Biomaterials in regenerative medicine

‘Synthetic materials’

Dr. Uwe Freudenberg
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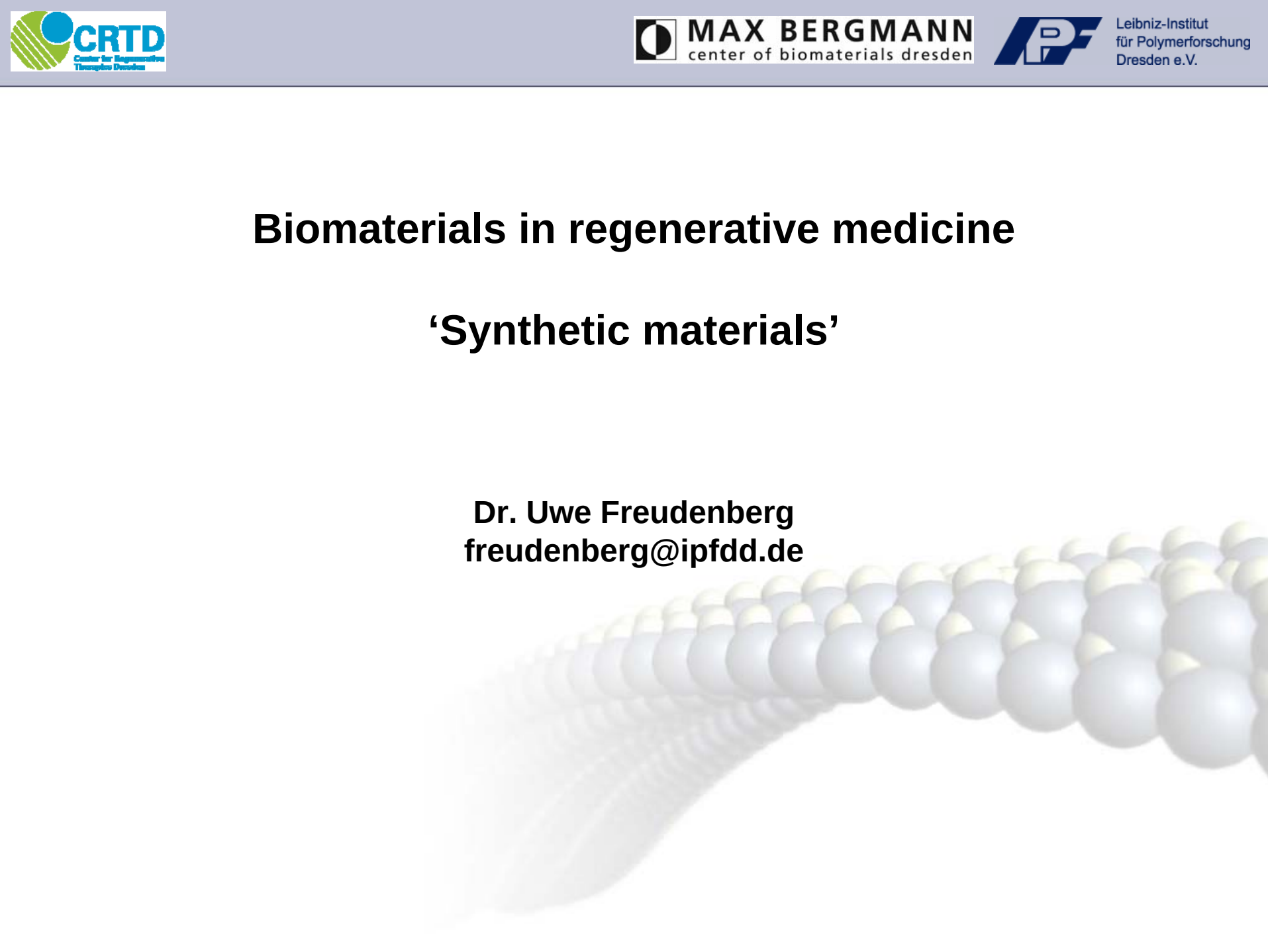
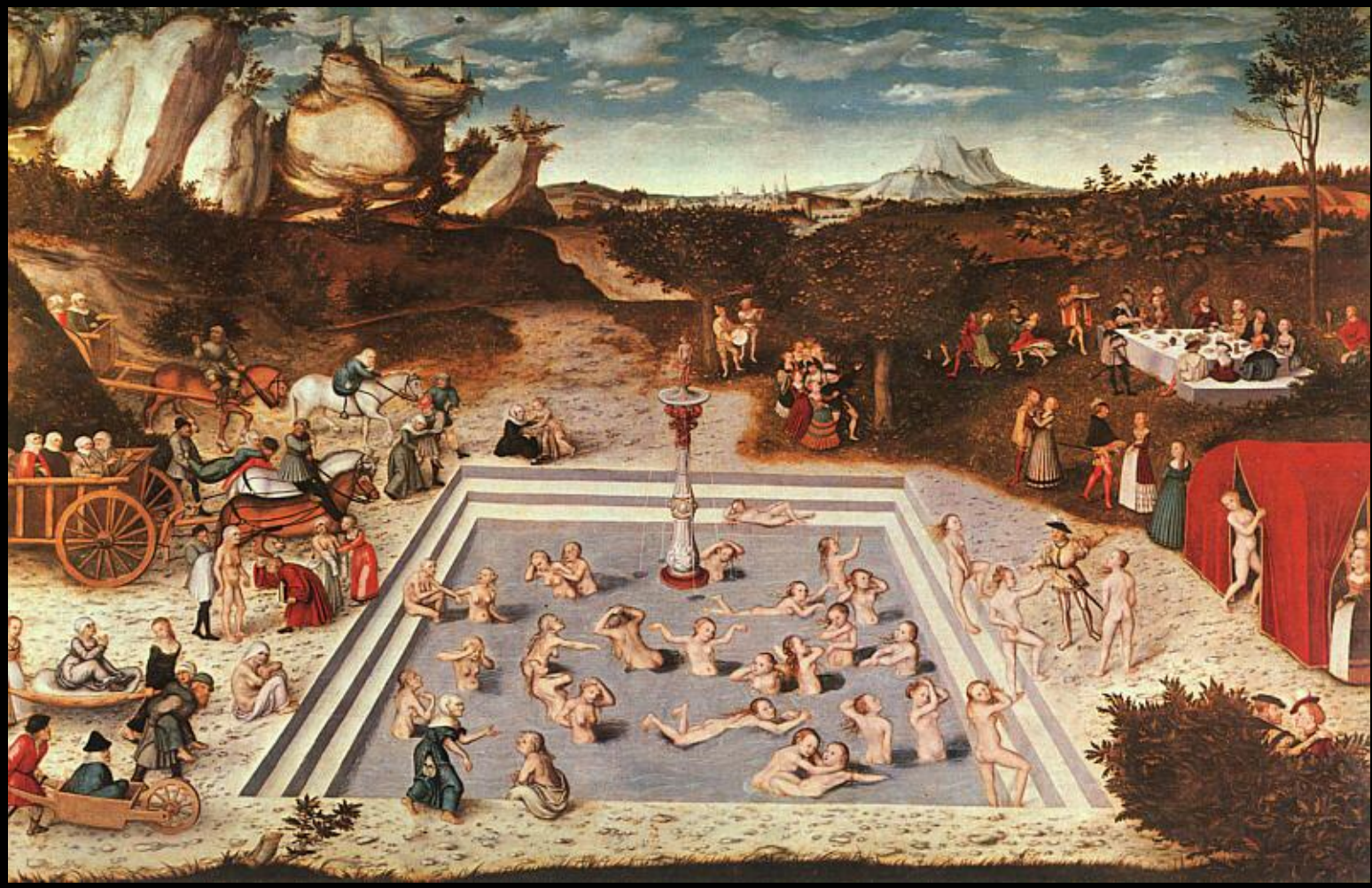


…. most of the current medical devices ***do not cure diseases*** *but only* ***treat symptoms.***

What is missing there? **Regeneration into fully functional tissue** - as, for instance, present in human during healing of fractured bone and …

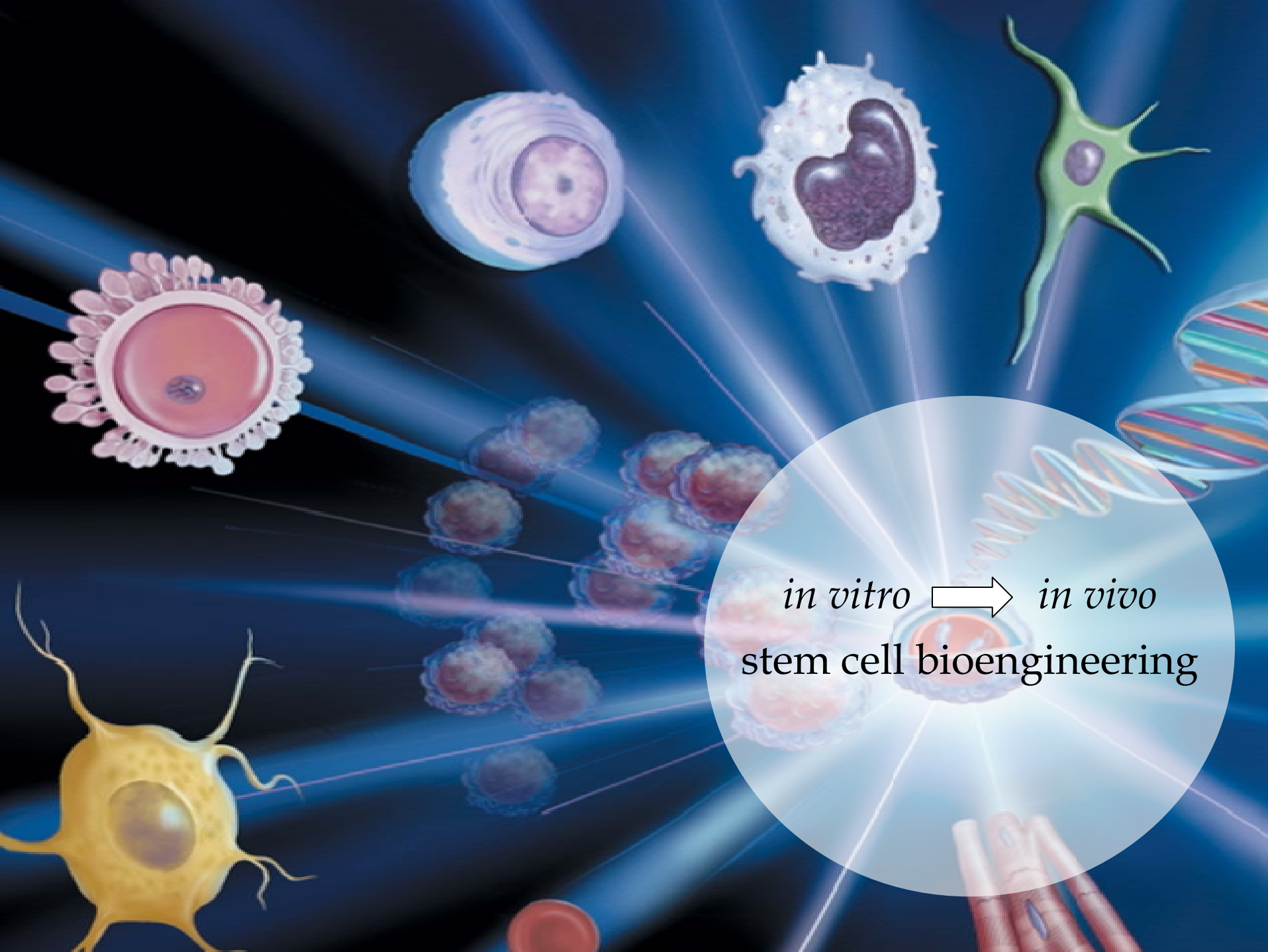
… as impressively shown by some amphibians.

Key question: How can we rekindle **tissue formation processes** in adult mammalians that normally occur during embryogenesis and development ?



Lucas Cranach The Fountain of Youth (1546)

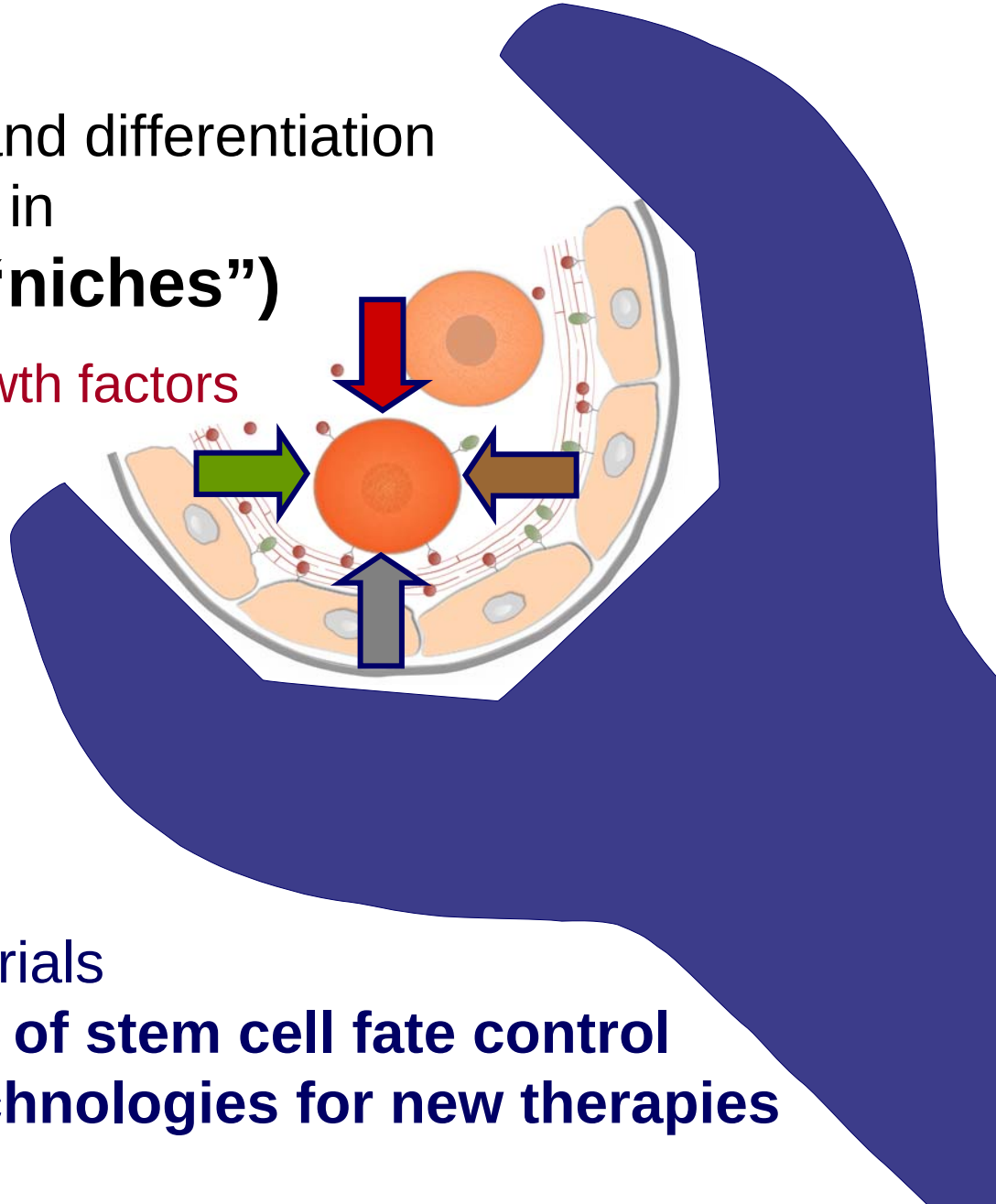
It‘s all about cells: Lack of regeneration is often caused by **competing cellular processes**
(e.g. wound healing/ scar formation dominating over recruitment of tissue specific cells...).


