Tissue engineered constructs for the replacement of laboratory animals:

Biomimetics and Microfluidics

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ANIMAL MODELS

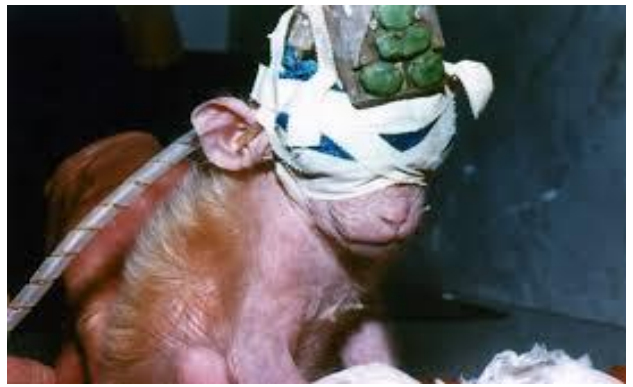
-The use of animals for biomedical research purposes it has been the subject of debate for many years.

-Identification of the importance of welfare for animals used in science has pushed towards the development of methods to replace animals in research.

-Animal models often show ***limits in the reproduction of specific human conditions***

-Human pathologies can be induced in animals models but the molecular mechanisms that guide their onset e progression are often significantly different

-The proven inefficacy in humans of some drugs, successfully tested on animals, are symptoms of the ***animal model's inability to reproduce effectively human physiology***



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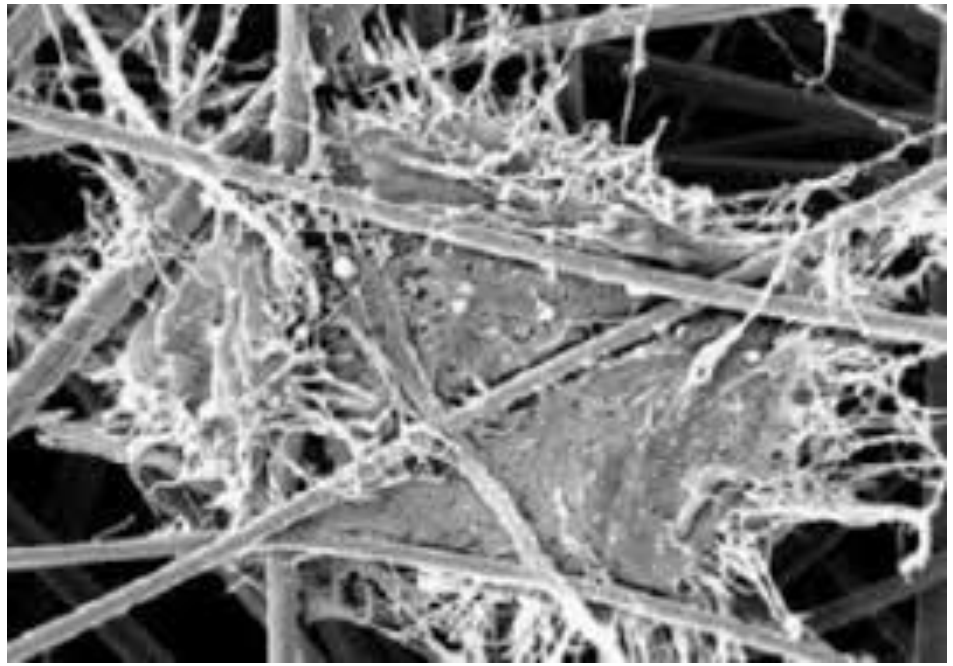
3D CELL CULTURES



The cells grow in vivo in a three-dimensional (3D) environment that allows them to actively interact with the surrounding cells and the extracellular matrix (ECM) that provides them with stimuli affecting their functions and gene expression profile.

In recent years, 3D cell culture techniques have received much attention as they represent the microenvironment in which cells thrive in vivo much more accurately than traditional 2D cultures.

A 3D in vitro model allows the cells to growth and interct to each other and with the ECM in all spatial dimentions.

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3D SCAFFOLD SYSTEMS

There are two main types of 3D cell culture systems:

-scaffold-based systems

-scaffold free systems

Most of the focus is on regular 3D scaffold systems

The 3D structure is achieved throught a 3D support matrix called «scaffold»

Two different scaffold categories can be found:

-on the one hand, there are in vitro 3D ***scaffolds for cell culture and experimental applications*** *(drug and cosmetic testing)*

-on the other hand ***tissue engineering scaffolds*** *are selected as support for tissues regeneration applications*

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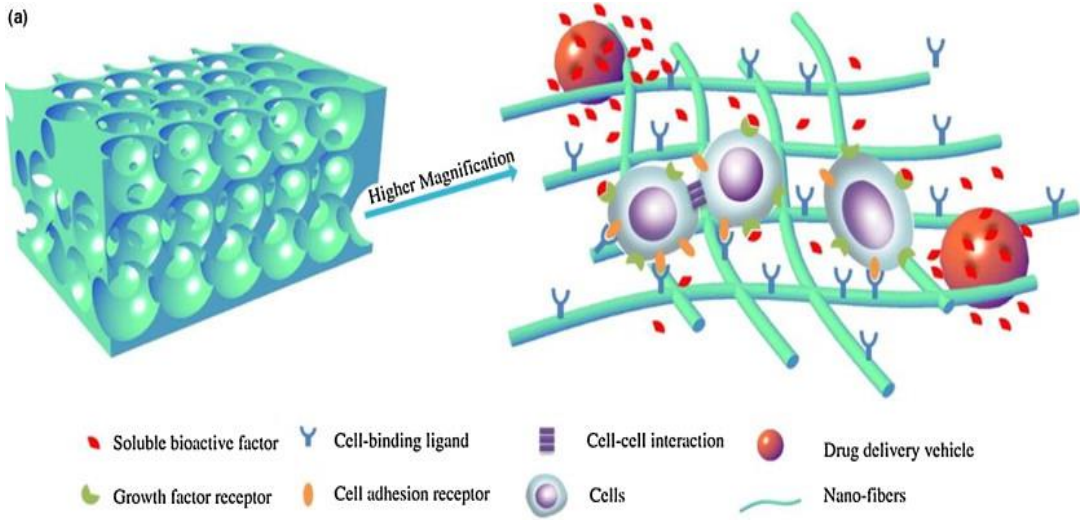
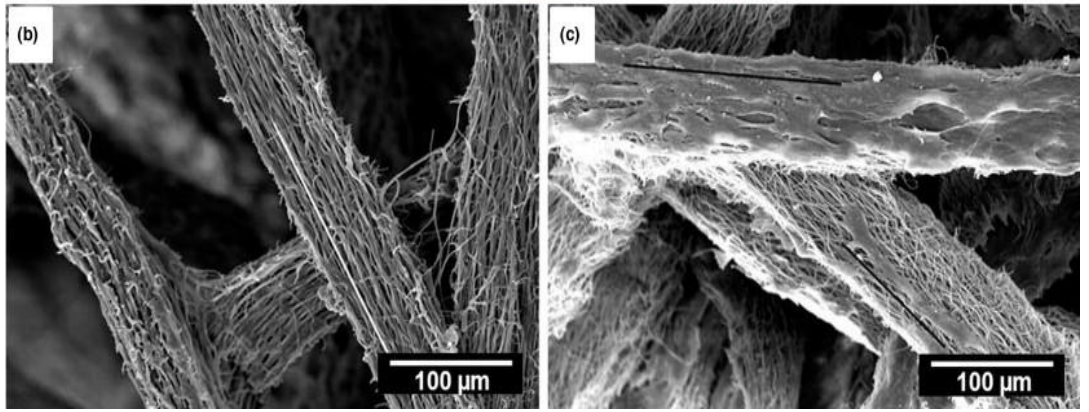
BIOMIMETIC SCAFFOLD:

A SYSTEM THAT MIMICS NATURE

***Biomimetics** is considered as the future for materials design and production.*

The history of biomimetics exploration by humans dates back to early fifteenth century by Leonardo da Vinci speculating the clues of possibility of human air travel following the mechanics of flight of birds.





BIOMIMETIC SCAFFOLD:

A SYSTEM THAT MIMICS NATURE

The creation of "biomimetic materials" for biomedical applications raises a question:

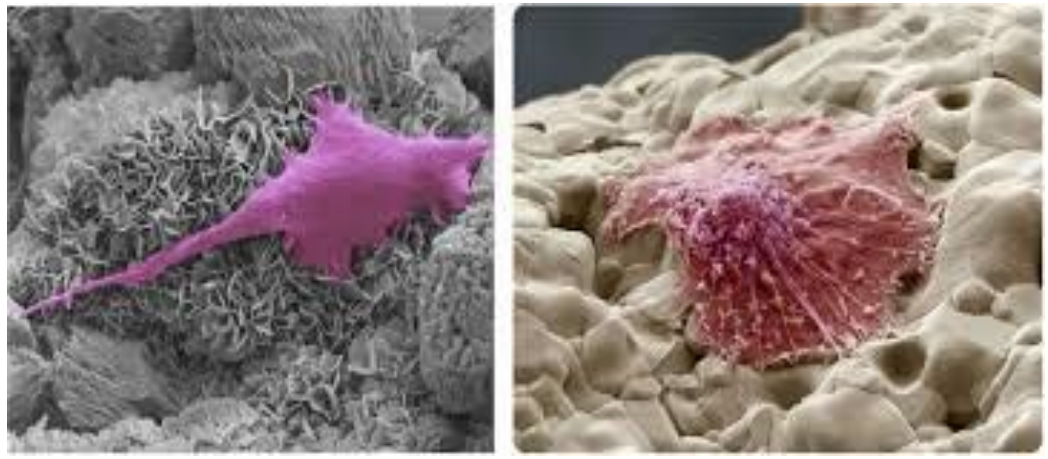
"HOW DOES NATURE DESIGN?

The natural evolution has allowed the creation of biological materials with specific performances often extraordinary to which synthetic systems must tend.

