# Implantable polymeric scaffolds for neural repair

*Laura Calzà & Maria Letizia Focarete*
*Health Sciences and Technologies—Interdepartmental Center for Industrial Research (HST-ICIR),*
*University of Bologna, Bologna, Italy*

# Technopoles

Activities of the
*Health Sciences and Technologies— Interdepartmental Center for Industrial Research* of the University of Bologna related to regenerative medicine and tissue engineering

THE MATERIAL SIDE

Polymer Science Group

Functional polymeric biomaterials

THE PRECLINICAL SIDE

JRL CIRI-SDV&IRET Foundation

In vitro and in vivo testing

Safety also according to GLP

Efficacy in disease models

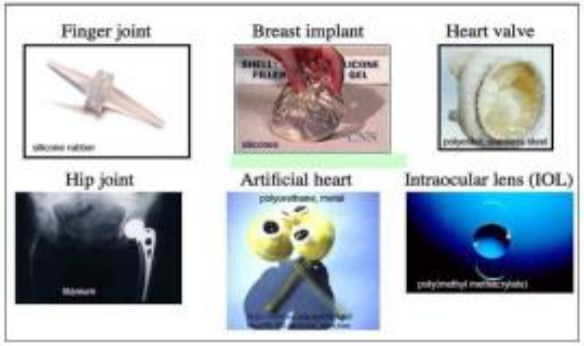
# Implantable polymeric scaffolds for neural repair:
# *The material side*

*Maria Letizia Focarete*
*Marialetizia.focarete@unibo.it*

# THE MATERIAL SIDE

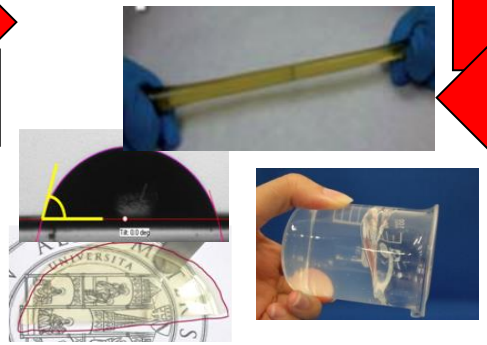
According to a specific task we are able to develop the **more suitable polymeric system**, on the basis of the required properties

## Medical demand

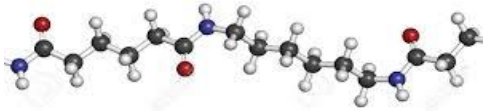



## Properties

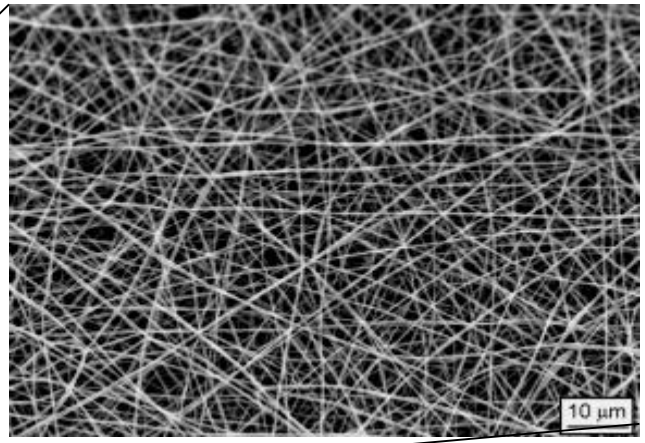
Chemical, Physical
Mechanical, Thermal



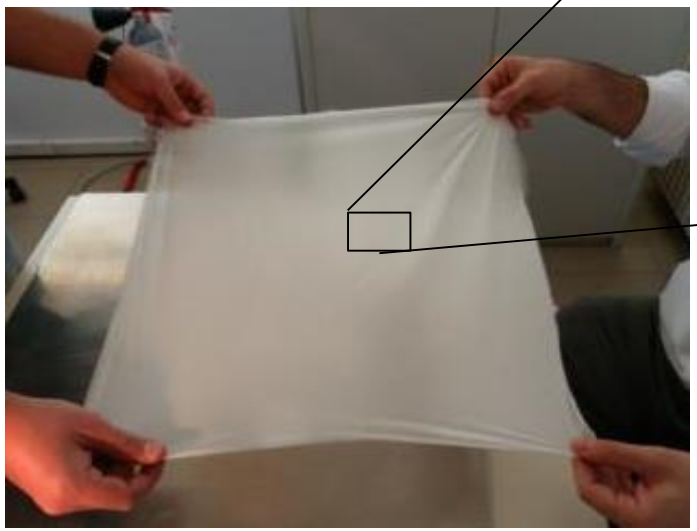
## Suitable polymeric system



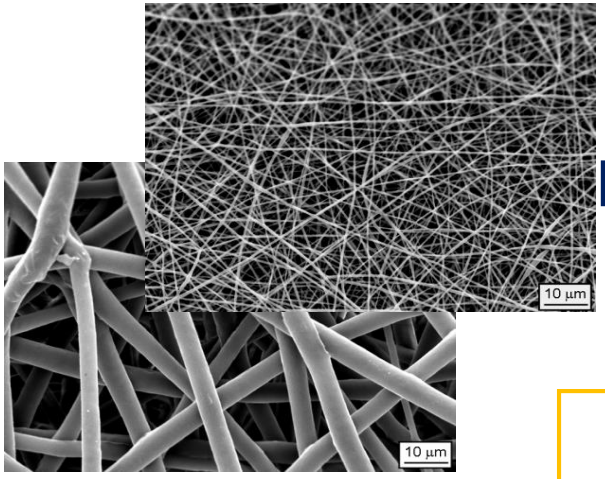
Randomly arranged fibers



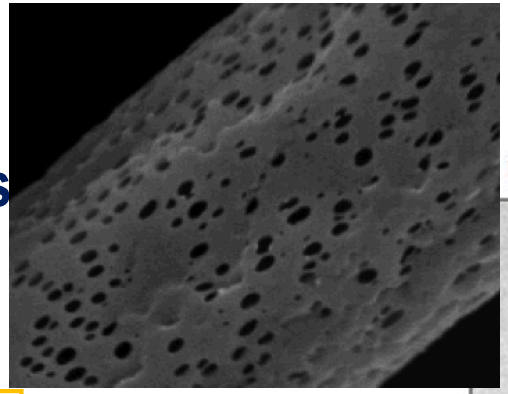

Self-standing
non-woven mats



- Micrometric and nanometric fibers (from hundreds of nm to a few µm)
- High porosity ~ 90%
- Pore size from a few µm to tens of µm

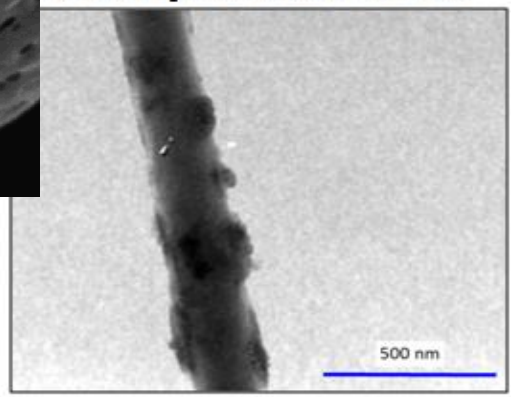



Random fibers
Micro- Nanofibers



Porous fibers

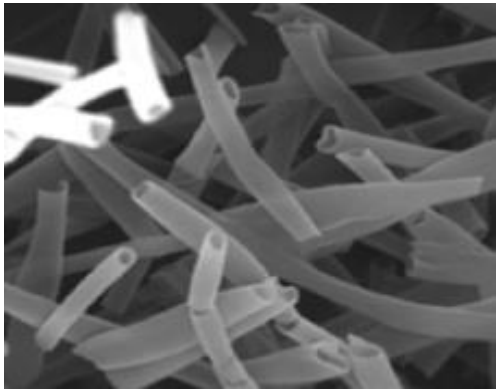
Composite fibers




*Highly tunable design*

Aligned fibers

Core shell fibers



Hollow fibers

# Functional polymeric materials

**3D microenvironment to mimik different tissues**

- **To study cell faith mimiking different pathologies**
- **To perform cell culture for cell transplant**

Drug delivery

- Tuning the material properties is possible to obtain the desired release kinetics of one/multi drug systems

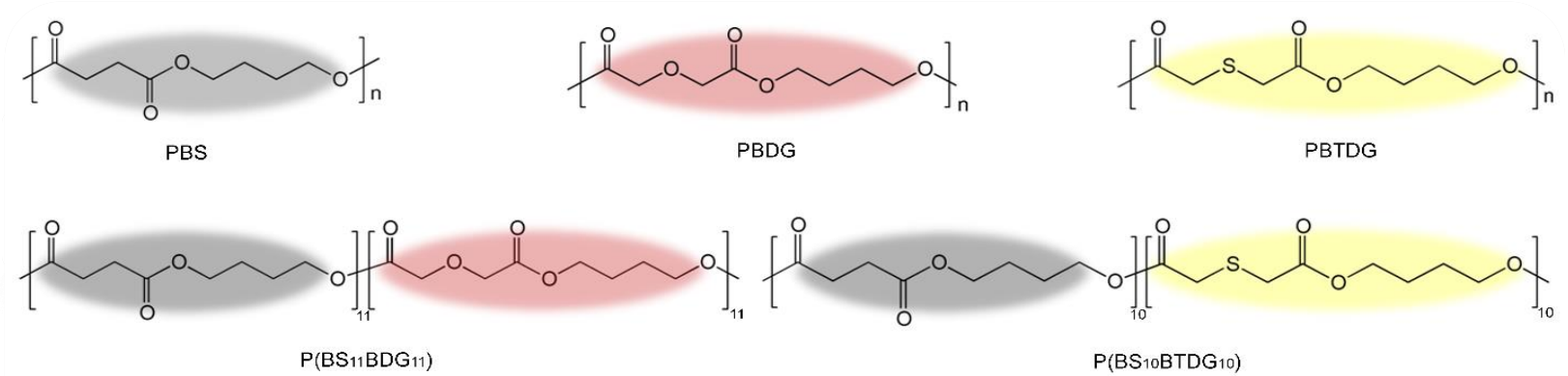
Bioactive Scaffolds (bioconjugation)

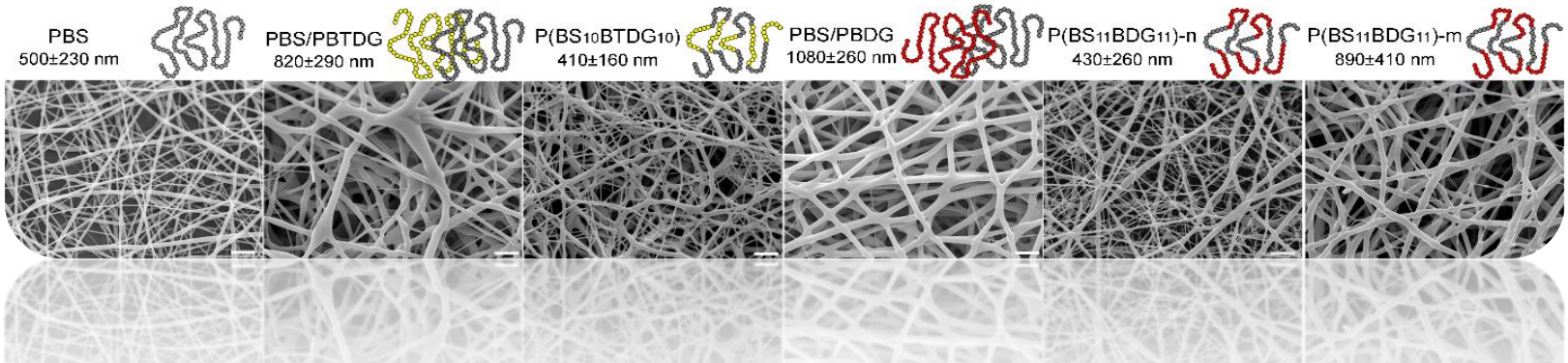
- Create link between the selected material and antibodies/peptides/ growth factors

# New polymeric systems for bioinspired scaffolds

Poly(butylene succinate) - PBS
Poly(butylene thiodiglycolate) - PBTDG
Poly(butylene diglycolate) - PBDG

Homopolymers
Blends
Multiblock copolymers




- Thermal properties
- Mechanical properties
- Hydrolitic degradation rate
- Wettability
- Osteogenic/ chondrogenic cell differentiation

