Recombinant Materials: Materials for the post-oil age

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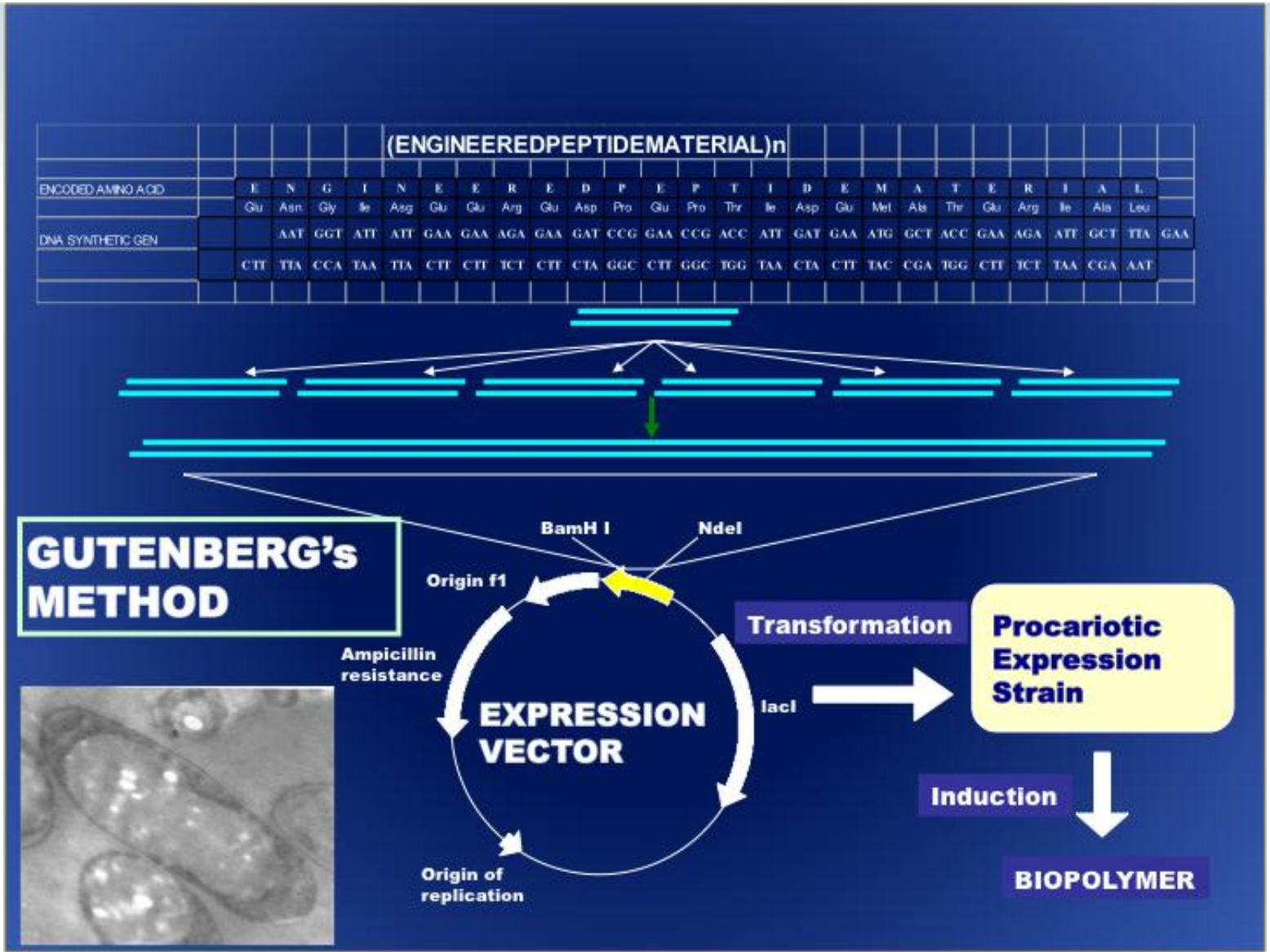
Recombinamers: Recombinant Protein Polymers



Recombinamers

Some Advantages of Recombinant Protein Polymers:

- They are able to incorporate any simple or complex function present in natural proteins
- They are able to show any other amino-acid-based functionallity of scientifical or technological interest that evolution has not been selected in natural proteins
- Absolute control in the polymer composition. That includes total absence of polydispersity and randomness.
- Huge number of possible compositions.
- They can be really complex in sequence, while an increase in complexity does not imply an increase in production costs.
- They are environmentally clean from production to waste
- They are produced exclusively from biomass (do not require oil-derived materials)
- ...

FUNCTIONALITY

Recombinant Technology:

- Direct Functionality given by (Bio)active peptide domains
- Functionality given by Nanometric design and control of Macromolecular composition

Holistic functionality by designing precise molecular architectures

Selected Examples:

- Injectable Hydrogels for Regenerative Medicine
- Smart Surfaces for Cell/Cell-sheet Harvesting
- Hierarchical morphogenesis

Regenerative Medicine: Material Designs

$(VPGIG)_2$ (VPGKG) $(VPGIG)_2$–(BIOACTIVE DOMAIN) $(VPGIG)_2$ (VPGKG) $(VPGIG)_2$ $(VGVAPG)_3$

(VPGIG): Elastin pentapeptide.
(VPGKG): Elastin like pentapeptide modified to include the amino-acid K for cross-linking purposes.

⇒

- **Mechanical Properties**
- **Extreme Biocompatibility**
- **Smart and Self-assembling behaviour**

(AVTGRGDSPASS): RGD loop of human fibronectin.
(QAASIKVAV): Neurite outgrowth peptide
(GTPGPQGIAGQRGVV): Osteoblast cell attachment
(EEIQIGHIPREDVDYHLYP): Endotelial cell attachment
(DPGYIGSR): Laminin-derived non integrin-dependent cell attachment
(DDDEEKFLRRIGRFG): Hydroxyapatite binding domain

⇒

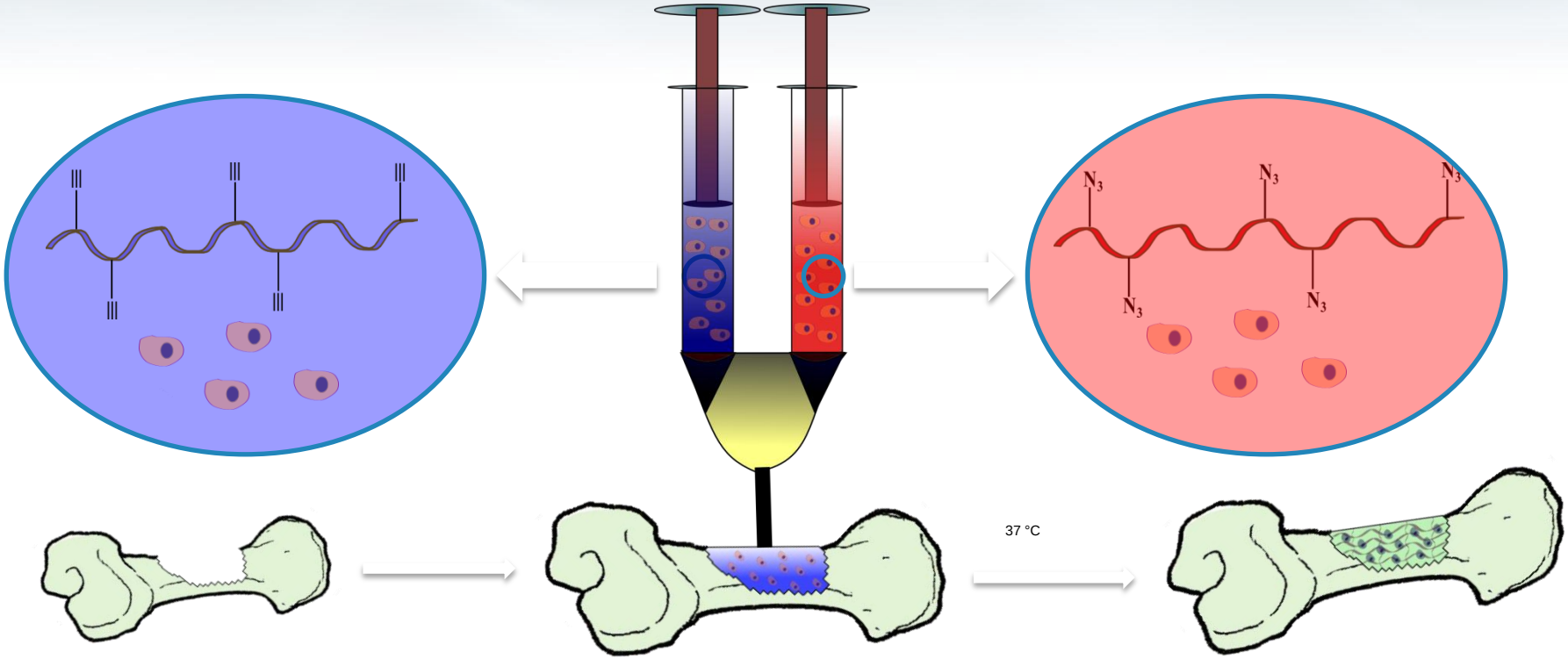
- **Specific and Efficient Cell Attachment functionality**

(VGVAPG): Human elastin bioactive hexapeptide. This is target of ECM proteases and, as released, promotes cell movement, proliferation and angiogenesis.