The production of scaffolds can not follow directly the rules of natural evolution from microstructure to the function, but it is necessary to follow a process of reverse decoding, it consists of:

1. Understanding of the processes that lead to the formation of a specific natural system

2. Understanding of the microstructure necessary to obtain the desired properties.



Only when these two basic steps have been performed it is possible **to start the design of a synthetic system** *for a possible substitution, or the search for technologies possible to get the system that,* **having the right microstructure**, *has the desired and* **suitable properties for the specific application**

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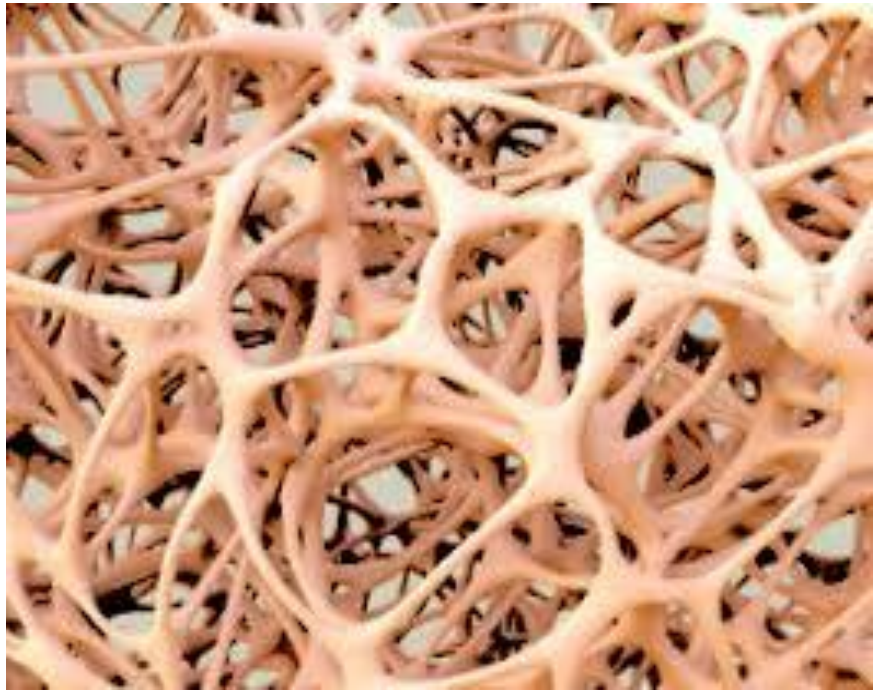
BIOMIMETIC SCAFFOLDS



Biomimetic scaffolds are necessary to recapitulate the natural environment and provide various cues to direct cell processes and differentiation.

Scaffolds characterized by chemical-physical signals and/or by a structure that ***mimics the extracellular matrix*** *(ECM) allow to control and influence the specific cellular response.*

It is fundamental ***replicate in the scaffold*** *the macro and nano* ***structure of the ECM.***

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Topics of our research activity

Engineering of biomimetic scaffolds

to support and guide tissue formation

-production of biomimetic polymeric systems by blending of different biological polymers

-production of biomimetic polymeric systems using traditional and innovative functionalization techniques

-characterization of the produced scaffolds

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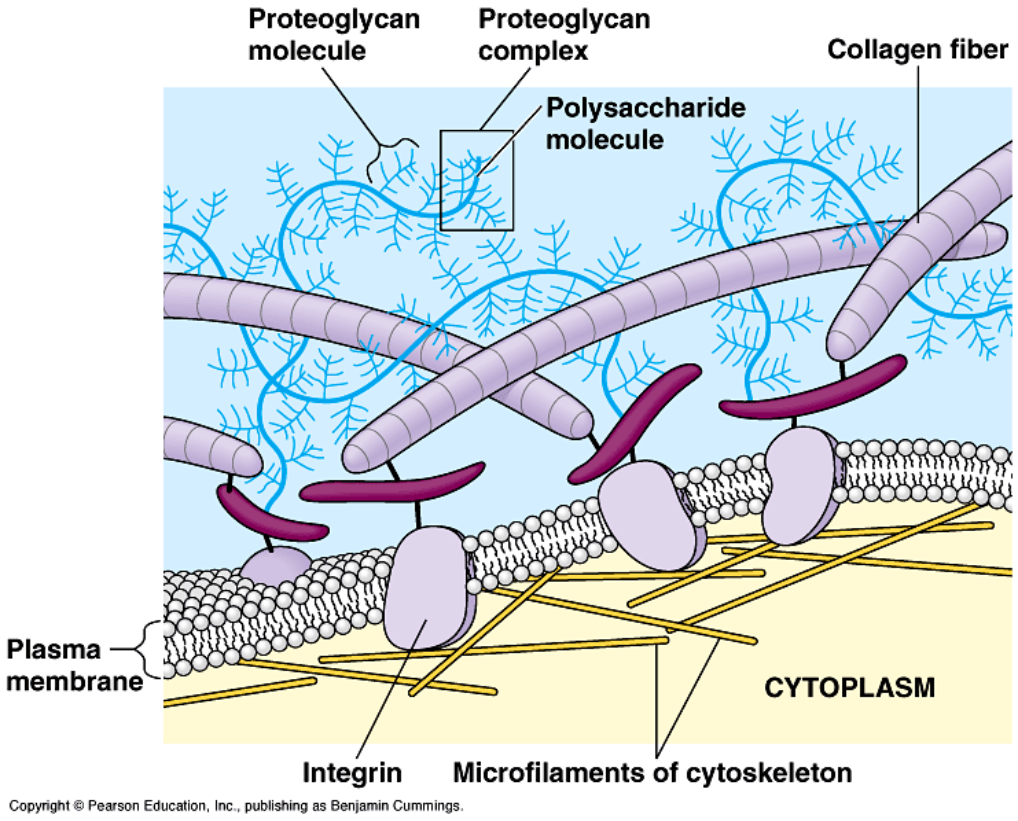
PRODUCTION OF BIOMIMETIC POLYMERIC SYSTEMS

BY BLENDING OF DIFFERENT BIOLOGICAL POLYMERS



Extracellular matrix: a blend of several biological components

Blends of natural polymers

Protein type components

Polysaccharide type components

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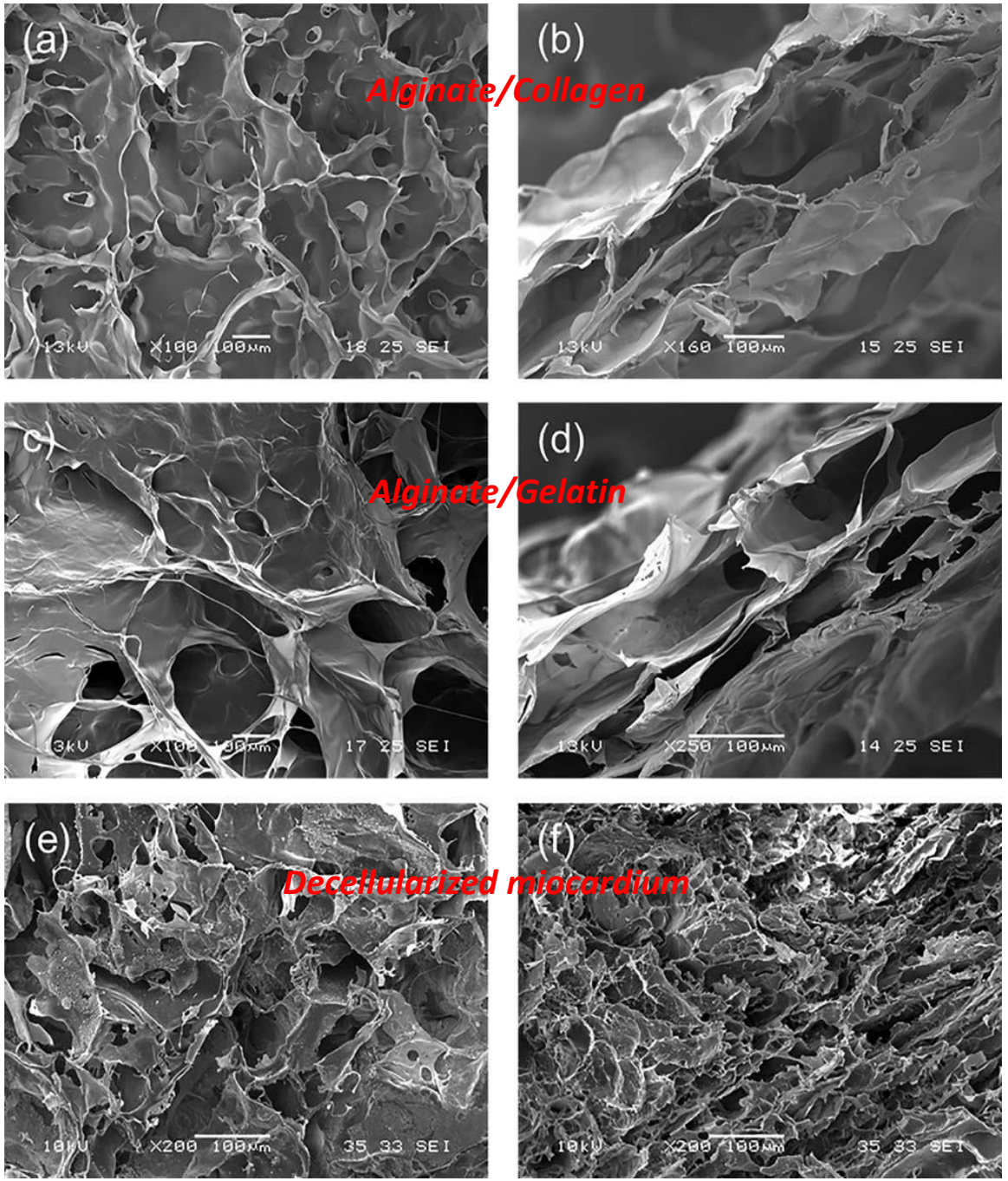
BLENDS OF NATURAL POLYMERS

The aim is to mimic not only the chemical composition but also the interactions between components that are present in the natural extracellular matrix.

Alginate, Gellan, Agarose: used to replace the polysaccharide components

Gelatin, Collagen, Elastin: used to replace the protein components



E. Rosellini, Y.S. Zhang , B. Migliori, N. Barbani, L. Lazzeri, S. R. Shin, M. R. Dokmeci, M.G. Cascone, Protein/polysaccharide-based scaffolds mimicking native extracellular matrix for cardiac tissue engineering applications. *Journal of Biomedical Materials Research Part A* 106A; 769-781; 2018.

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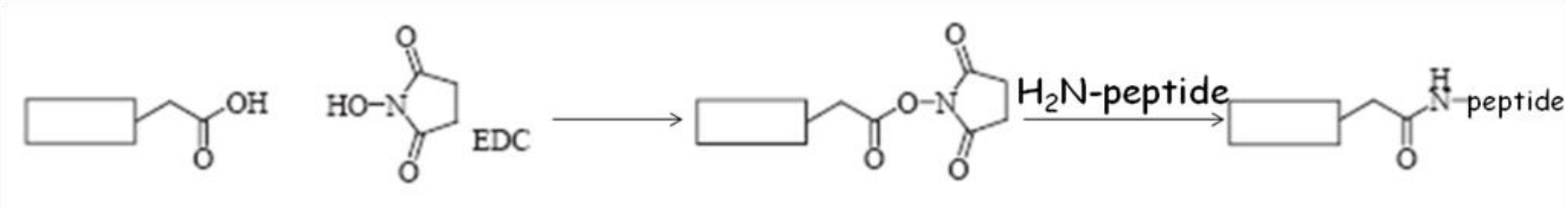
PRODUCTION OF BIOACTIVE POLYMER SYSTEMS USING TRADITIONAL AND INNOVATIVE FUNCTIONALIZATION TECHNIQUES

- *Covalent bonding of peptide sequences (RGD, YIGSR, REDV etc)*
- *Direct loading with active agents*
- *Loading with micro- / nano-particles containing active agents*
- *Molecular Imprinting*

SURFACE FUNCTIONALIZATION VIA ALKALINE HYDROLYSIS AND PEPTIDE ATTACHMENT

I. *Preparation of scaffolds by using synthetic polymers*
II. *Optimization of alkaline hydrolysis conditions, with regards to hydrolysis time, temperature and sodium hydroxide concentration*
III. *Protonation with HCl to yield polymer surfaces bearing carboxylic groups*
IV. *Activation in EDC/NHS*
V. *Coupling*



Rosellini E *et al.*, *Surface chemical immobilization of bioactive peptides on bioresorbable synthetic polymers for cardiac tissue engineering*, J Biomater Sci Polym Ed 2015; 26: 515-533.