in vitro ⇨ *in vivo*
stem cell bioengineering

Quiescence, self-renewal and differentiation of stem cells are controlled in

microenvironments (“niches”)

- chemokines, cytokines/growth factors
- extracellular matrix
- cell-cell interactions
- physical constraints

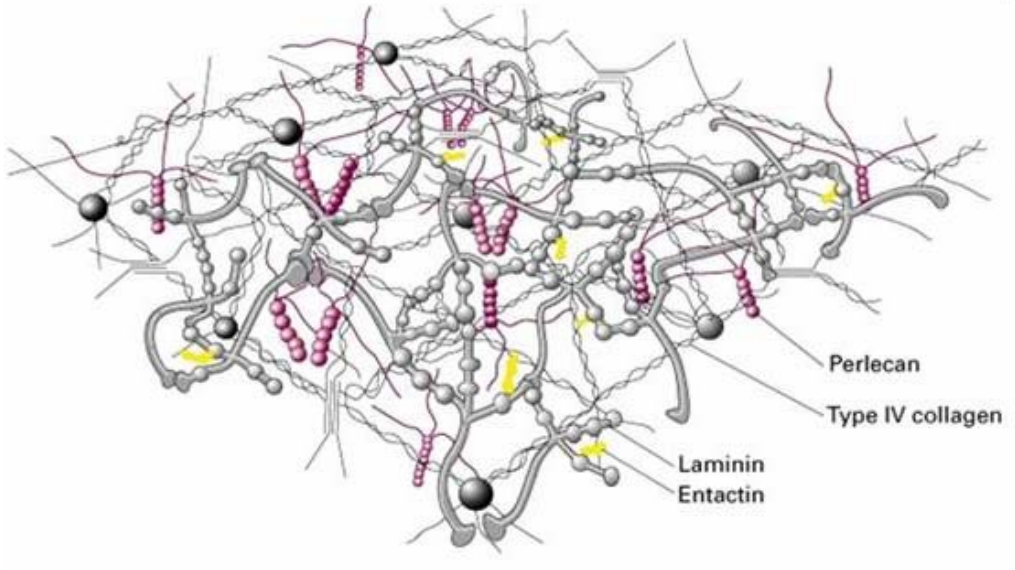


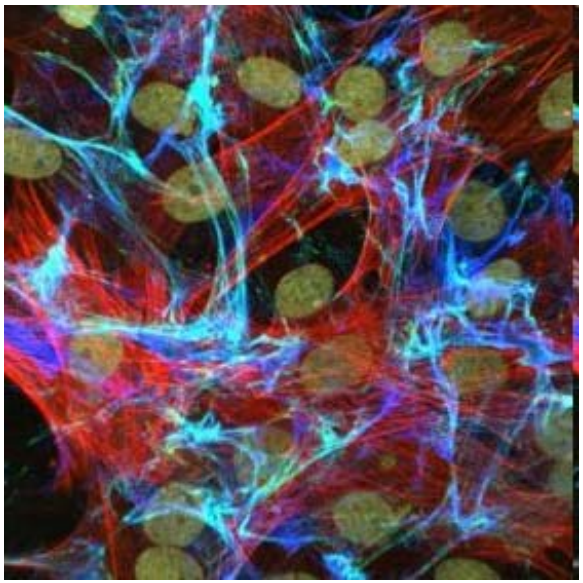
⇨ utilizing bioartificial materials

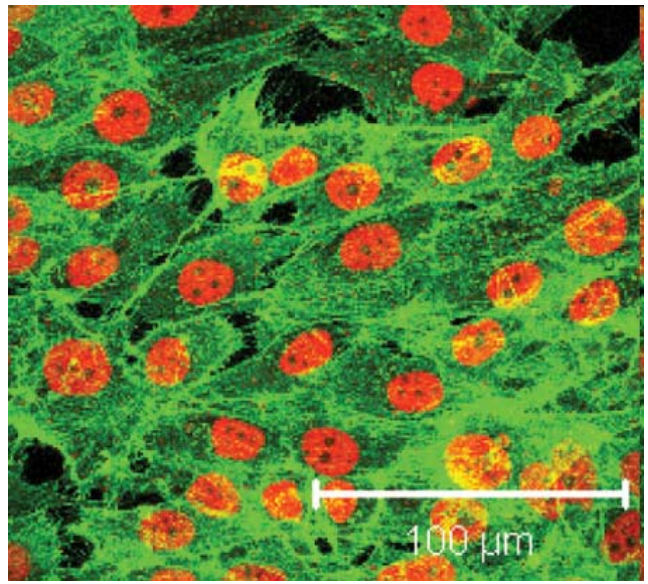
- **to explore mechanisms of stem cell fate control**
- **to apply the latter in technologies for new therapies**

… **intrinsic** (genetic) and/or (**micro)enivronmental signals** can direct cells to restore and rejuvenate tissues and organs.

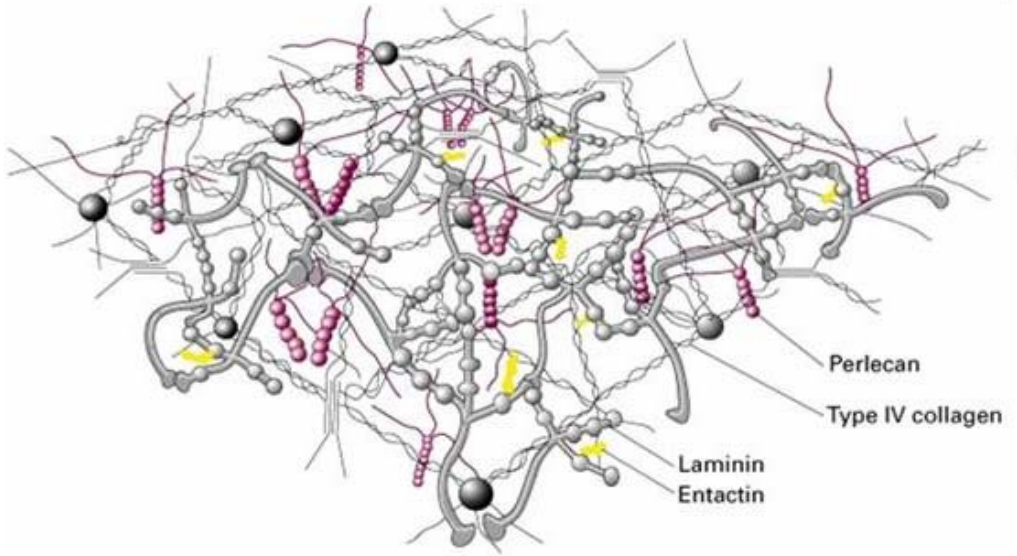
(micro)enivronment ⟹ extracellular matrix







(micro)enivronment → extracellular matrix



limitations: matrigel/collagen materials

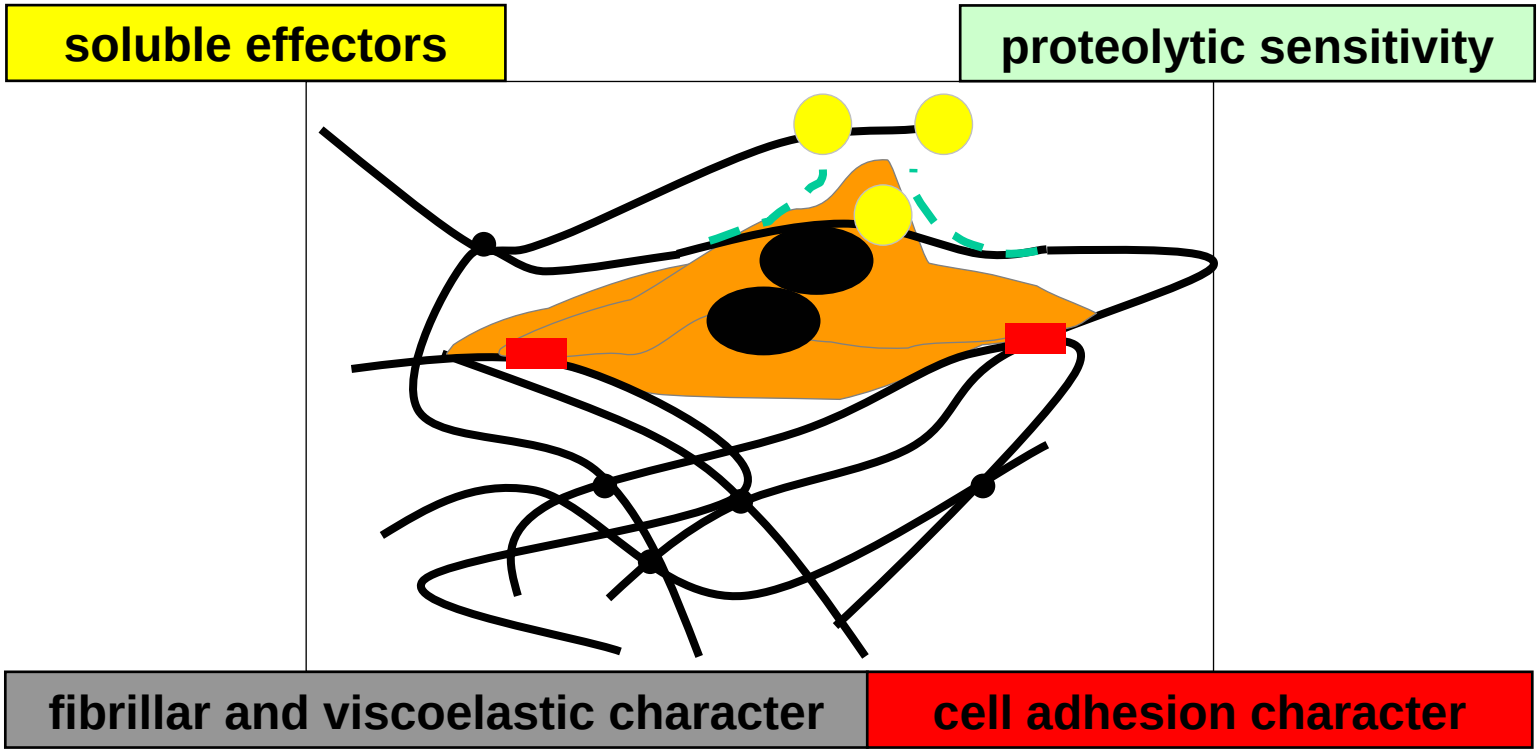
-natural components (source dependence)

-difficult purification procedures/carry the risks of immunogenicity and disease transmission

-restricted variation of physical and biological parameters

→ modulation/over expression of signals to rekindle lost regeneration potential

What kind of signals? - identification of important biofunctional building blocks



soluble effectors

proteolytic sensitivity

fibrillar and viscoelastic character

cell adhesion character

translation to artificial matrices

⇨soluble effectors

⇨structural scaffold: network characteristics highly hydrated meshwork

⇨enzymatic cleavable crosslinks

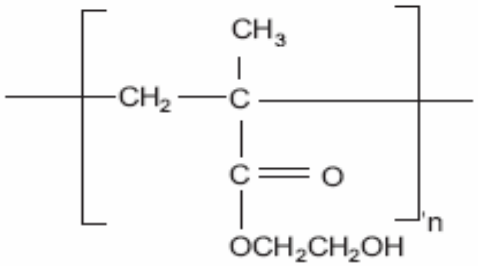
⇨adhesion ligands

I. Synthetic hydrogels

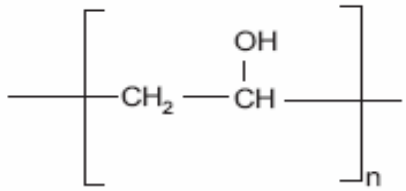
I. synthetic hydrogels – building blocks

-nontoxic, non-immunogenic, FDA-approved
-inert to proteins

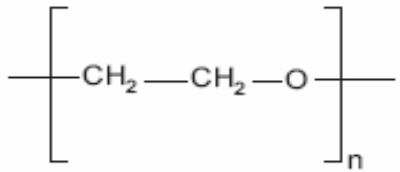
Neutral polymers



Poly(hydroxyethyl methacrylate)
(PHEMA)

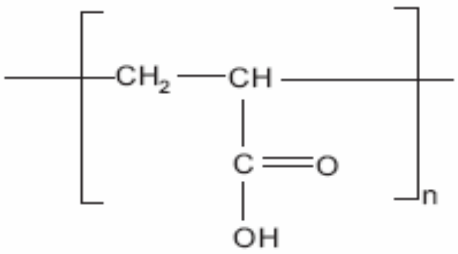


Poly(vinyl alcohol)
(PVA)

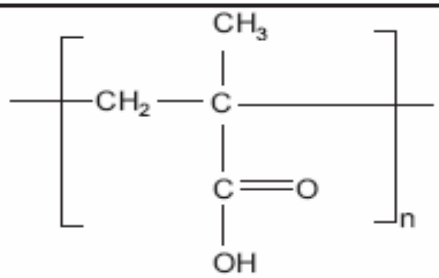


Poly(ethylene glycol)
(PEG)

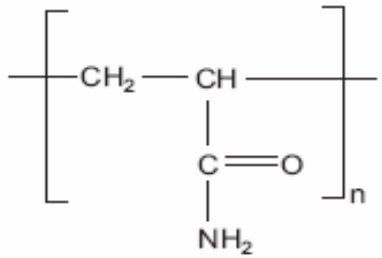
Ionic polymers



Poly(acrylic acid)
(PAA)

