

**Regeneration may  
greatly benefit of biomaterials**

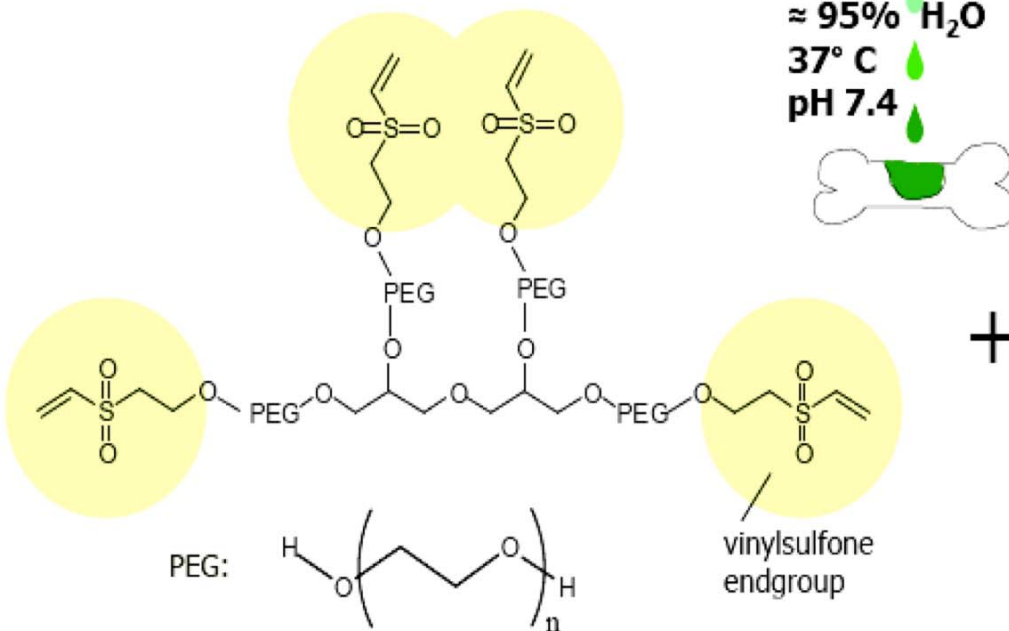
# Synthetic building blocks cross-linked by a mild addition reaction into cell-responsive 3-D gel networks

## Poly(ethylene glycol)

- hydrophilic, „biocompatible“ (inert)
- cross-linker (functionality > 2)
- structural properties

## Oligopeptides/proteins

- „biological information“
- cell-responsiveness (protease sensitivity)