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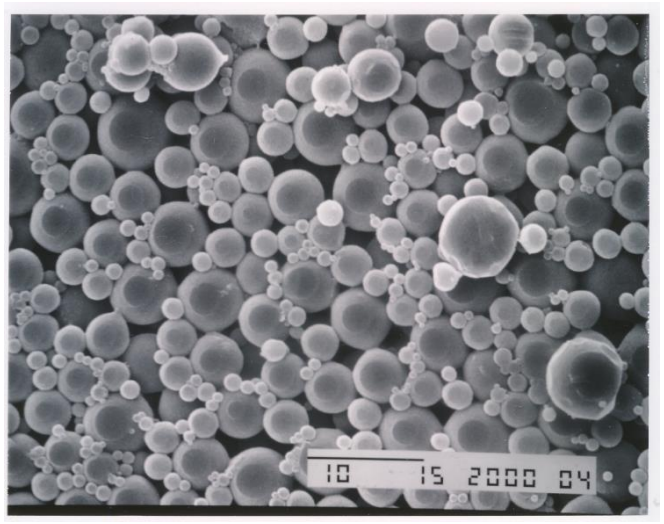
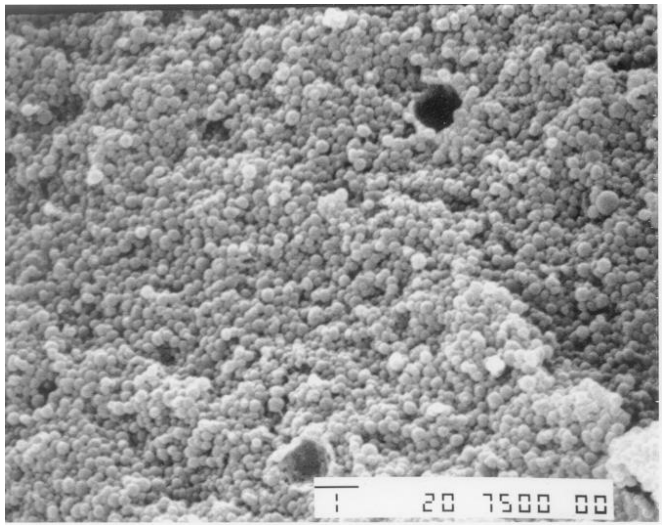
LOADING WITH MICRO-/NANO-PARTICLES CONTAINING ACTIVE AGENTS

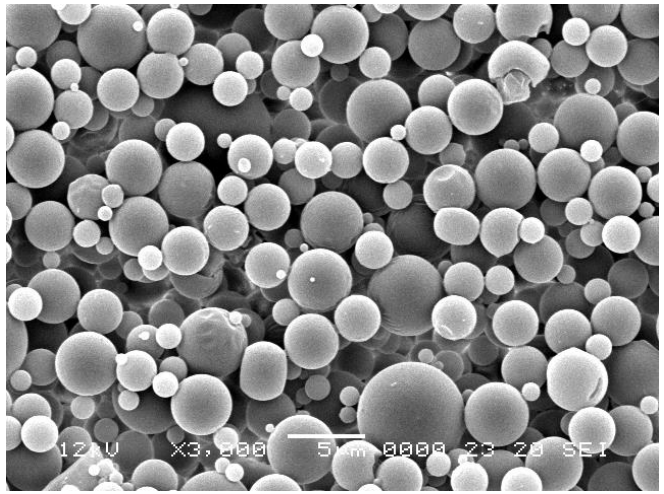
Biodegradable Particle Systems



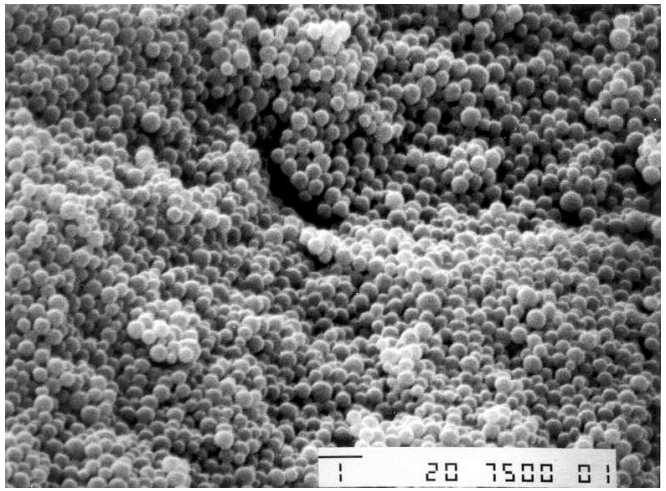
Alginate Microparticles



Gelatin Nanoparticles



PLGA Microparticles



PLGA Nanoparticles

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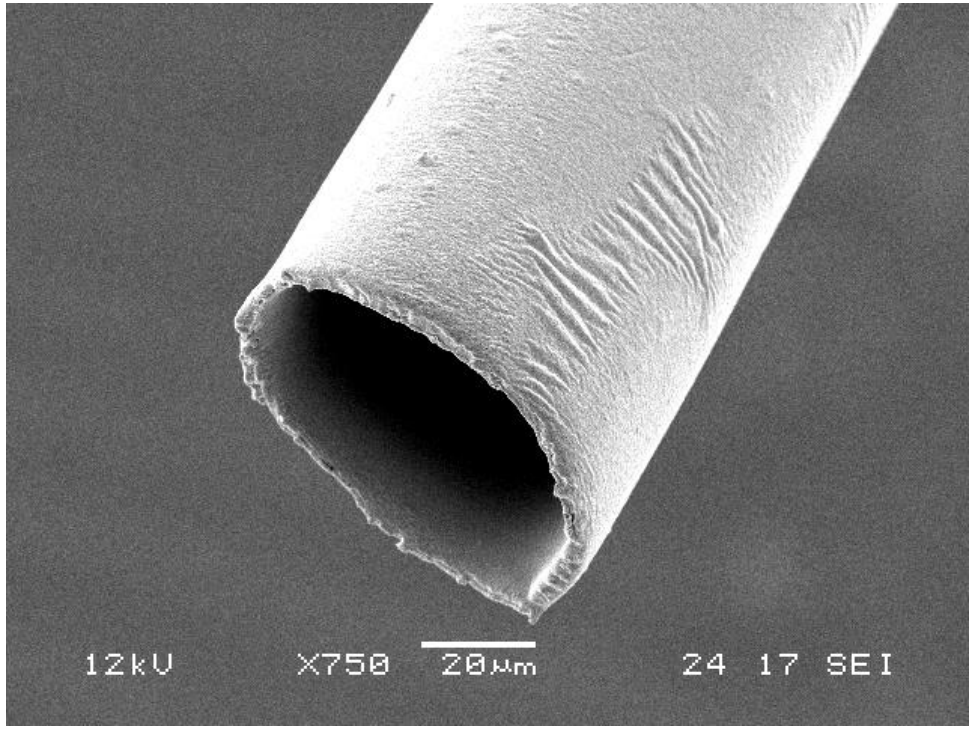
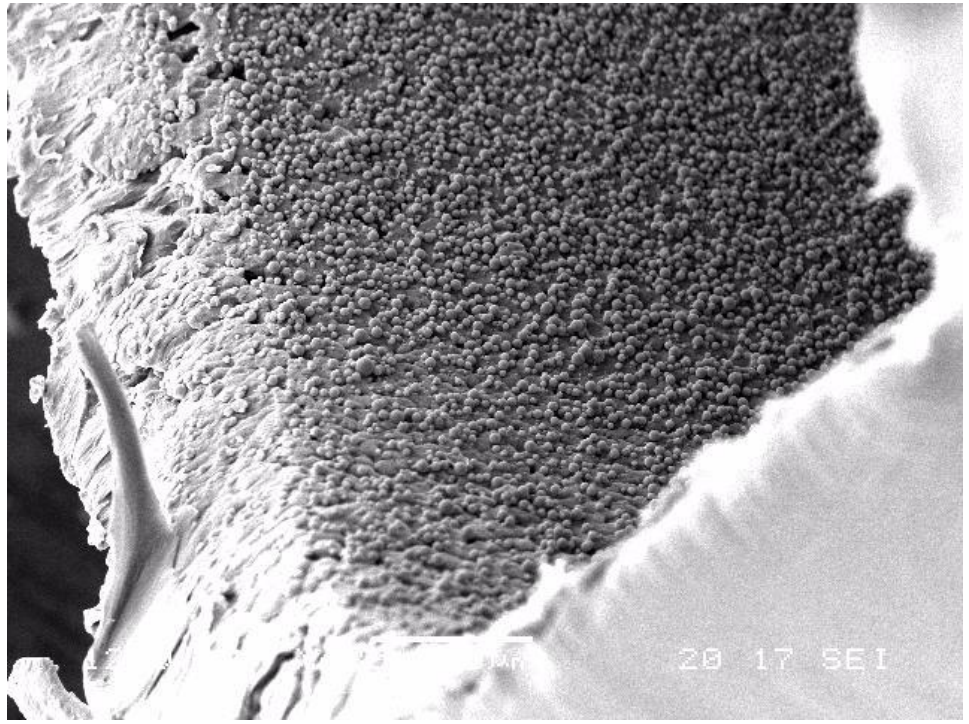
LOADING WITH MICRO- / NANO-PARTICLES CONTAINING ACTIVE AGENTS