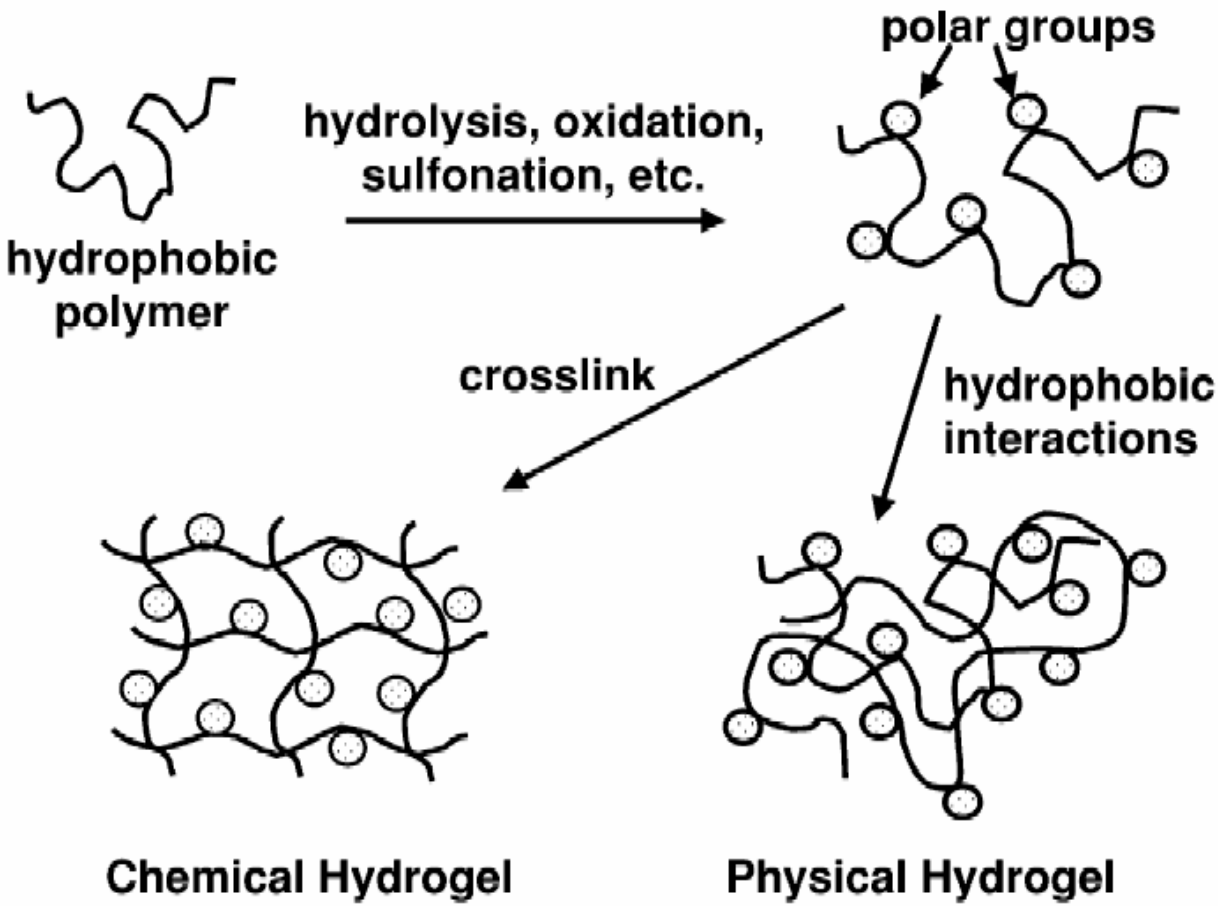


Poly(methacrylic acid)
(PMMA)



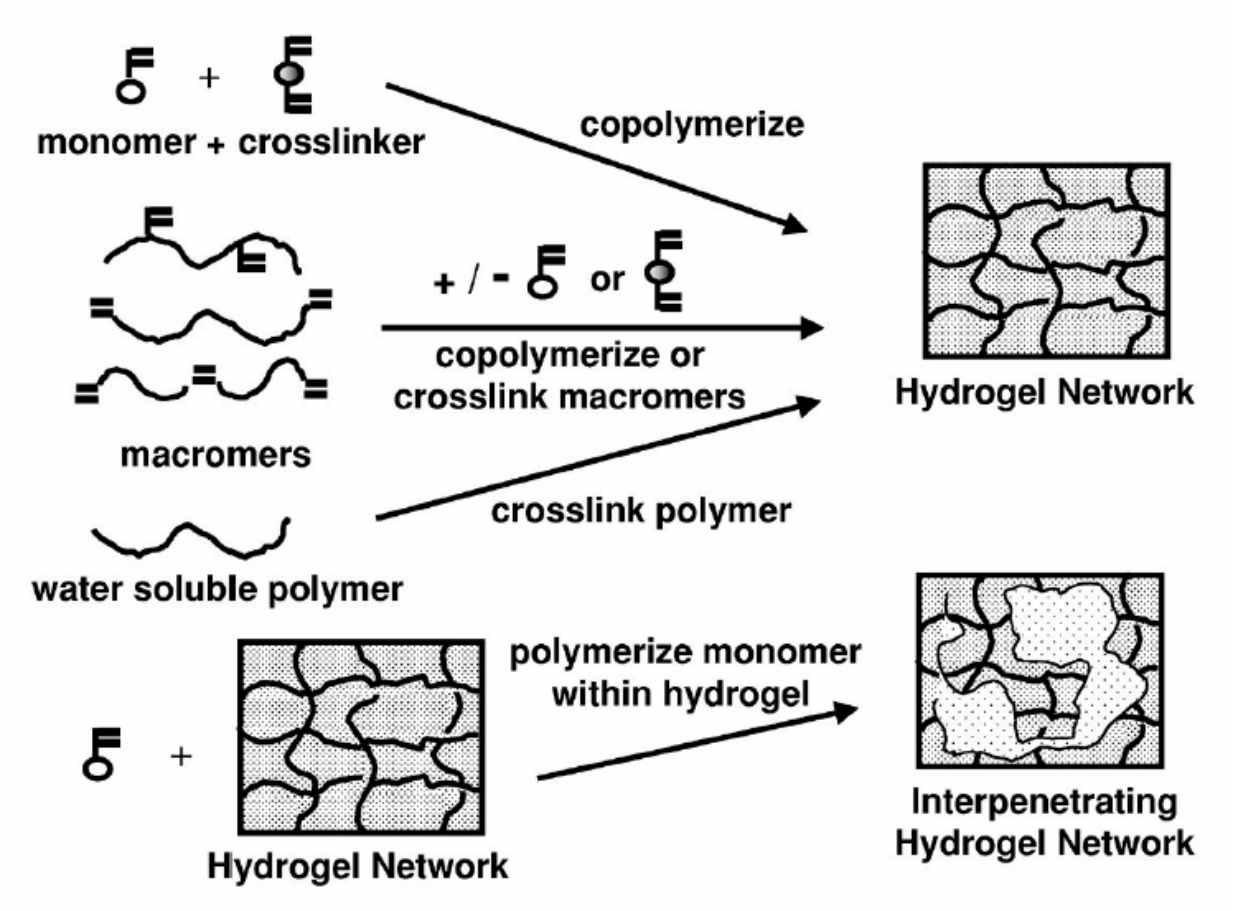
Polyacrylamide
(PAAm)

I. synthetic hydrogels – mechanisms



A **B**

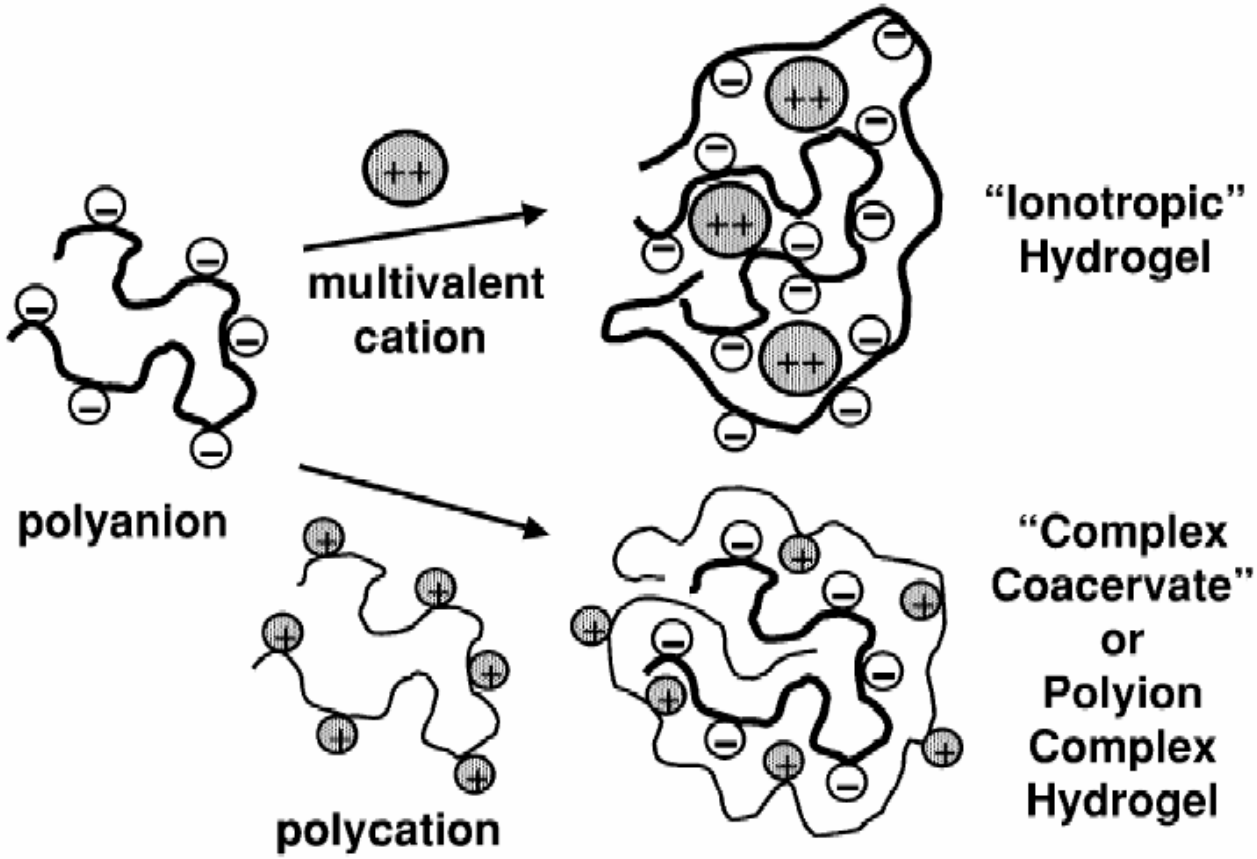
I. synthetic hydrogels – A: chemical crosslinks



advantages: -wide field of mechanical properties (storage modulus, water content …)

disadvantage: often cytotoxic crosslinking chemicals/reaction

I. synthetic hydrogels – B: physical crosslinks

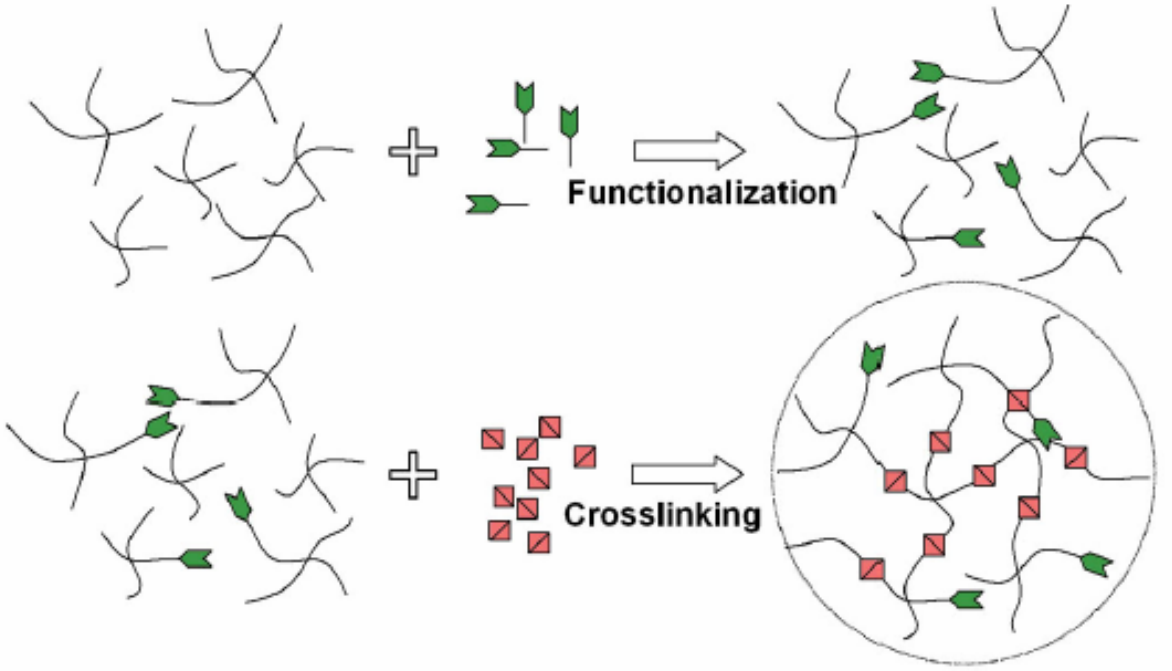


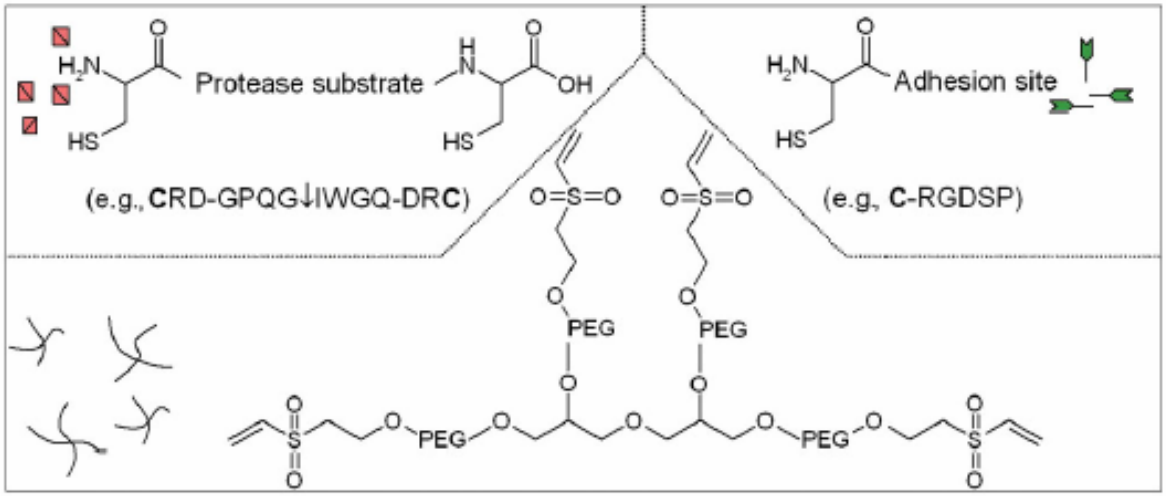
'reversible', or 'physical' gels:

advantages: -in situ crosslinking (no cytotoxic chemicals/reaction)