⇒

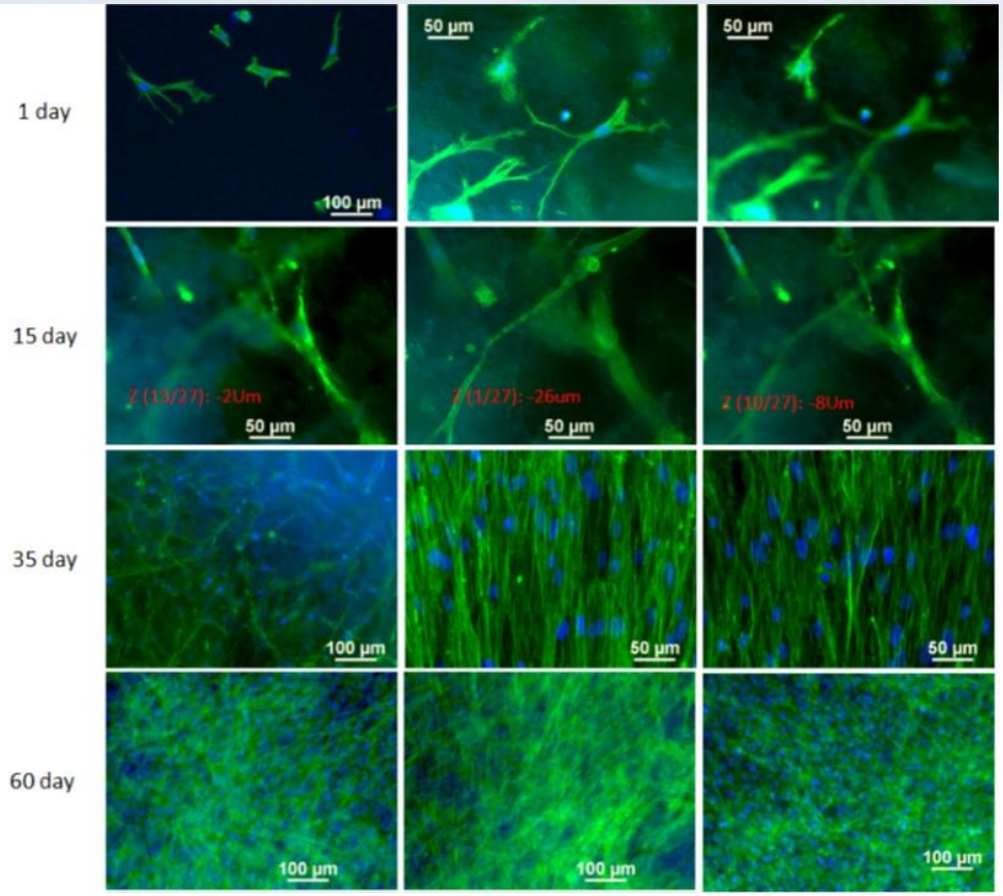
- **Adequate bioresobability and positive "Side Effects"**