No-woven meshes based on PLLA hollow microfibers loaded with PLGA microparticles



PLLA fiber
outer diameter: 80-100 μm
wall thickness: 5-10 μm



Detail of a PLLA hollow micro-fiber loaded with PLGA micro-particles

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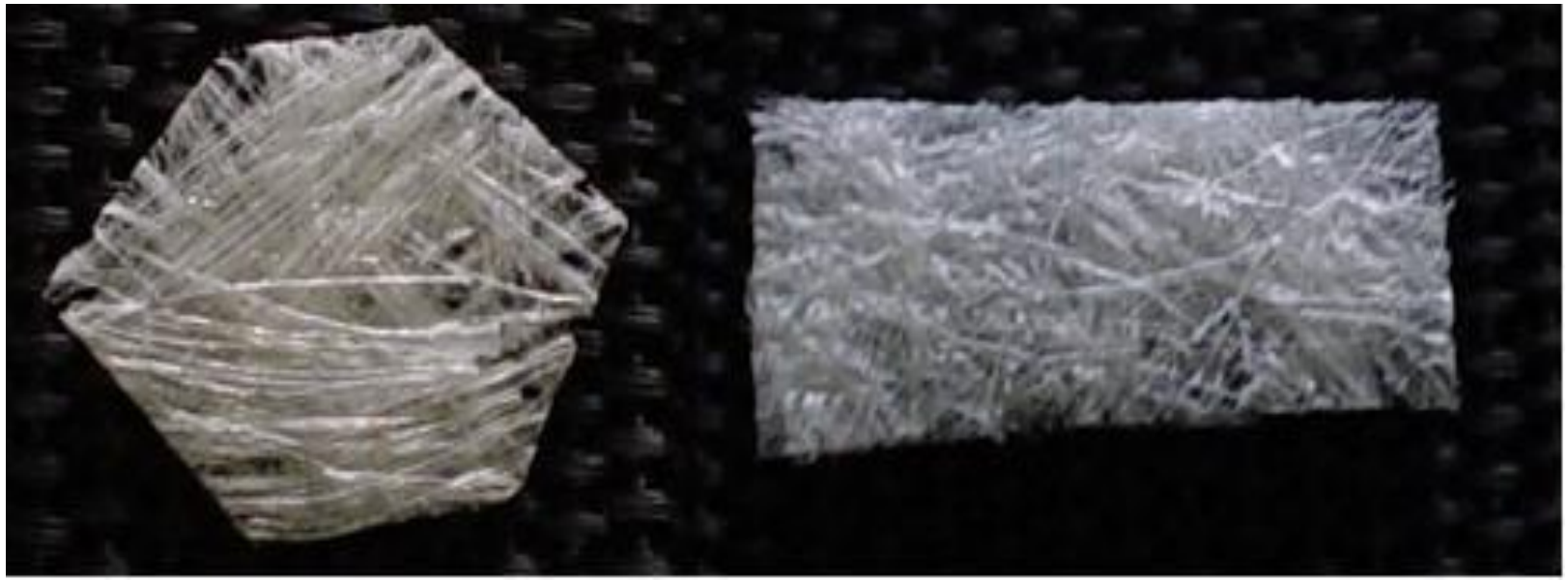
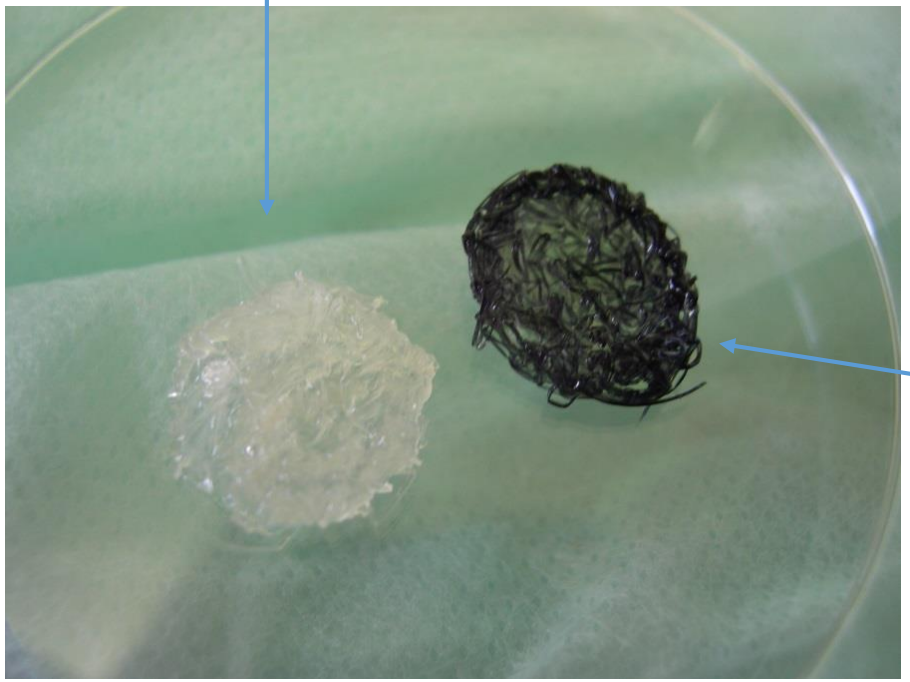
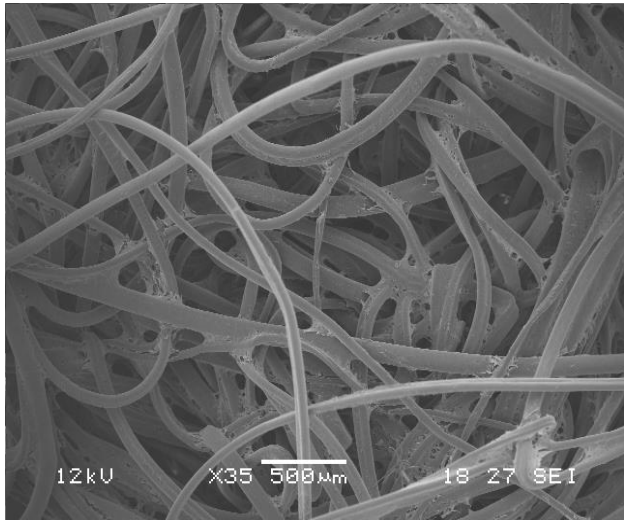
NO-WOVEN MESHES BASED ON PLLA HOLLOW MICROFIBERS



Control



Cell seeded scaffold (MTT test)



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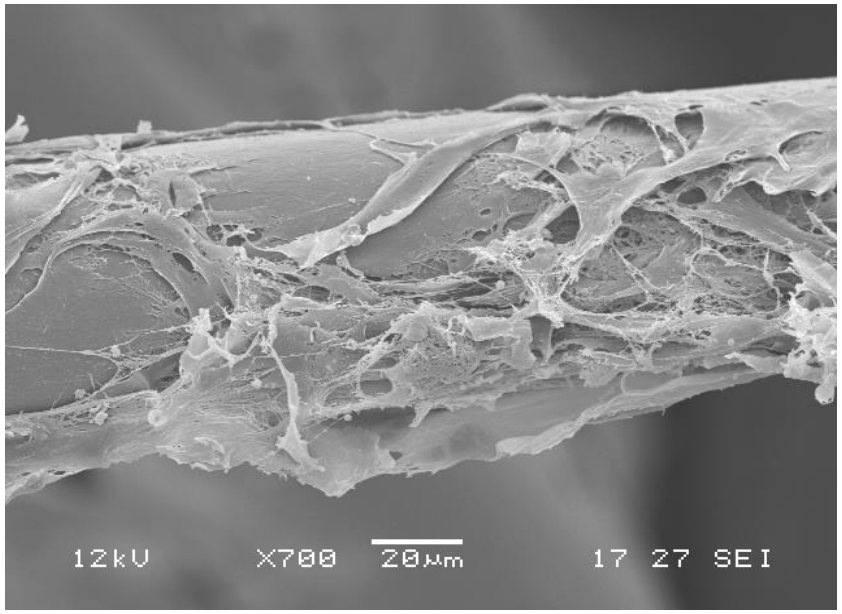
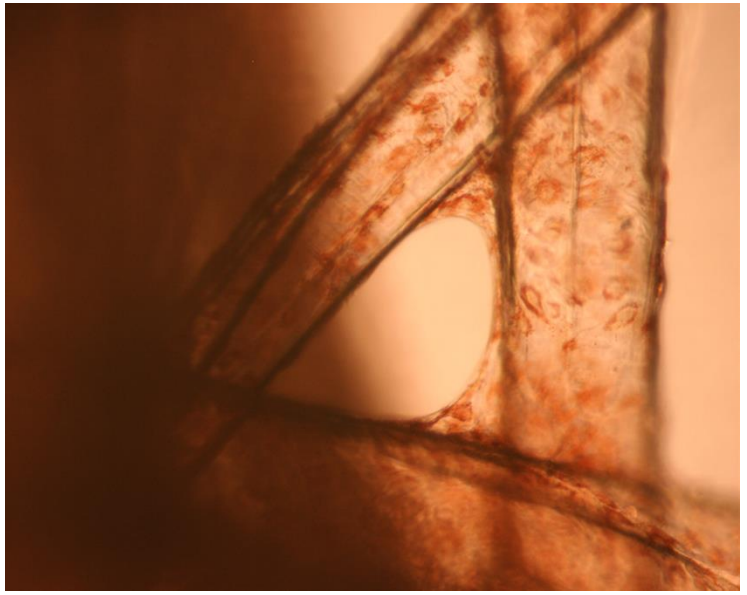
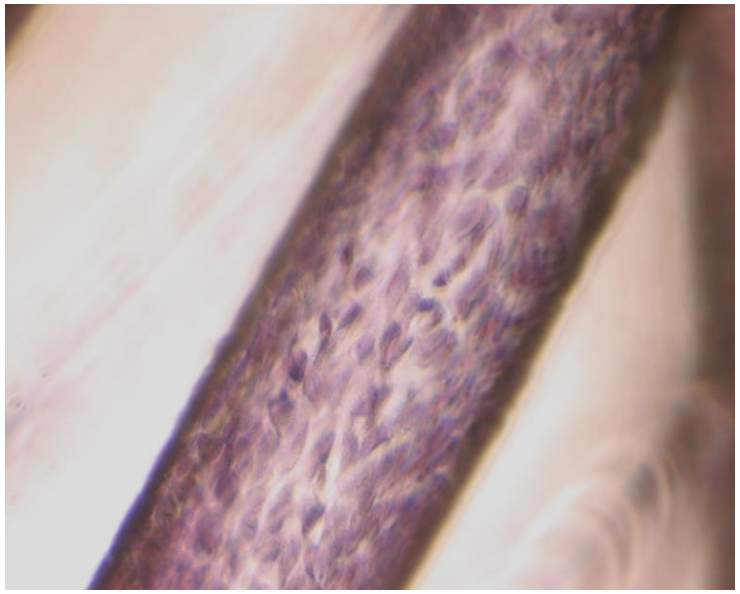
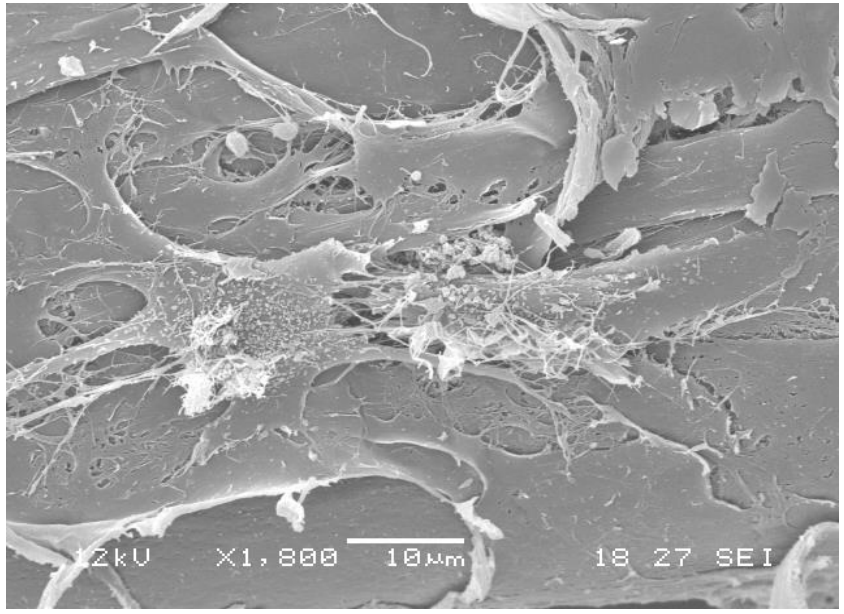
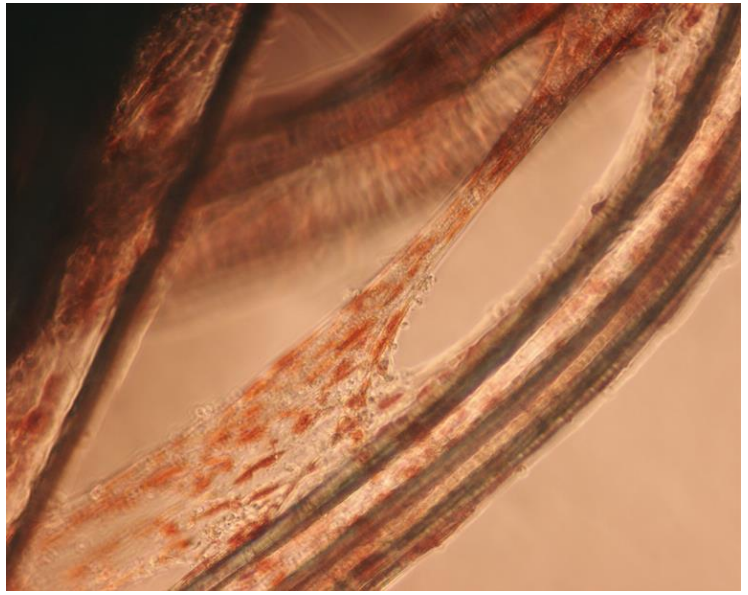
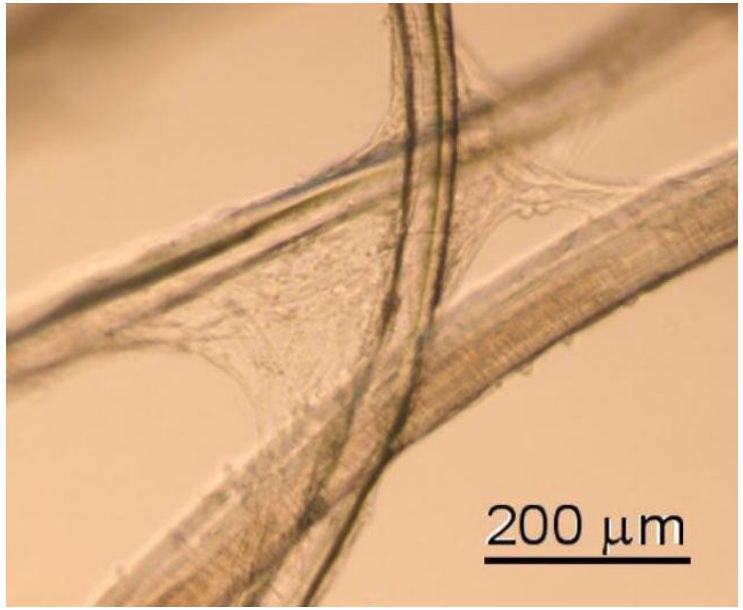