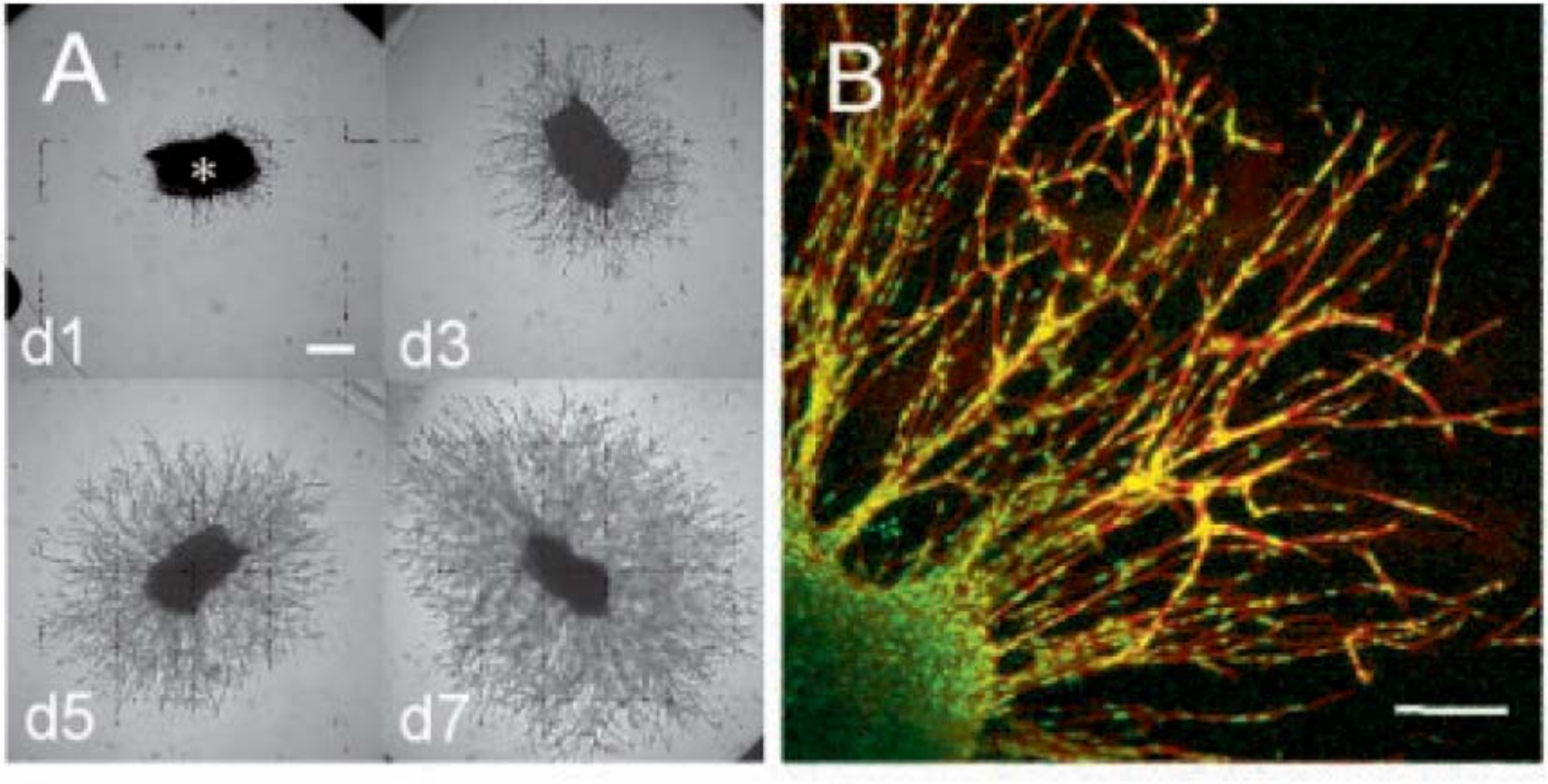
disadvantage: often poor stability (dependent of ionic strength/temperature)

I. synthetic hydrogels – examples





Repair of bone defects using synthetic mimetics of collagenous extracellular matrices.
Lutolf et al.
***Nature Biotechnology* 2003, 21, 513-518**



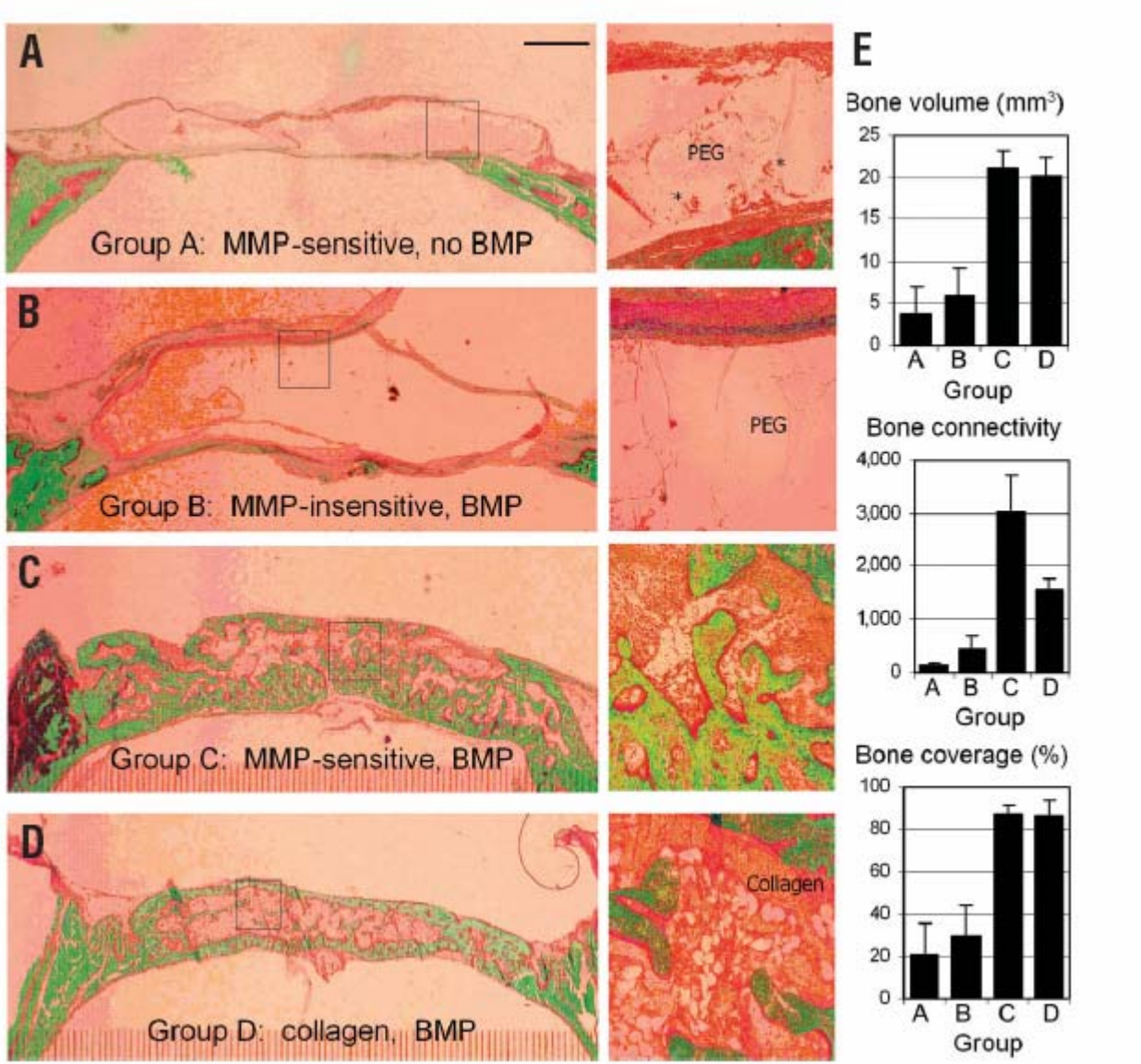
(*A*) Fibroblasts radially invaded the adhesive and MMP-sensitive synthetic hydrogel matrix (bar = 250 μm). (*B*) Migration of spindle-like-shaped fibroblasts occurred in a cohort manner (bar = 150 μm).

Synthetic matrix metalloproteinase-sensitive hydrogels for the conduction of tissue regeneration: Engineering cell-invasion characteristics

M. P. Lutolf, J. L. Lauer-Fields, H. G. Schmoekel, A. T. Metters, F. E. Weber, G. B. Fields, and J. A. Hubbell

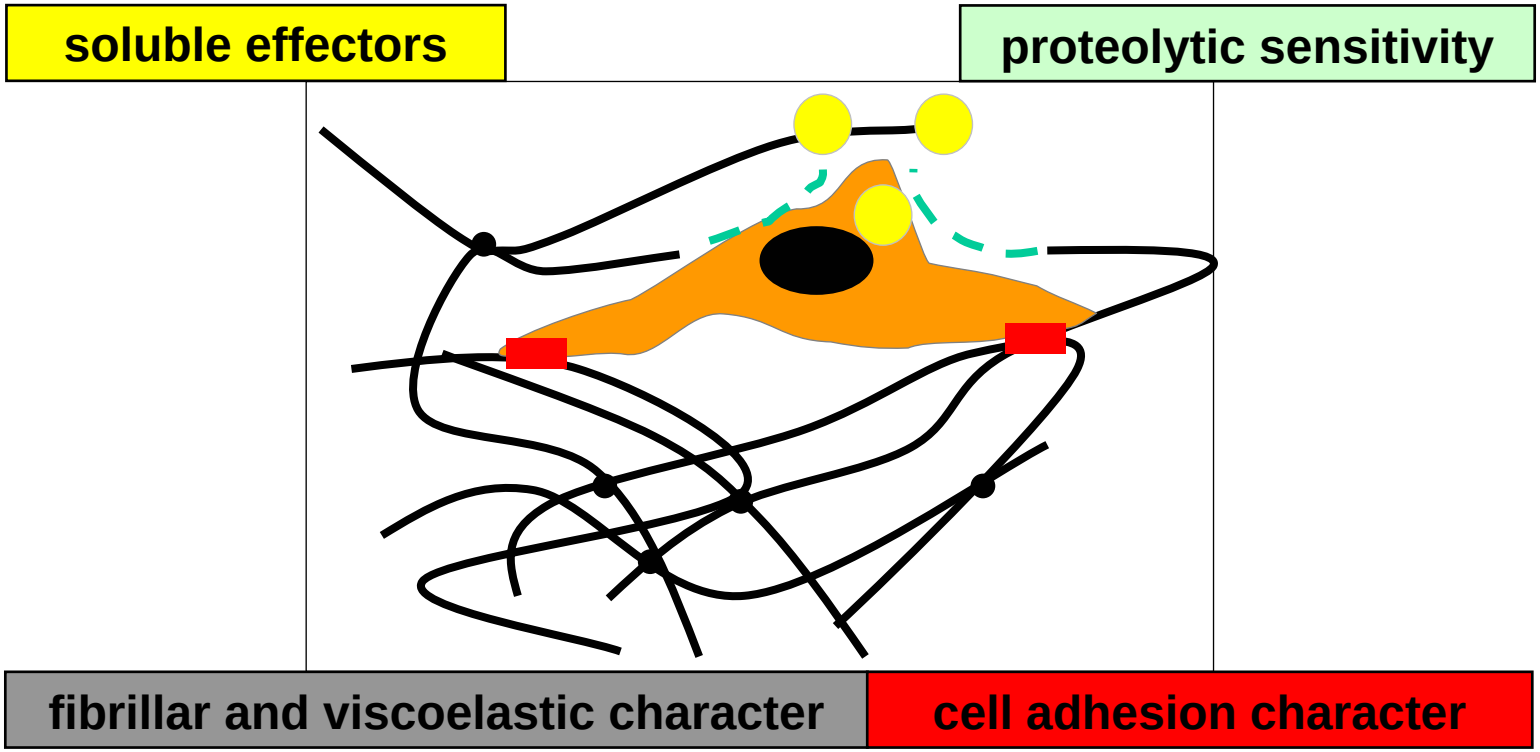
PNAS **2003**, 100, 5413-5418

in vivo test: healing of critical size rat calvarial defects 4 wk after implantation.



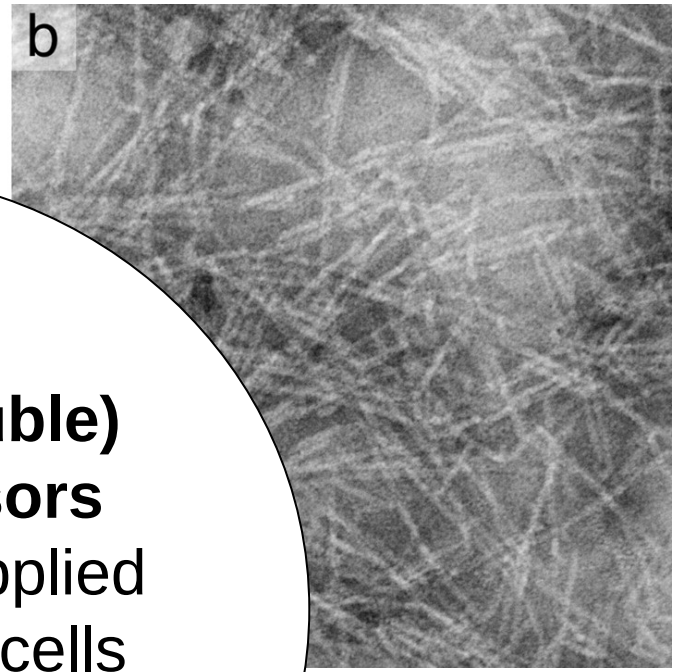
Lutolf et al. *Nature Biotechnology* **2003**, 21, 513-518

What kind of signals? - identification of important biofunctional building blocks

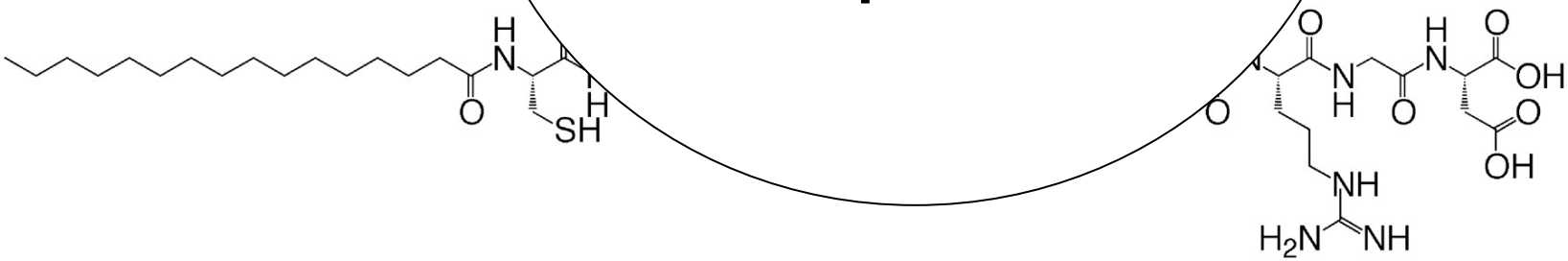


Injectable matrices

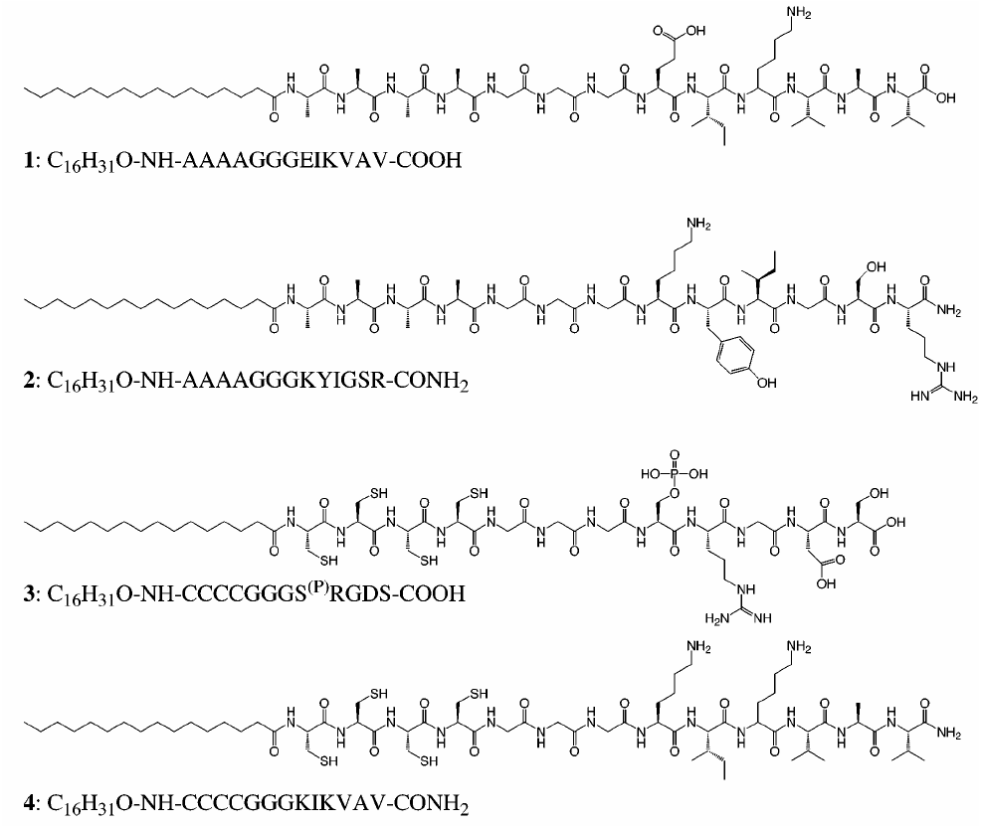
- **synthetic peptide-amphiphile molecules self-assemble into nanofibers**
- amino acid selection and all… yield nanofibers varying i… surface chemistry, and …
- self-assembly induced… ion induction, and cor…