≈ 95% H<sub>2</sub>O  
 37° C  
 pH 7.4

+

SH  
|  
C adhesion ligand

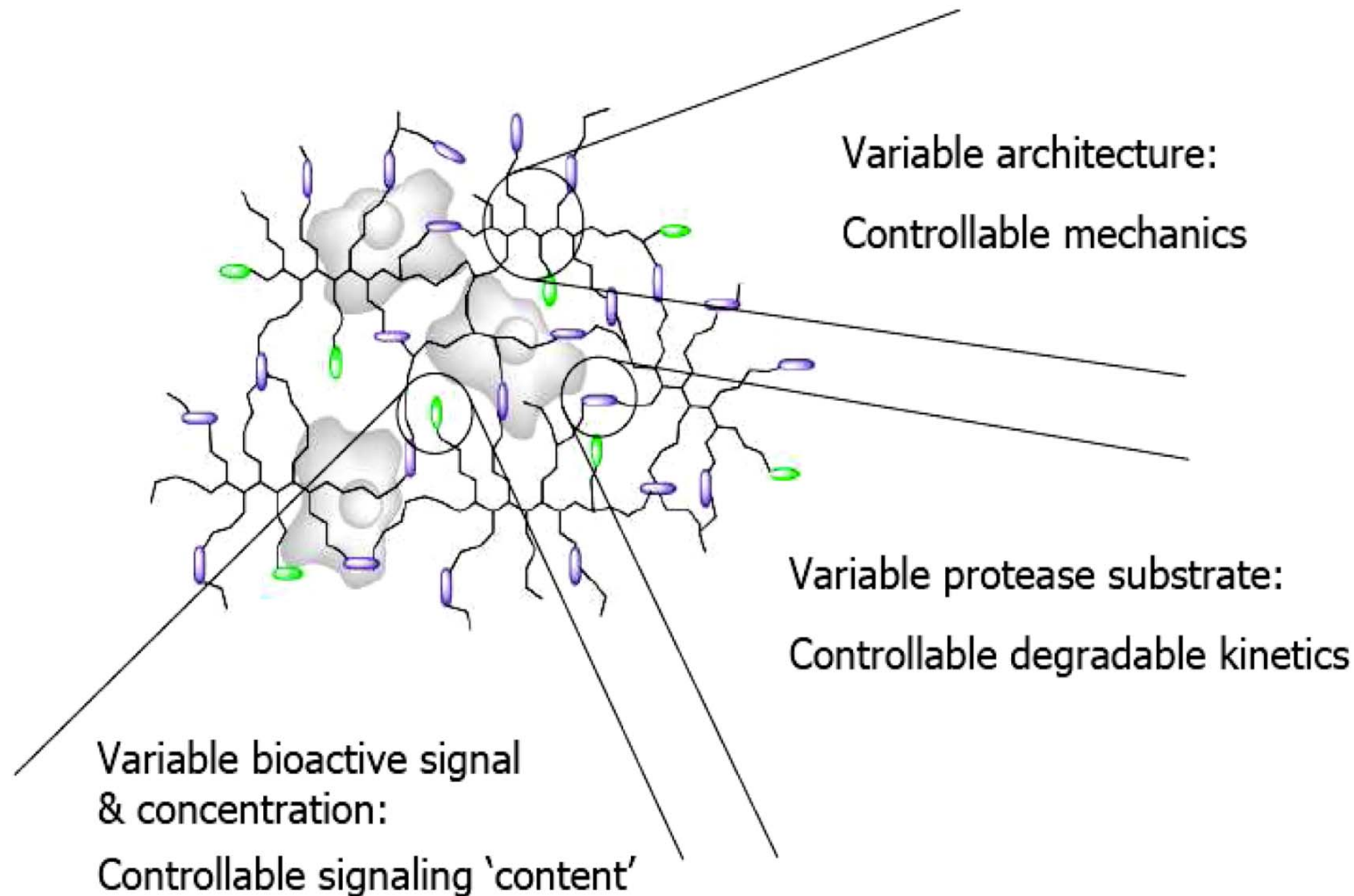
e.g. Cys-RGDSP

e.g. Cys-GPQG↓IWGQ-Cys

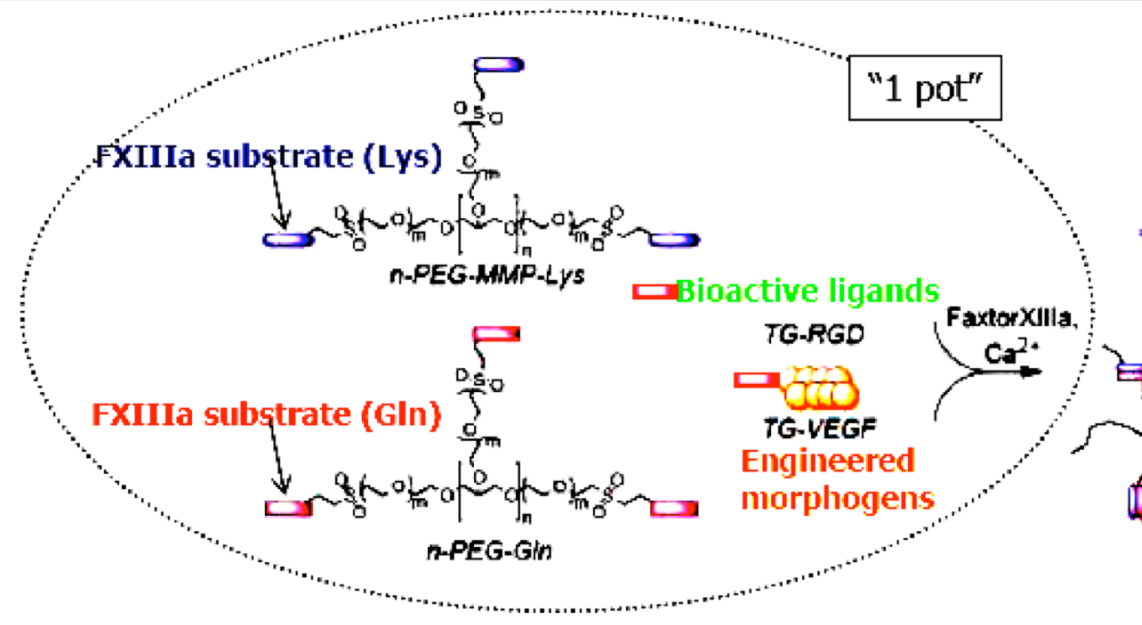
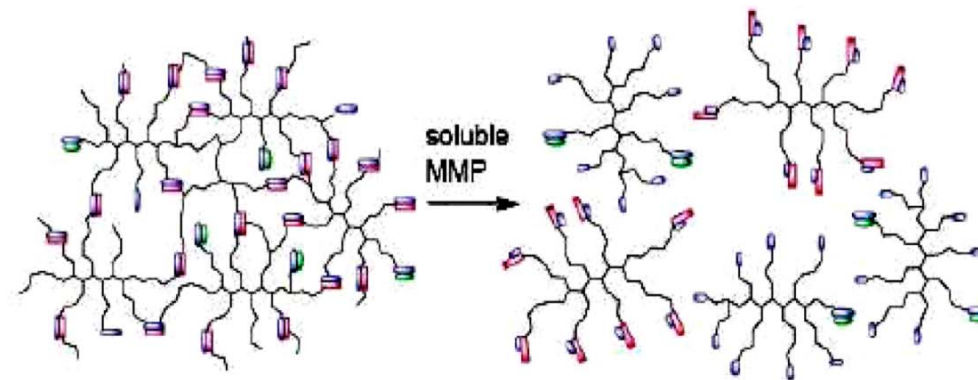


# Molecular toolbox to engineer cell-matrix interactions

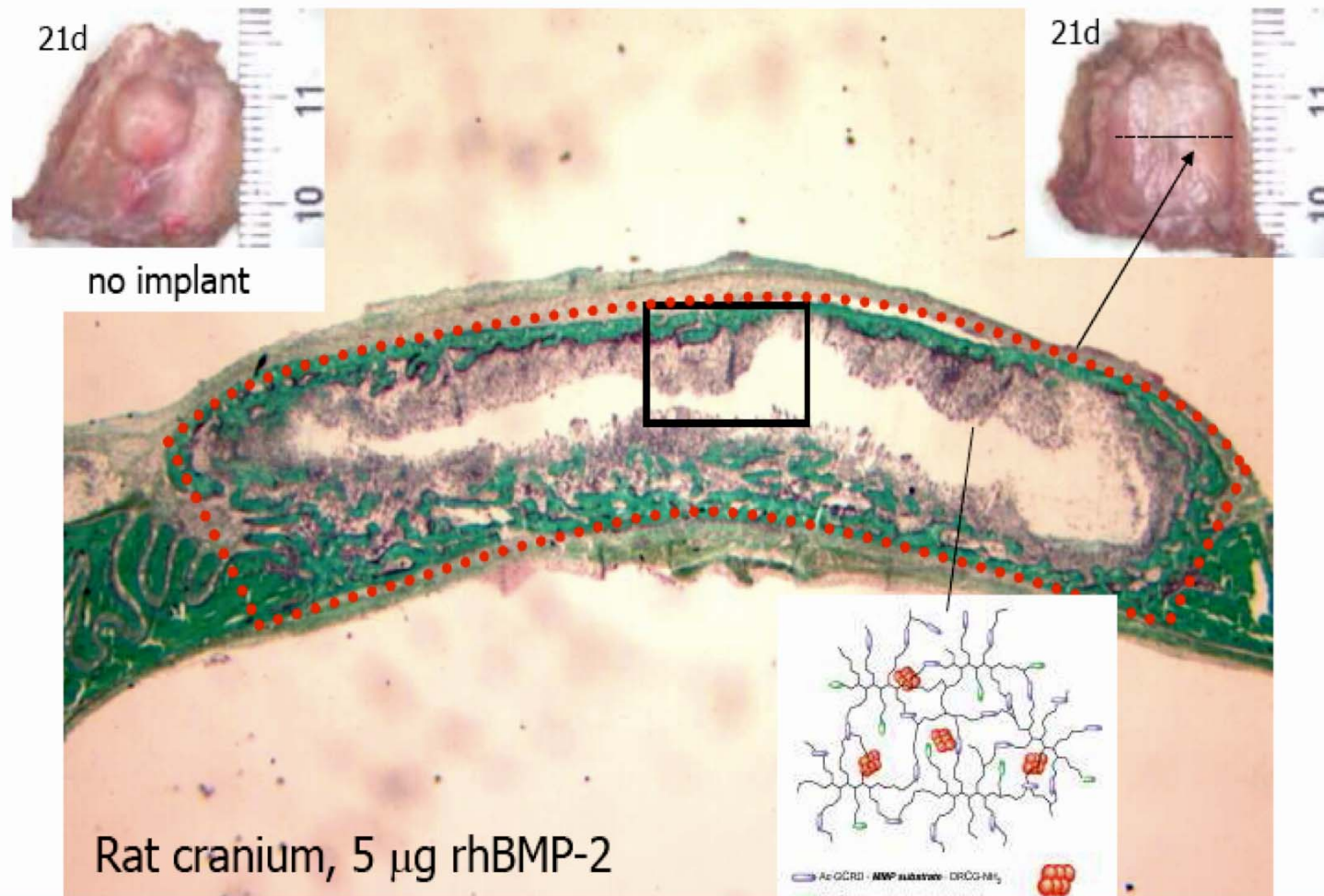
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# Gels can be rendered susceptible to proteolytic degradation by MMPs or other proteases