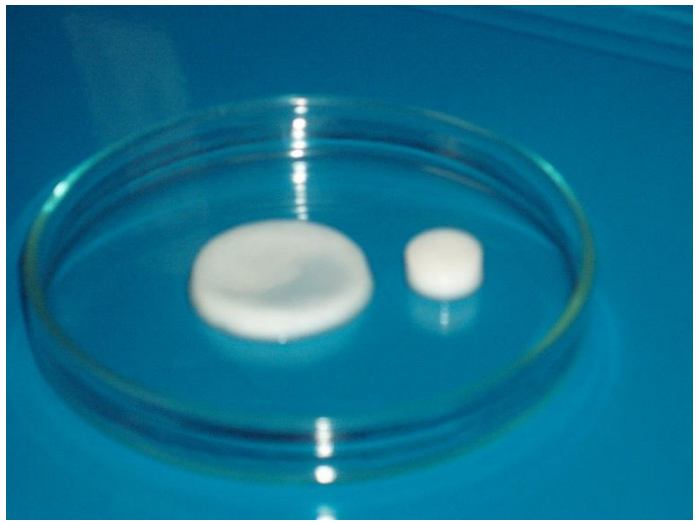


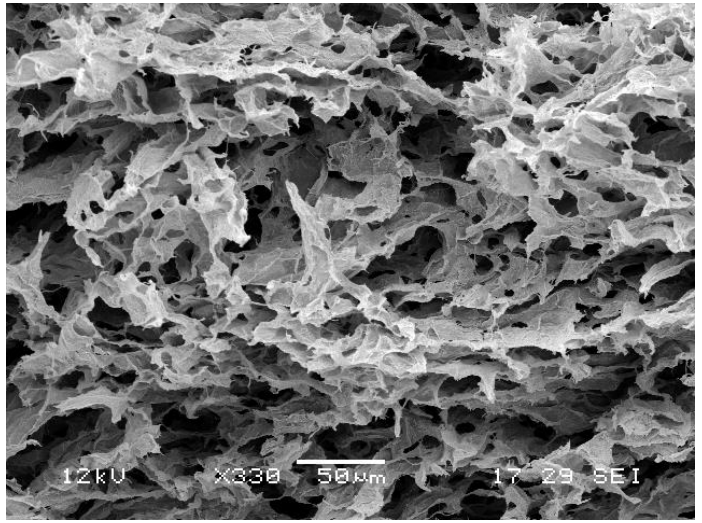




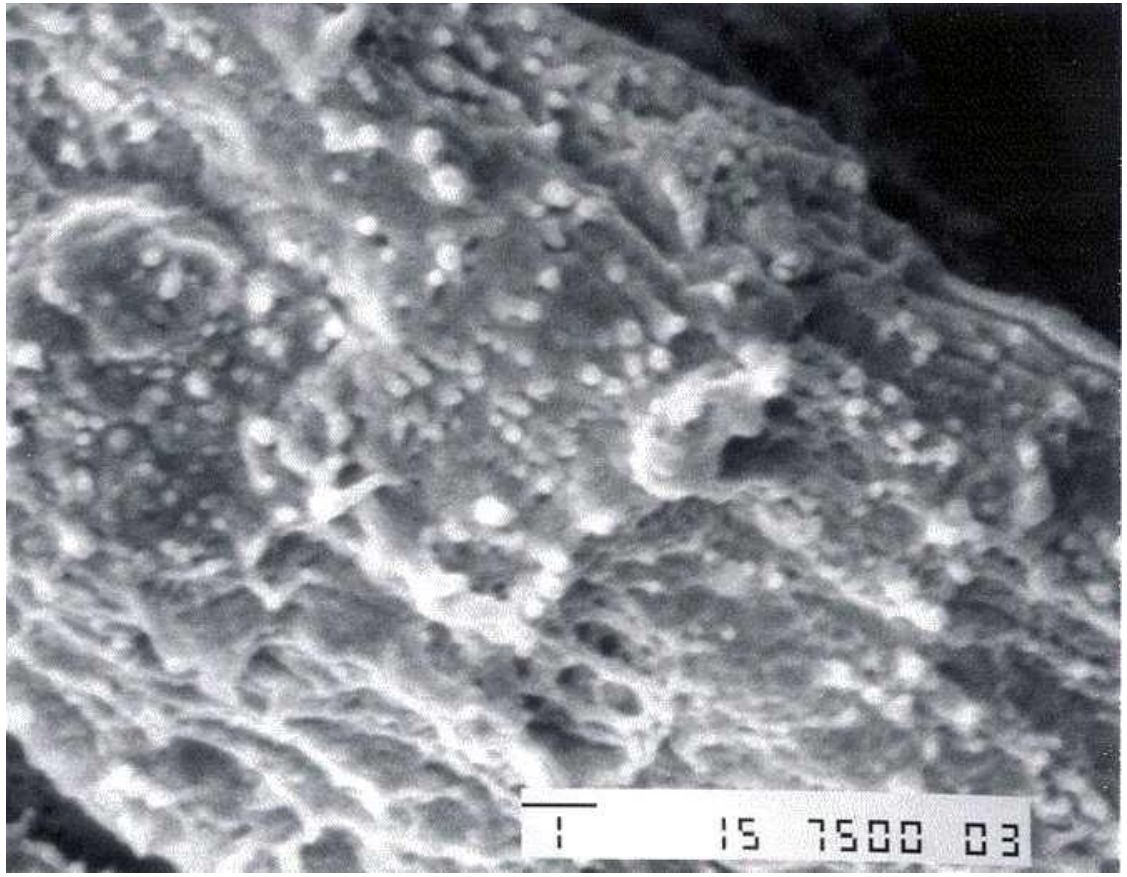
LOADING WITH MICRO- / NANO-PARTICLES CONTAINING ACTIVE AGENTS

PVA hydrogel loaded with PLGA microparticles



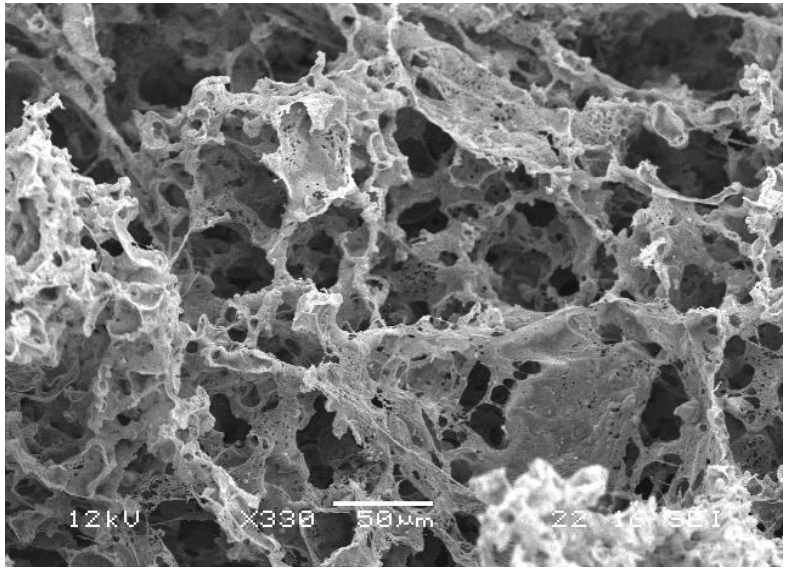


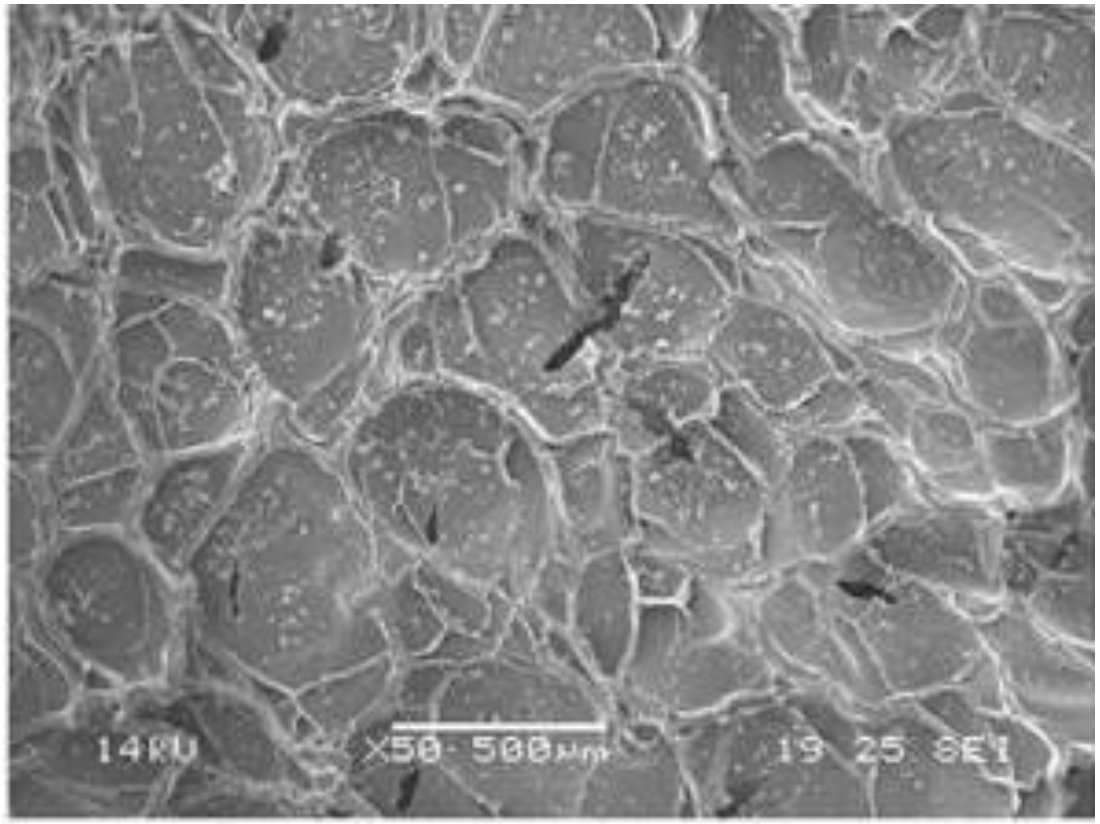
(freeze-thawing method)



LOADING WITH MICRO- / NANO-PARTICLES CONTAINING ACTIVE AGENTS

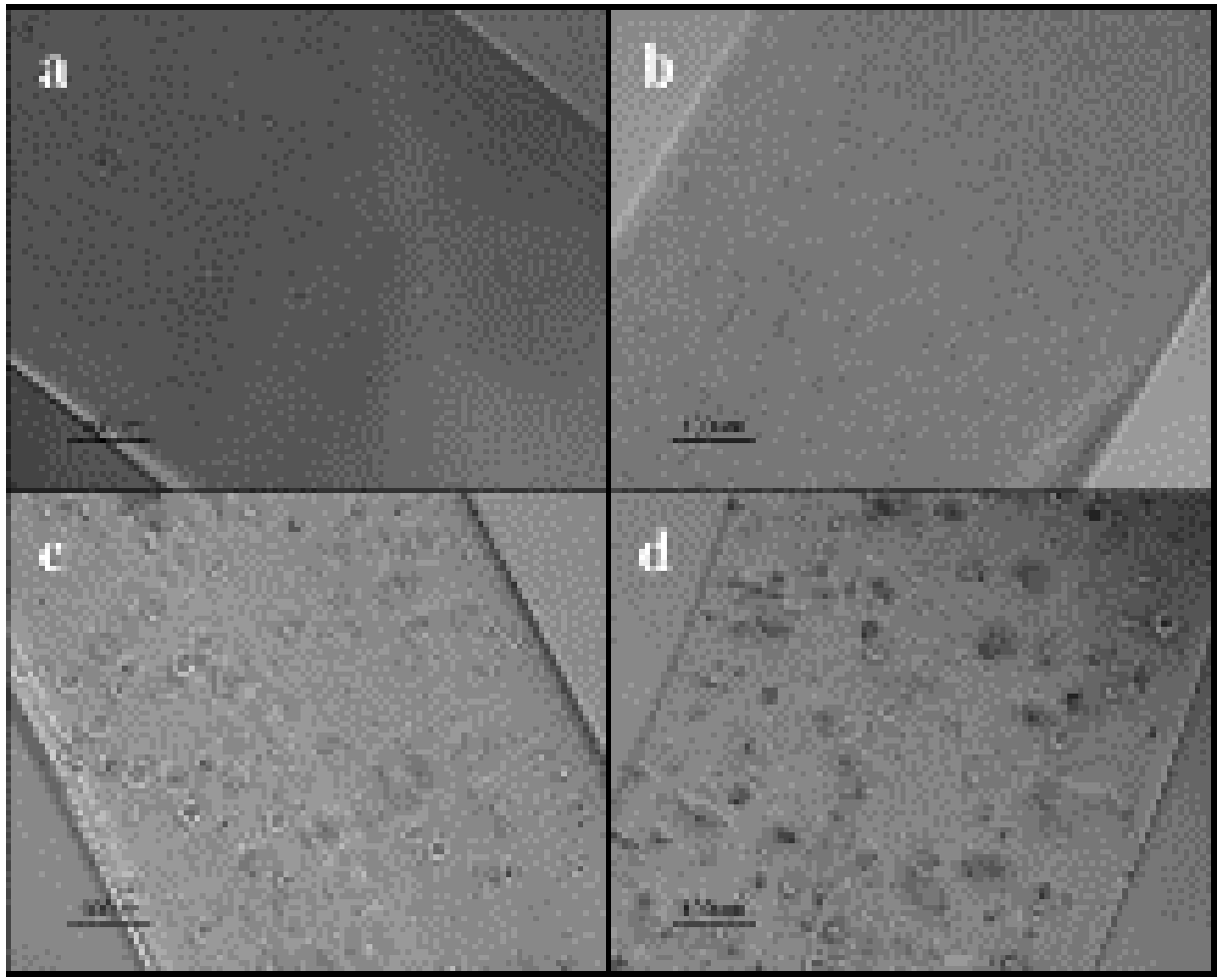
Gelatine/PLLA sponges loaded with PLGA microparticles





CELL LADEN ALGINATE/ALBUMIN HYDROGEL FIBERS

for potential skin tissue engineering applications



