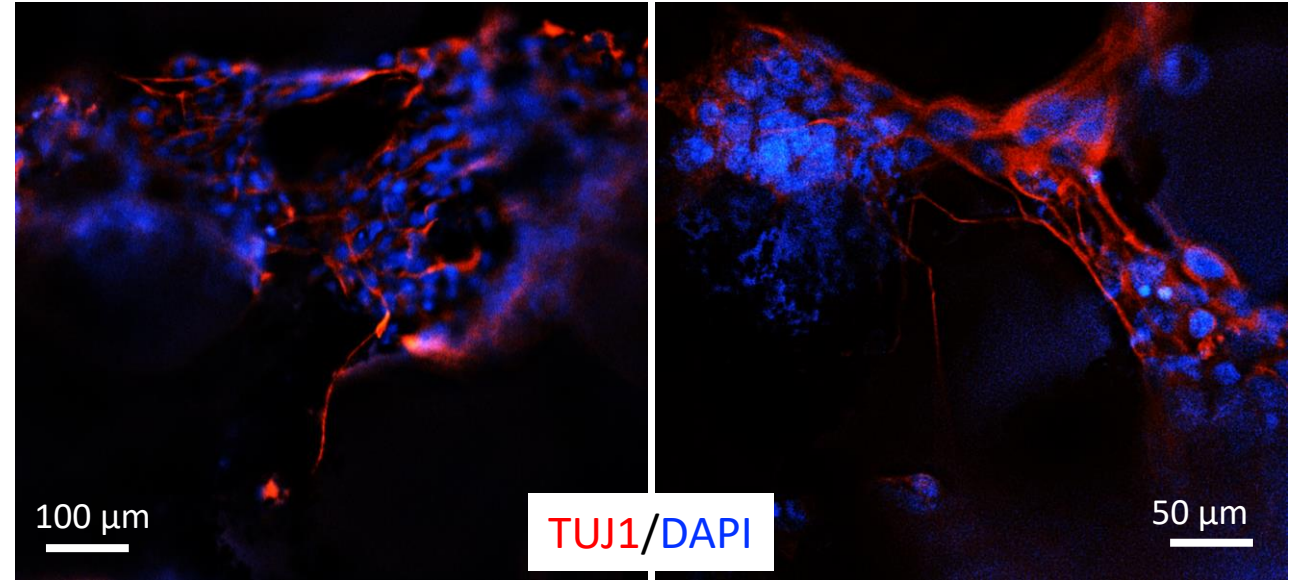
Cell proliferation



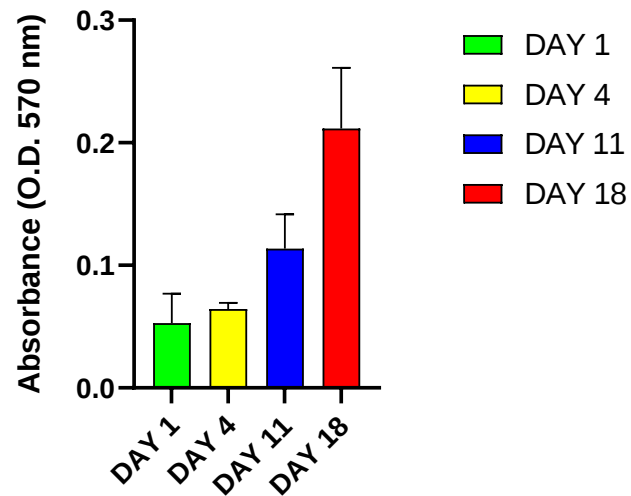
PIEZOELECTRIC EGG-WHITE SCAFFOLDS



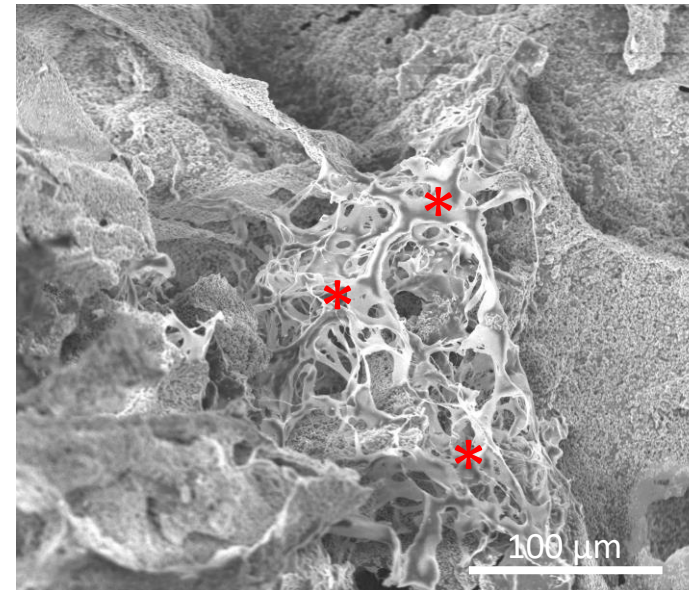
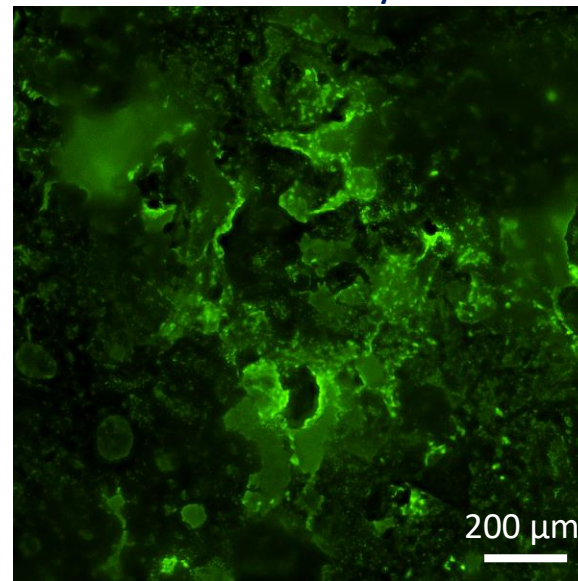
○ NEURONAL CELLS (SH-SY5Y cell line)