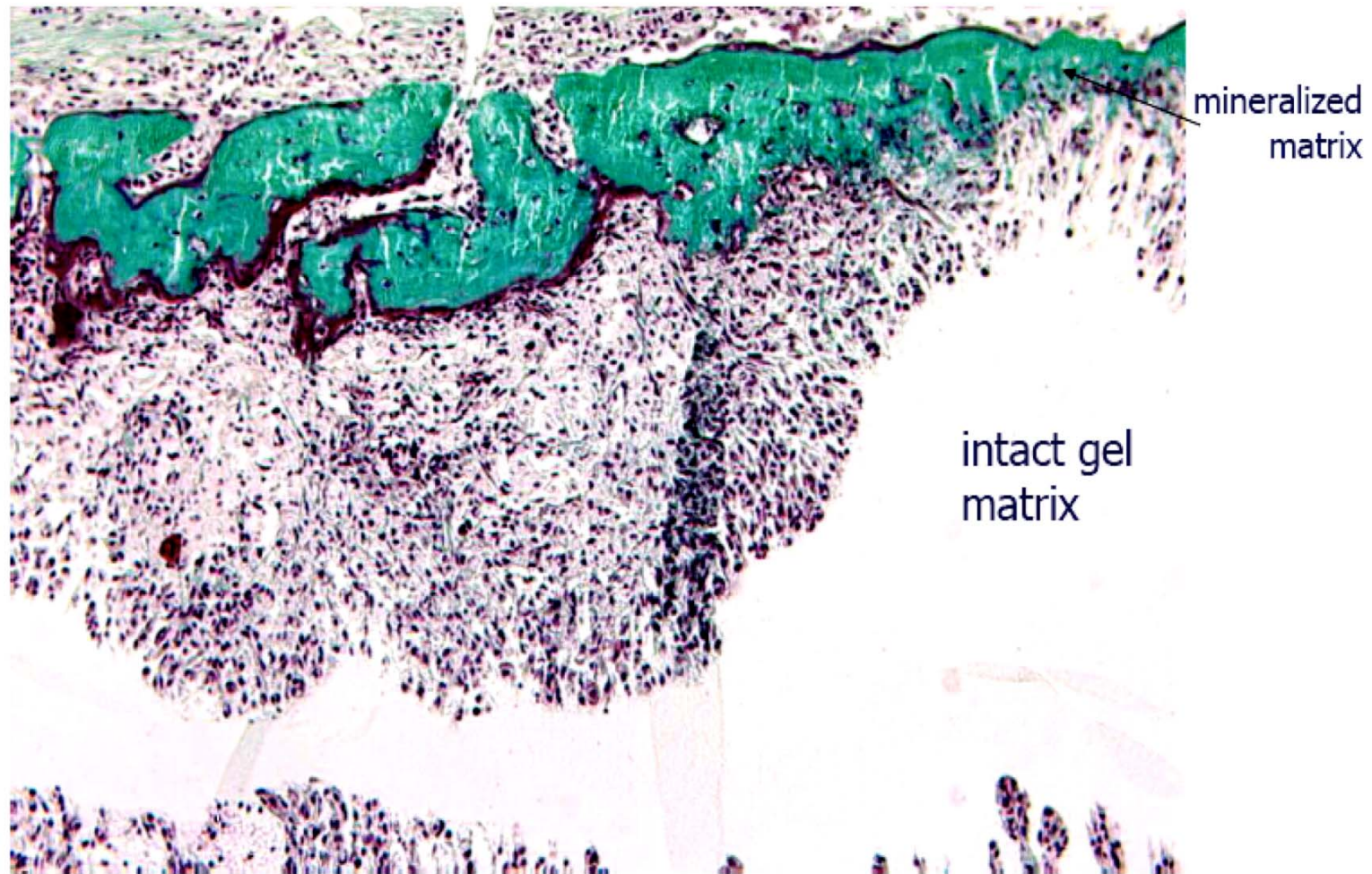


# Endogenous progenitor cells invade gels and regenerate bone by remodeling gels (3wks)

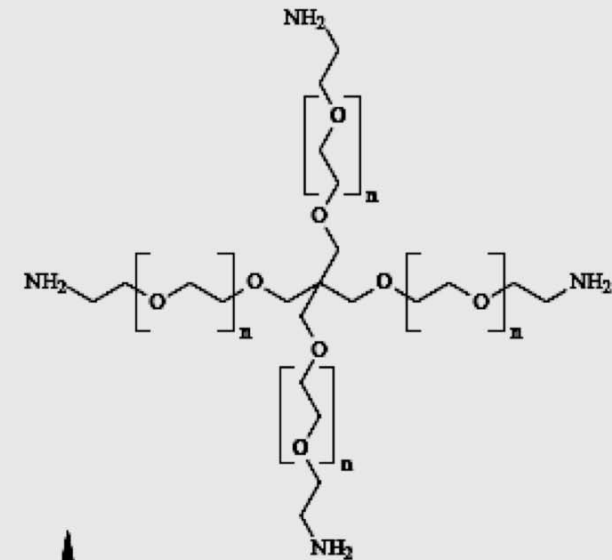
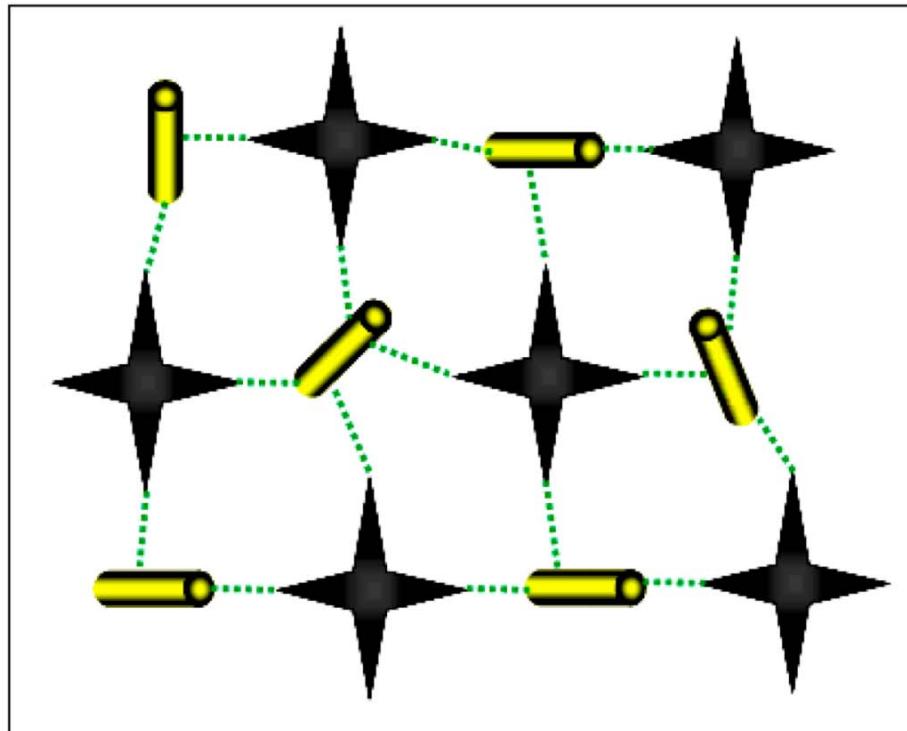