M.G. Cascone, E. Rosellini, S. Maltinti. A. Baldassare, L. Lazzeri, Cell laden alginate/albumin hydrogel fibers for potentil skin tissue engineering applications, *Biomedical Engineering: Applications, Basis and Communications*, 30(6). 1850045 (2018)

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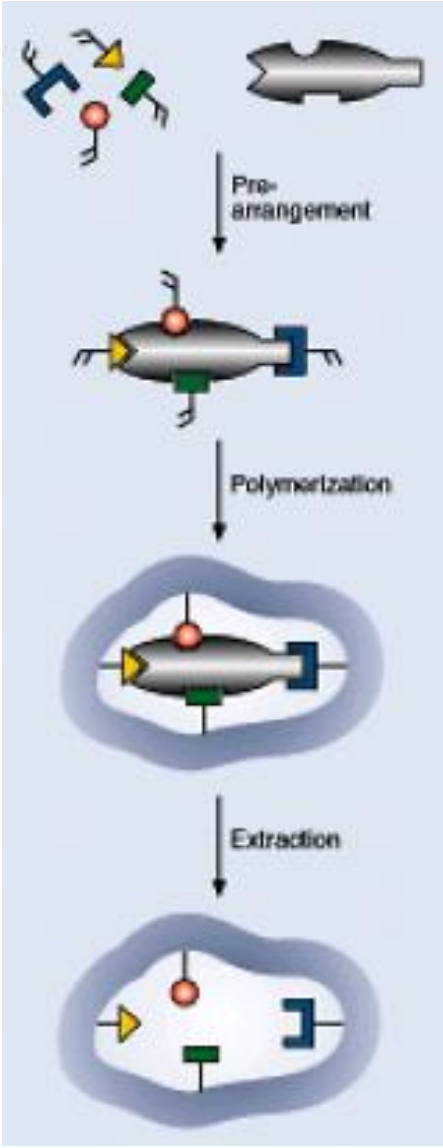
SCAFFOLD FUNCTIONALIZATION THROUGH MOLECULAR IMPRINTING

Molecular imprinting technology permits the **production of synthetic polymers capable of** *selectively* **linking themselves to a** *specific substance, called* **template**

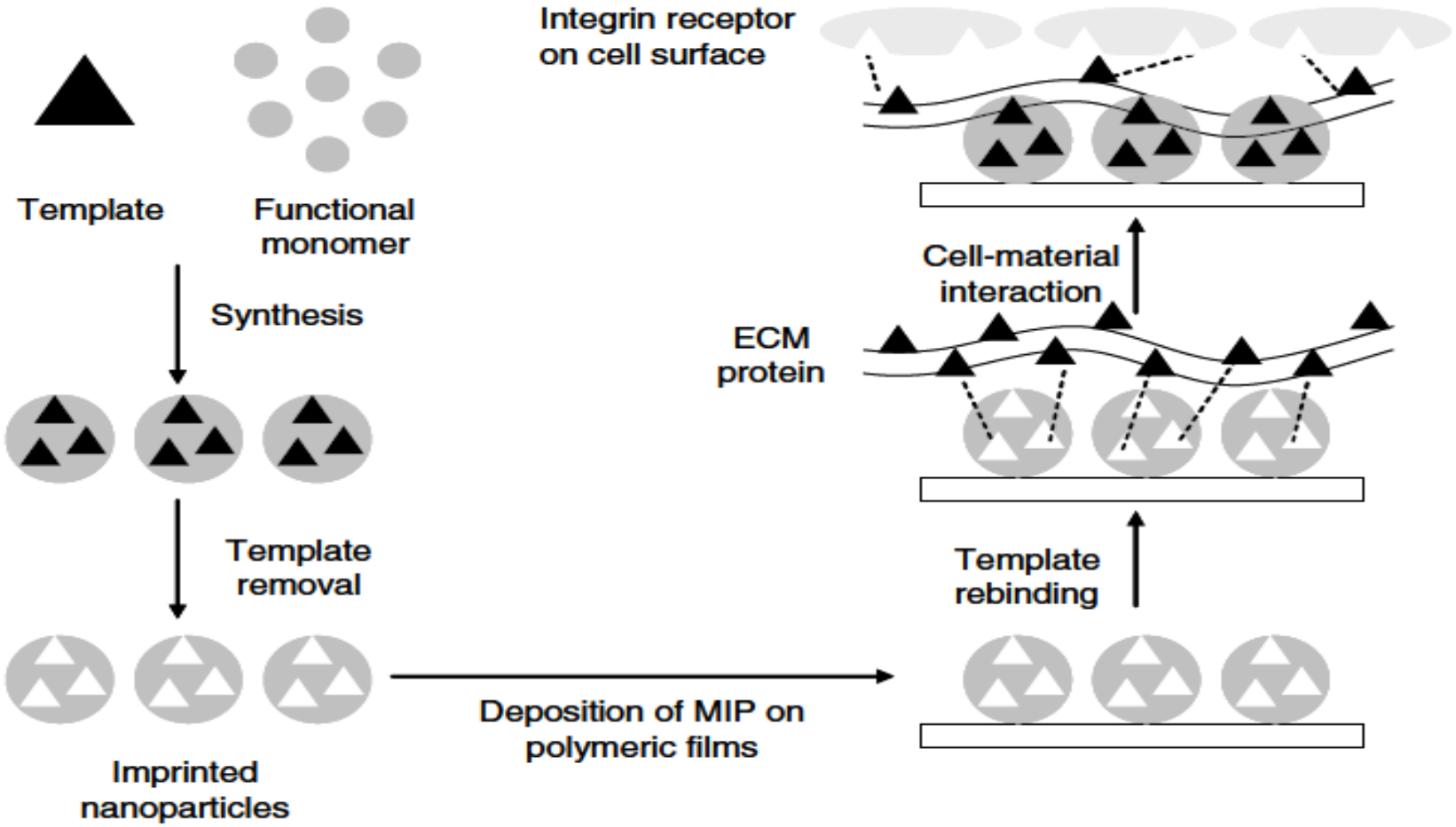
Production of molecularly imprinted polymers (MIP)