Biomaterials 76 (2016) 261–272

Contents lists available at ScienceDirect

Biomaterials

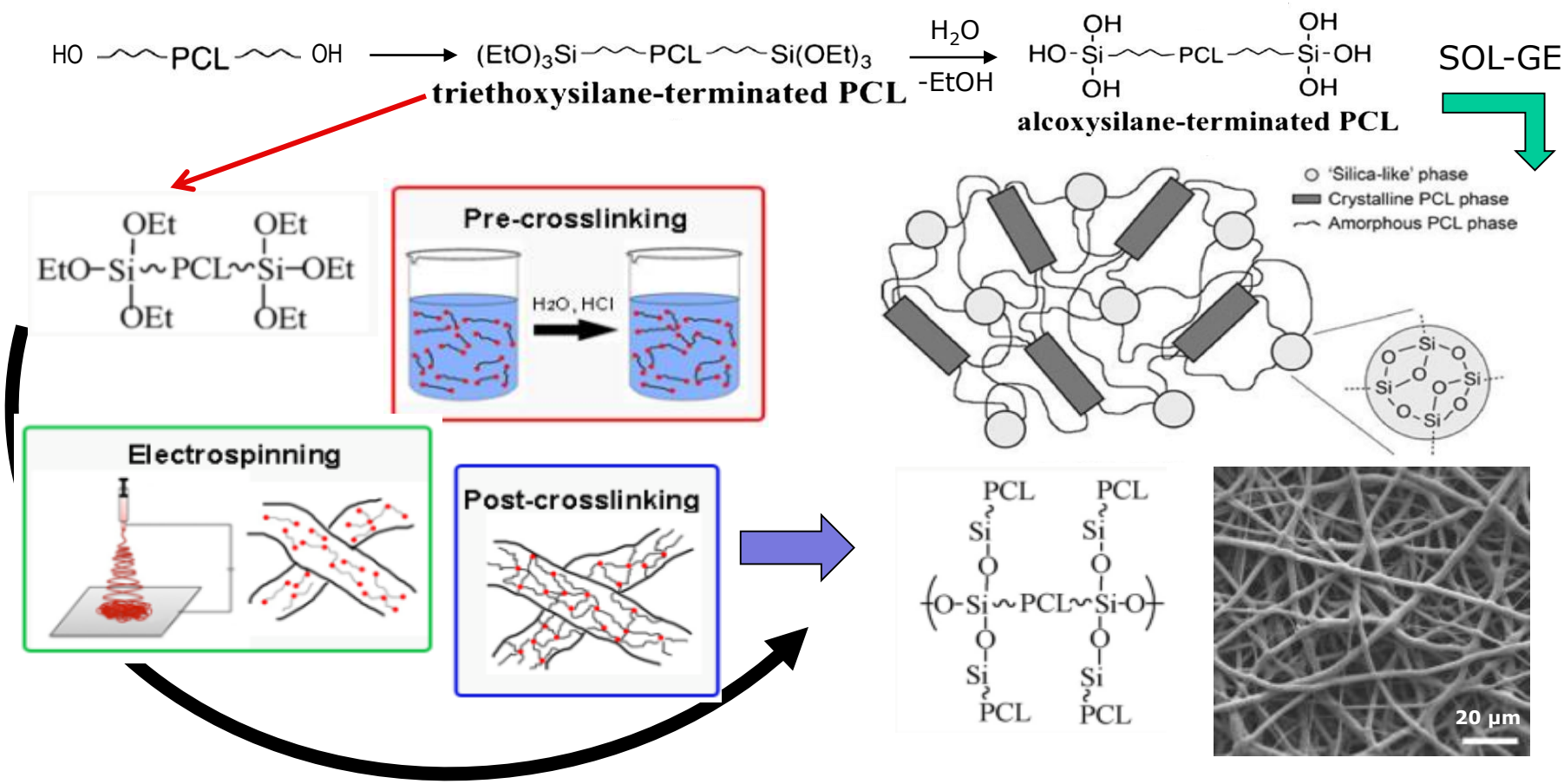
journal homepage: www.elsevier.com/locate/biomaterials

# Tailoring chemical and physical properties of fibrous scaffolds from block copolyesters containing ether and thio-ether linkages for skeletal differentiation of human mesenchymal stromal cells

Honglin Chen [a, 1], Matteo Gigli [b, 1], Chiara Gualandi [c], Roman Truckenmüller [a], Clemens van Blitterswijk [a], Nadia Lotti [b], Andrea Munari [b], Maria Letizia Focarete [c, d, **], Lorenzo Moroni [a, *]

# Shape memory meshes

## PCL-based through Sol-Gel Electrospinning



RSC Advances

PAPER

CrossMark
click for updates

Cite this: *RSC Adv.*, 2016, 6, 43964

## Facile fabrication of shape memory poly(ε-caprolactone) non-woven mat by combining electrospinning and sol–gel reaction

Andrea Merlettini,[a] Stefano Pandini,*[b] Silvia Agnelli,[b] Chiara Gualandi,[a] Katia Paderni,[c] Massimo Messori,[c] Maurizio Toselli[d] and Maria Letizia Focarete*[a]

Full Paper

Macromolecular Journals

Macromolecular Materials and Engineering

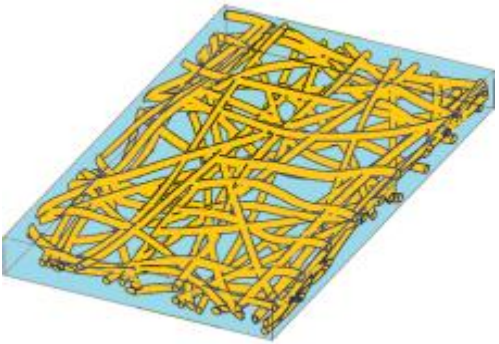
## Mutifunctional Electrospun Nonwoven Mats with Two-Way Shape Memory Behavior Prepared from Sol–Gel Crosslinked Poly(ε-Caprolactone)

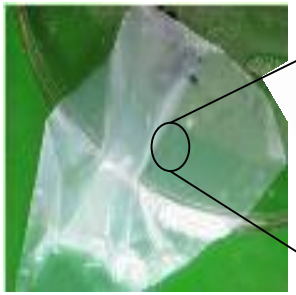
Stefano Pandini,* Silvia Agnelli, Andrea Merlettini, Federica Chiellini, Chiara Gualandi, Katia Paderni, Maria Letizia Focarete, Massimo Messori, Maurizio Toselli

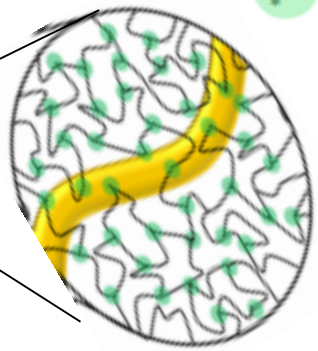
# Hydrogel-fiber 3D composite system for multipotent stem cell culture

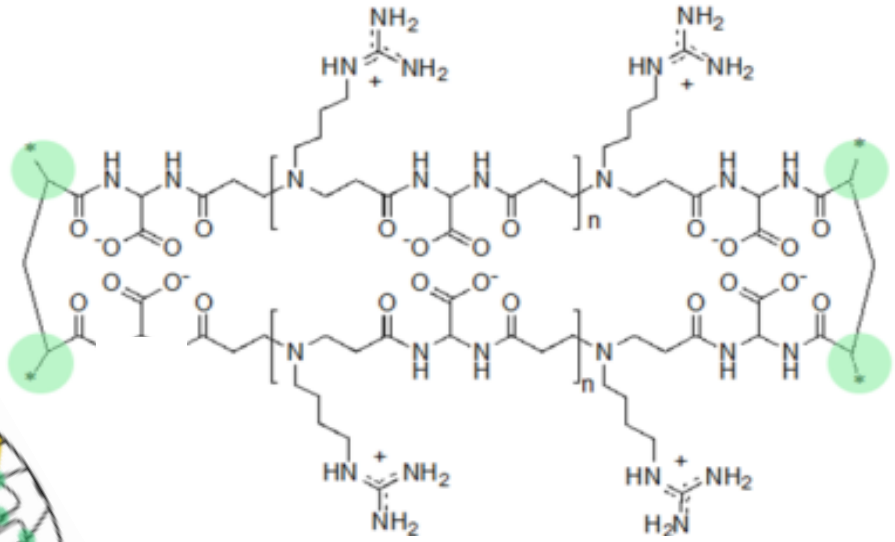
**FIBROUS COMPONENT**

Plasma treated Poly(L-lactide) fibers







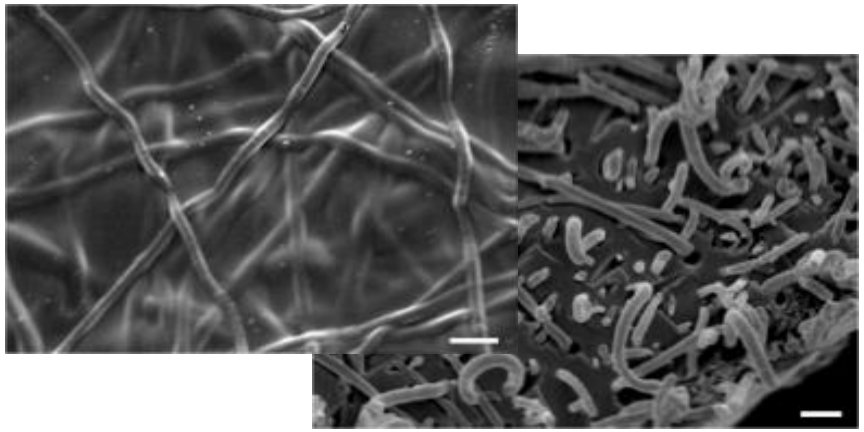


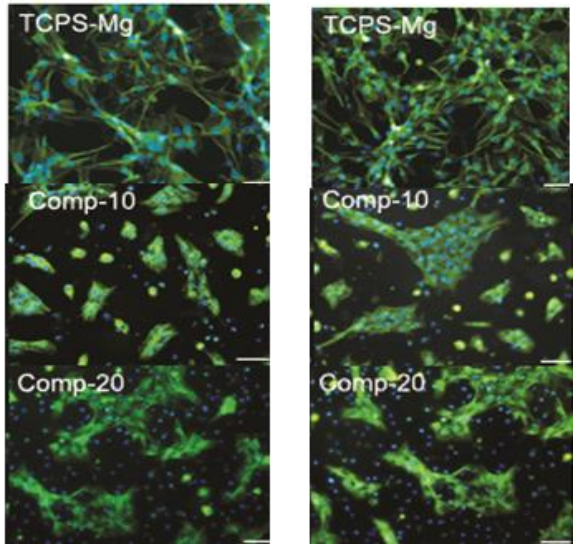
Human embryonic SCs

Human induced pluripotent SCs

**GEL COMPONENT**

RGD-like hydrogel





# Hydrogel-fiber 3D composite system for multipotent stem cell culture

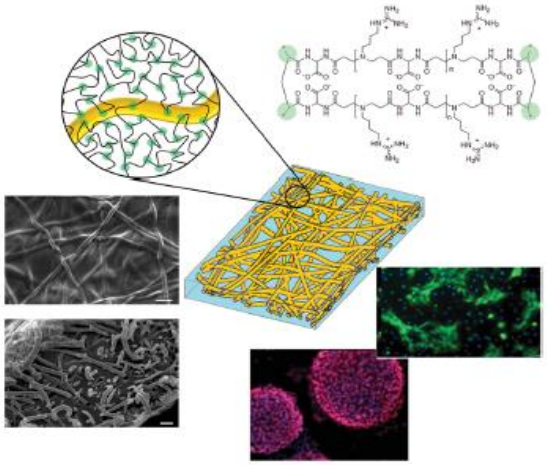
Full Paper

Macromolecular Journals

Macromolecular Bioscience

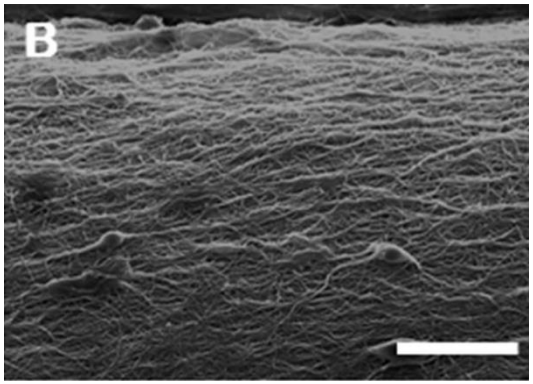
## Poly-L-Lactic Acid Nanofiber–Polyamidoamine Hydrogel Composites: Preparation, Properties, and Preliminary Evaluation as Scaffolds for Human Pluripotent Stem Cell Culturing

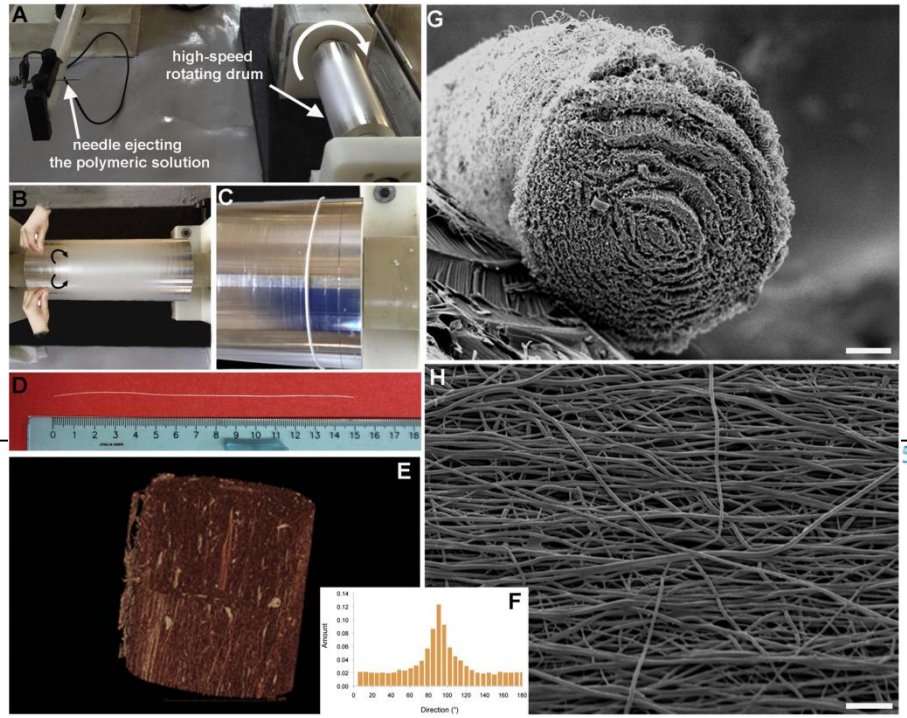
ɔra Bloise, Nicolò Mauro, Paolo Ferruti, Amedea Manfredi,
si, Anna Liguori, Romolo Laurita, Matteo Gherardi,
ivia Visai, Maria Letizia Focarete,* Elisabetta Ranucci*