## Endogenous progenitor cells invade gels and regenerate bone by remodeling gels (3wks)

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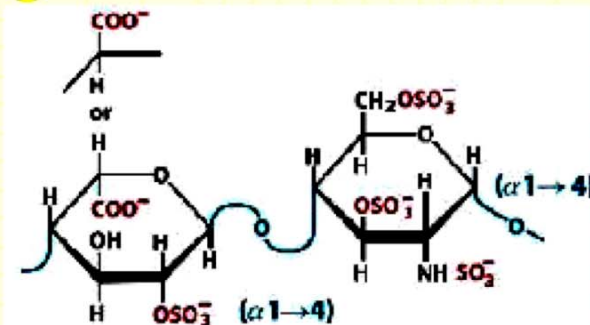
modular **biohybrid network** of  
end-functionalized, **multi-armed**  
(,star`)-PEG and **heparin**



multi-armed amino-  
(end)-functionalized  
PEG

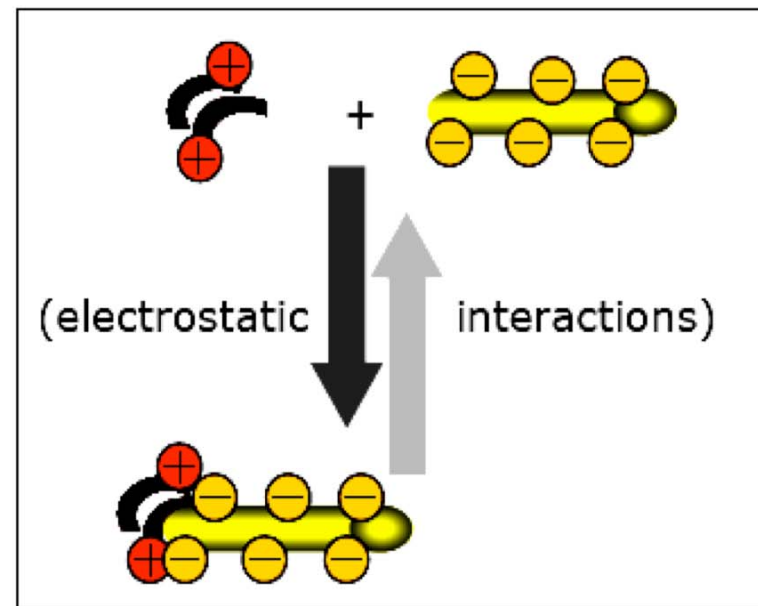
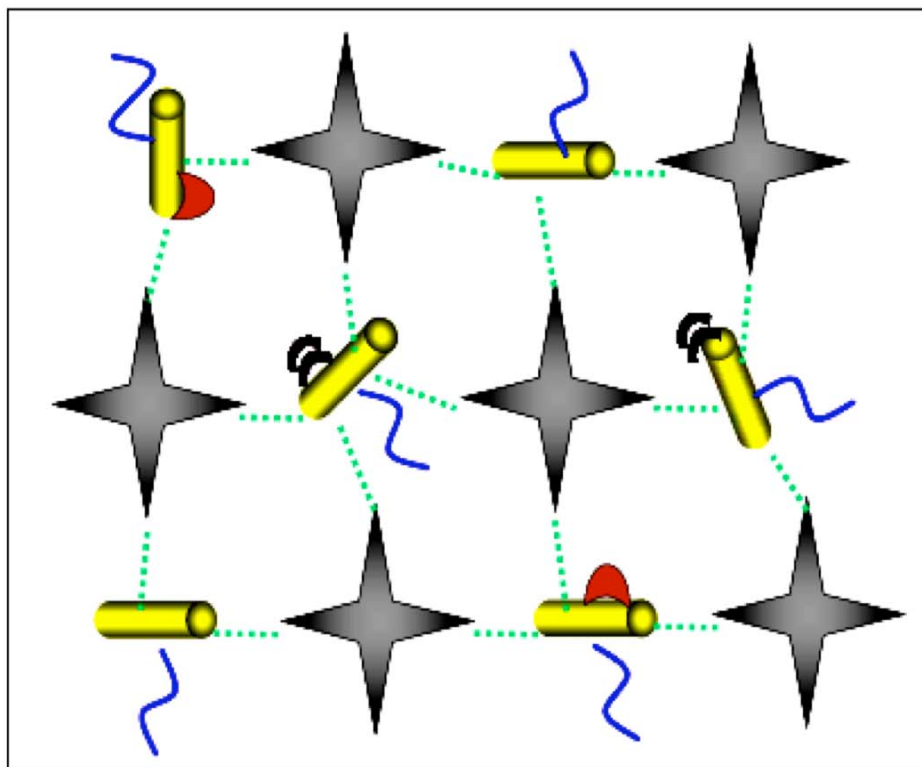


heparin



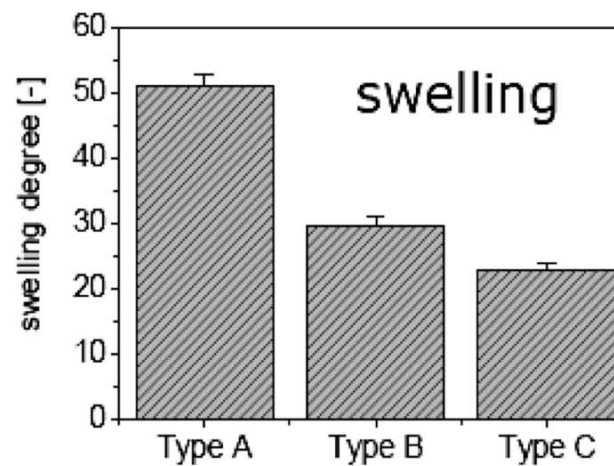
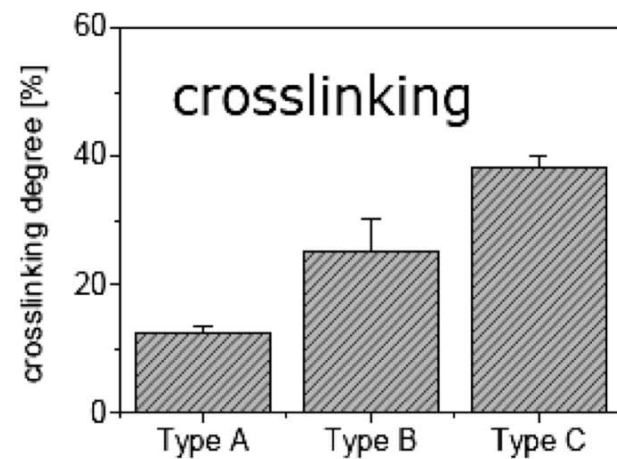
## biofunctionalization:

- **peptide-based crosslinker**
- **reactive conversion of heparin**
- **loading of soluble factors**

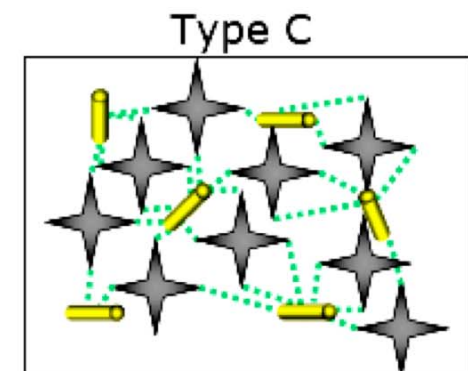
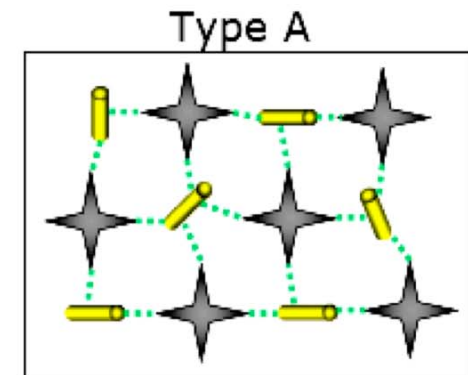
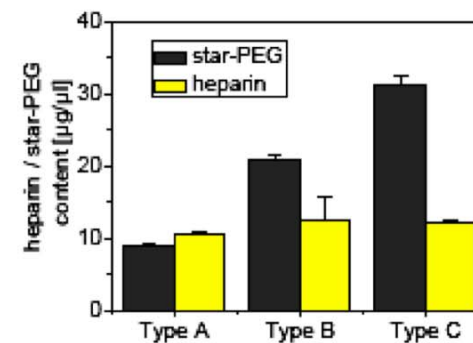