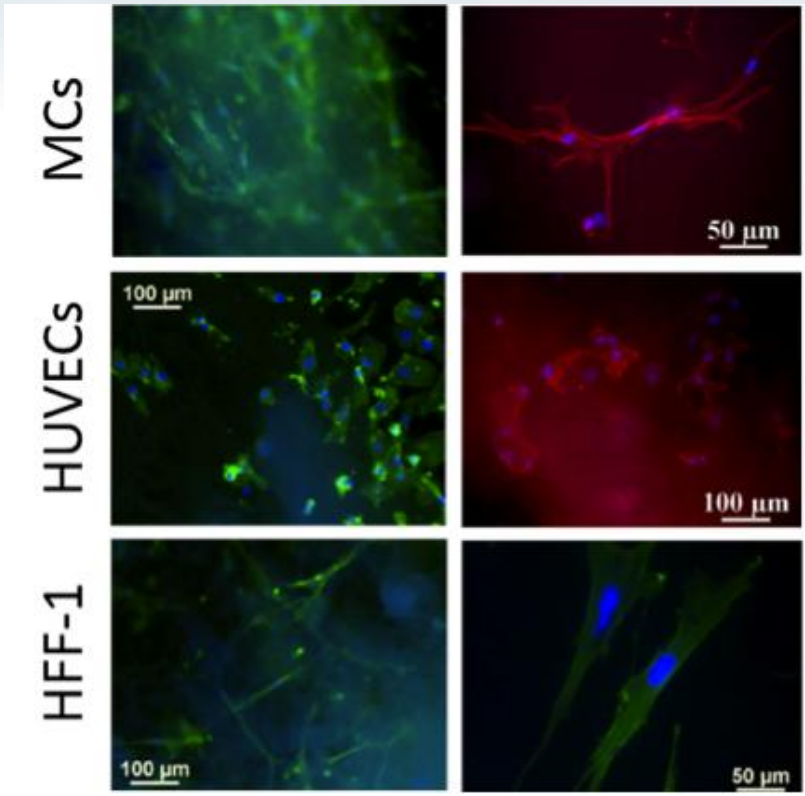
Cell Therapy: Injectable systems



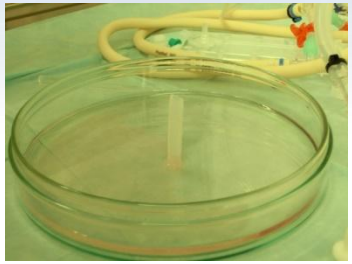
Cell Therapy: Injectable systems

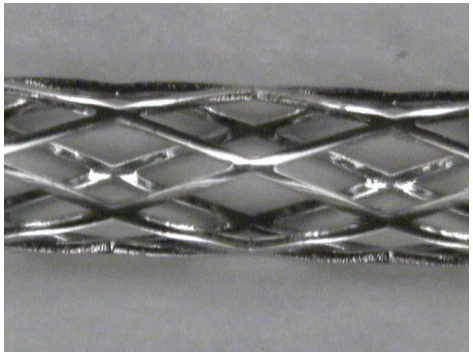
HFF-1

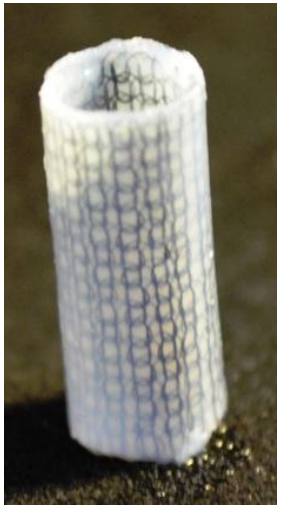




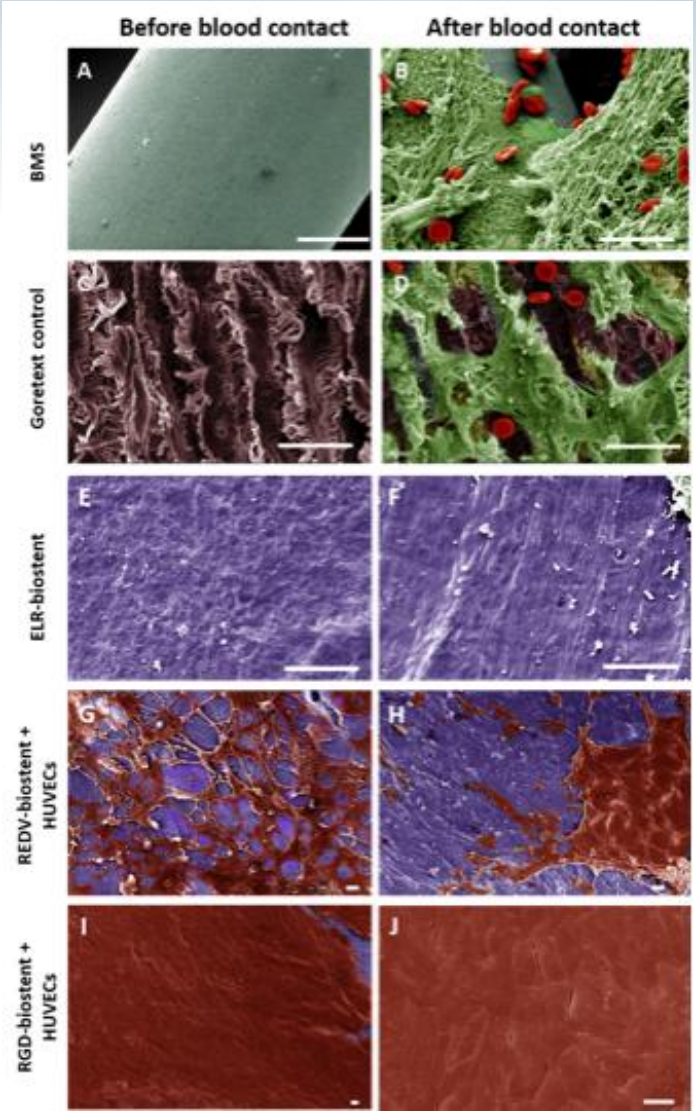
Bioactive Stents



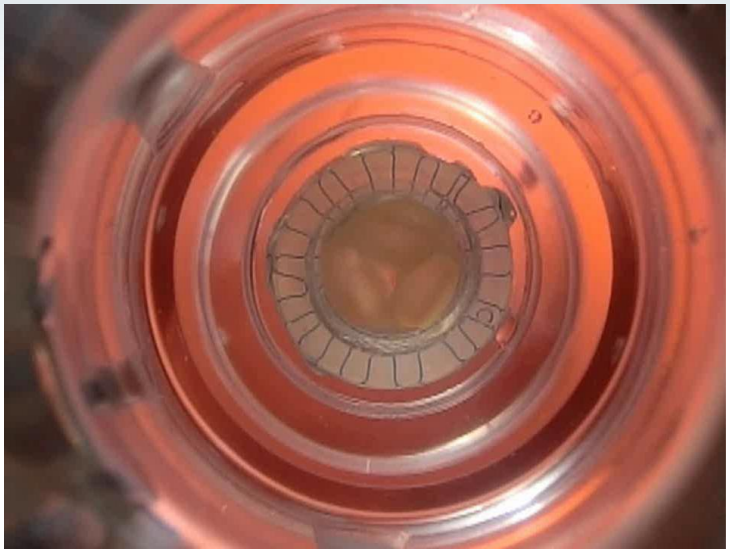


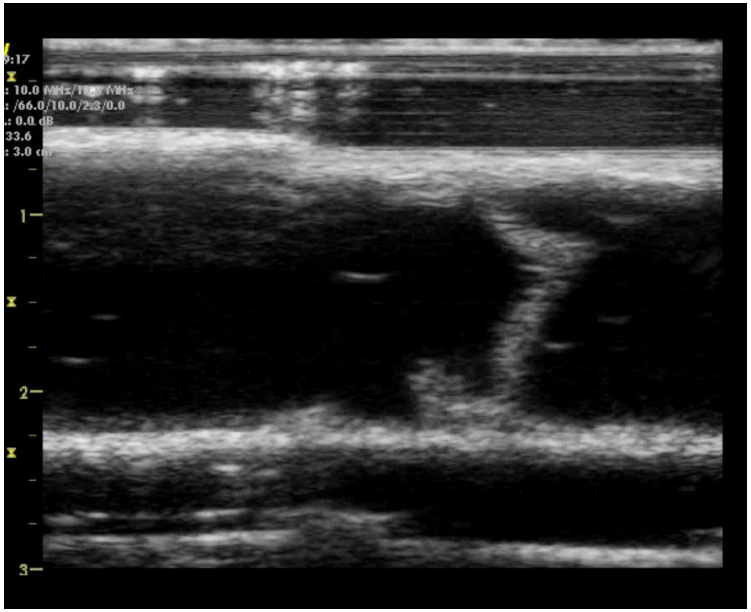


Bioactive Stents



Cell Therapy: Injectable systems





Amphiphilic multiblocks

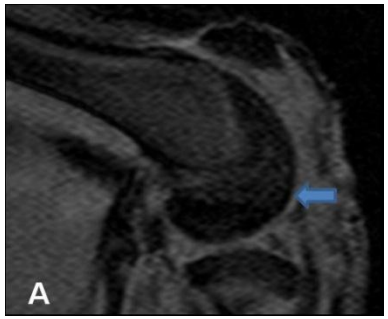
E50	I60	E50	I60

E50 **Hydrophilic block**

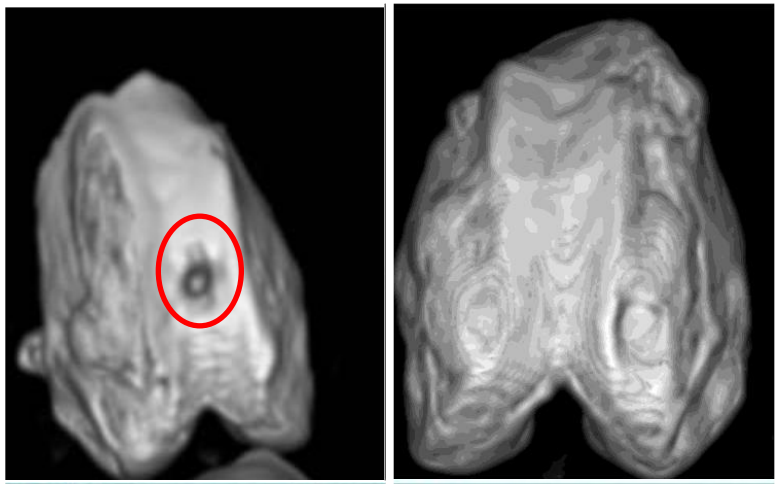
I60 **T-Driven Hydrophobic block**

Left Knees **Right Knees**

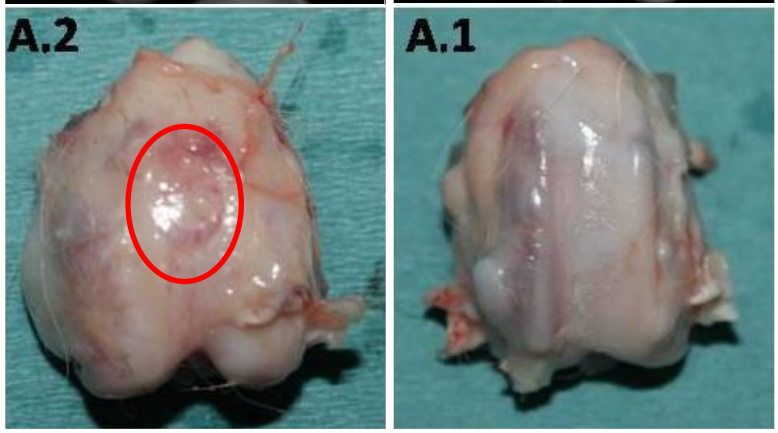
Hydrogel **Hydrogel with MSC**



Nuclear Magnetic Resonance (NMR) -3D SPGR-

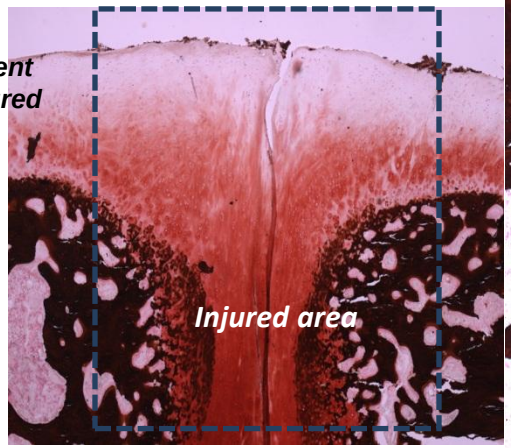


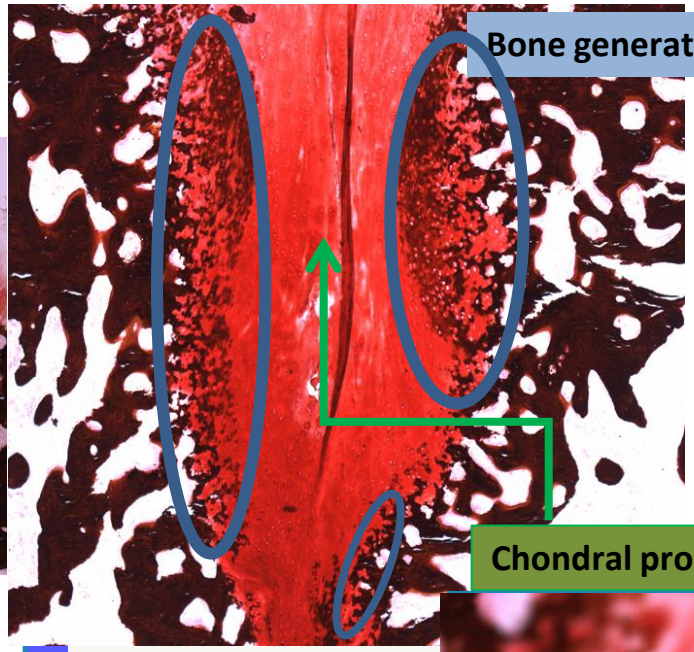
3D Computed Tomography (CT)