*Macromol. Biosci.* **2016**, *16*, 1533–1544

# Bioinspired 3D matrices: Tendon substitutes




IOP Publishing Biofabrication 9 (2017) 015025 5090/aa6204

Biofabrication

PAPER

# Biofabrication of bundles of poly(lactic acid)-collagen blends mimicking the fascicles of the human Achille tendon

RECEIVED
25 January 2017

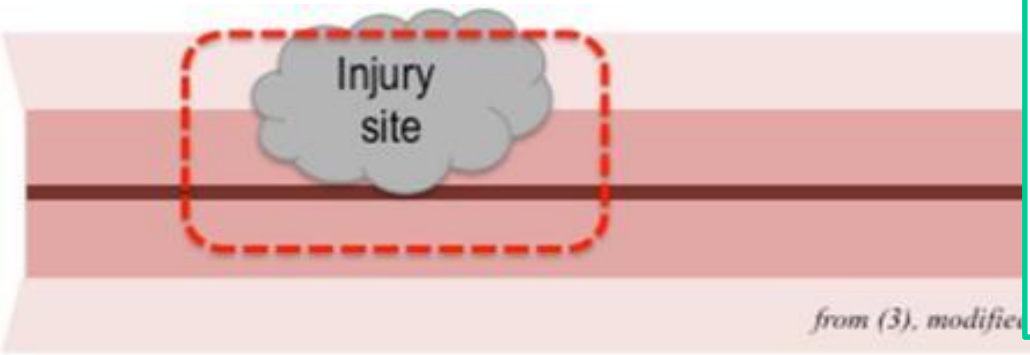
ACCEPTED FOR PUBLICATION
22 February 2017

PUBLISHED
8 March 2017

Alberto Sensini[1], Chiara Gualandi[2], Luca Cristofolini[1,3], Gianluca Tozzi[4], Manuela Dicarlo[5], Gabriella Teti[6], Monica Mattioli-Belmonte[5] and Maria Letizia Focarete[2,3]

"Step-by--step: integrated approach for the patient with acute neurologic lesions"
Prof. Laura Calzà

AIM: Development and testing of **devices made of electrospun fibers** for the **controlled release of two drugs** to favore the riemilinization process and to locally control the inflammation in neurological traumatic lesions



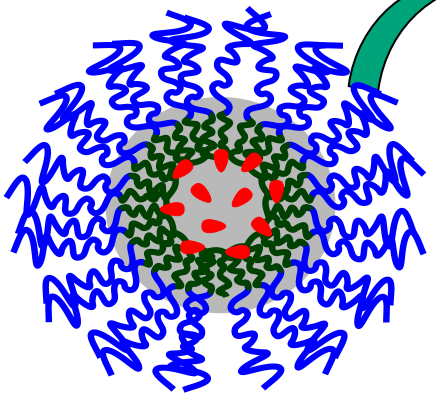

**Primary damage:**
- impact
- hemorrhage
- inflammation

→ Glutamate release + Ca++ influx + BBB breakdown + O2 free radicals formation + ARA cascade+ PGs production + necrosis

→ **Secondary damage:**
- inflammatory and immune cells infiltration
- cytokines release
- myelin degeneration (and remyelination failure)
- apoptosis

*hours* >>>>>>>>>>>>>>>> *days* >>>>>>>> *weeks* >>>>>>>>>>>>>>>>> *months*

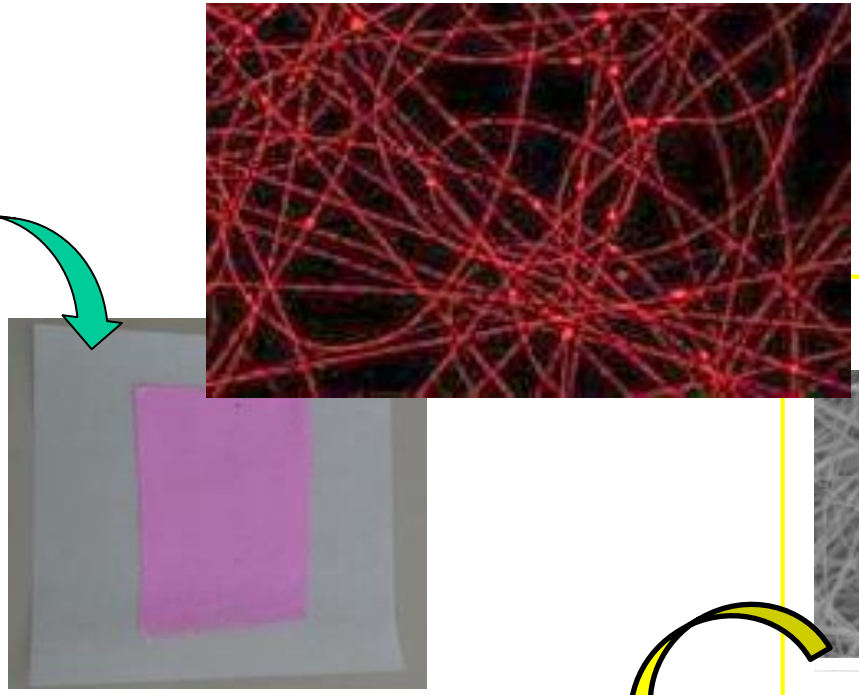
# Controlled *in situ* release of luminescent nanoparticles

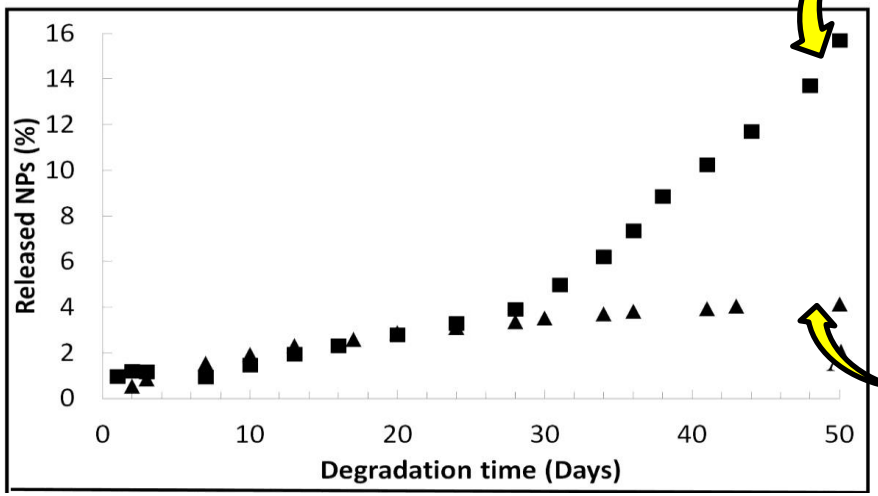


**SILICA core ~ 10 nm**

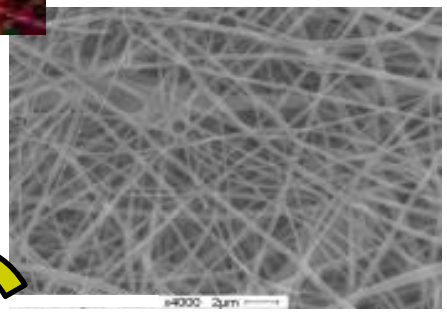
**NPs size ~ 25 n**

*Rampazzo et al., Nanoscale, 2*

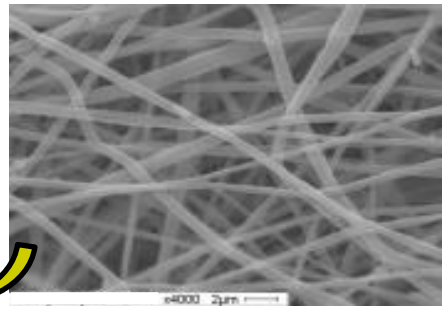





NPs + PLGA **50:50**

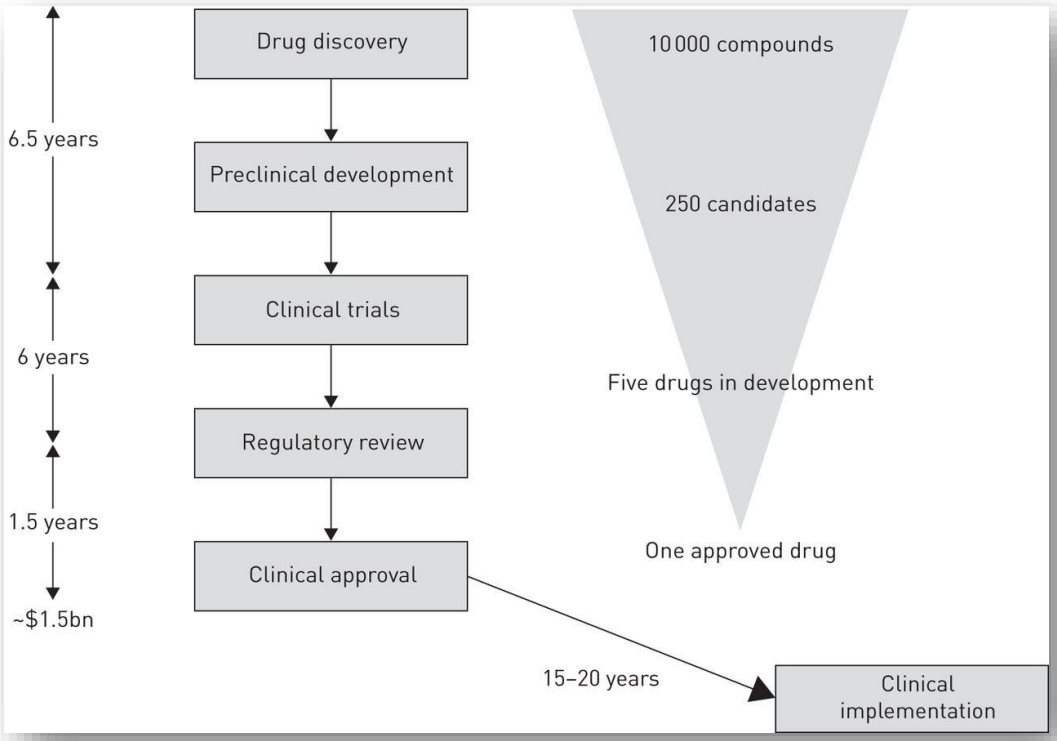


NPs + PLGA **75:25**



# Implantable polymeric scaffolds for neural repair:
## *In vitro testing for safety and efficacy.*
## *The preclinical side.*