➡ incorporation of various heparin-binding factors (reversible, presentation comparable to natural ECM)

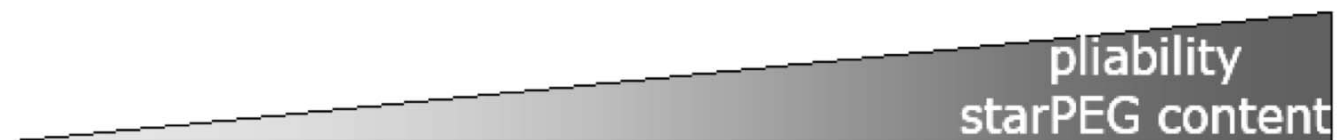
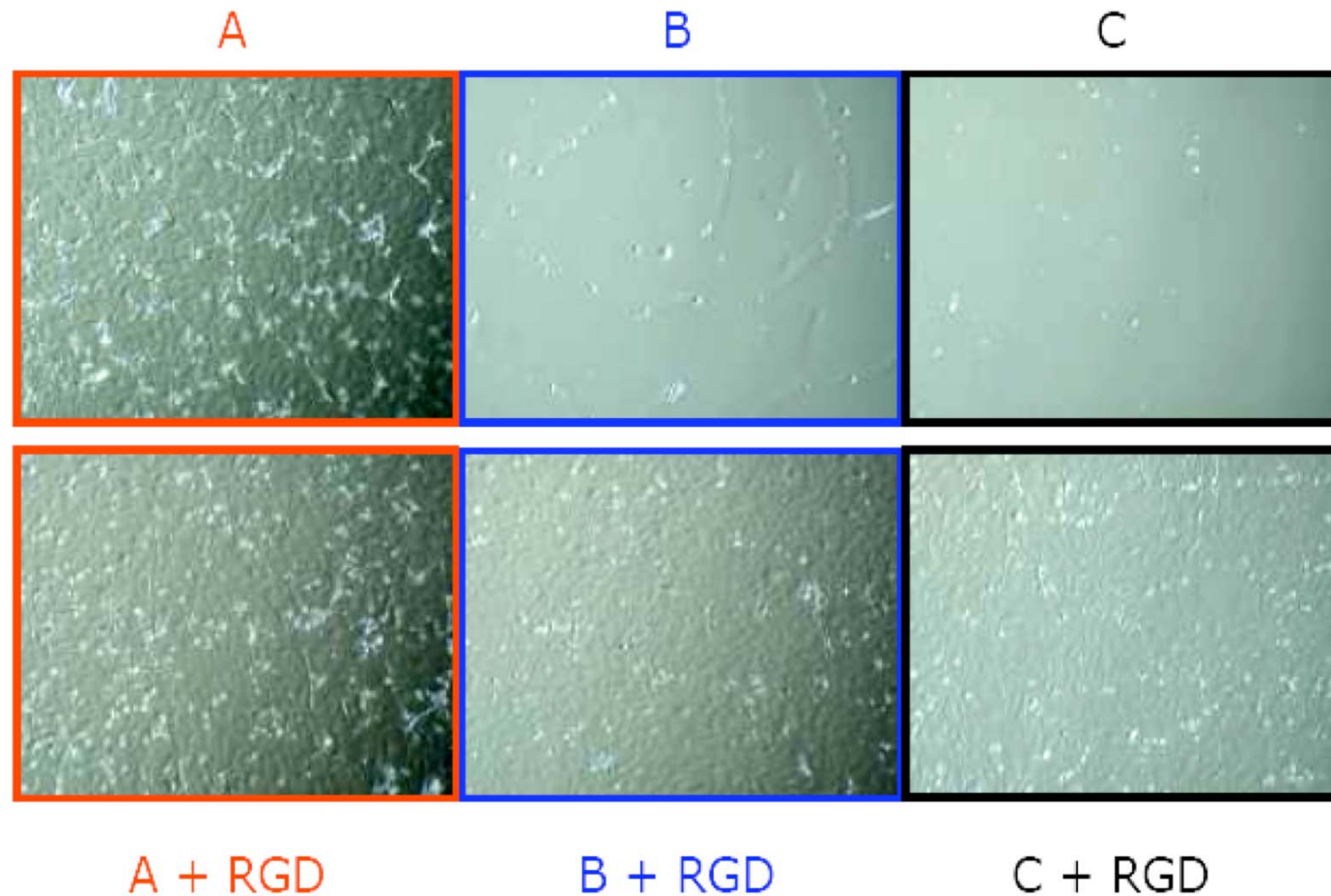
## tunable material properties: **crosslinking degree**



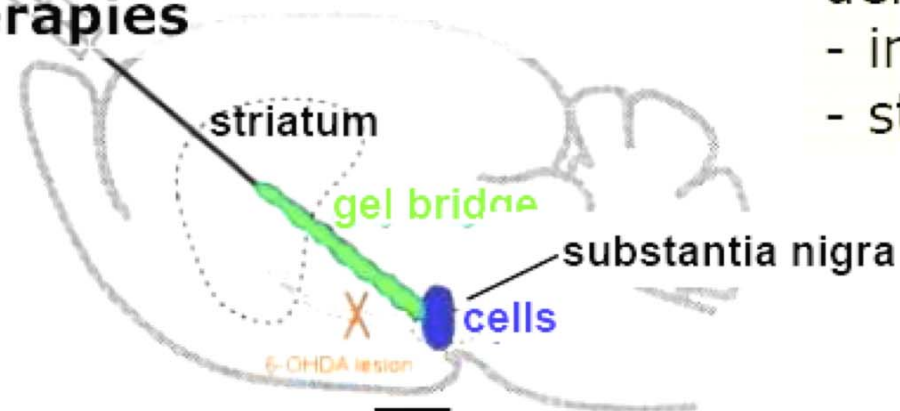
- different degree of crosslinking (varied starPEG incorporation)
- constant heparin content



dissecting the role of **mechanical characteristics** and integrin **ligands** in the **adhesion of endothelial cells** (HUVEC; after 5 days culture)