1. *Formation of complexes consisting of a template molecule, which we want to have recognized by the polymer, and monomers with functional groups which are complementary to the template*
2. *Polymerization in the presence of a cross-linking agent and a solvent having a porogen action*
3. *Removal of the template molecule*

MOST COMMON APPLICATIONS: chromatography, artificial antibodies, catalysis, biosensors



SCAFFOLD FUNCTIONALIZATION THROUGH MOLECULARLY IMPRINTED POLYMERS



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Rosellini E et al., *Molecularly Imprinted Nanoparticles with Recognition Properties Towards a Laminin H–Tyr–Ile–Gly–Ser–Arg–OH Sequence for Tissue Engineering Applications*, Biomed Mater 2010; 5: 065007

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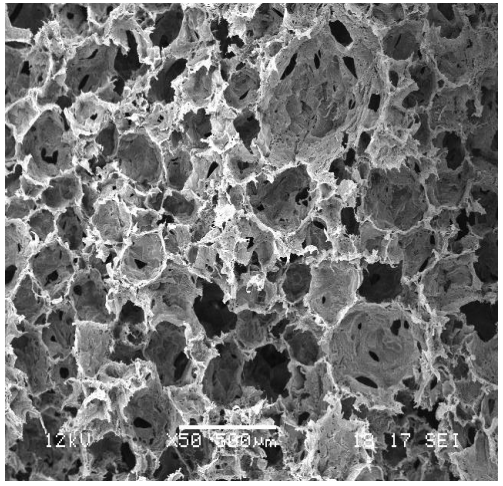
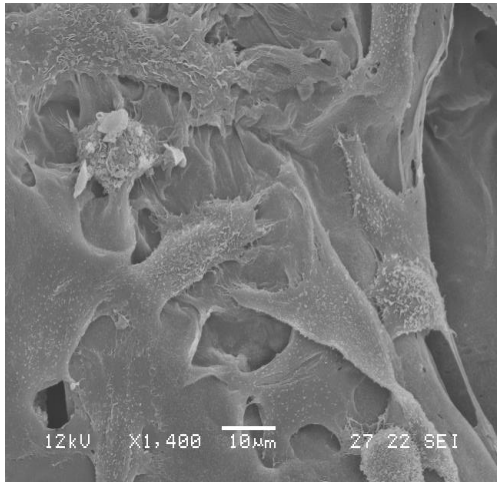
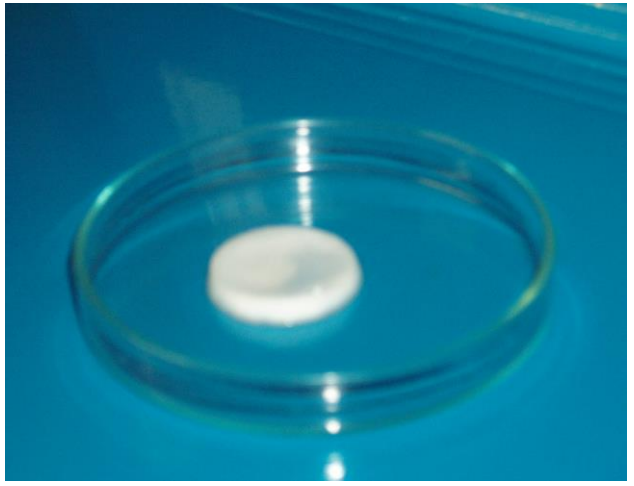
BIOMIMETIC SCAFFOLDS

Sponge-like systems

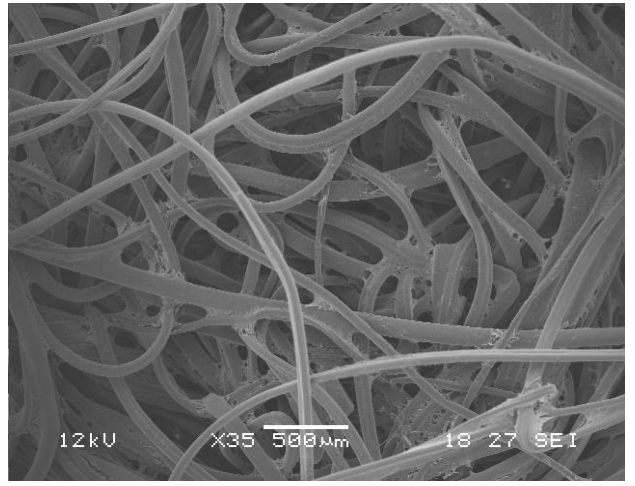




Hydrogels



Fiber meshes



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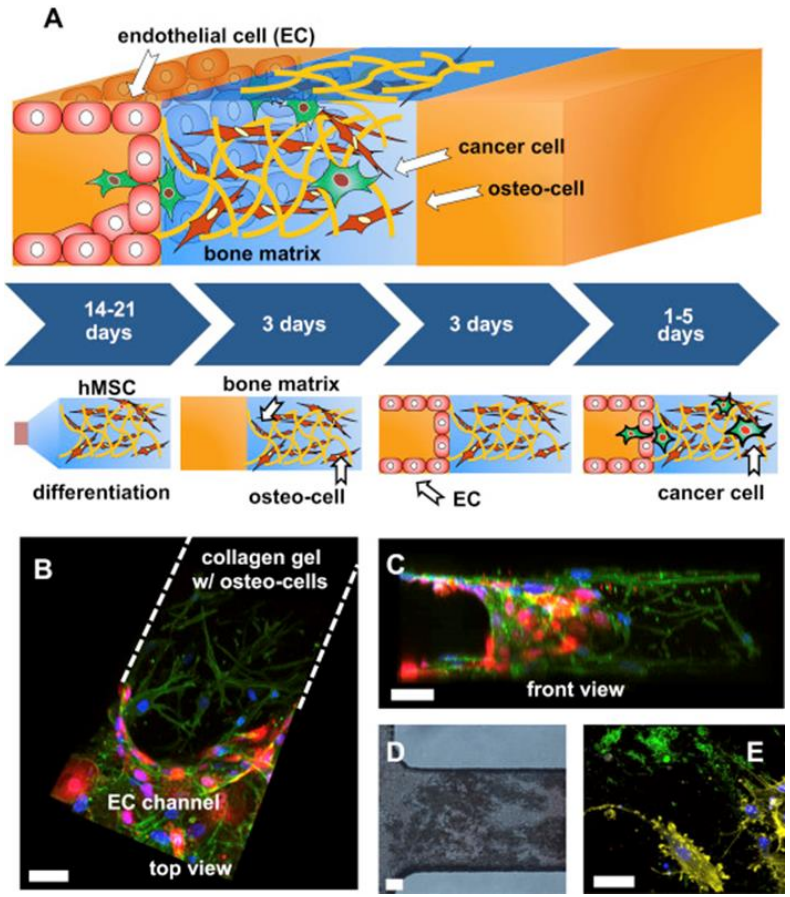
MICROFLUIDIC PLATFORMS

The microfluidic technology can provide ***micro-scale complex structures and well-controlled parameters to mimic the in vivo environment of cells.***
The combination of microfluidic technology with 3D cell culture offers great potential for in vivo-like tissue-based applications

The microfluidic technology also called ***Lab-on-a-chip*** *developed in the 1990s* ***offers a unique opportunity for*** *3D cell culture and cell-based assays,* ***creating a platform for engineering highly complex and dynamic microenvironments*** *that are controllable, reproducible, and optimizable.*



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MICROFLUIDIC PLATFORMS

The microfluidic technology has five significant features:

(1) Its ***micro-scale dimensions are compatible with those of many microstructures and environments native to in vivo systems****. For example, the mean free path length between adjacent capillaries in many in vivo animal tissue models is in the micro-scale region*

(2) Microfluidic devices can readily ***create complex dynamic micro-scale environments to mimic 3D in vivo environments****, such as a complex chemical gradient*

(3) It requires only a ***small amount of samples****, and the reagent consumption is low, which significantly reduces costs*

(4) ***Some substrates*** *like polydimethylsiloxane (PDMS)* ***used in microfluidic devices are permeable to O2****, an important factor influencing cell proliferation*

(5)Microfluidic technology ***can integrate multiple steps*** *such as cell culture, cell sampling, fluid control, cell capture, cell lysis, mixing, and detection* ***on a single device****.*