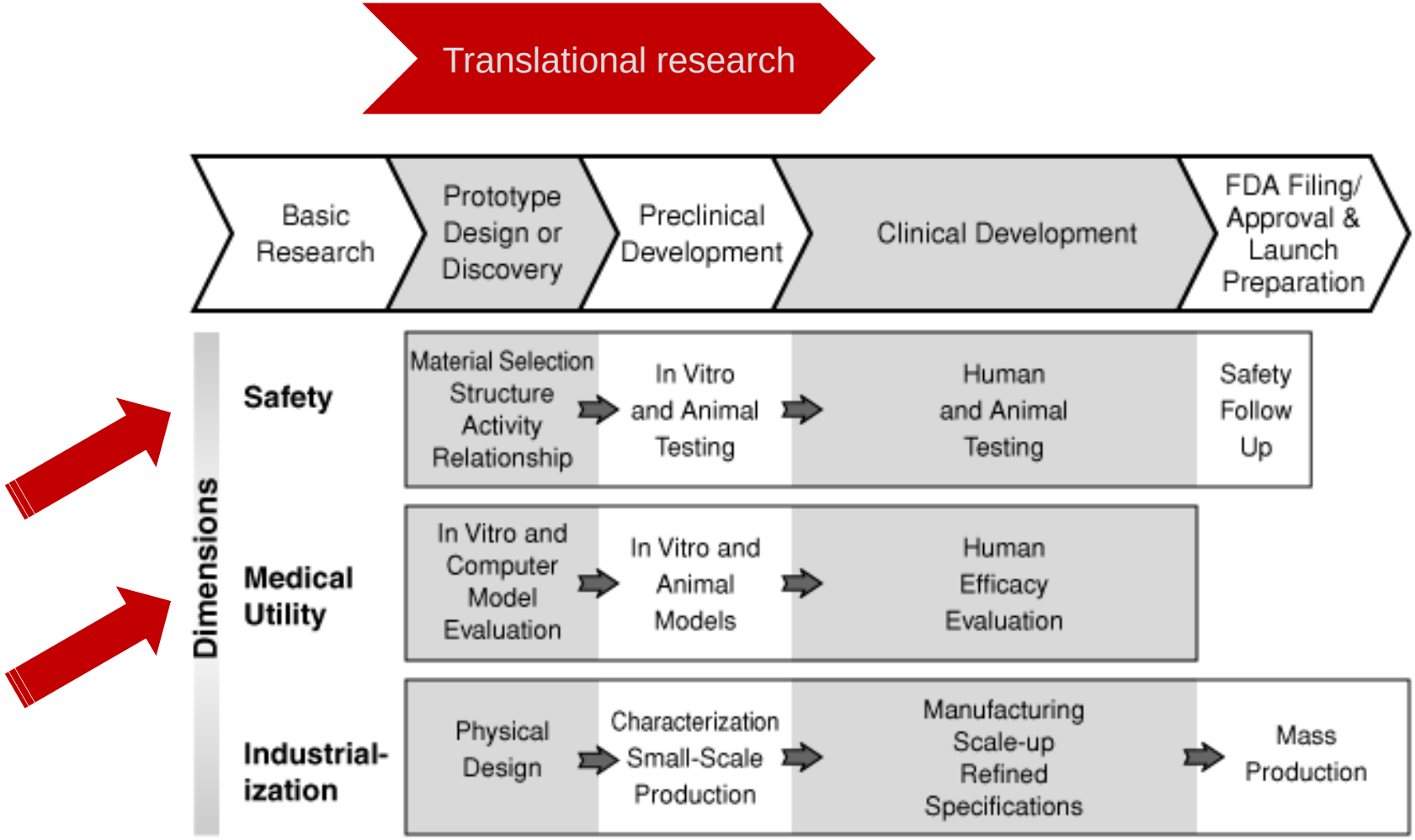
*Laura Calzà*
Laura.calza@unibo.it



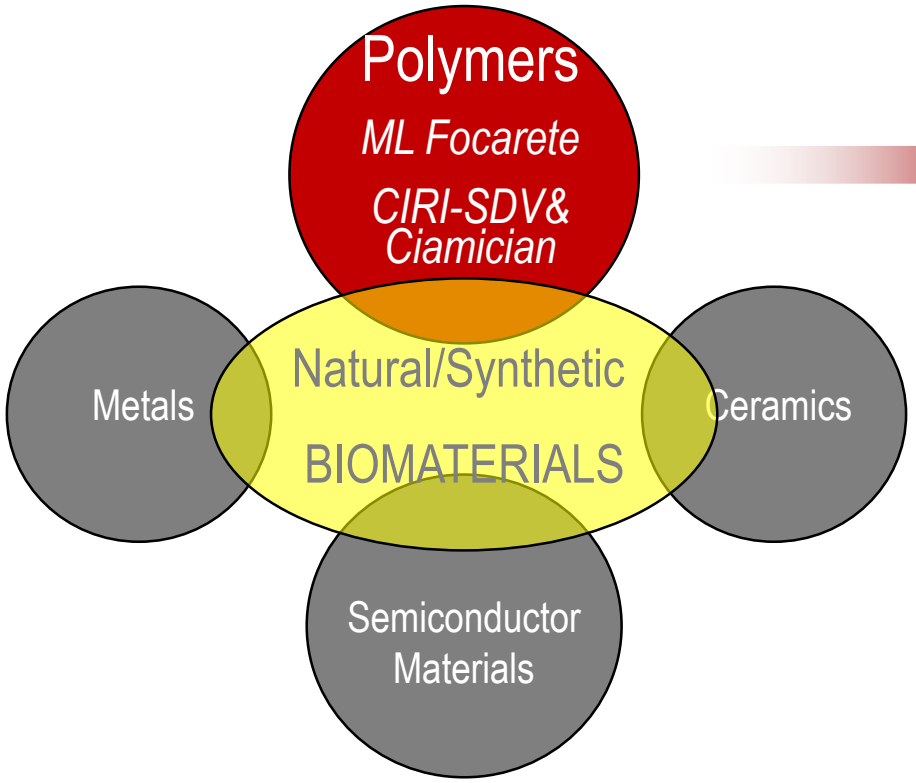

FDA U.S. FOOD & DRUG ADMINISTRATION

“implantable scaffolds”:

***How** to keep **close** PoC **efficacy** studies and FDA compliant **safety** tests*


Translational research
Basic Research
Prototype Design or Discovery
Preclinical Development
Clinical Development
FDA Filing/ Approval & Launch Preparation
Dimensions
Safety
Material Selection Structure Activity Relationship
In Vitro and Animal Testing
Human and Animal Testing
Safety Follow Up
Medical Utility
In Vitro and Computer Model Evaluation
In Vitro and Animal Models
Human Efficacy Evaluation
Industrial-ization
Physical Design
Characterization Small-Scale Production
Manufacturing Scale-up Refined Specifications
Mass Production

# CNS repair: a challenge for material science




3D mimicking

Self-healing

Drug delivery devices

Cell-scaffolds devices

Neurobiology and translational point of view

ALISEI
Advanced Life Sciences in Italy

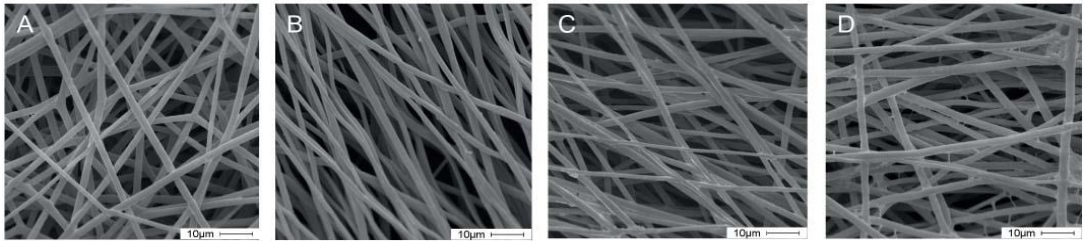
Italian
REGENERATIVE
MEDICINE
Infrastructure

POR FESR
EMILIA-ROMAGNA
2014/2020

# In vitro testing of biomaterials

*FDA-approved polymers (PLLA, PLGA)*


*Electrospun artificial polymers*




*Flexible, permeable, implantable biological reservoirs*

IRMI, technological cluster ALISEI (MIUR)
*scaffolds for self-healing improvement in the CNS*

Step-by-Step, POR-FESR (RER)
*scaffolds for localized drug delivery in the CNS*

# Toward clinical application: how we design a translational study: *in vitro experiments*

| | |
|---|---|
| Primary end-points | Toxicity<br>efficacy |
| Readout | cell viability<br>neurite elongation |
| Statistical power: | conventional vs high-troughput technologies |
| Regulatory: | GLP/ISO compliance |

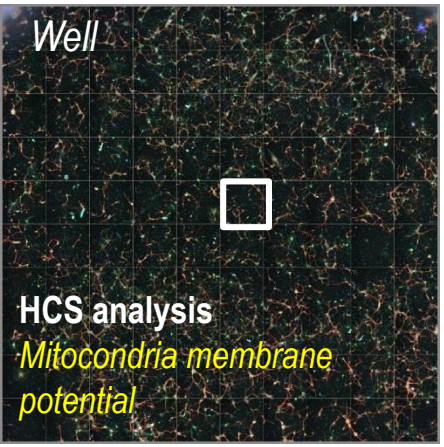
| | |
|---|---|
| materials | Glass<br>Plastic<br>PLLA |
| topography | 2D<br>Random semi 3D<br>Aligned semi 3D |
| Chemical functionalization | No-coating<br>Lamin<br>ECM extract |
| Cell type | SY5Y cell line<br>Primary neurons<br>Neural stem cells |

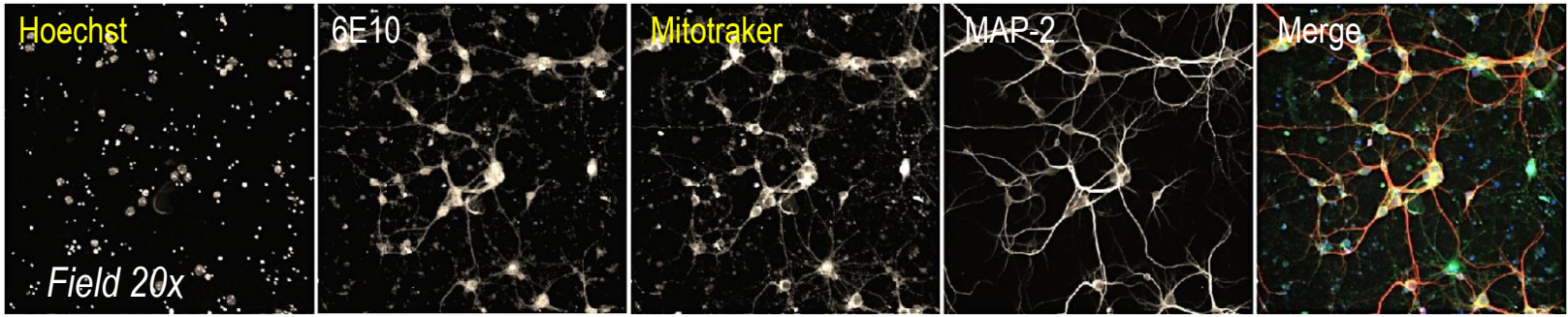
*Baldassarro et al., Biores Open Access. 2016*

| | description | PRO | CONTRA |
|---|---|---|---|
| **SY5Y (and other cell lines)** | neuroblastoma cell line derived from human tissue | • can be easily differentiated toward a neuronal-like cell<br>• widely available<br>• easy-to-handle<br>• highly reproducible system<br>• Human<br>• Maturation in 7DIV | • Tumor cells<br>• The sensitivity to a wide range of toxic stimuli is lower compared to primary neurons<br>• neurite outgrowth may be different from that occurring in primary neurons |
| **Primary neurons** | From fetal and neonatal brain | • physiologically significant<br>• Low possibility of false positives and negatives<br>• Maturation in 15/21DIV | • Animal (and human)<br>• require selective skills for handling and result interpretation<br>• mixed |
| **NSCs** | From fetal, neonatal and adult brain | • physiologically significant<br>• Low possibility of false positives and negatives<br>• Maturation in 15DIV (lineage)<br>• Mixed cell composition | • Animal (and human)<br>• require selective skills for handling and result interpretation<br>• Mixed cell composition |

*Baldassarro et al., Biores Open Access. 2016*

# Cell-based high content screening




Toxicity:

- Cell adhesion
- Mitochondria membrane potential
- Nuclear morphology
- LDH

Efficacy:

- Neurite elongation

Statistical design:

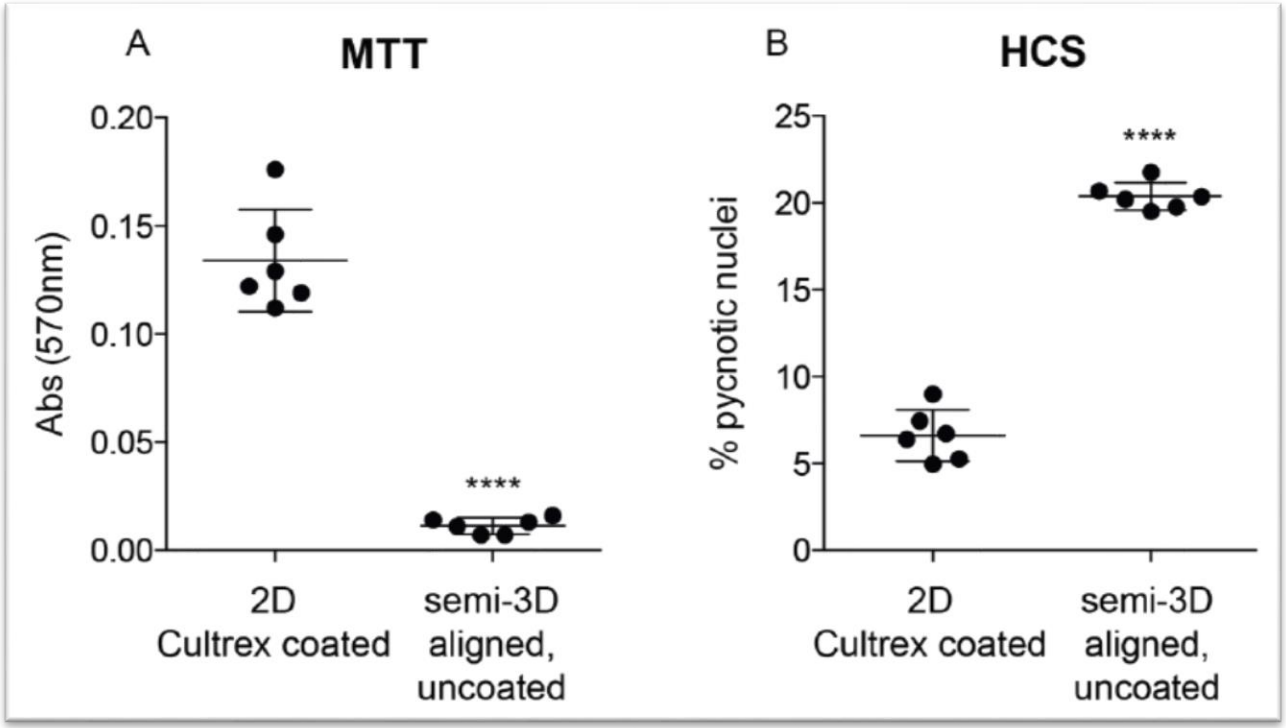
- 6 technical replicates
- 3 biological replicates

Statistical design:experimental variables:

- Material topography
- Material chemical functionalization
- Neural cell type

*Baldassarro et al., Microchem J, 2017, in press*

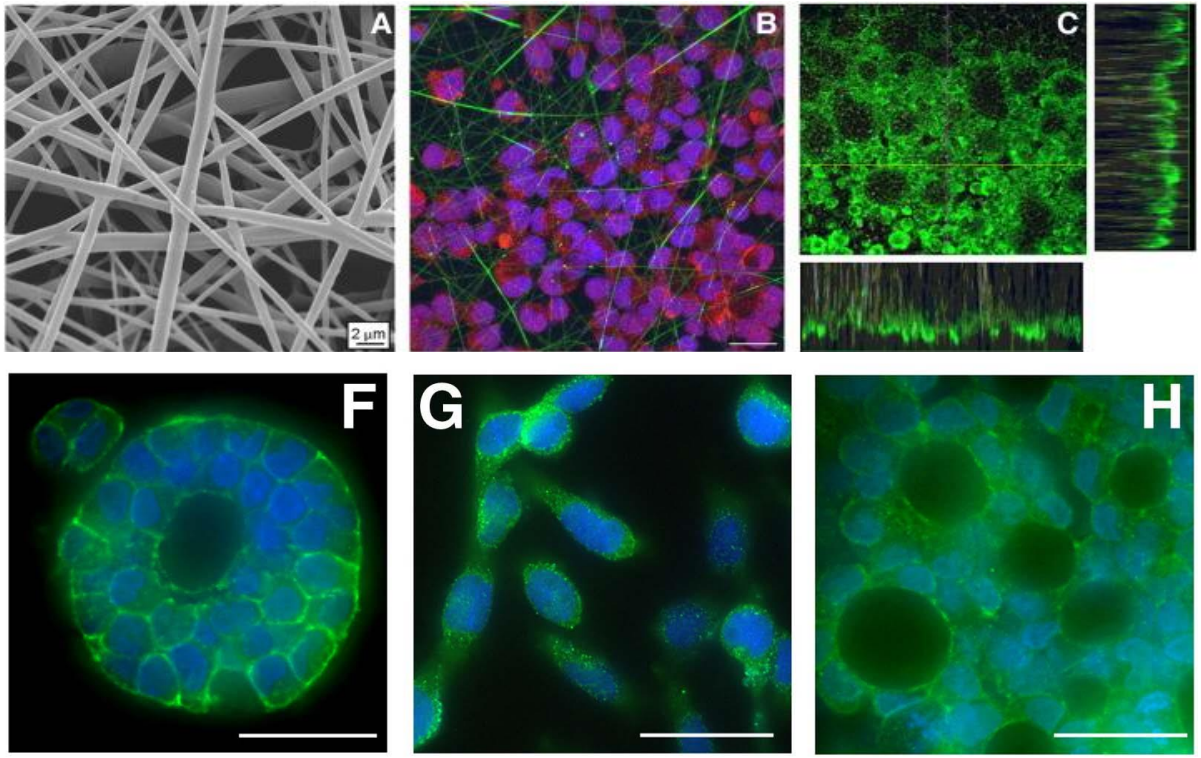
# Cell-based high content screening




coefficient of variation:

| | | |
|---|---|---|
| 2D cultrex coated, | MTT = 17.62% | HCS = 22.35%; |
| semi-3D aligned uncoated, | MTT = 32.86% | HCS = 3.90%) |