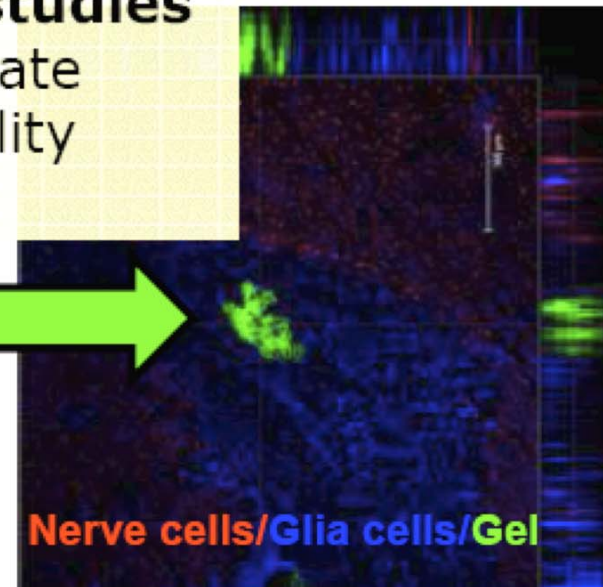


## implantation studies to explore cell replacement therapies



## *ex vivo* studies

- demonstrate
- injectibility
- stability



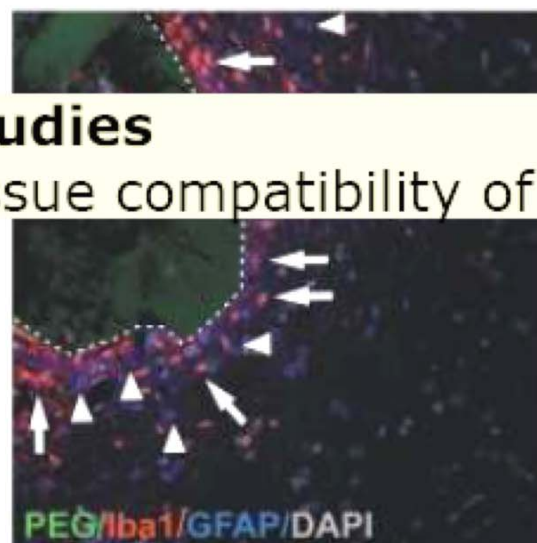
3 days

7 days

21 days

## *in vivo* studies

confirm tissue compatibility of gel materials



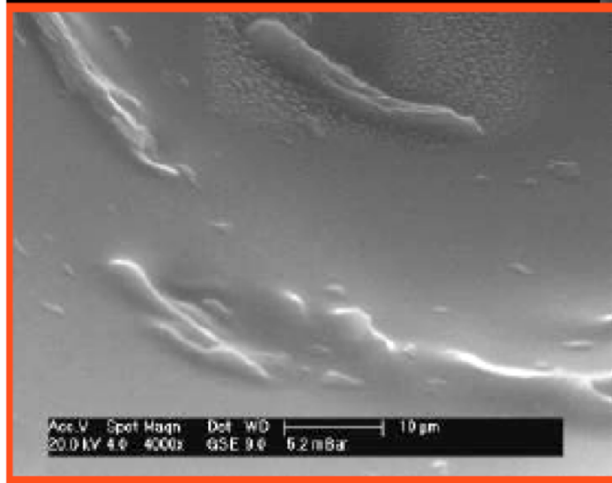
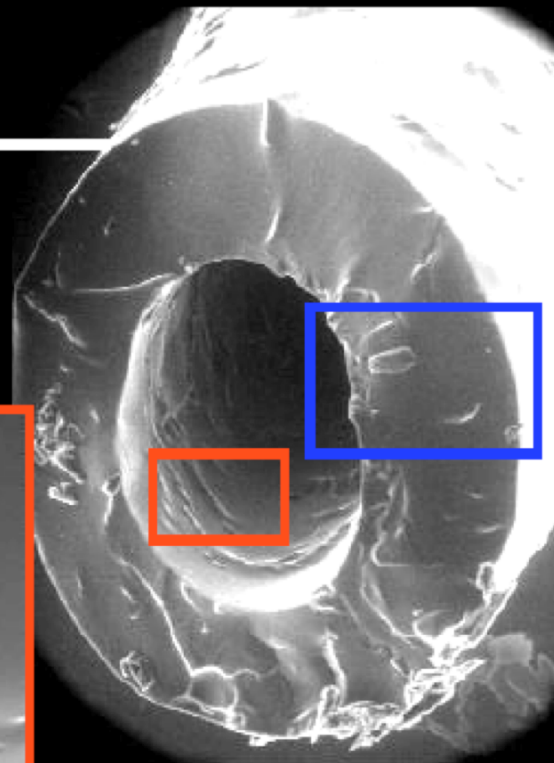
## processing into various shapes

example: tubular gel-bodies

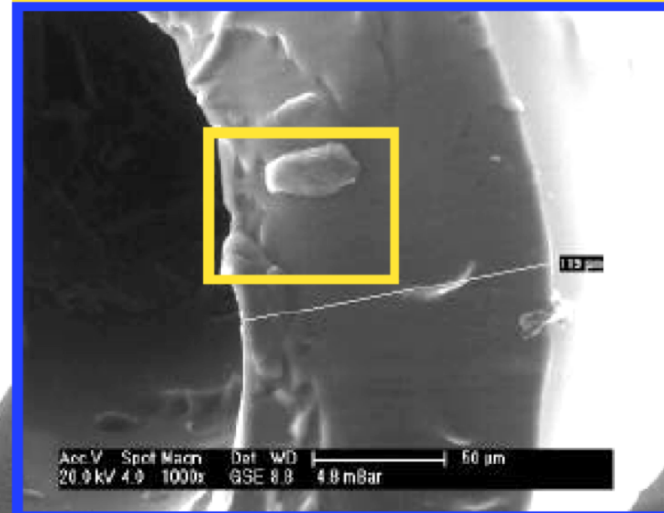
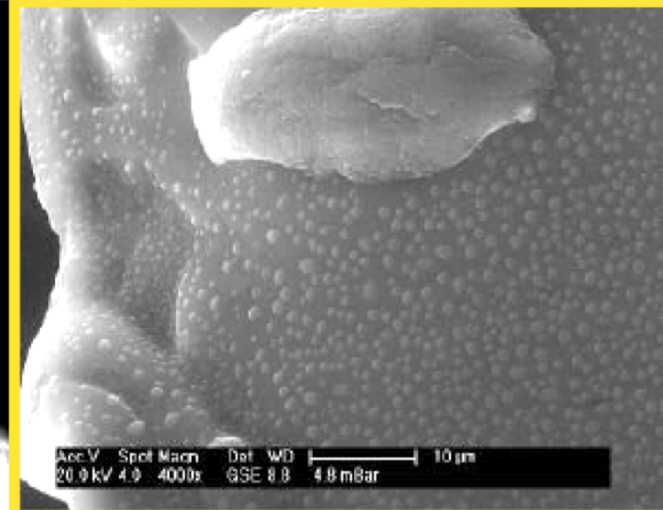
-length: up to 6 cm