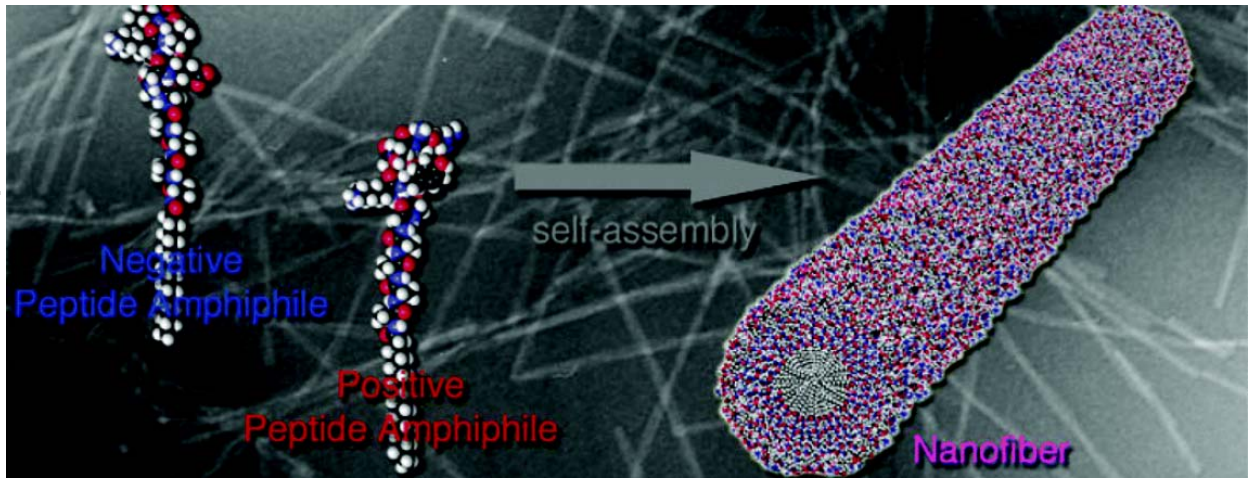
⇨ need for
injectable (soluble)
matrix precursors
which can be supplied
with suspended cells
and **solidify**
in the recipient‘s tissue



J.D. Hartgerink, E. Beniash, and S.I. Stupp
PNAS 99 (2002) 5133-5138

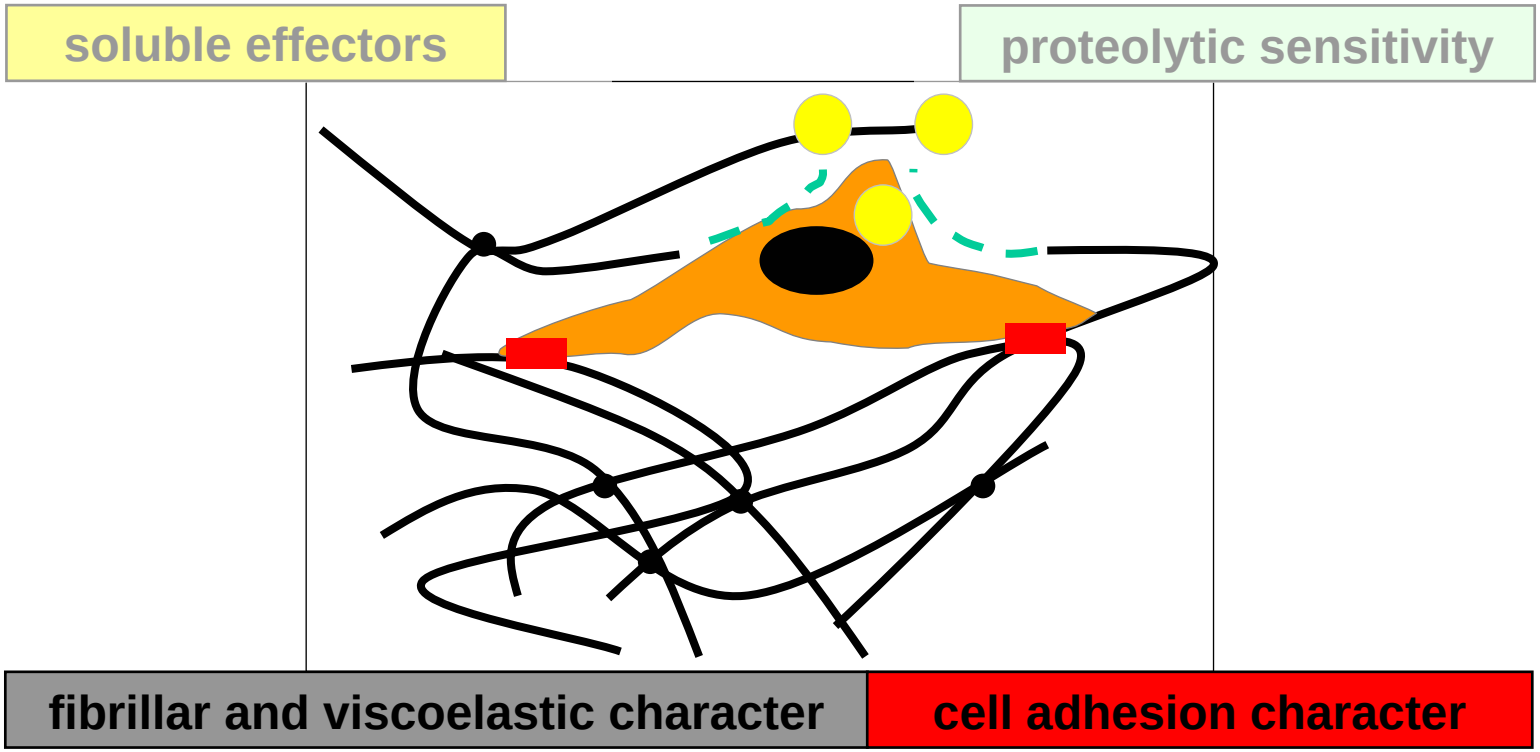


Molecule **1** and **3** self-assemble at acidic pH, and molecule **2** and **4** self-assemble at basic pH, while molecule pairs **1/2** and **3/4** co-assemble at neutral pH



K.L. Niece, J.D. Hartgerink, J. J. M. Donners, and S. I. Stupp
Self-Assembly Combining Two Bioactive Peptide-Amphiphile Molecules into Nanofibers by Electrostatic Attraction
JACS 2003, 125, 7146-7147

What kind of signals? - identification of important biofunctional building blocks



II. Biohybrid hydrogels

II. Biohybrid hydrogels– building blocks

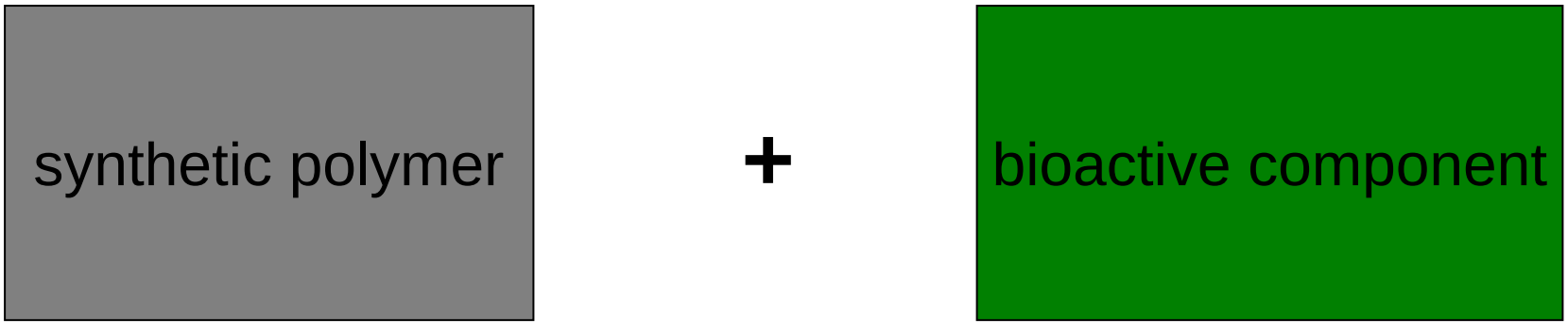
-combining synthetic polymers (PLA, PEG …) with proteins/GAGs (natural ECM-components) to enable:

-in situ crosslinking

-proteolytic sensitivity

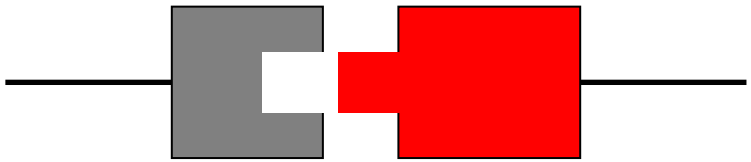
-delivery of soluble effectors

-presentation of adhesive ligands



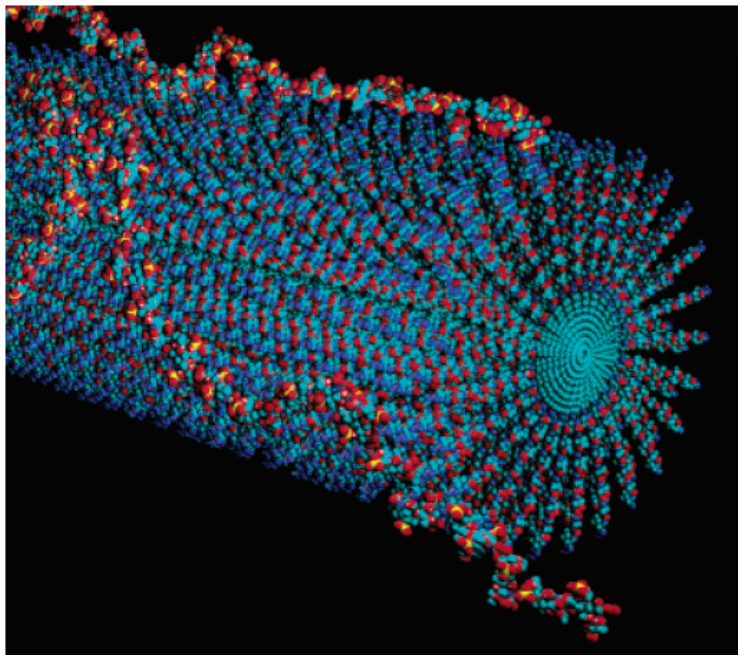
II. biohybrid hydrogels – A/B: chemical/physical crosslinks

Physical gels can form from biospecific recognitions:
-avidin with a polymeric biotin
-leucine zipper
-adsorption of GAGs/peptides



‘permanent’ or ‘chemical’ gels: covalently-crosslinked networks: -photo-polymerization, …

Rajangam K et al.
Heparin binding nanostructures to promote growth of blood vessels.
Nano Lett 2006;13:2086–90.