*Baldassarro et al., Microchem J, 2017 in press*




**2D, semi3D systems:**
A: SEM micrograph of PLLA electrospun scaffold
B: RESC cells on es-PLLA scaffold; actin, red, nuclear Hoechst 33258blue
C: 3D Oct4-IR RESCs (green

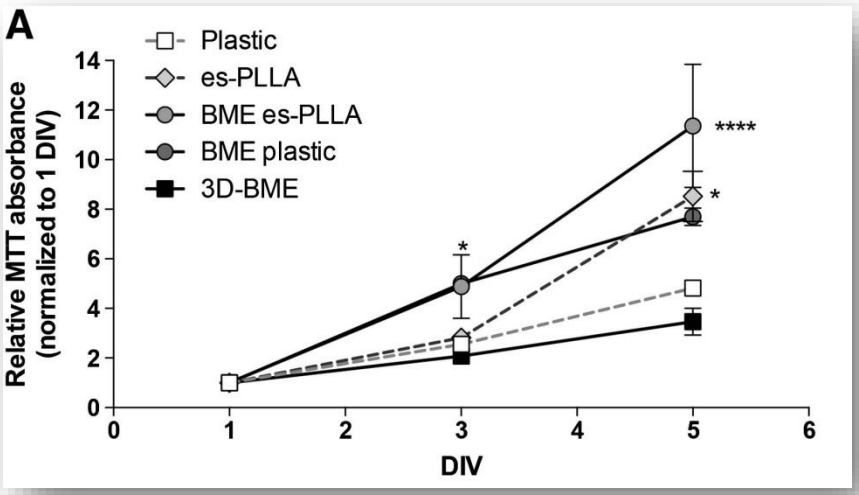
**actin** immunostaining (green)

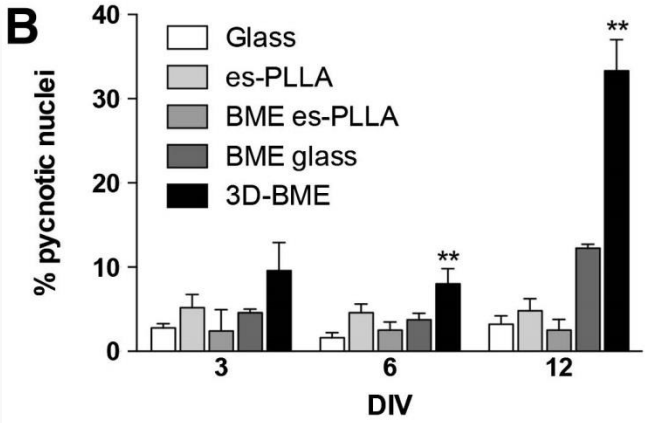
F: 3D-BME
G: glass
H: es-PLLA

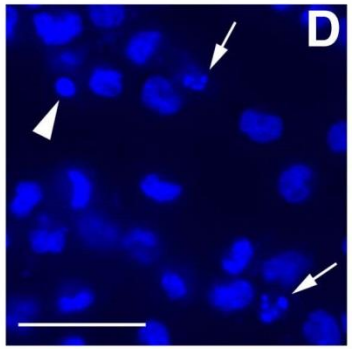
| material | functionalization | Cellular test system |
|---|---|---|
| chemistry | Physical | Proliferation/viability |
| topography | chemical | differentiation |
| 2D vs 3D | pharmacological | Biological properties |

*Alessandri et al., Matrix Biol, 2014*

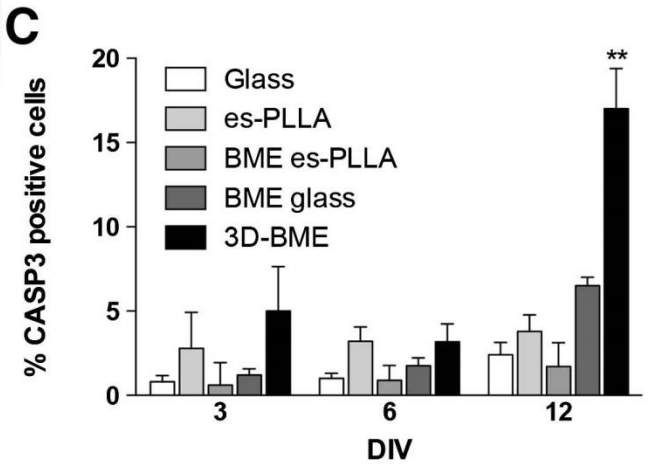
# Ex 1: RESC, proliferation & viability

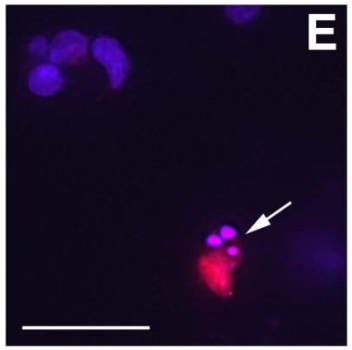



Hoechst33258
caspase3

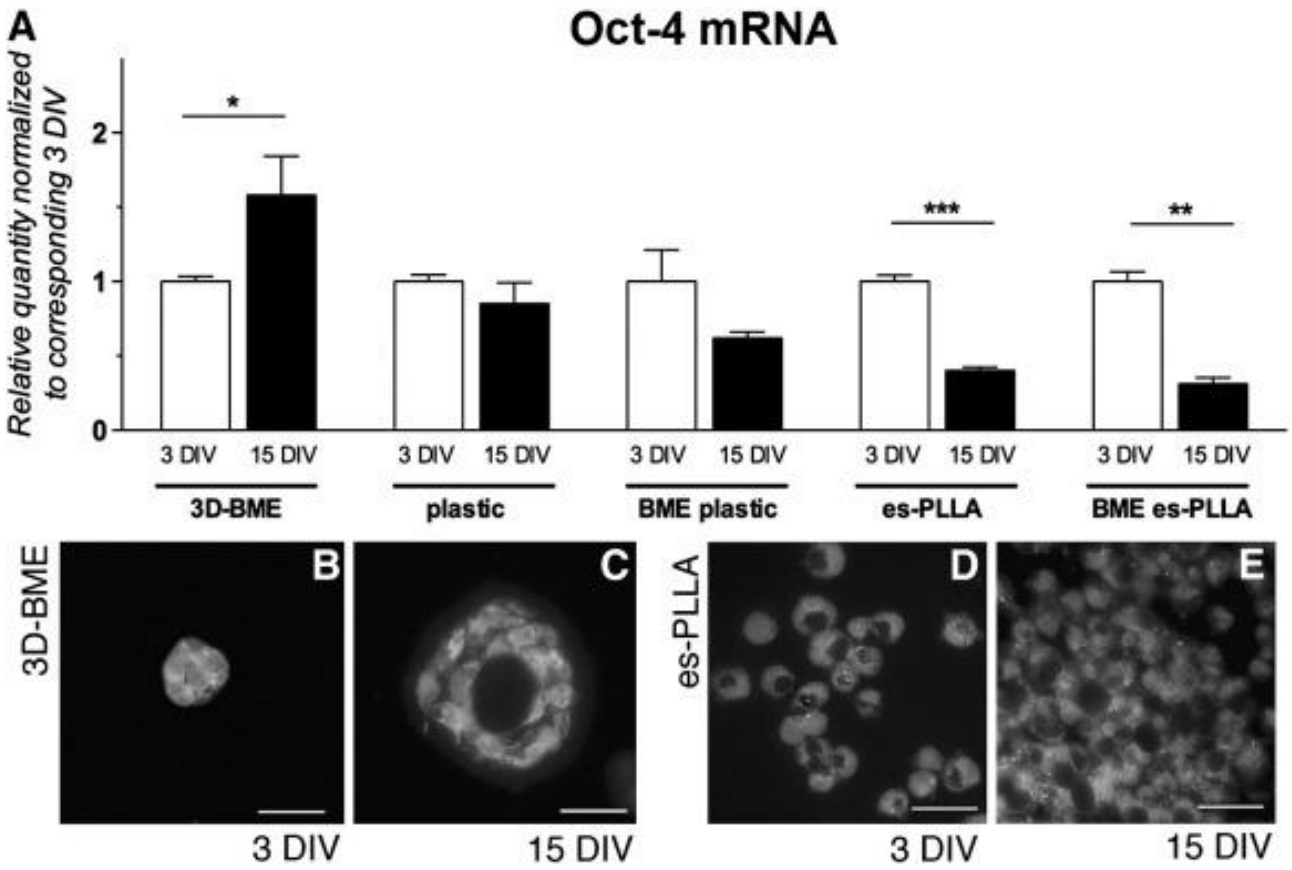



Proliferation and viability assays of RESCs cultured on different 2D and 3D surfaces