-inner diameter (swollen): 300-600  $\mu\text{m}$

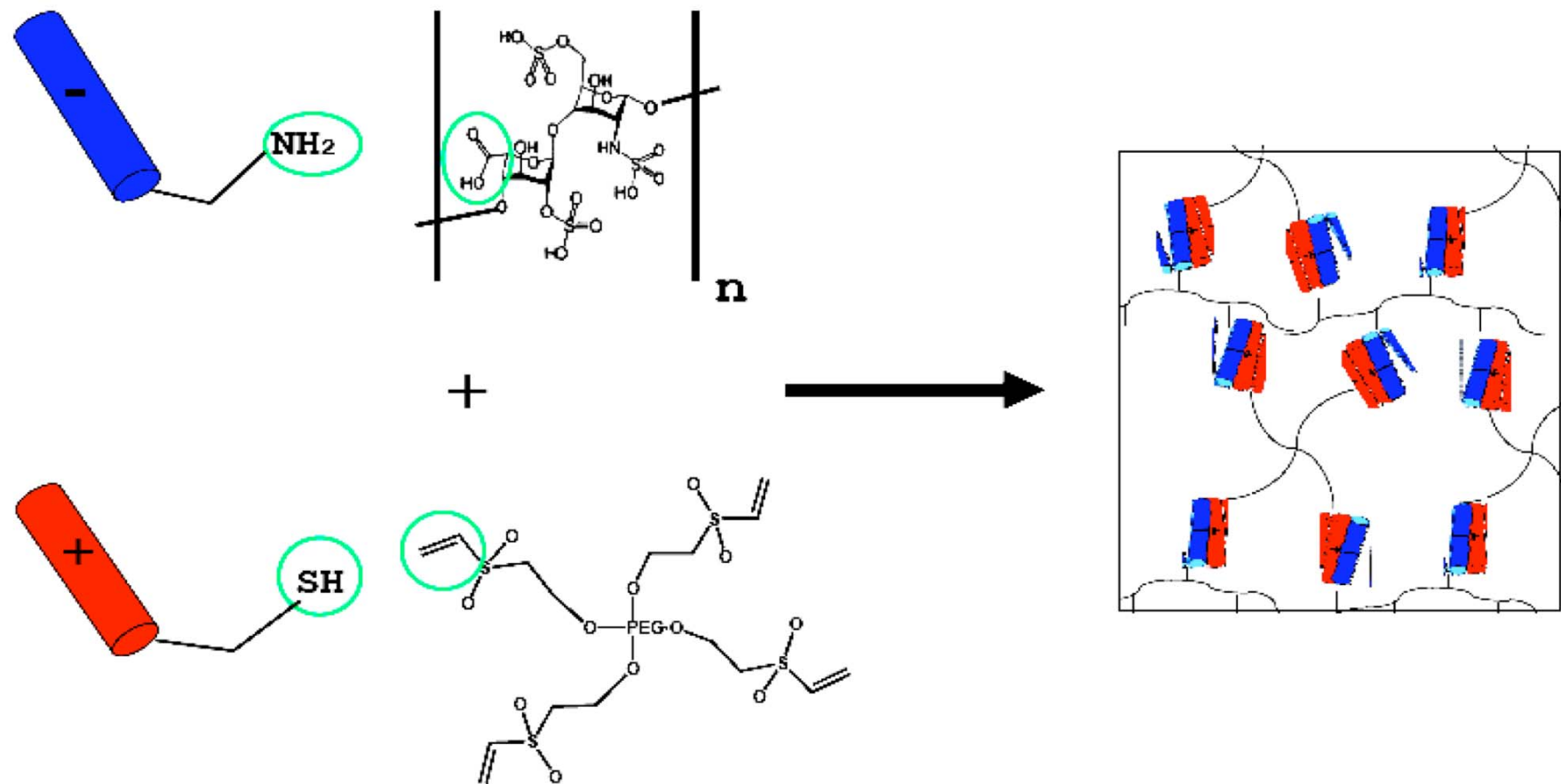
-pore size: 10-20 nm



Det WD |-----| 200  $\mu\text{m}$   
GSE 9.0 4.5 mBar

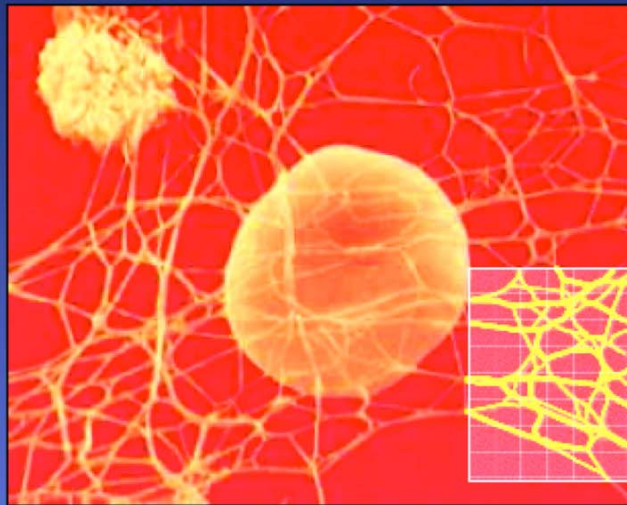


## *in situ* crosslinking: leucine zipper

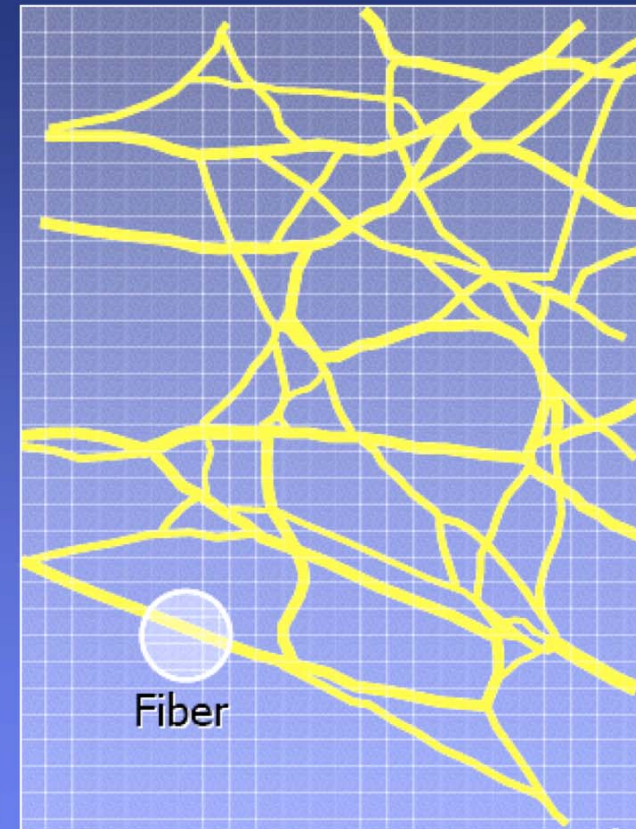
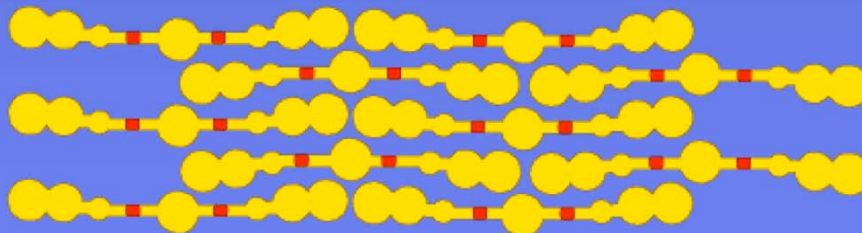