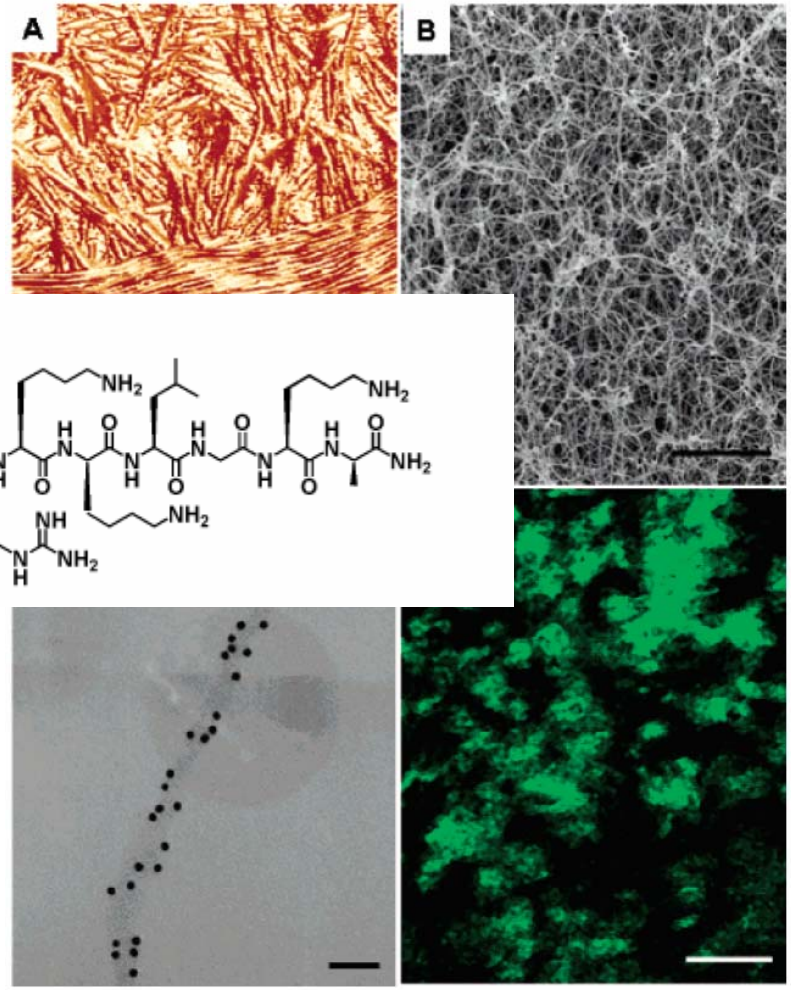
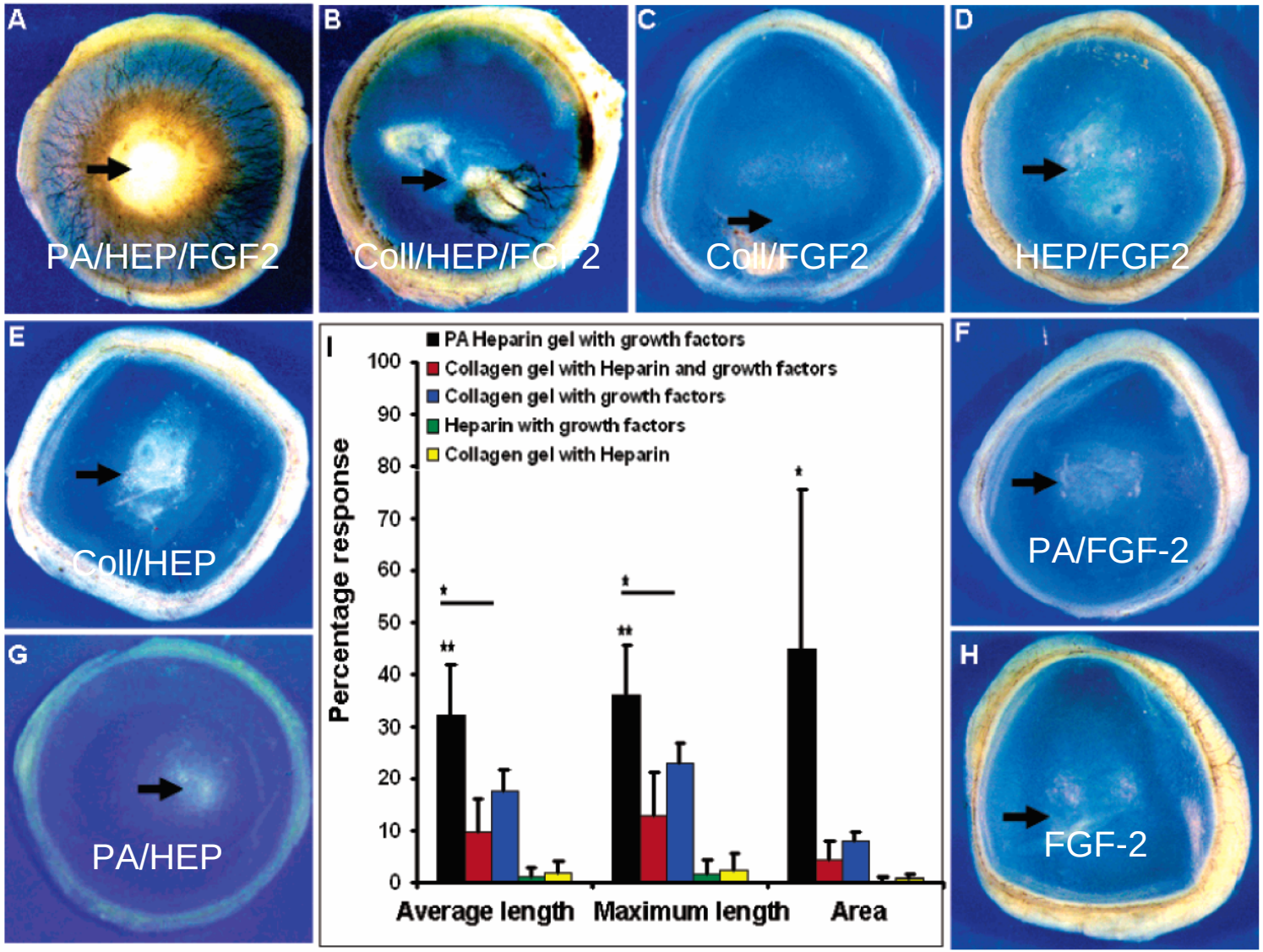
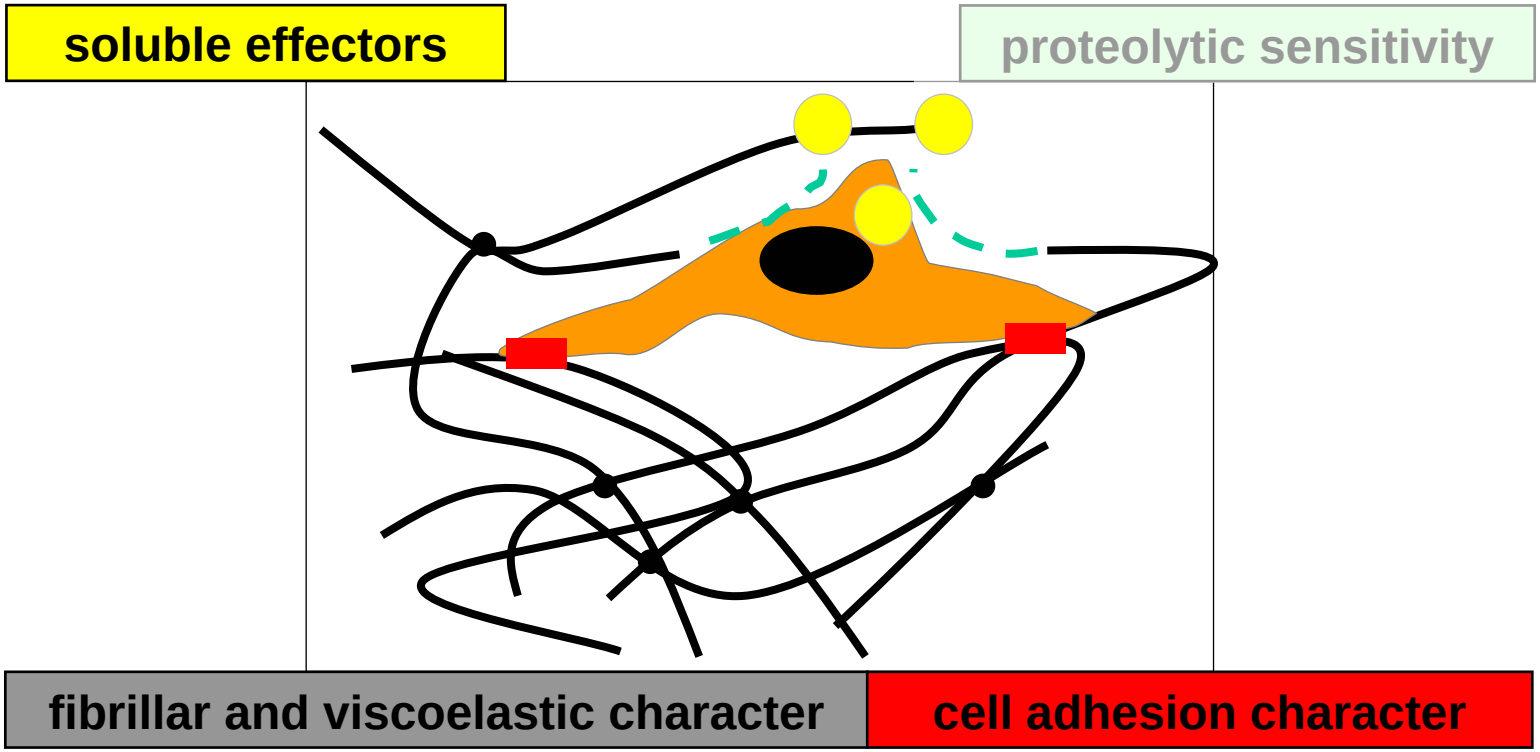


Figure 1. Structure of the nanostructure matrix. (A) Atomic force micrograph of the nanofibers (scale bar = 20 nm). (B) Scanning electron micrograph of the heparin-nucleated PA nanostructures showing entangled fiber bundles (scale bar = 2 μm). (C) Transmission electron micrograph showing bundles of nanofibers covered by 10 nm heparin−gold particles (black dots) (scale bar = 40 nm). (D) Confocal micrograph of PA−fluorescein heparin gel (scale bar = 100 μm) showing extensive heparin staining.

Rajangam K et al. Heparin binding nanostructures to promote growth of blood vessels. Nano Lett 2006;13:2086–90.



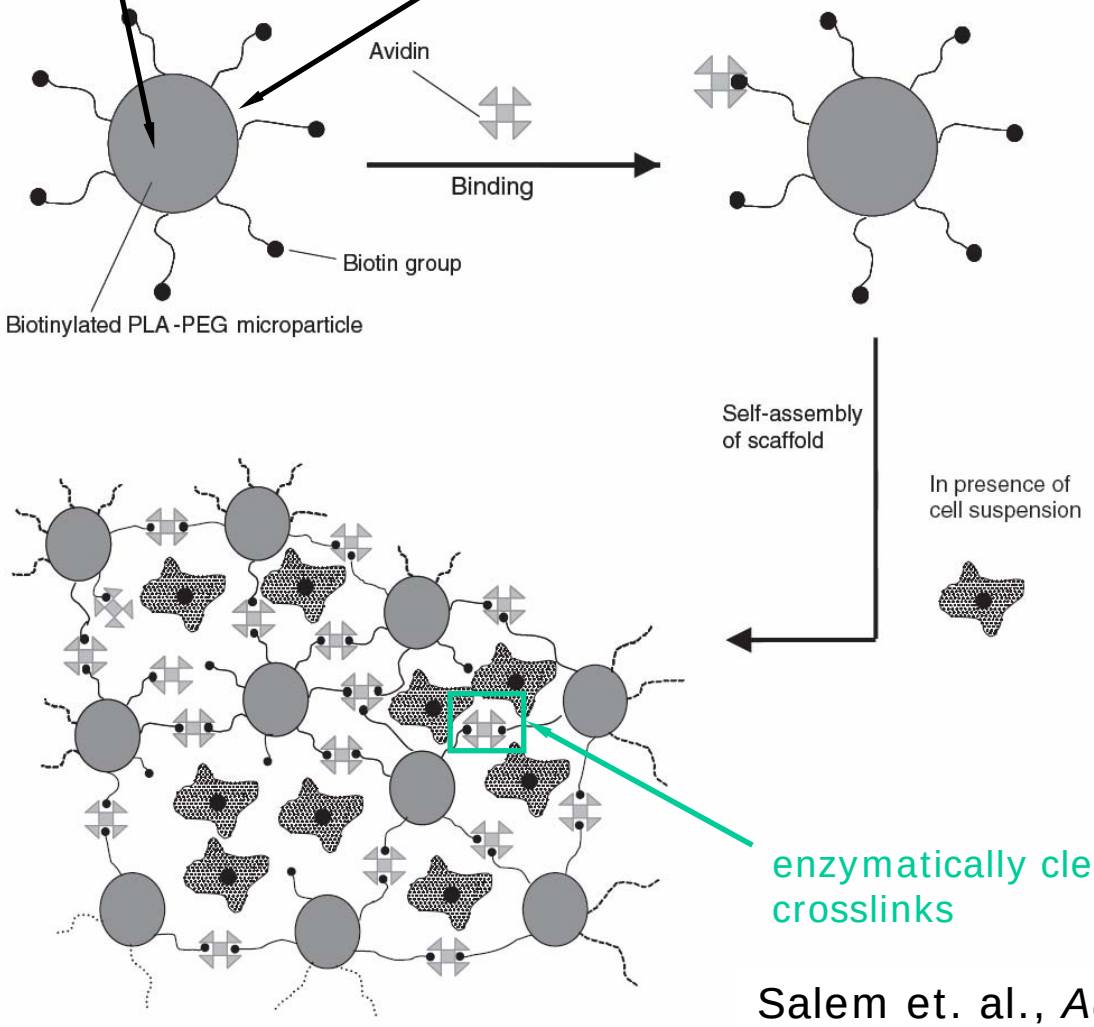
What kind of signals? - identification of important biofunctional building blocks