*BME: Basement Membrane Extract*
*es-PLLA: submicrometric elctrospun fibres*

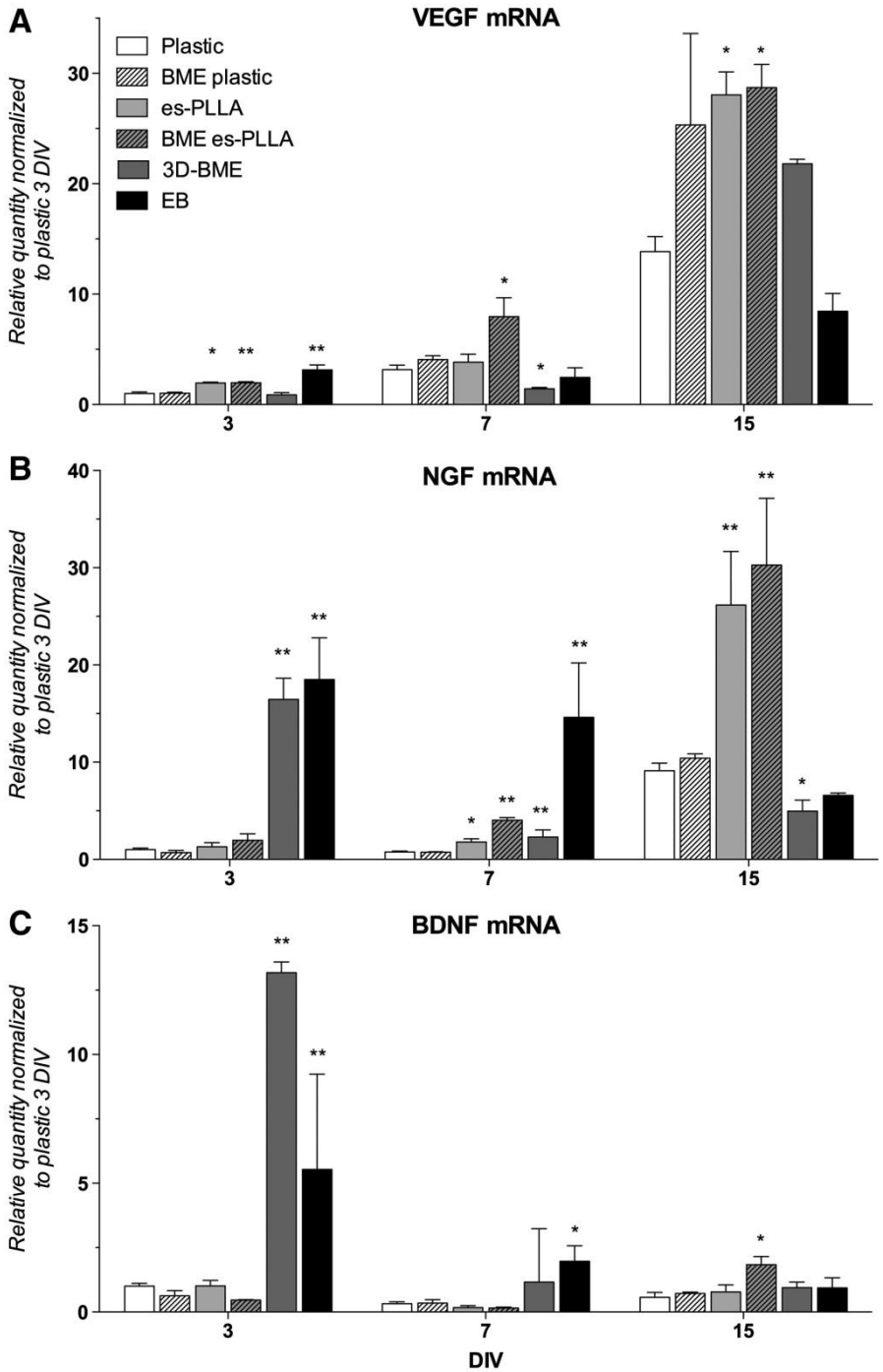
*Alessandri et al., Matrix Biol, 2014*

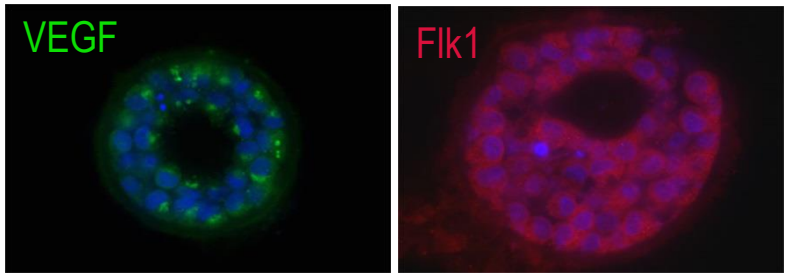


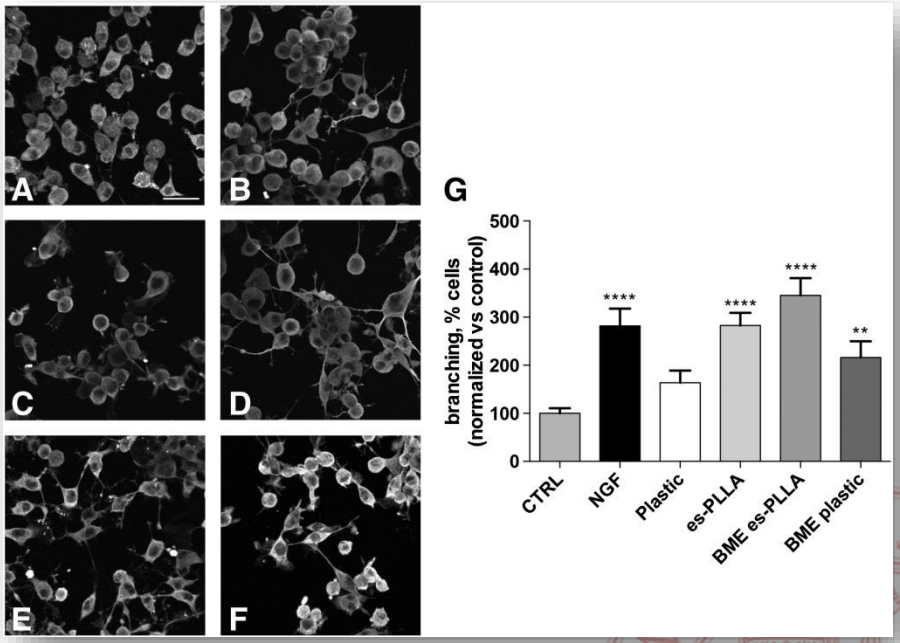

Oct4 expression in RESCs cultured on different surfaces.
A: Real-time PCR analysis of Oct4 mRNA expression in RESCs grown on 3D-BME, plastic, BME-coated plastic, es-PLLA scaffold, BME-coated es-PLLA scaffold at two different time points (3 and 15 DIV)

*Alessandri et al., Matrix Biol, 2014*

# Ex 1: RESC, differentiation

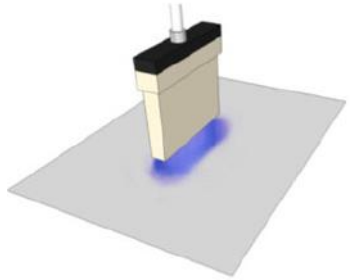


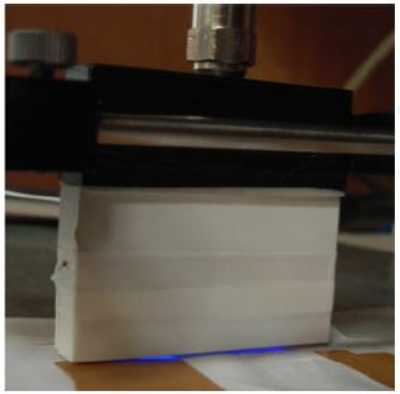



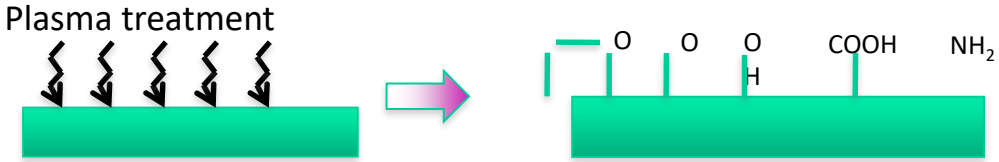

*Alessandri et al., Matrix Biol, 2014*

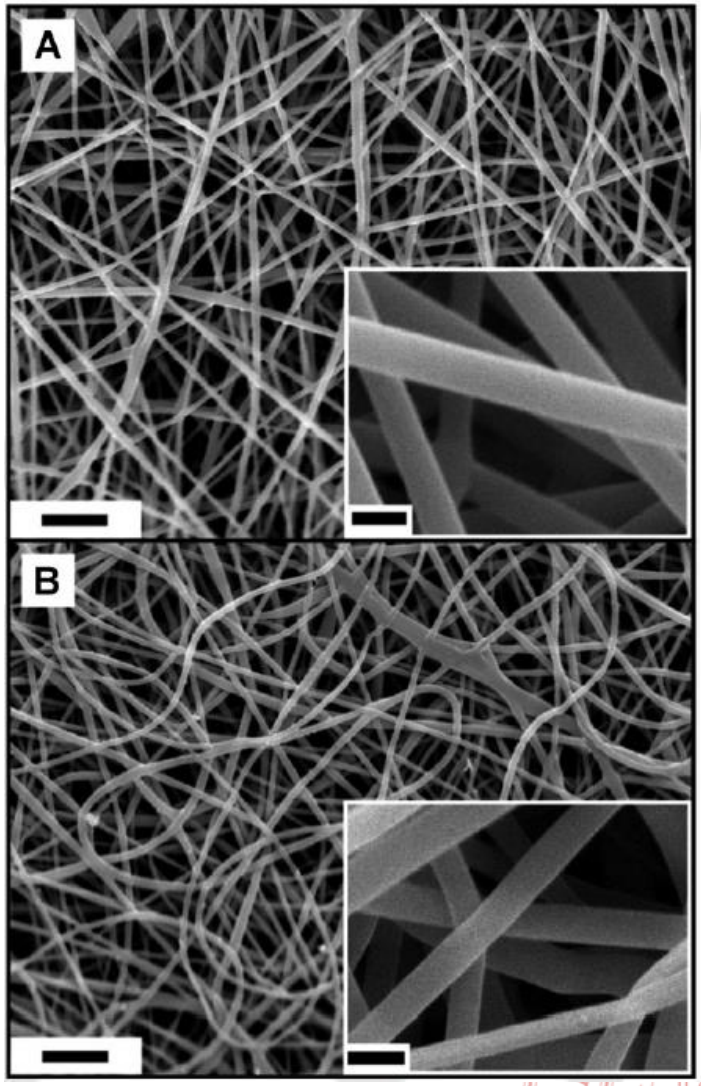
# Ex 2: Plasma treatment of polymers





*" the fourth state of matter": Heating a gas may ionize its molecules or atoms (reducing or increasing the number of electrons in them), thus turning it into a plasma, which contains charged particles: positive ions and negative electrons or ions*




*Dolci et al., Plasma Process. Polym, 2013*

# Ex 2: Plasma treatment of polymers

*Table 2.* Effect of ageing time at RT on WCA values of PLLA electrospun scaffolds and films.

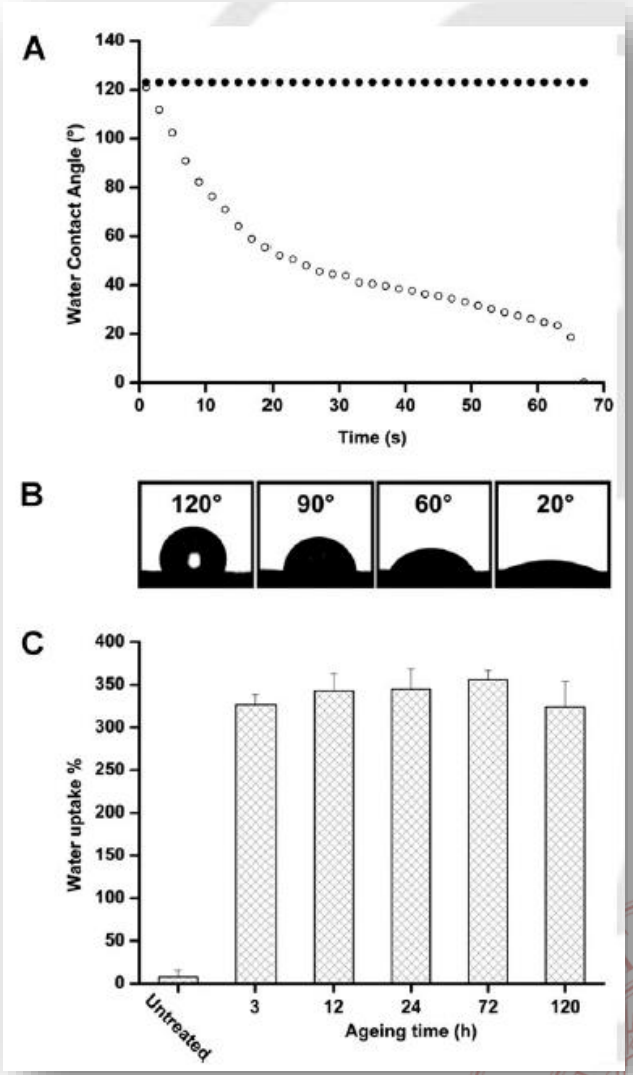
| Sample | Time after plasma treatment [h] | WCA [°] |
|---|---|---|
| ES-PLLA-untreated | – | 121.5 ± 1.7 |
| ES-PLLA-LC | 3 | a) |
| | 24 | a) |
| | 48 | a) |
| PLLA-film-untreated | – | 90.3 ± 5.8 |
| PLLA-film-LC | 3 | 46.4 ± 3.9 |
| | 24 | 48.6 ± 3.4 |
| | 48 | 45.8 ± 4.0 |

a) Instantaneous water penetration.

*Table 1.* Thermal and mechanical properties of ES-PLLA-untreated and ES-PLLA-LC scaffolds[Q4].

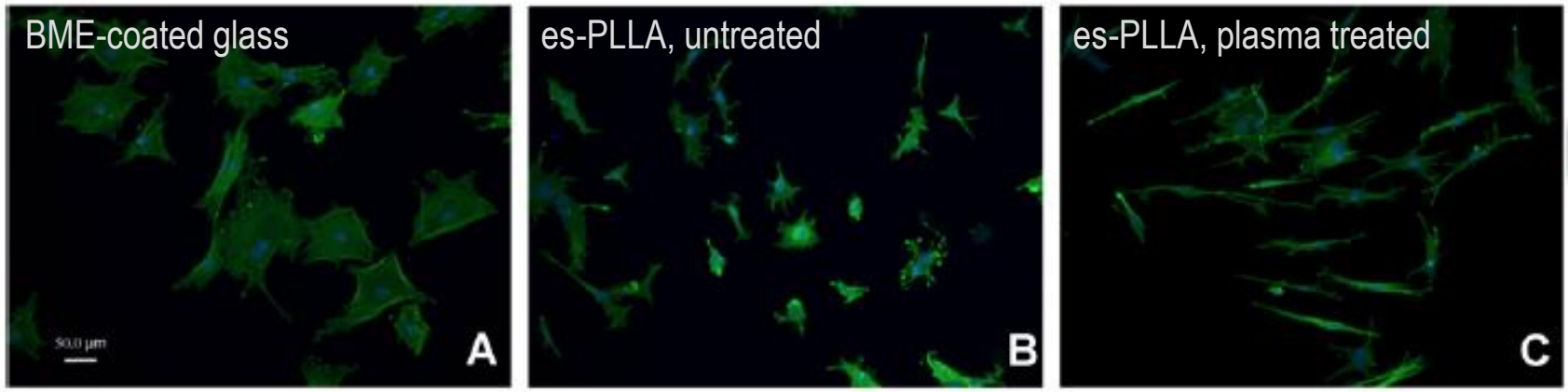
| Sample | $T_g$ [°C] | $\Delta C_p$ [J g$^{-1}$ °C$^{-1}$] | $T_c$ [°C] | $\Delta H_c$ [J g$^{-1}$] | $T_m$ [°C] | $\Delta H_m$ [J g$^{-1}$] | $\sigma_b$ a) [MPa] | $\varepsilon_b$ b) [%] | $E$ c) [MPa] |
|---|---|---|---|---|---|---|---|---|---|
| ES-PLLA-untreated | 65 | 0.73 | 126 | 40 | 163 | 41 | 3.4 ± 0.5 | 56 ± 5 | 86 ± 13 |
| ES-PLLA-LC | 64 | 0.75 | 126 | 39 | 162 | 40 | 2.3 ± 0.3 | 71 ± 9 | 64 ± 8 |

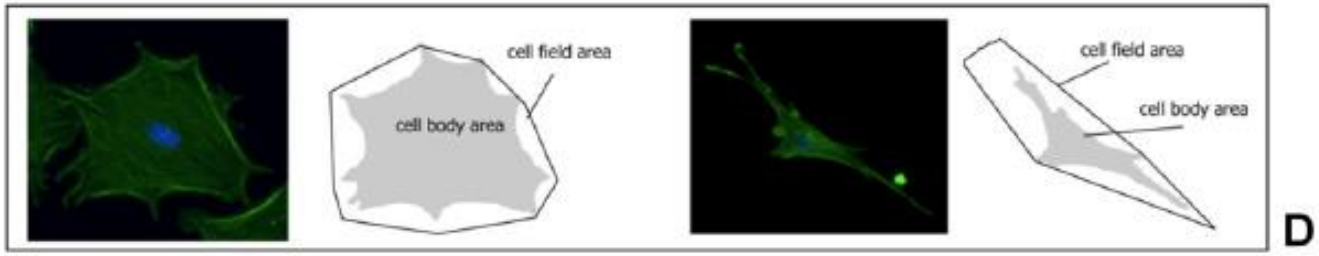
a) Elongation at break; b) Tensile modulus; c) Tensile strength.