Cell proliferation

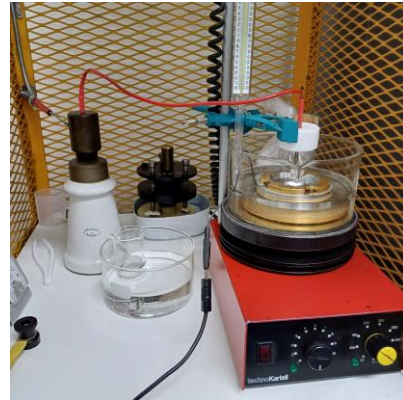
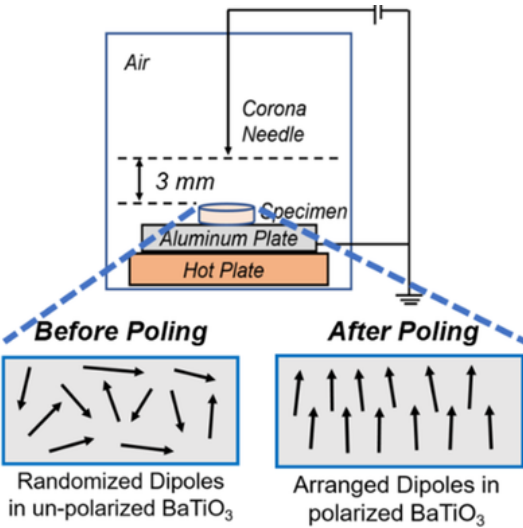


Cell Viability

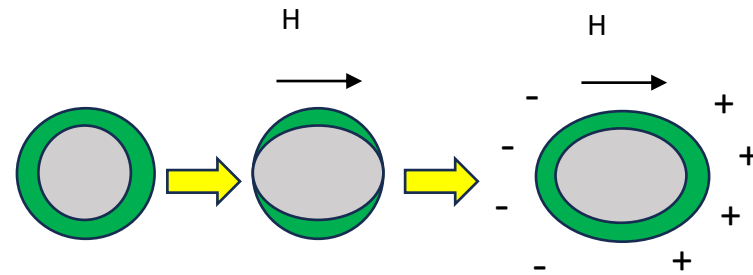


CONCLUSIONS and FUTURE PERSPECTIVES

CORONA POLING

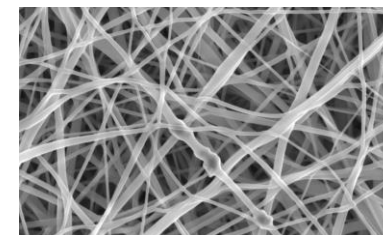
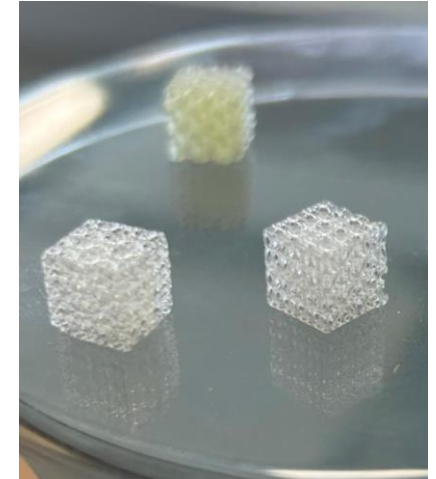
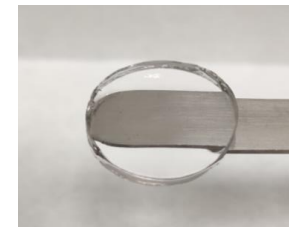


Piezoelectric nanoparticles Magnetoelectric nanoparticles (BaTiO_3 + Magnetite)



Magnetostrictive and piezoelectric phases coupled via a common interface

**CAUTION
SCIENCE IN
PROGRESS**



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Ministero della Difesa





STIMULI-RESPONSIVE BIOENGINEERED BIOMATERIALS: 3D *IN VITRO* MODELS & REGENERATIVE MEDICINE THERAPIES



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dei Materiali Ceramici

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PHILADELPHIA, PA
JULY 13-18, 2025

**NEXT-GENERATION
DELIVERY INNOVATIONS**