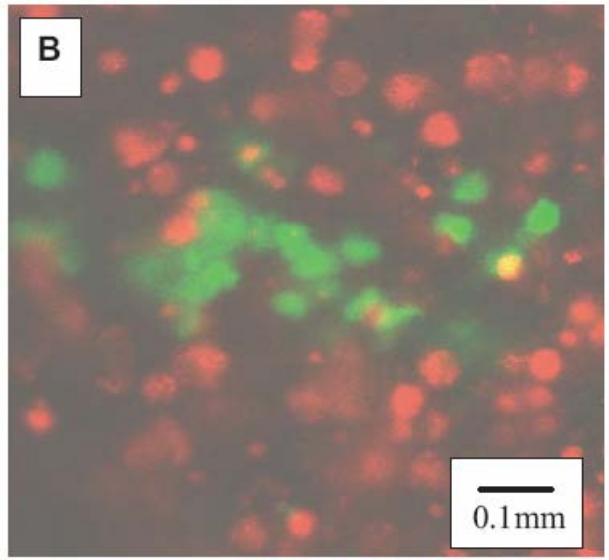


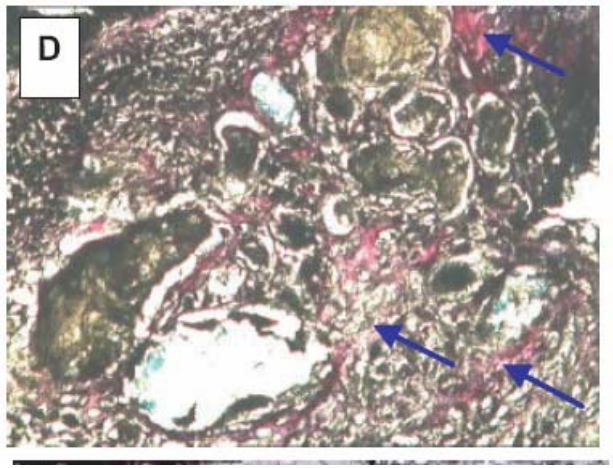
II. biohybrid hydrogels – examples

particles could be loaded with growth factors

structural scaffold: Biotinylated PLA-PEG microparticels

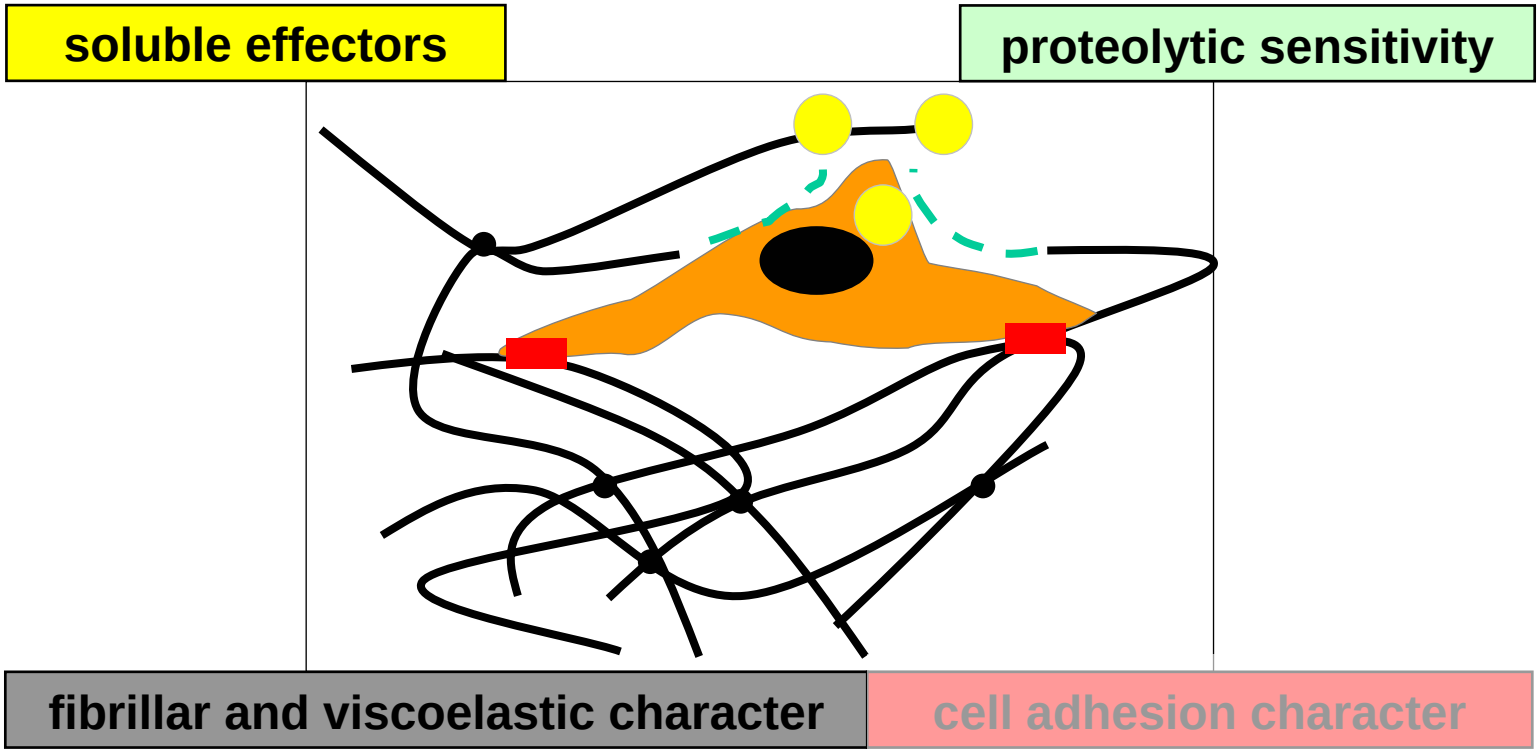


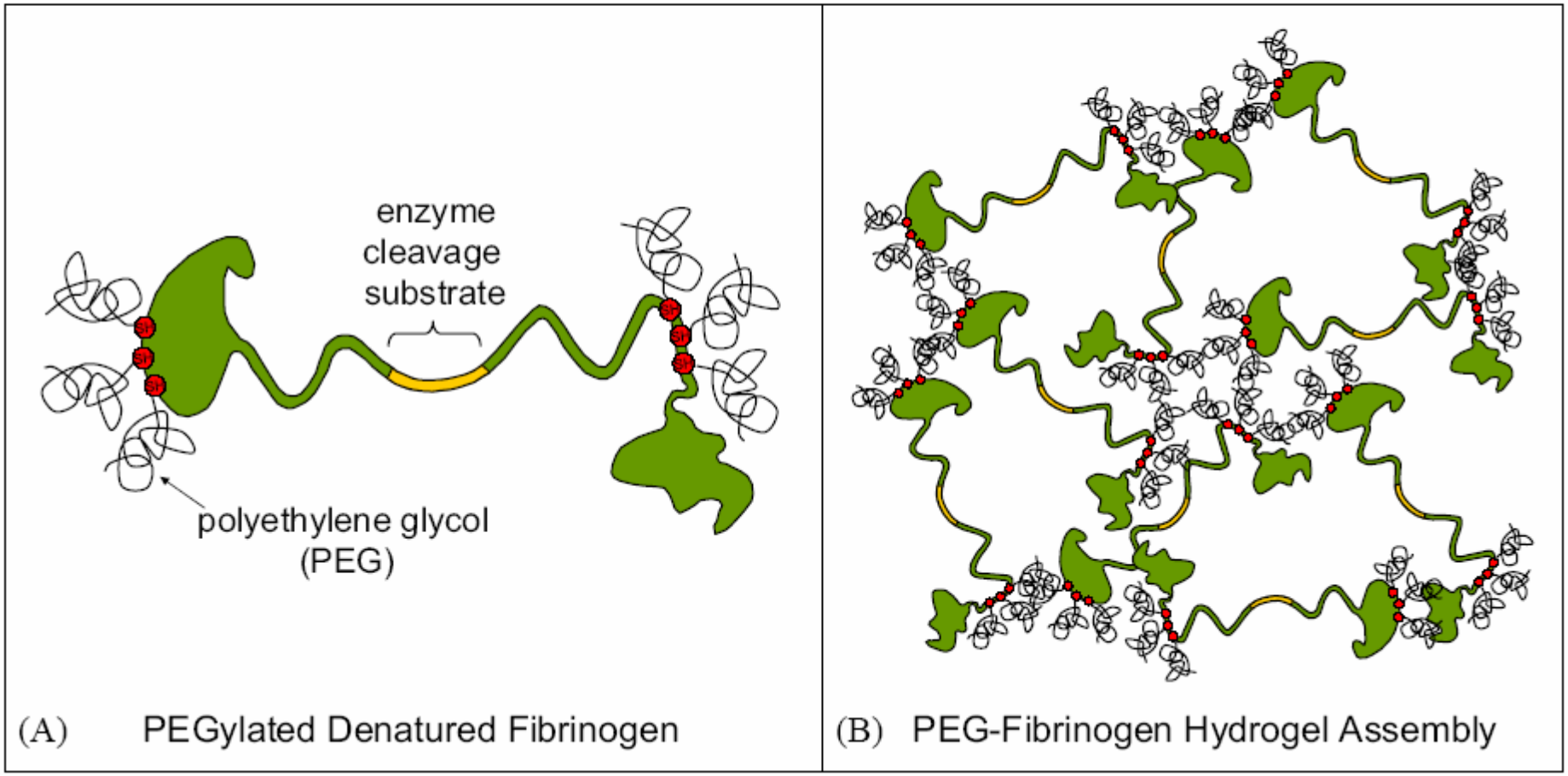




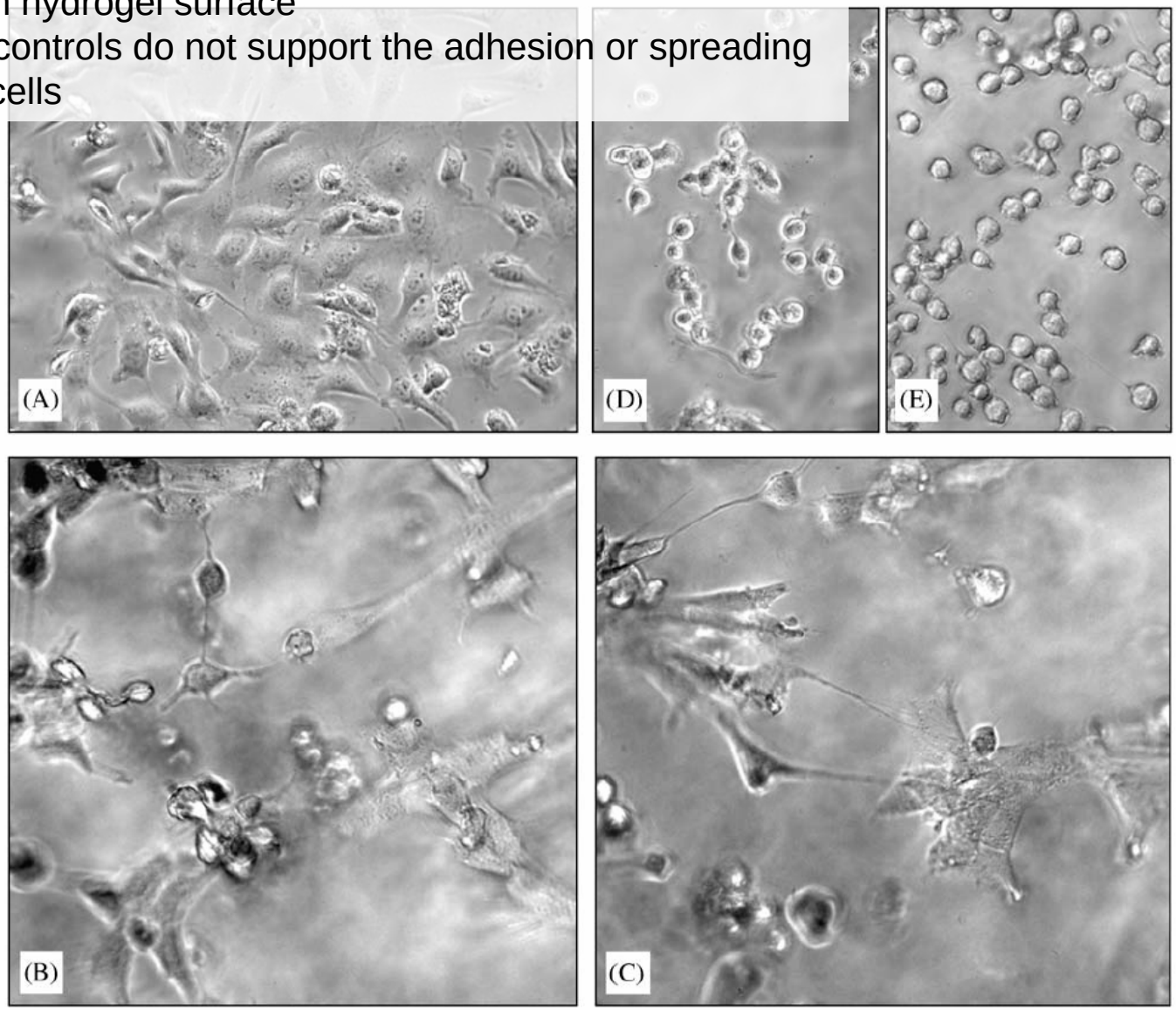
Salem et. al., *Adv. Mater.* 15, 2003, 210-213

What kind of signals? - identification of important biofunctional building blocks




enzyme
cleavage
substrate
polyethylene glycol
(PEG)
(A) PEGylated Denatured Fibrinogen
(B) PEG-Fibrinogen Hydrogel Assembly

A: monolayer of bovine aortic endothelial cells growing on a PEG-fibrinogen hydrogel surface
D: PEG–PEG controls do not support the adhesion or spreading of endothelial cells



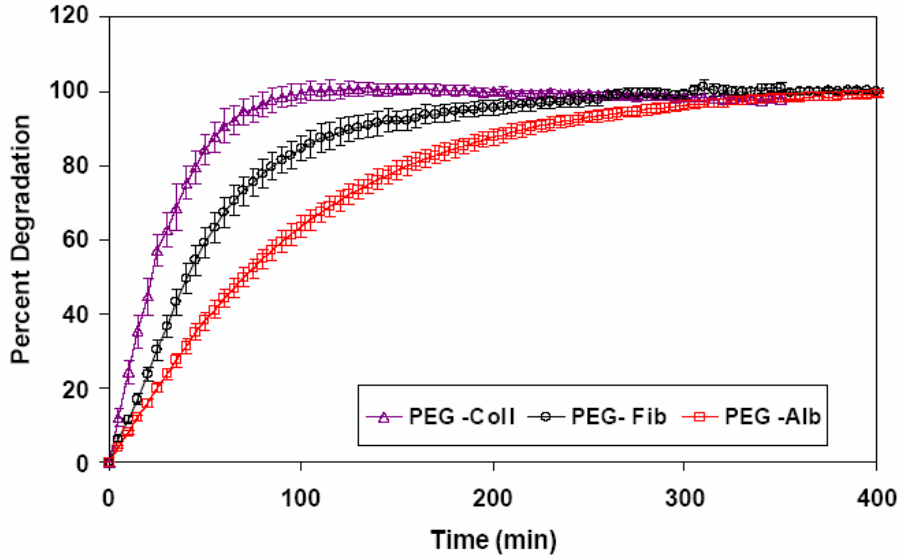
B+ C: Bovine aortic smooth muscle cells cultured inside PEG-fibrinogen hydrogels (are seen extending three-dimensionally in two separate z-slices of the same gel (B, C).

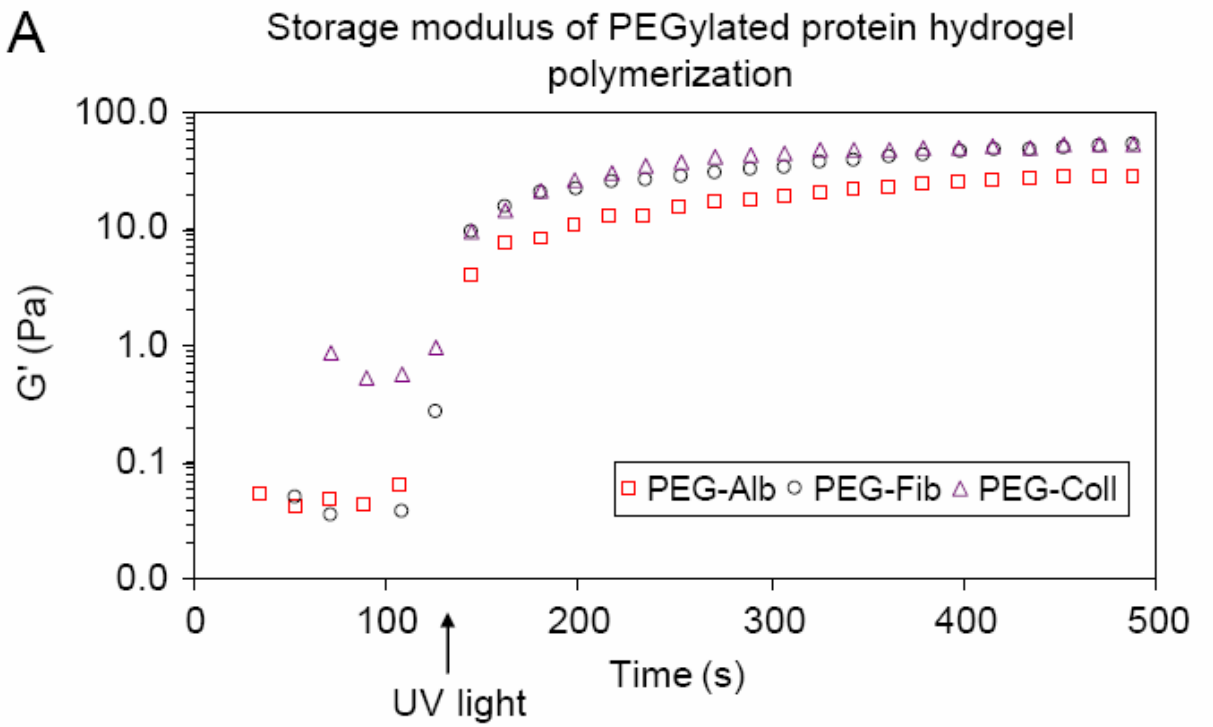
Biomaterials 28 (2007) 3876–3886

Protein–polymer conjugates for forming photopolymerizable biomimetic hydrogels for tissue engineering

Maya Gonen-Wadmany, Liat Oss-Ronen, **Dror Seliktar**

⇨ biomedical proteins collagen, fibrin(ogen), and albumin were PEGylated and formed into hydrogel networks by free-radical photopolymerization.