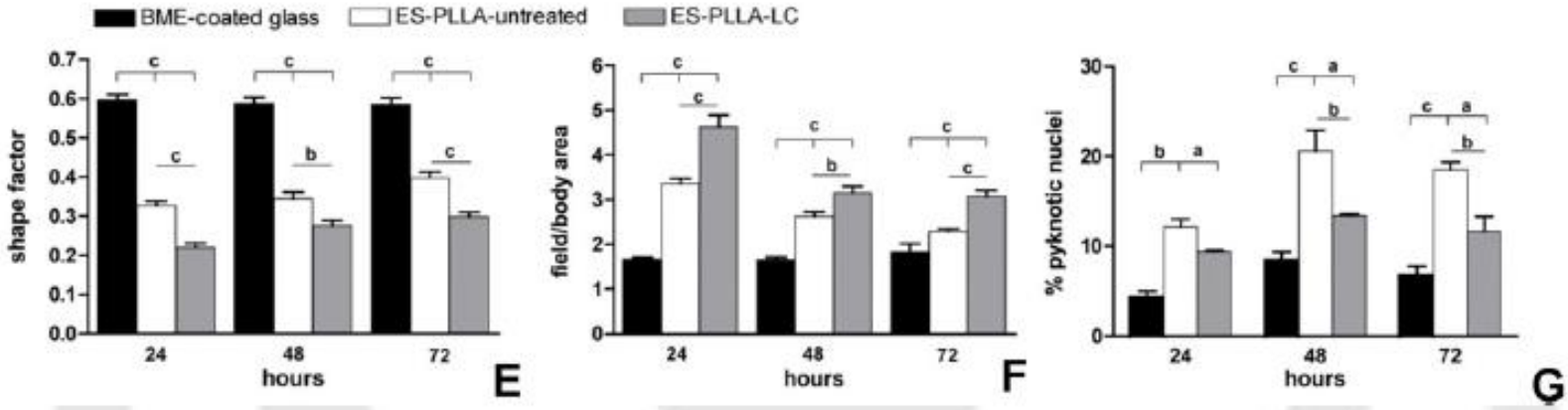



*Dolci et al., Plasma Process. Polym, 2013*

# Ex 2: Plasma treatment of polymers

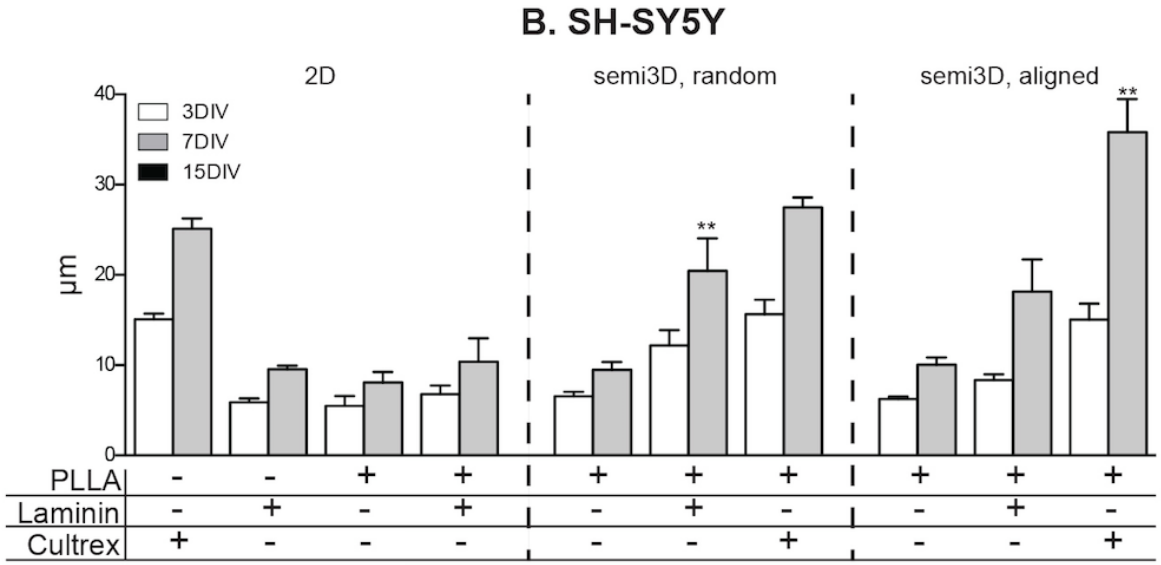


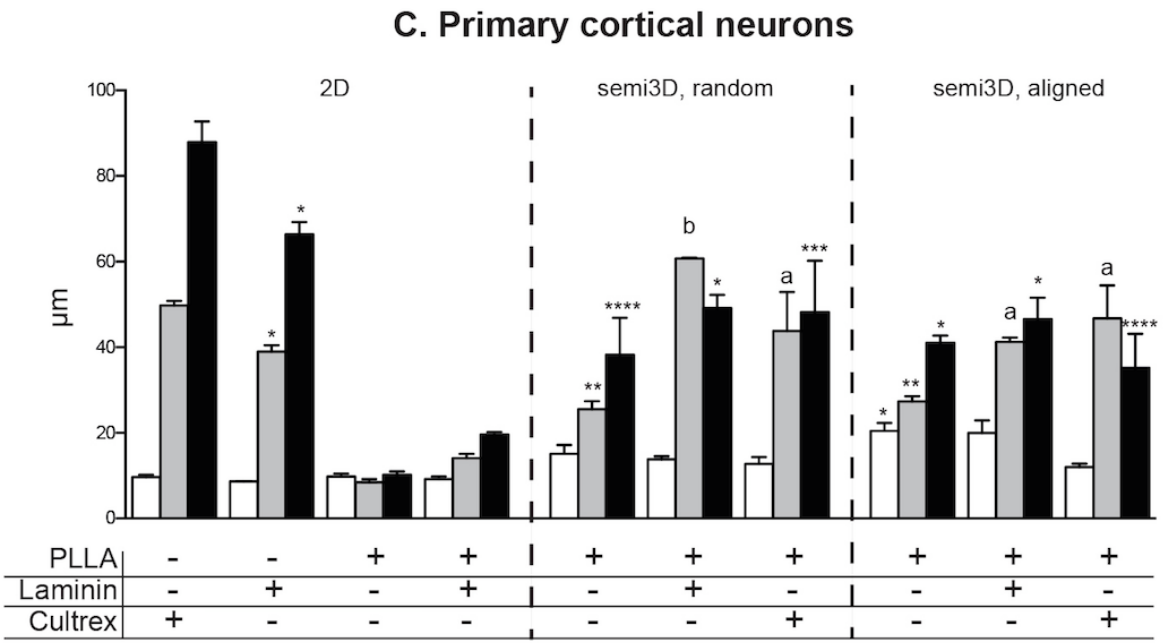




*Dolci et al., Plasma Process. Polym, 2013*

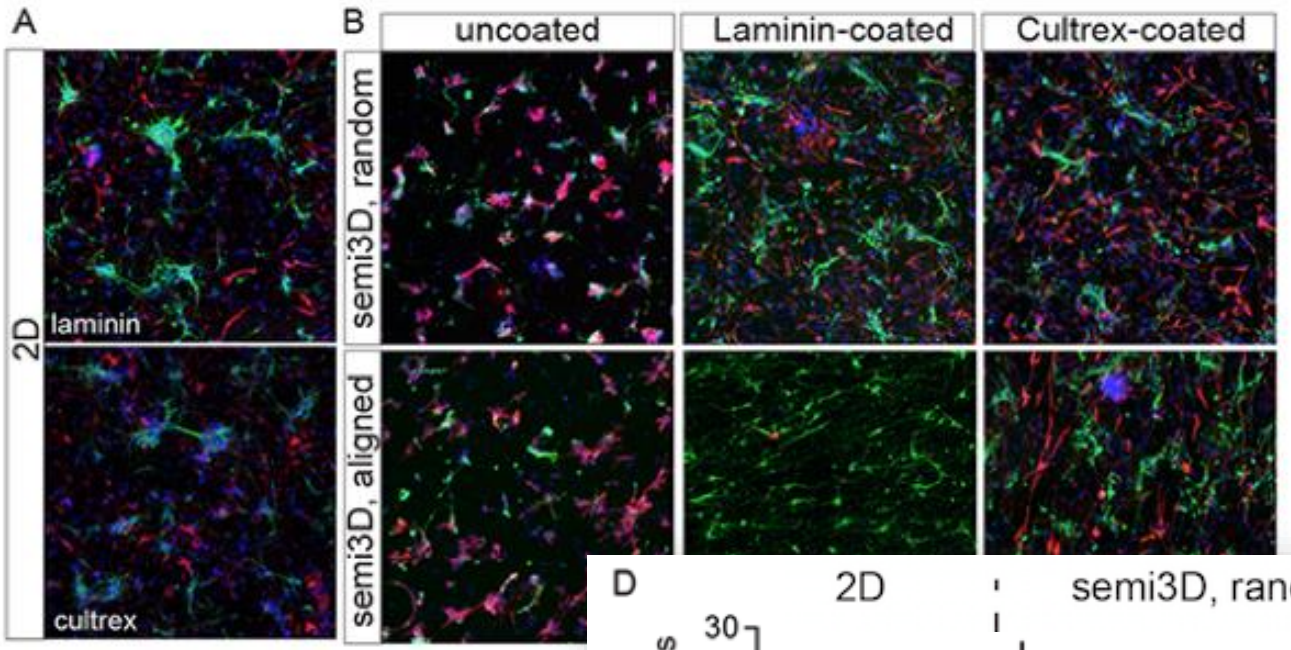
# Ex 3: Efficacy, neurite elongation

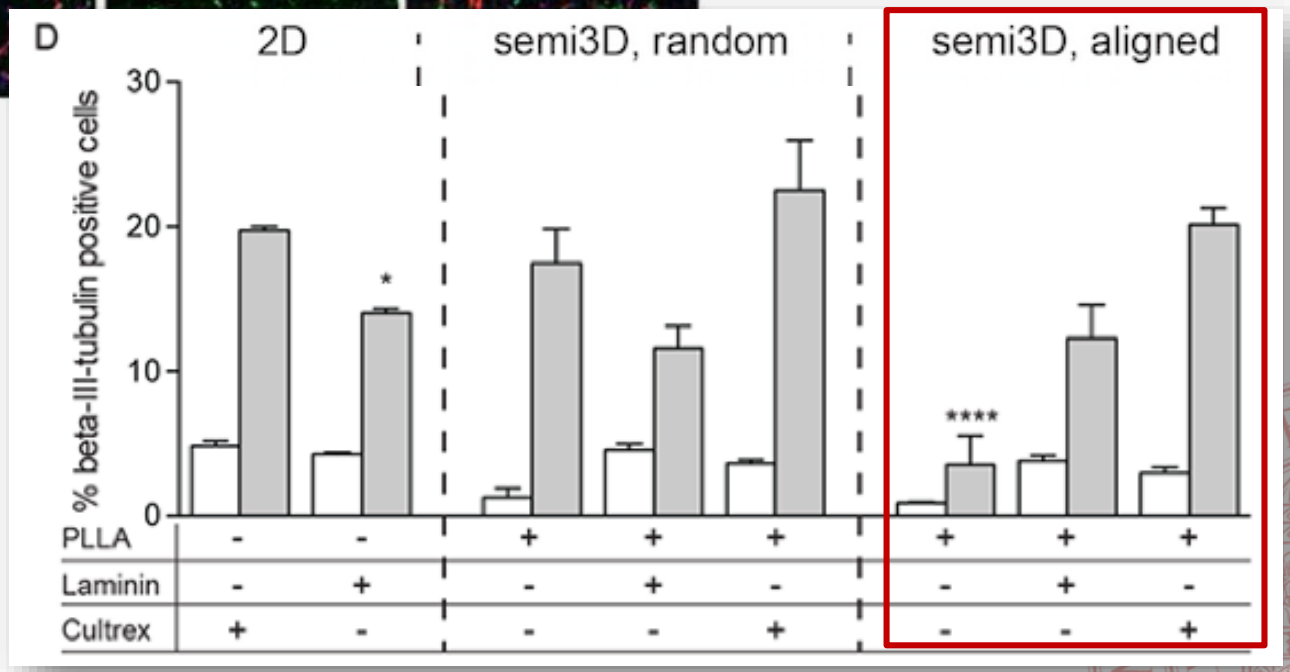



Baldassarro et al., Biores Open Access. 2016

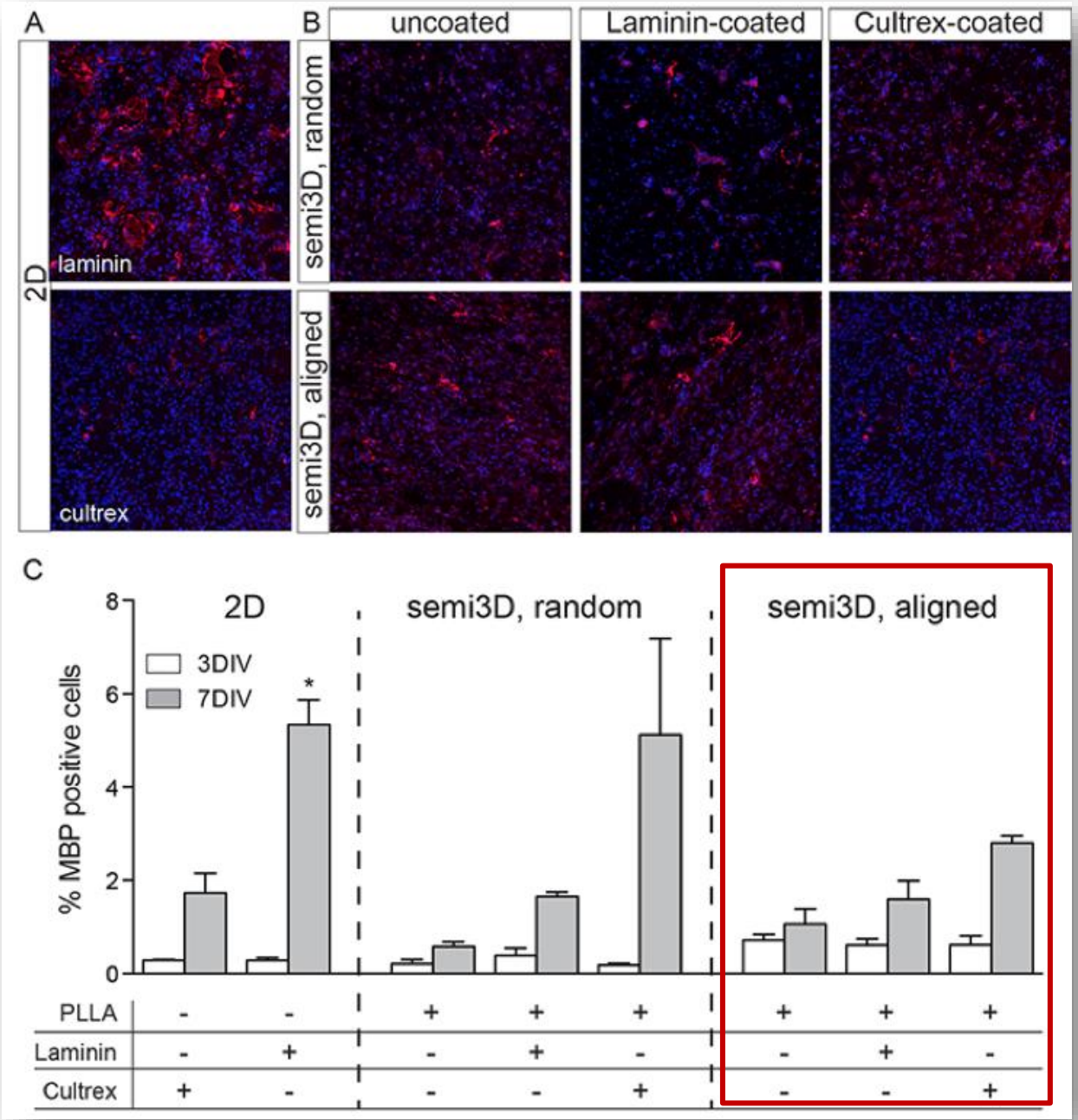
# Ex 3: Efficacy, lineage



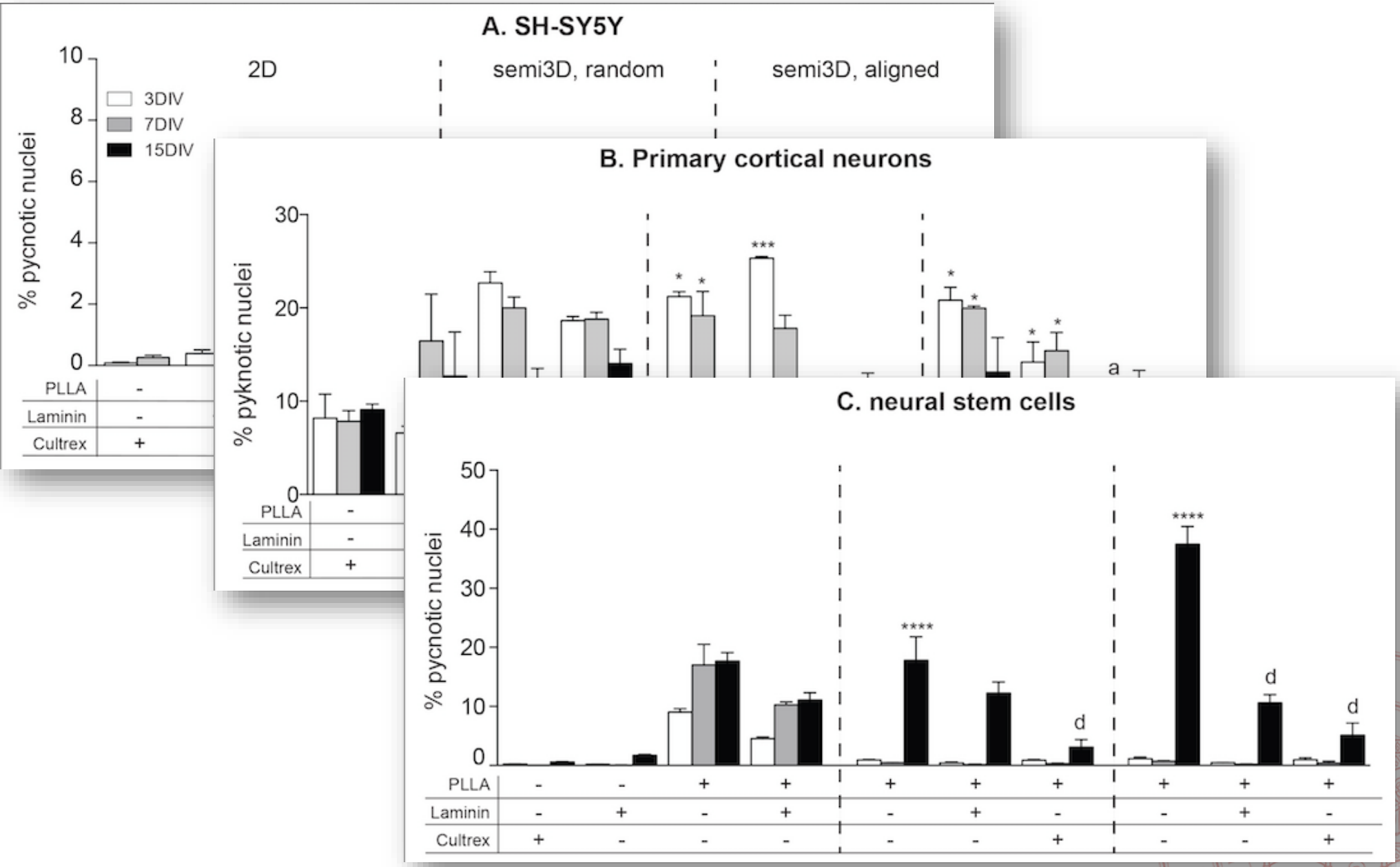

*Baldassarro et al., Biores Open Access. 2016*

# Ex 3: Efficacy, lineage




Baldassarro et al., Biores Open Access. 2016

# Ex 3: Efficacy, safety/toxicology




*Baldassarro et al., Biores Open Access. 2016*

*Chemical selection*
*Material design*
*Biomaterial functionalization*
*Cell selection*
*Test readout*
*Test robustness*

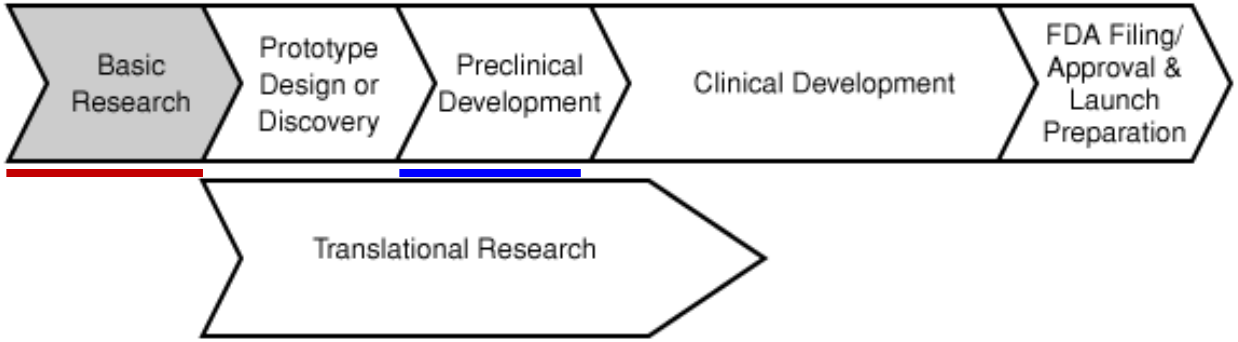
TISSUE ENGINEERING
Volume 11, Number 9/10, 2005


Editorial

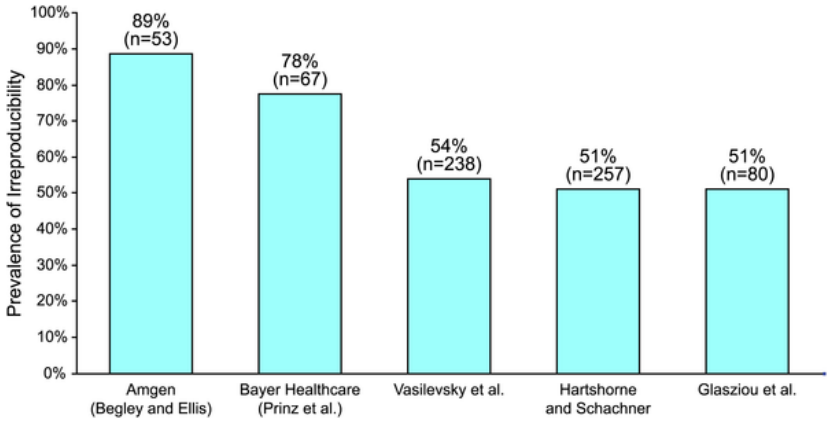
Standardized Experimental Procedures in Tissue Engineering: Cure or Curse?

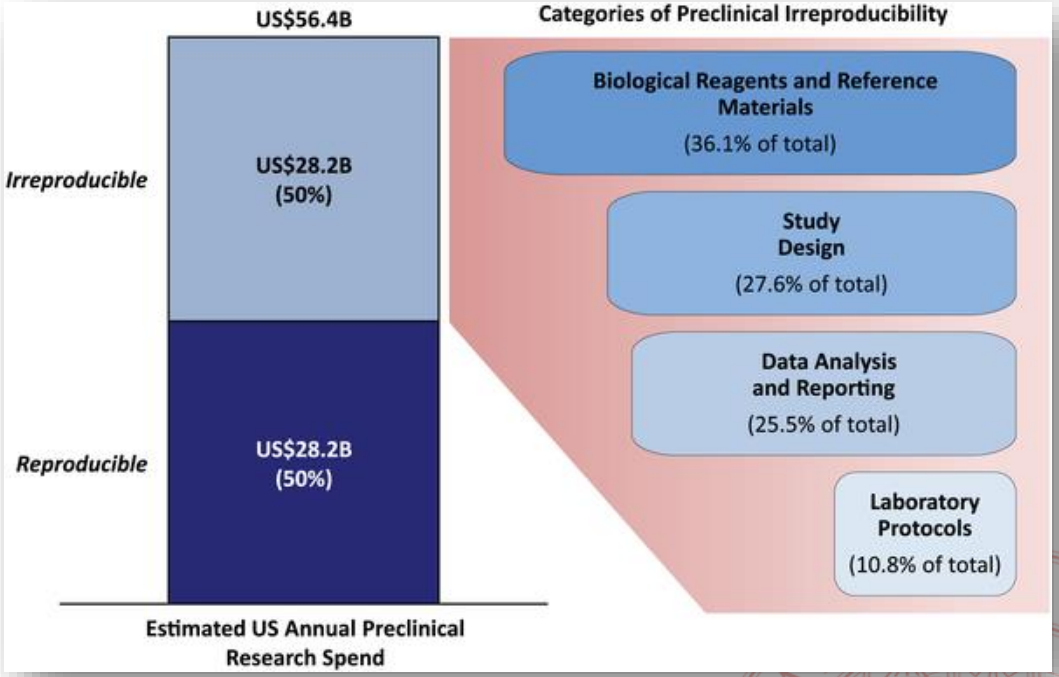
ward. What has not occurred, however, is the adoption of these, or indeed any, standards by academic researchers in the field. As a result, it remains very difficult to compare published data, since each laboratory uses its own experimental approach. I do not suggest that creative new experimental systems are not valuable, but simply that without all laboratories using a set of minimum standards as part of their comprehensive strategy the field will suffer.