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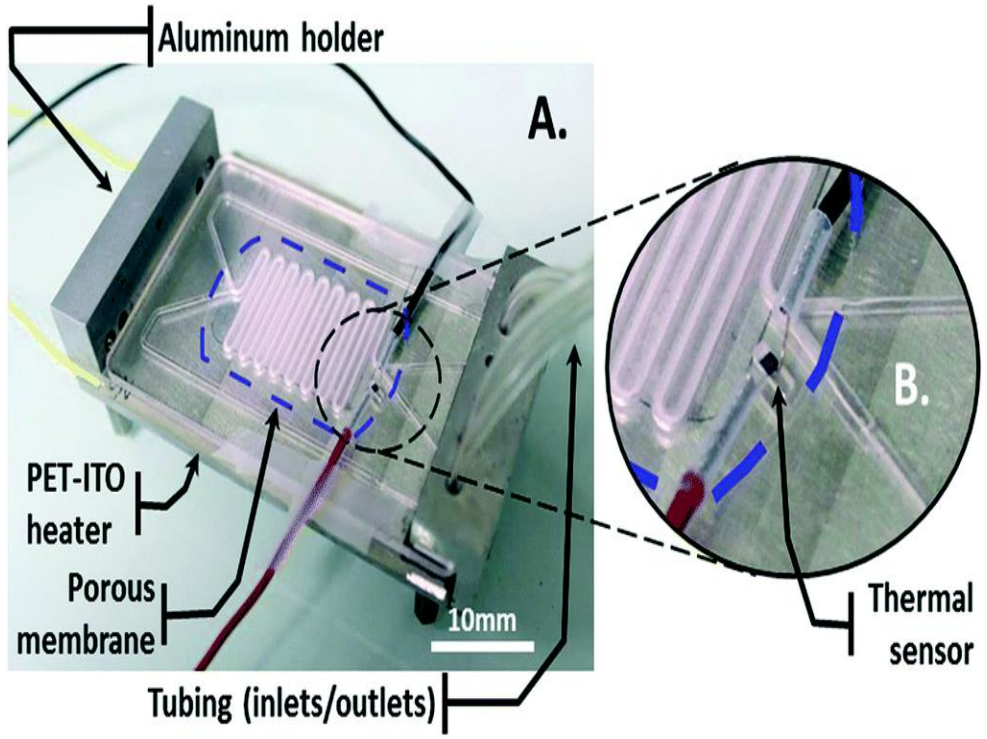
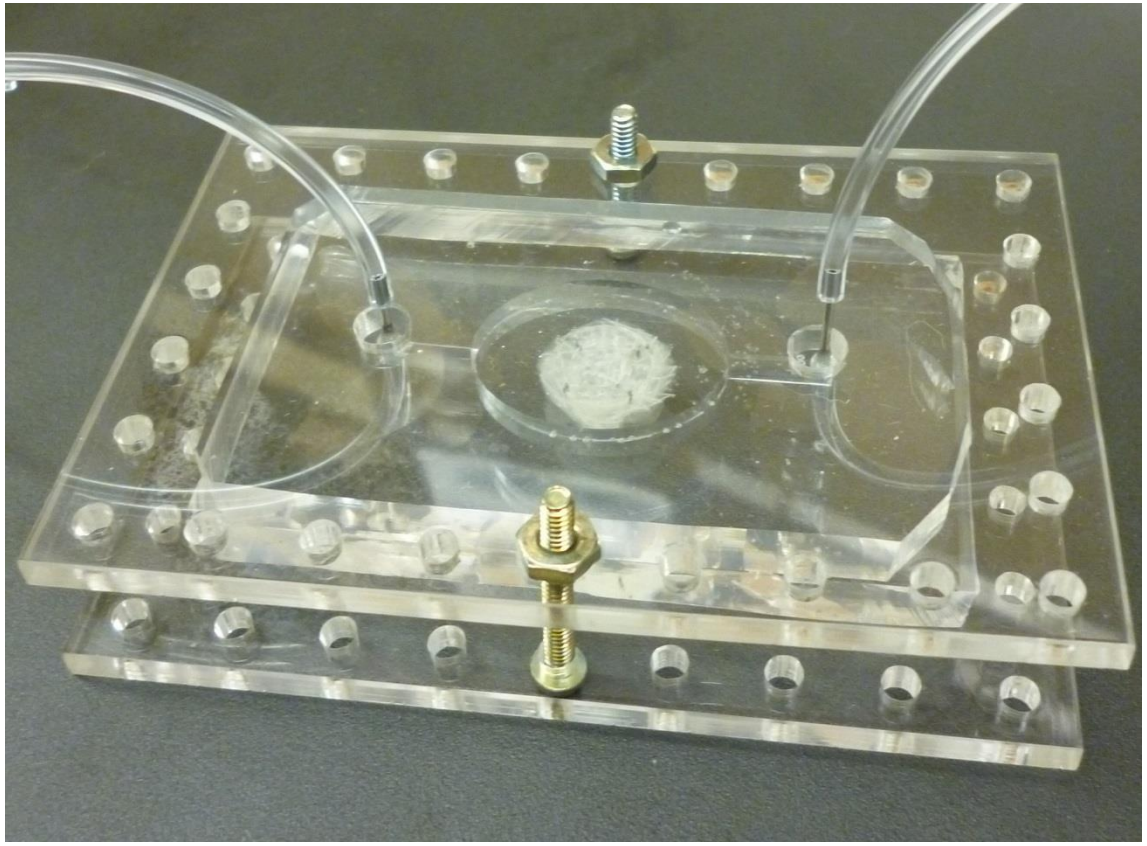
BIOMIMETIC SCAFFOLDS IN MICROFLUIDIC PLATFORMS

- *Microfluidic bioreactors can be used for cell culture on biomimetic scaffolds as in vitro tissue and organ models with the aim to develope alternative methods to in vivo experiments*

- *Microfluidics can provide useful model systems to investigate complex phenomena under combination of multiple controllable biochemical and biophysical microenvironments*



BIOMIMETIC SCAFFOLDS IN MICROFLUIDIC PLATFORMS



A ***microfluidic*** *bioreactor was designed for perfusion culture of cardiomiocytes seeded onto a* ***biomimetic*** *scaffold (alginate/gelatin sponge)*

E. Rosellini, Y.S. Zhang , B. Migliori, N. Barbani, L. Lazzeri, S. R. Shin, M. R. Dokmeci, M.G. Cascone, Protein/polysaccharide-based scaffolds mimicking native extracellular matrix for cardiac tissue engineering applications. *Journal of Biomedical Materials Research Part A* 106A; 769-781; 2018.

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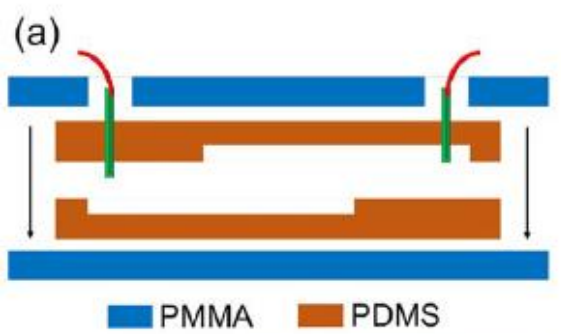
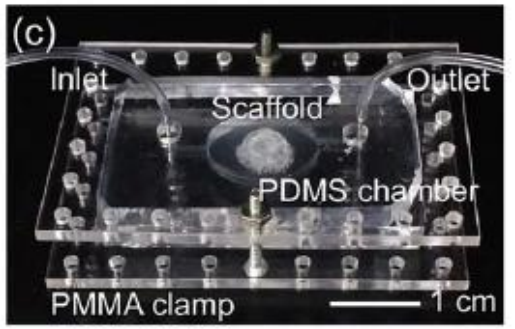
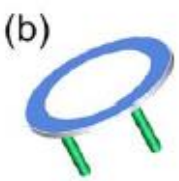
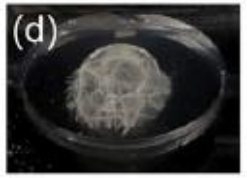
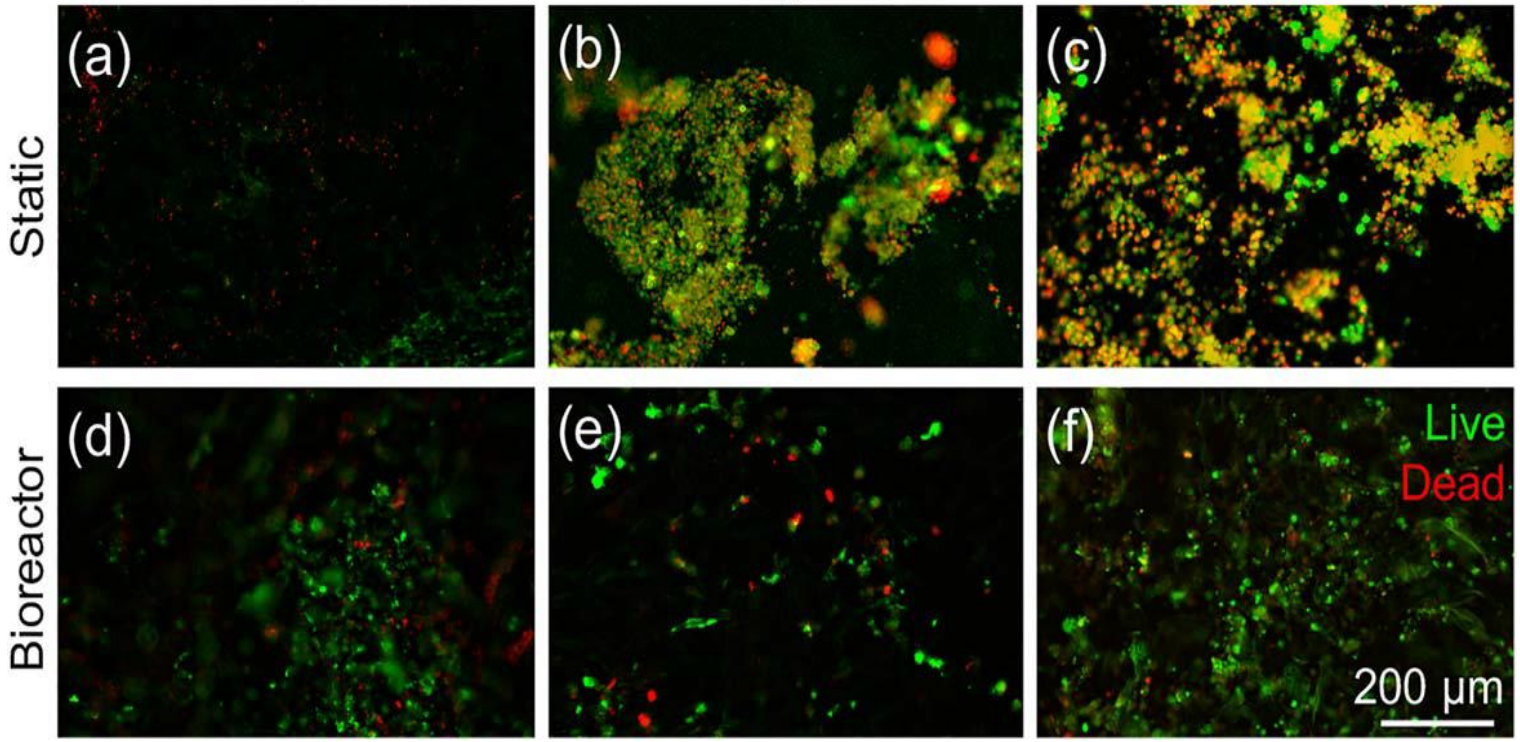
BIOMIMETIC SCAFFOLDS IN MICROFLUIDIC PLATFORMS











High viability of the resulting cardiac constructs, under dynamic flow culture was observed

Conclusions

- ***Biomimetic Scaffolds** characterized by chemical-physical signals and/or by a structure that mimics the extracellular matrix allow to control and influence the specific cellular response*

- ***Microfluidic devices** can readily create complex dynamic micro-scale environments able to mimic 3D in vivo environments*

- *The use of **Microfluidic Bioreactors** for cell culture on Biomimetic Scaffolds allow to develope in vitro tissue and organ models usable as alternative methods to in vivo experiments*

Thank you