Advantage: PEG-mediated degradation:

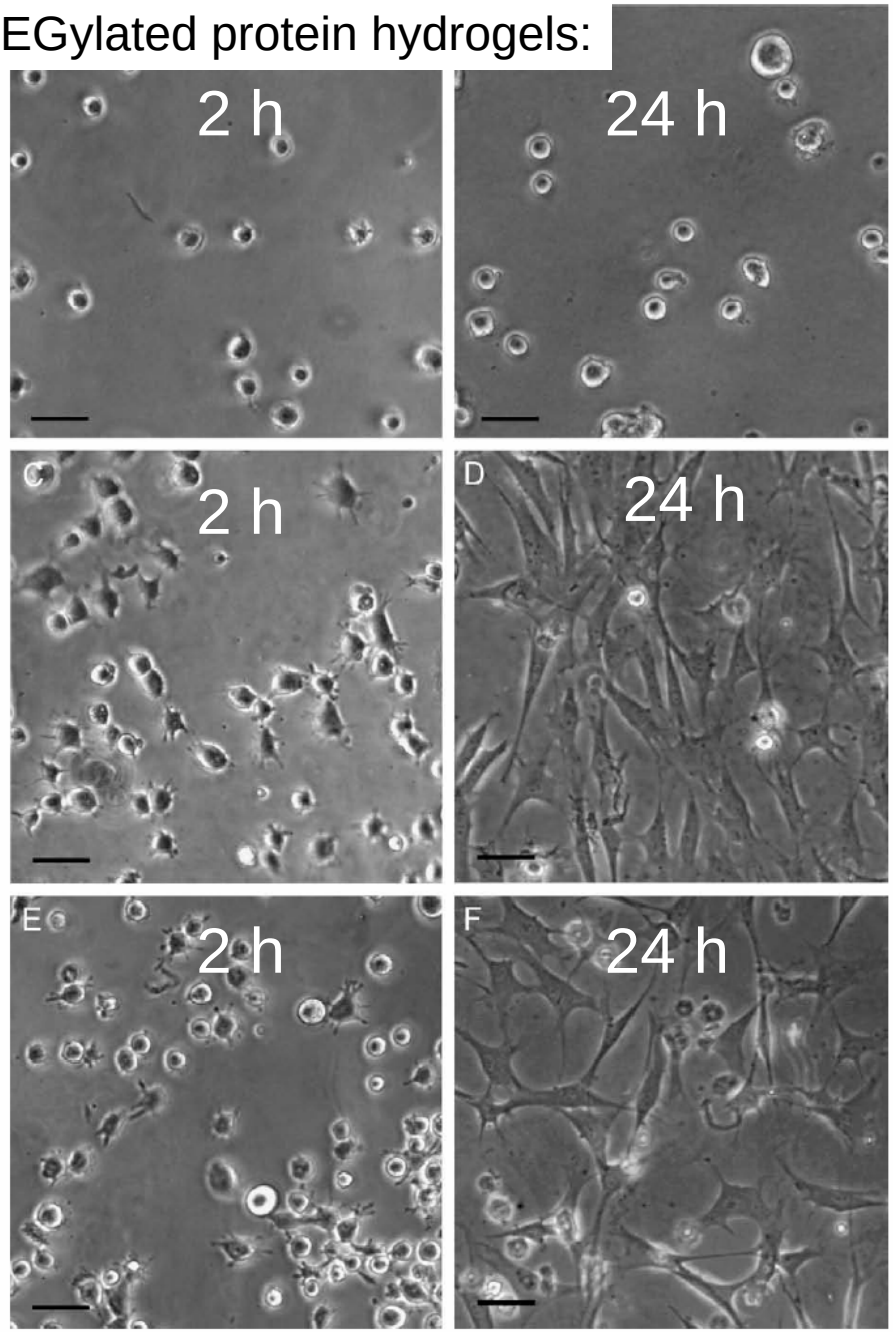
PEG shields the protein from enzymes through steric hindrances without blocking all the natural biological function of the structural protein molecule

Smooth muscle cells (SMCs) adhering to PEGylated protein hydrogels:

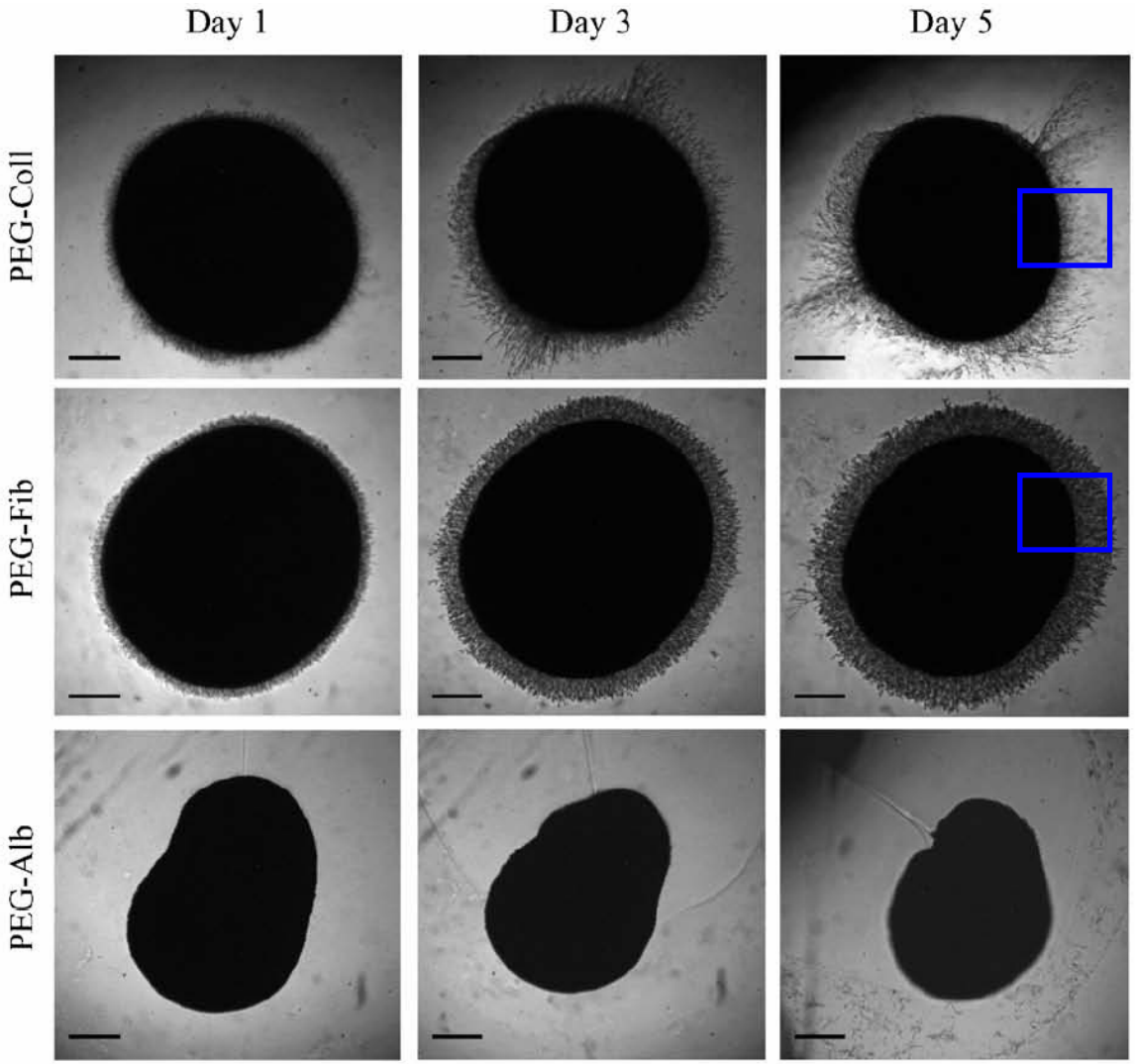
PEG-Albumin: no adhesive sites
⇨ no cell spreading

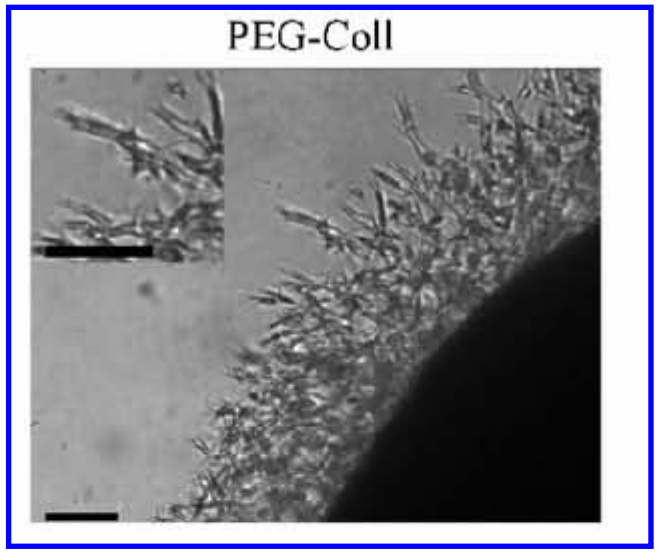
PEG-Collagen: adhesive sites
⇨ cell spreading

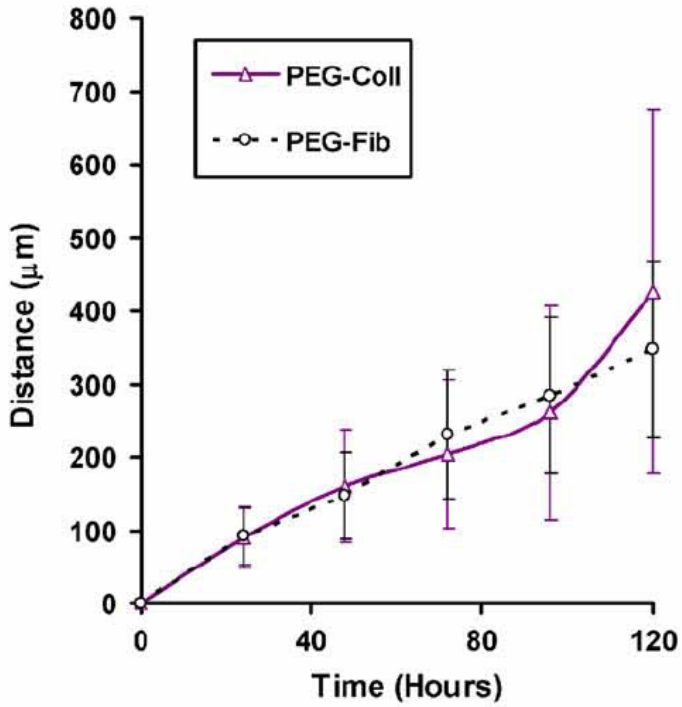
PEG-Fibrinogen: adhesive sites
⇨ cell spreading



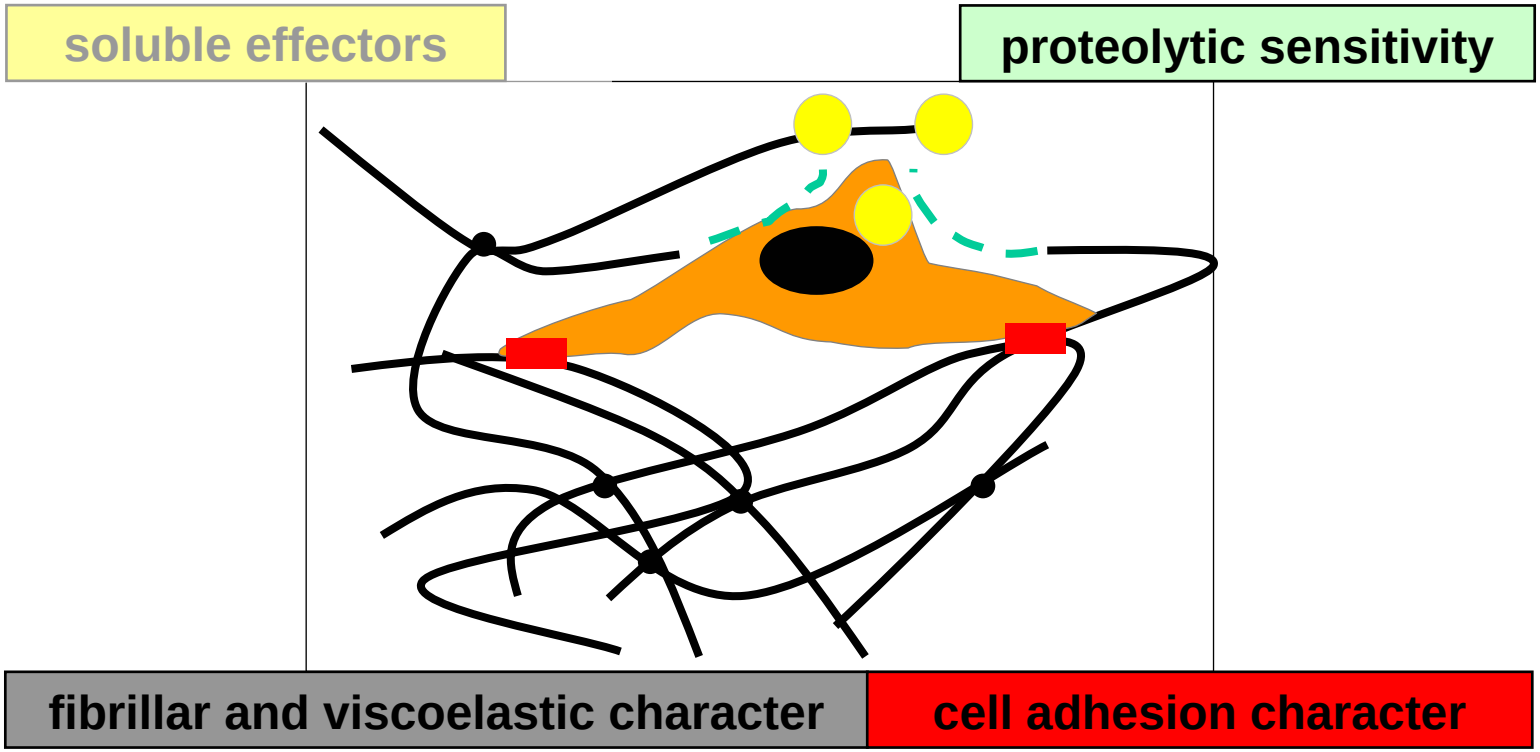
Cellular outgrowth experiments document smooth muscle cell (SMC) invasion into PEGylated protein hydrogels from a dense tissue construct (dark).



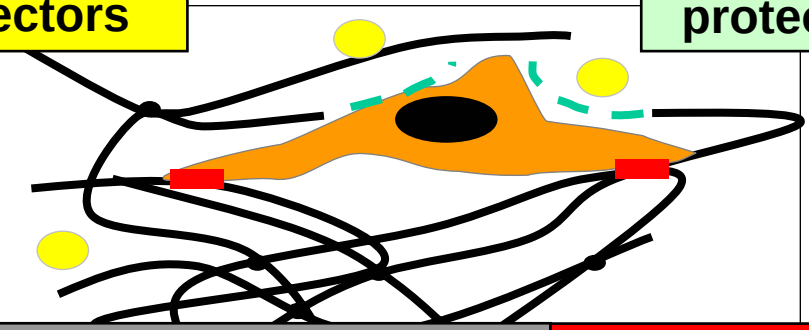




What kind of signals? - identification of important biofunctional building blocks



taking home:



translation to artificial matrices

⇨soluble effectors

⇨adhesion ligands

⇨structural scaffold: network characteristics

⇨enzymatic cleavable crosslinks

***taking home*:**

„simply polymers“...

- Polymer scaffolds for regenerative medicine have to provide **physical, chemical and biomolecular signals** for tissue formation.

- Often, scaffolds have to be **biodegradable *and* bioactive** in order to support the desired tissue organisation.

- Degradation and signalling of ideal scaffolds have to be readily adjusted to environmental (biological) demands which may **vary during time**.

***taking home*:**

Polymers for Regenerative Medicine

Regenerative medicine and tissue engineering promise more **permanent solutions for many frequent diseases** and are currently moving from science fiction to the clinics.