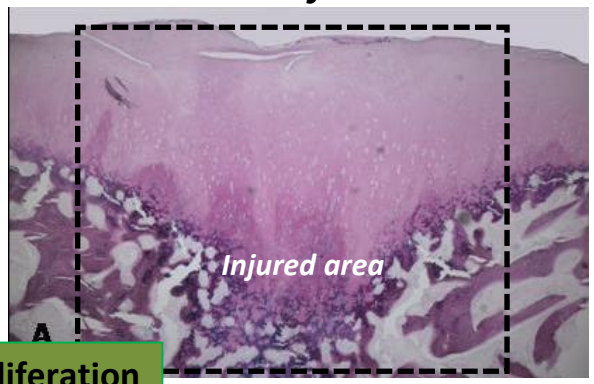
Macroscopic Observation

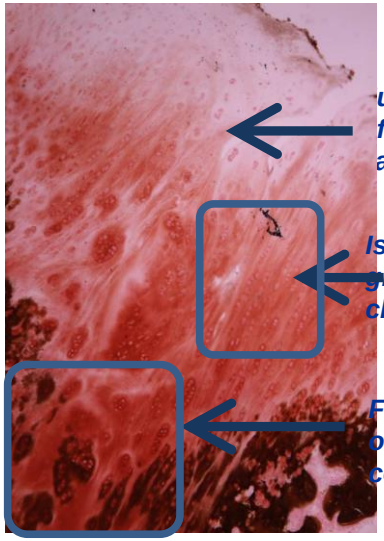
Von Kossa stain

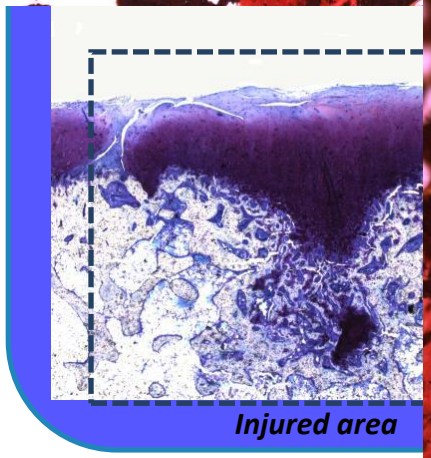


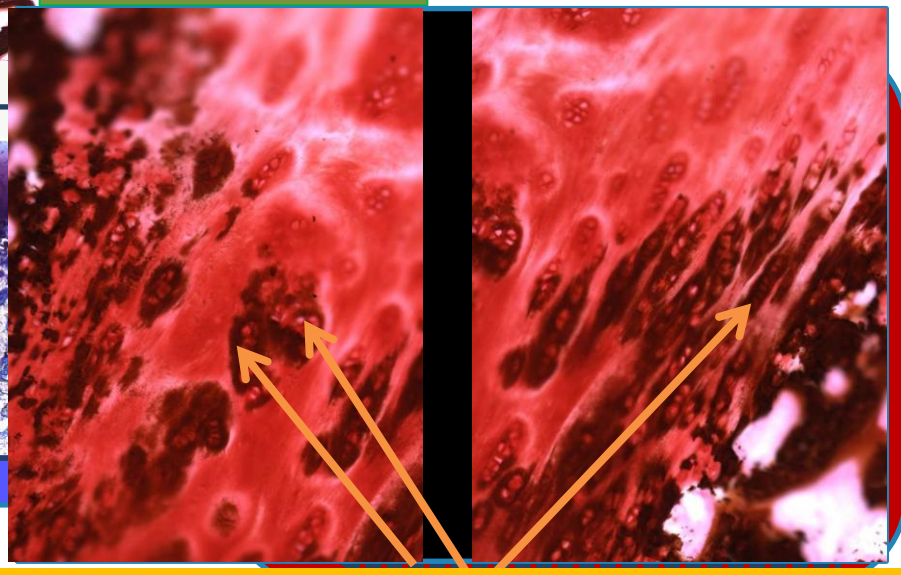


Alcian Blue-PAS-hematoxylin stain



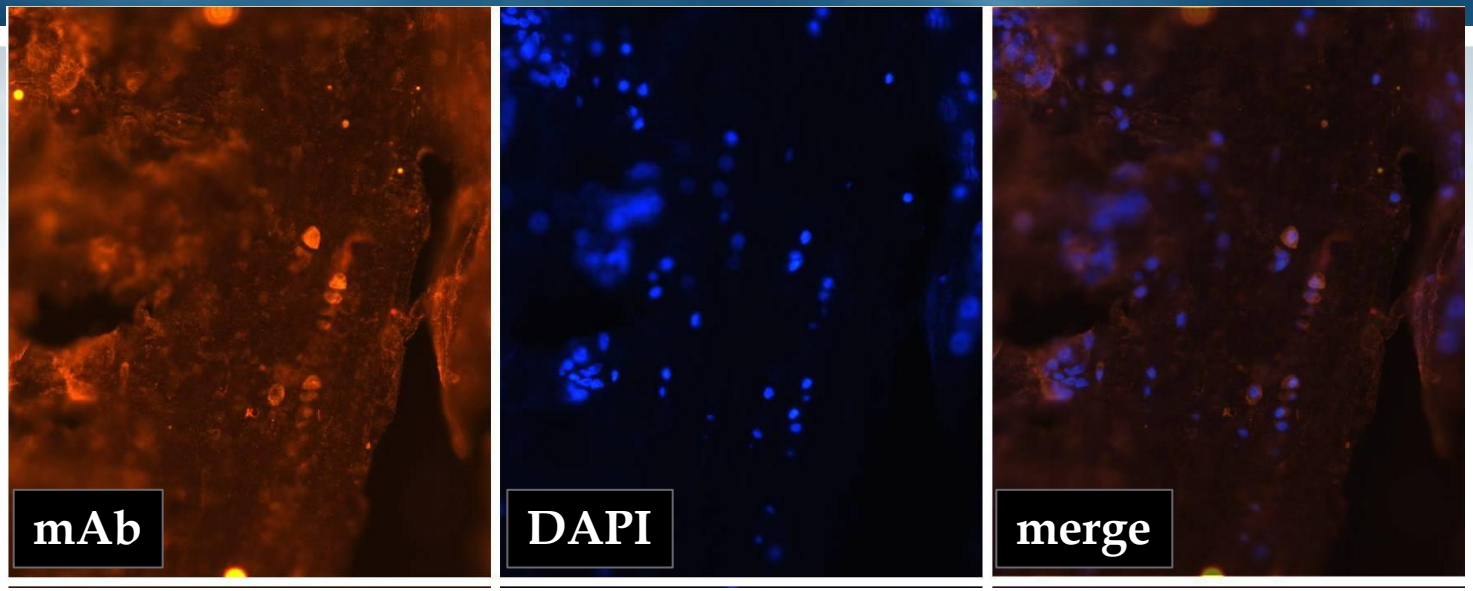






Endochondral ossification and Isogeneous groups of chondrocytes in the injured zone