**Use of International Standard ISO-10993, "Biological Evaluation of Medical Devices Part 1: Evaluation and Testing"**




## Studies reporting the prevalence of irreproducibility




*Freedman et al., PlosBiol, 2015*

***Ready to work together for research programs, collaboration with companies, third party research***

Topics:

- ✓ scaffold fabrication (natural and synthetic polymers) and bio-conjugation
- ✓ shape memory polymers and functional polymeric materials
- ✓ *in vitro* testing using neural cell lines, primary neurons and glial cells, neural stem cells, embryonic stem cells
- ✓ - *in vivo* testing in rodent models for multiple sclerosis, neonatal hypoxia-ischemia, traumatic spinal cord injury, Alzheimer's disease
- ✓ - GLP and ISO service for *in vitro* and *in vivo* safety

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RegioneEmilia-Romagna


# for your attention!

*CONTACTS*:

Prof. Laura Calzà
CIRI-SDV and Fabit, University of Bologna
IRET Foundation
phone: +39 051 798776
mail: laura.calza@unibo.it

Prof. Maria Letizia Focarete
University of Bologna - Department of Chemistry "G. Ciamician“
Via Selmi 2 - 40126 Bologna - Italy
Ph: +39-051-2099572
Email: marialetizia.focarete@unibo.it