Quantitative Analysis



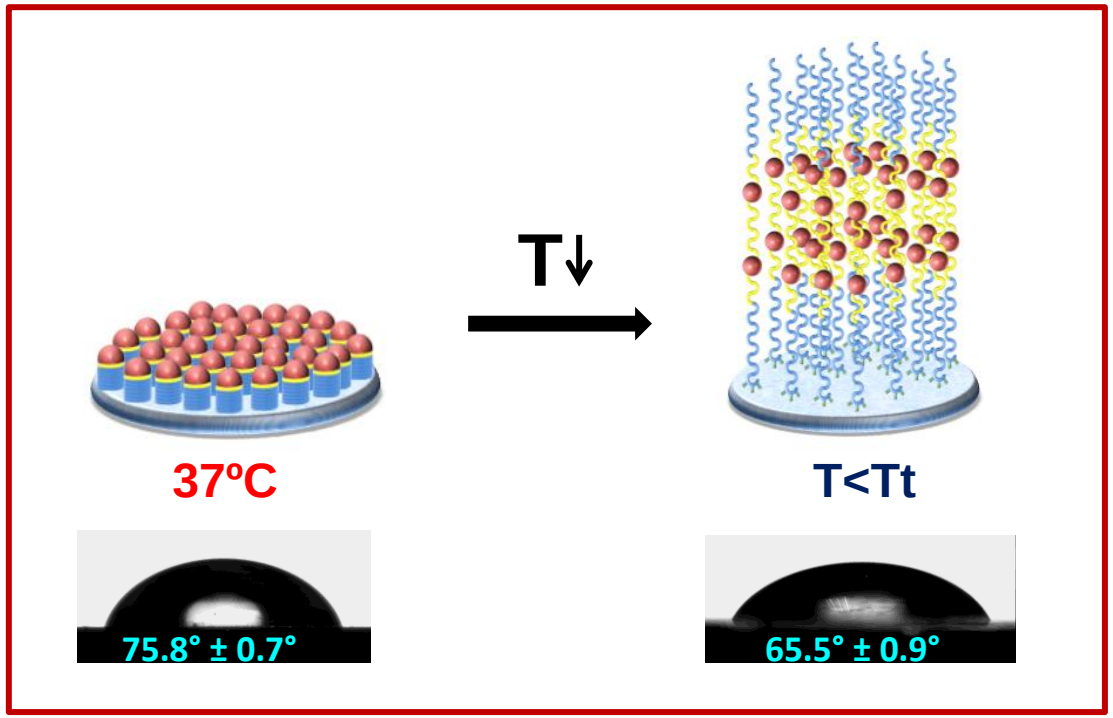
Immunohistochemistry with mAb against a human mitochondria marker and DAPI stain.

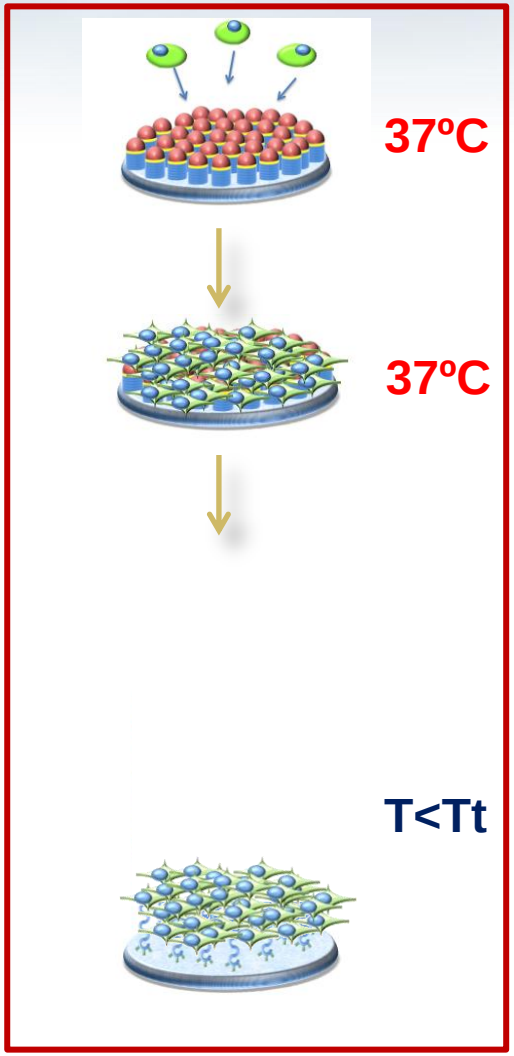
Histological scoring of cartilage repair according to modified Wakitani score.

	Hydrogel (left knees)						Hydrogel with MSC (right knees)					
↓ CATEGORY # RABBIT →	*1*	*2*	*3*	*4*	*5*	*6*	*1*	*2*	*3*	*4*	*5*	*6*
CELL MORPHOLOGY (0-4) *	2	2	1	1	2	2	4	4	4	4	4	4
MATRIX-STAINING (METACHROMASIA) (0-3) *	1	2	1	1	2	2	3	3	2	3	3	2
SURFACE REGULARITY (0-3) *	2	2	2	1	2	2	3	3	2	2	3	2
THICKNESS OF CARTILAGE (0-3) *	1	1	1	0	1	1	3	3	3	3	3	2
INTEGRATION WITH HOST ADJACENT CARTILAGE (0-2)	2	2	1	0	1	1	2	2	1	2	2	1
TOTAL (0-15)	**8**	**9**	**6**	**3**	**8**	**8**	**15**	**15**	**12**	**13**	**15**	**11**
AVERAGE (0-15)	5.6						13.5					

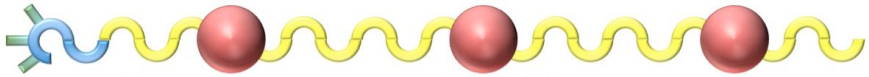
* Significant differences in the medians according to Kruskal-Wallis analysis.

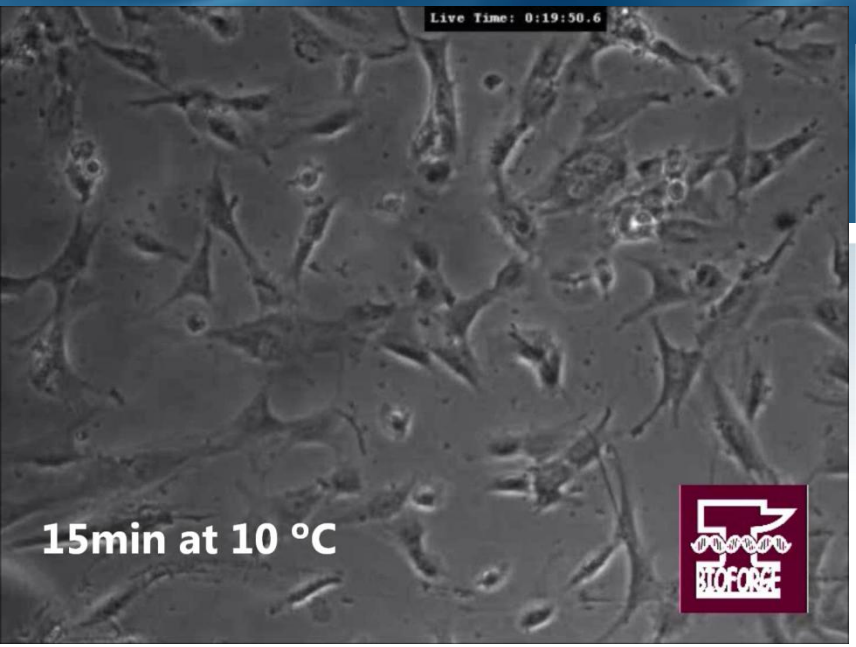
Thermosensitive ELR Surfaces for Cell Harvesting

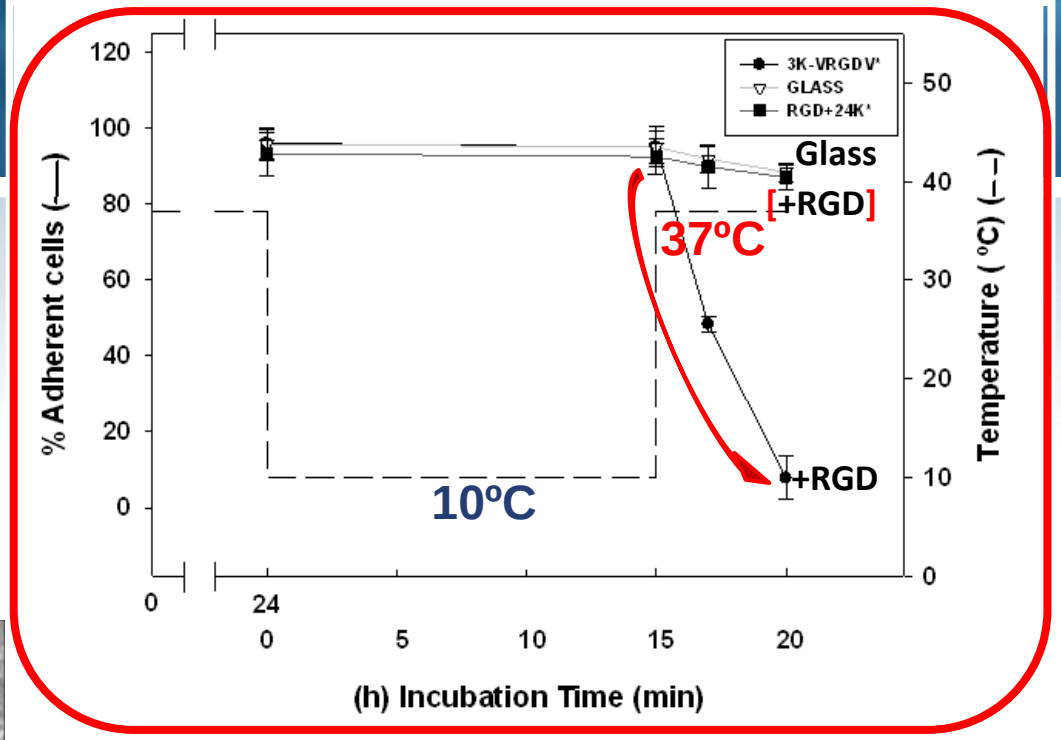


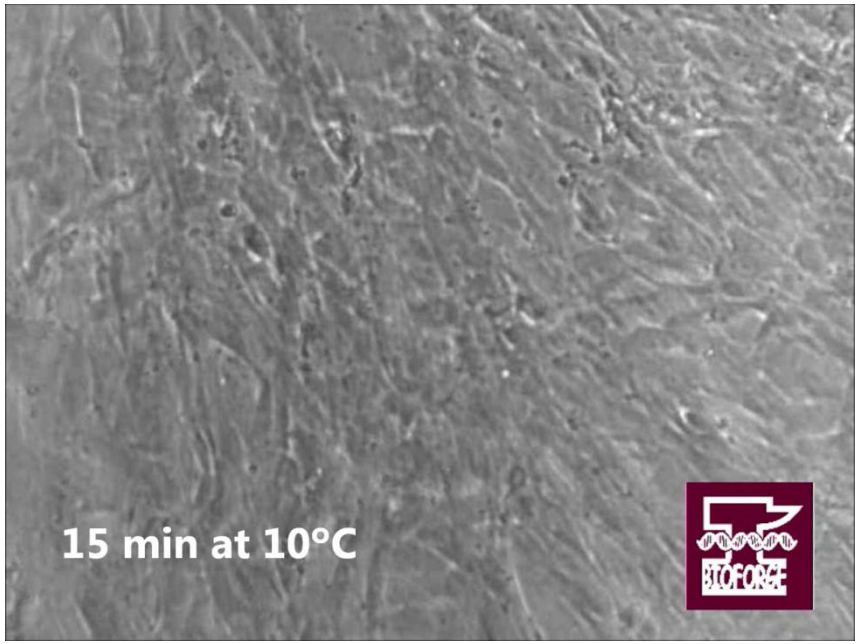


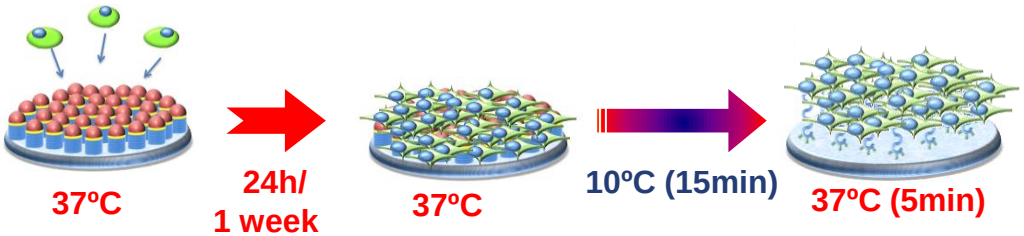
Molecular Designs

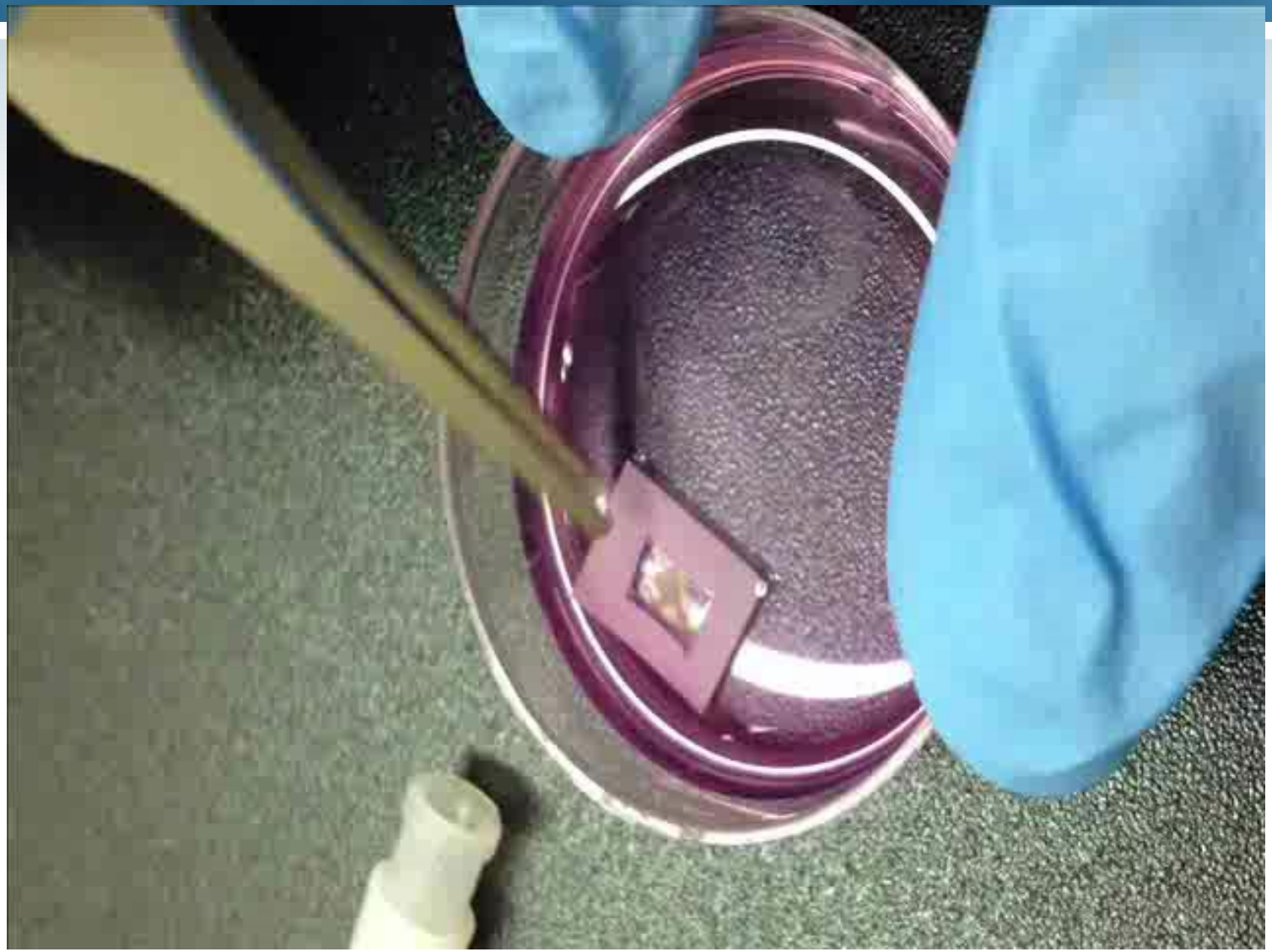
RECOMBINAMER	AMINO-ACID SEQUENCE + SCHEMATIC CARTOON OF THE MOLECULE	T_t/ºC
3K-RGD (1)	 MG**KKK**P-$(VPGVG)_{14}[(VPGIG)_{10}$AVTG**RGD**SPASS$(VPGIG)_{10}]_2(VPGVG)_{14}$V	24.2
3K-RDG (2)	 MG**KKK**P-$(VPGVG)_{14}[(VPGIG)_{10}$AVTG**RDG**SPASS$(VPGIG)_{10}]_2(VPGVG)_{14}$V	23.5
3K-V84 (3)	 MG**KKK**P-$(VPGVG)_{84}$V	29.4
N-RGD (4)	 MESLLP-$(VPGVG)_{14}[(VPGIG)_{10}$AVTG**RGD**SPASS$(VPGIG)_{10}]_2$ $(VPGVG)_{14}$V	23.9
RGD+24K (5)	 MESLLP$[(VPGIG)_2(VPG$**K**$G)(VPGIG)_2$AVTG**RGD**SPASS$(VPGIG)_2(VPG$**K**$G)(VPGIG)_2]_6$V	32.2
3K-RGD3 (6)	 MG**KKK**P-$[(VPGIG)_{10}$AVTG**RGD**SPASS$(VPGIG)_{10}]_3$V	23.0


Live Time: 0:19:50.6
15min at 10 °C
BIOFORGE

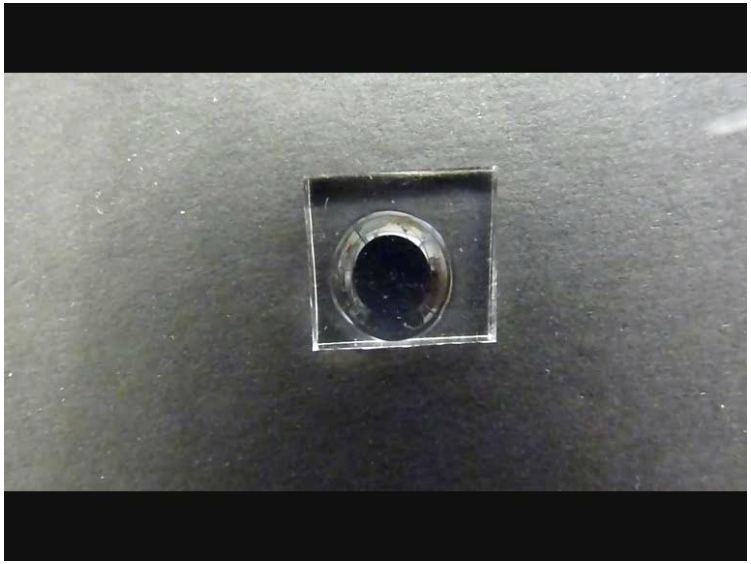

3K-VRGDV'
GLASS
RGD+24K'
Glass
[+RGD]
37ºC
+RGD
10ºC
% Adherent cells (—)
Temperature (ºC) (– –)
(h) Incubation Time (min)
120
100
80
60
40
20
0
50
40
30
20
10
0
24
0
5
10
15
20

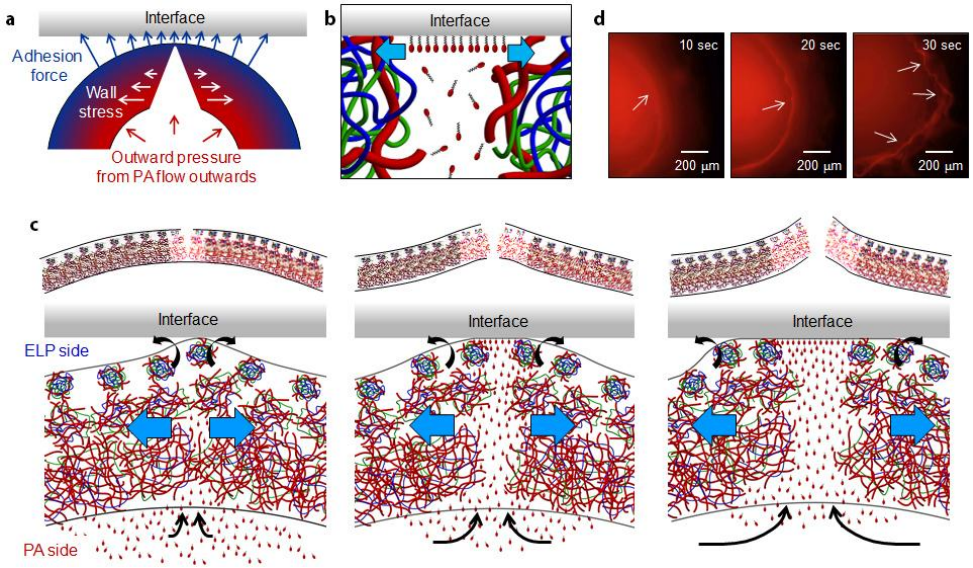

15 min at 10ºC
BIOFORGE


37ºC
24h/
1 week
37ºC
10ºC (15min)
37ºC (5min)

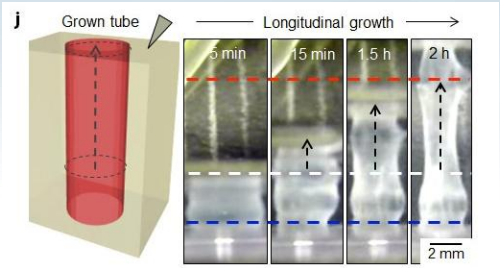


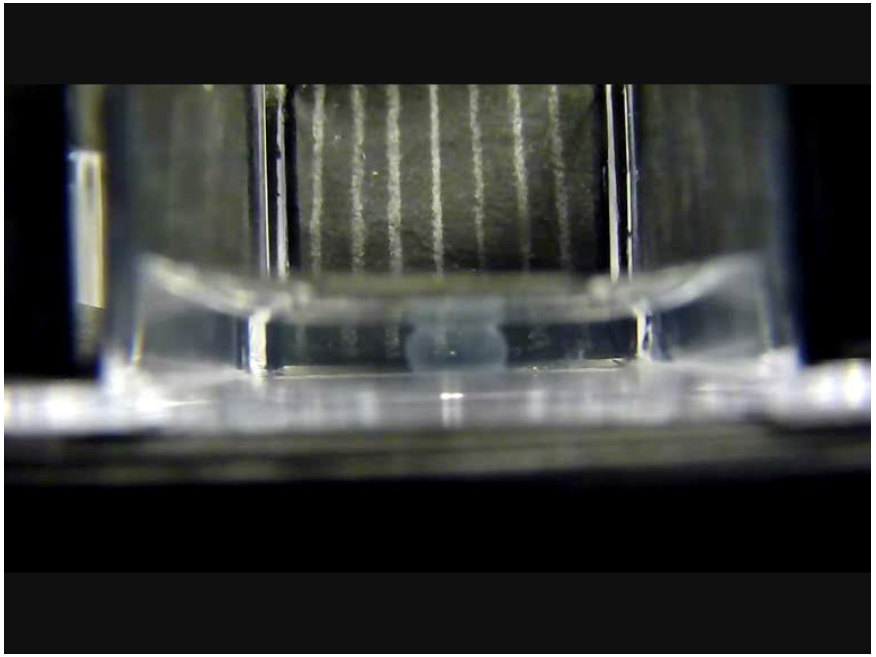
Hierarchical Morphogenesis of a hybrid peptide amphiphile/ELR system

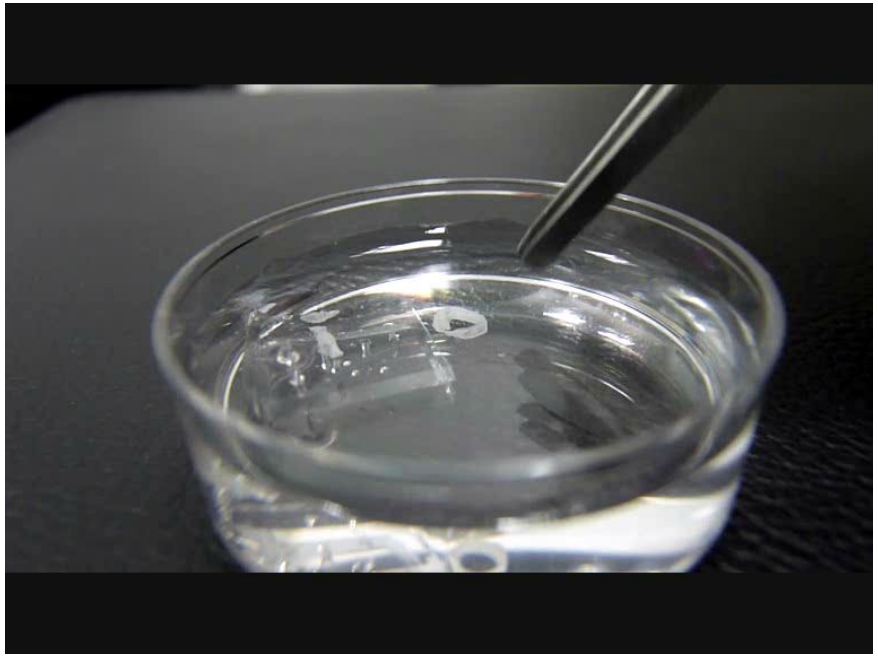


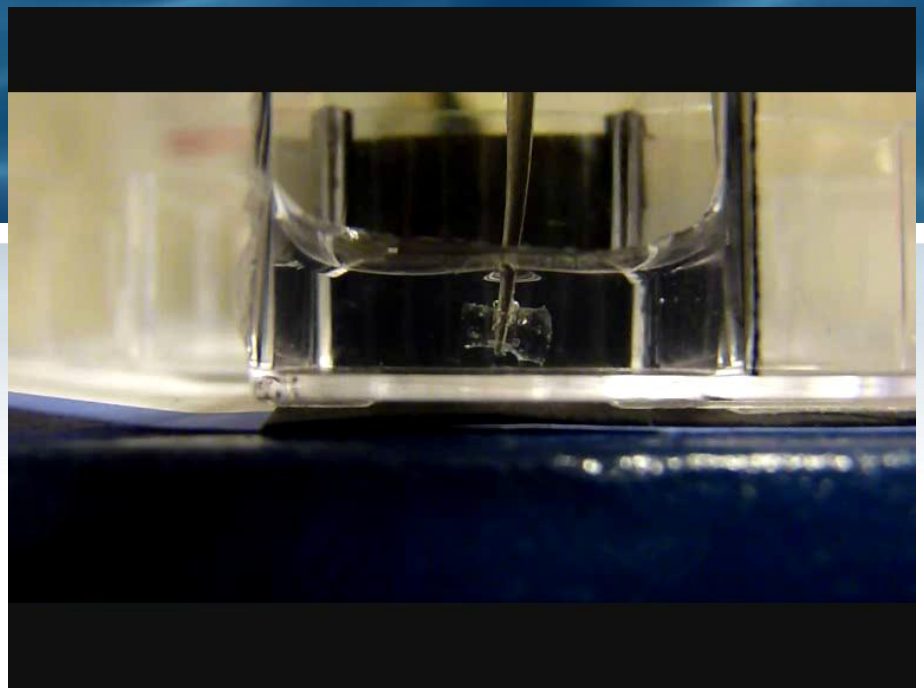


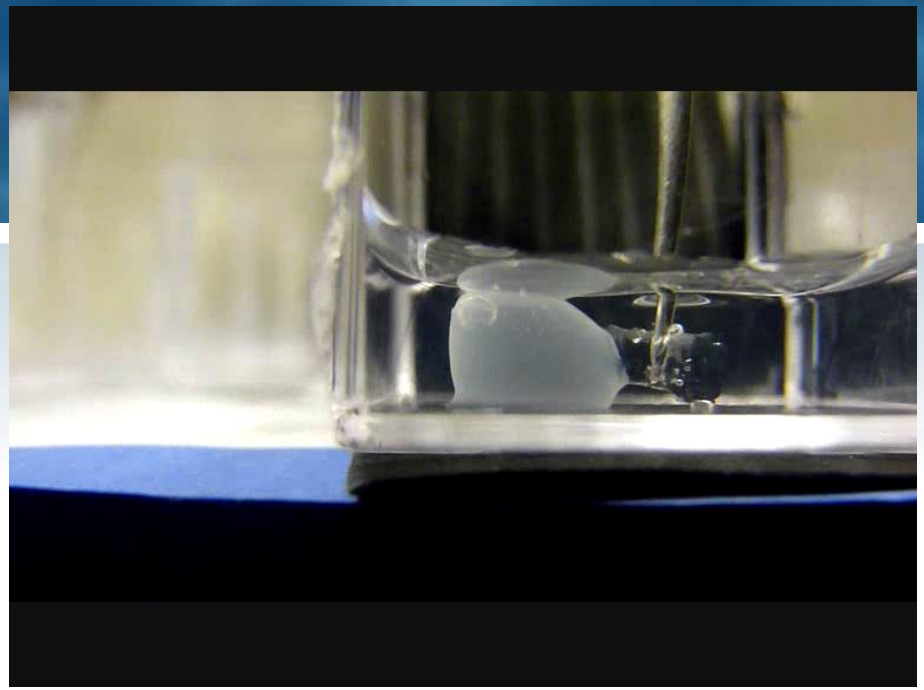
Tube growing

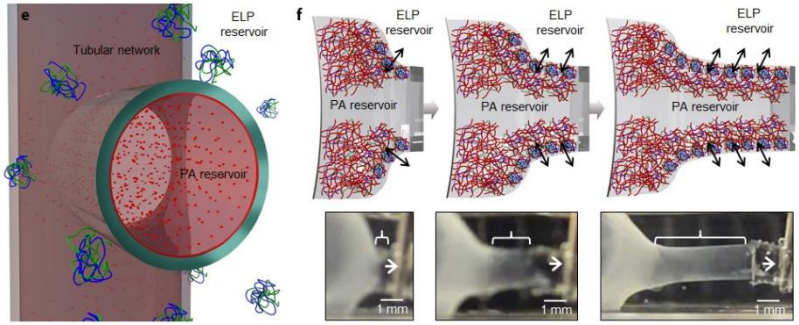


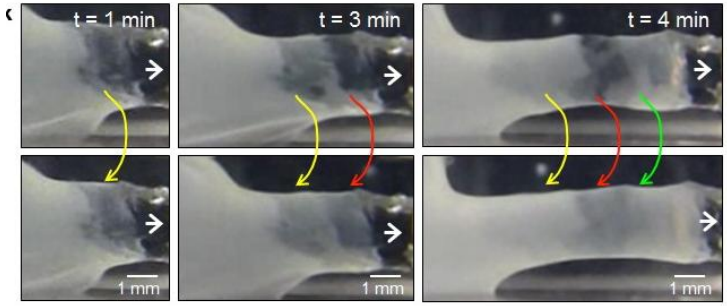


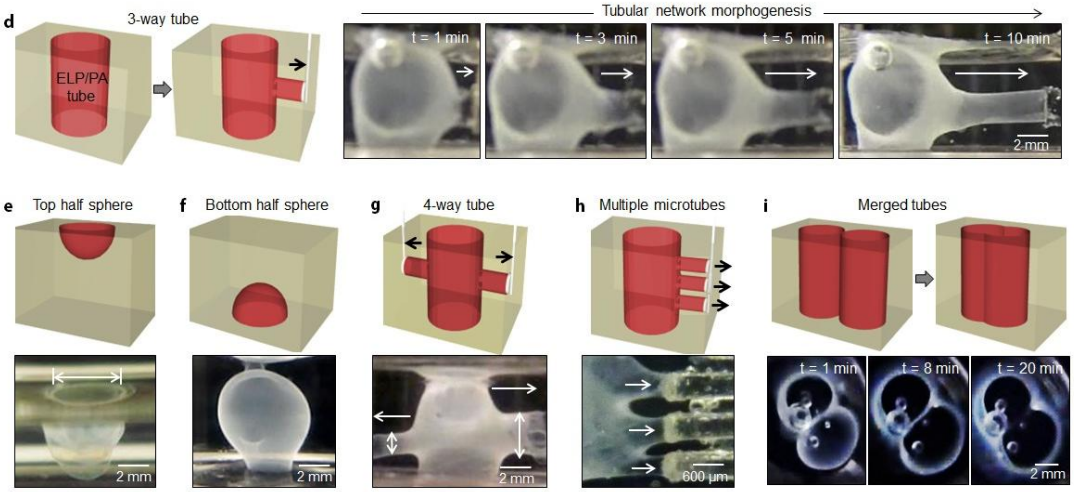







e
ELP reservoir
Tubular network
PA reservoir
f
ELP reservoir
PA reservoir
1 mm


c
t = 1 min
t = 3 min
t = 4 min
1 mm


d
3-way tube
ELP/PA tube
Tubular network morphogenesis
t = 1 min
t = 3 min
t = 5 min
t = 10 min
2 mm
e Top half sphere
f Bottom half sphere
g 4-way tube
h Multiple microtubes
i Merged tubes
600 µm
t = 1 min
t = 8 min
t = 20 min

Acknowledgements

Layered hierarchical structured scaffolds with injectable self setting bioactive gel for clinical bone tissue repair
(FP7-NMP-2010-2.2.3)

Tissue in Host Engineering Guided Regeneration of Arterial Intimal Layer
FP7-Health-2011-278557

Development of biomaterial-based delivery systems for ischemic conditions; an integrated pan-European approach. FP7-PEOPLE-2012-ITN

Engineering responsive and biomimetic hydrogels for biomedical therapeutic and diagnostic applications. MSCA-ITN-2014-ETN

Tailored Elastin-like recombinamers as advanced systems for cell therapies in Diabetes Mellitus: as synthetic biology approach towards a bioeffective and immunoisolated biosimilar islet/cell niche. MMP-2014-642687

THANK YOU

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BIOFORGE

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Universidad de Valladolid

Genoma España

Junta de Castilla y León

ciber-bbn

THANK YOU

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