# The Blood Clot Fibrin Network

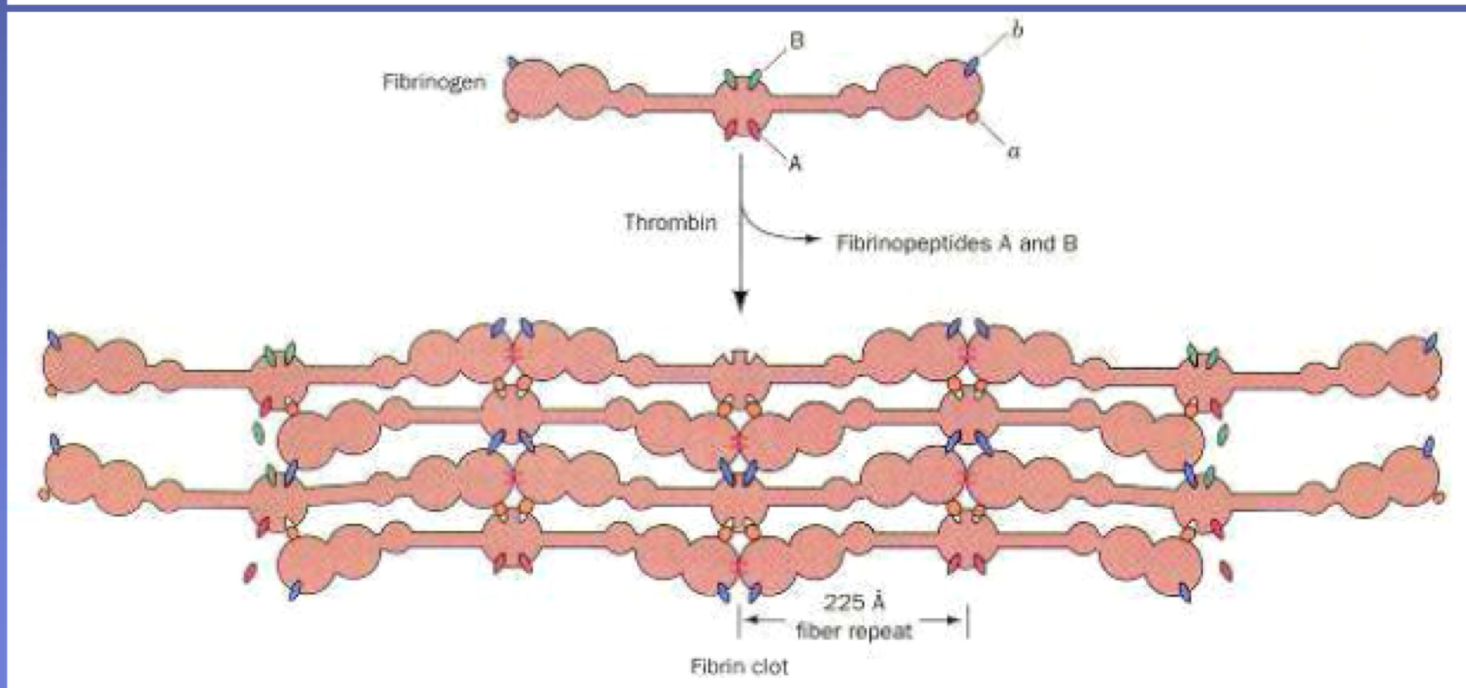
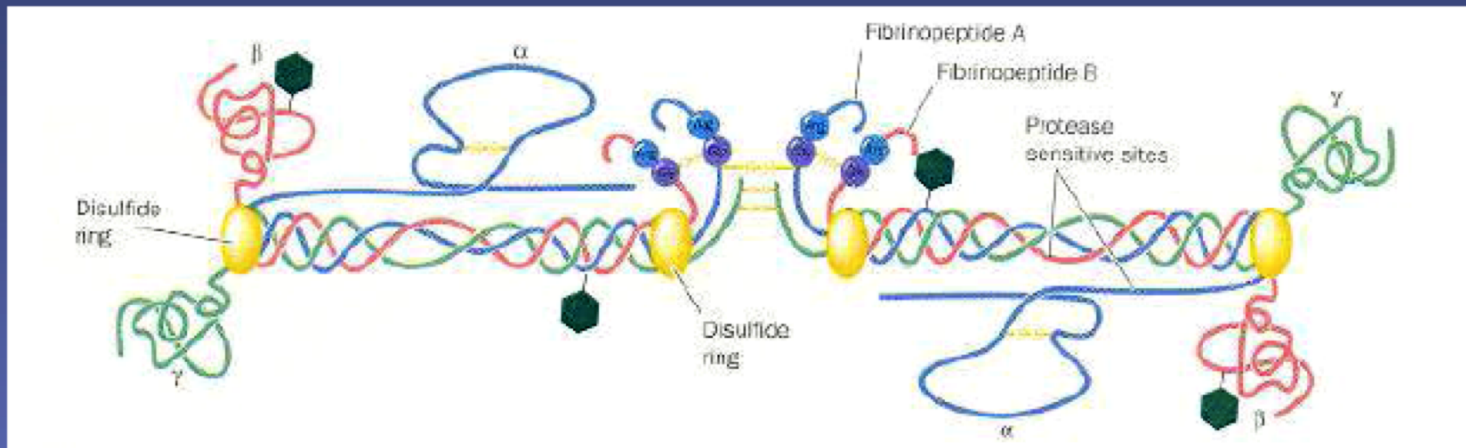
## A Natural Healing Substrate



Fibrin Polymer

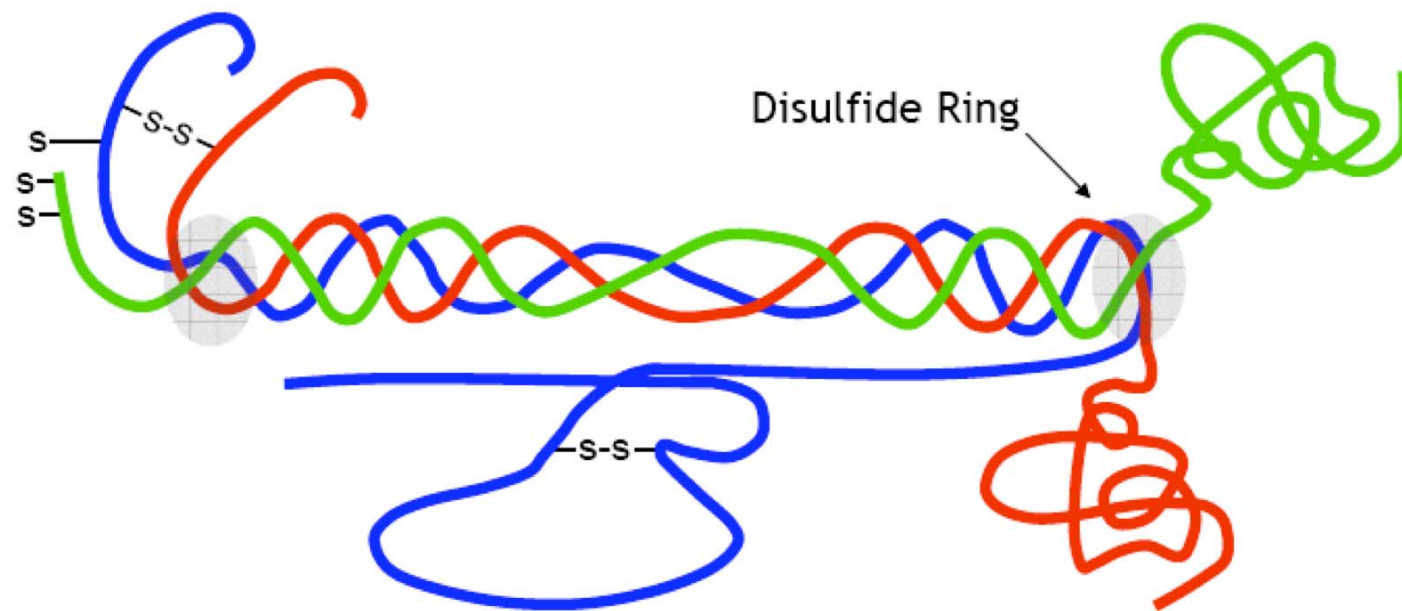


# Natural Fibrin(ogen)

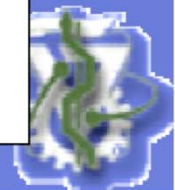


# Making a Biosynthetic Material

## Part 1: Purified Fibrinogen Molecule

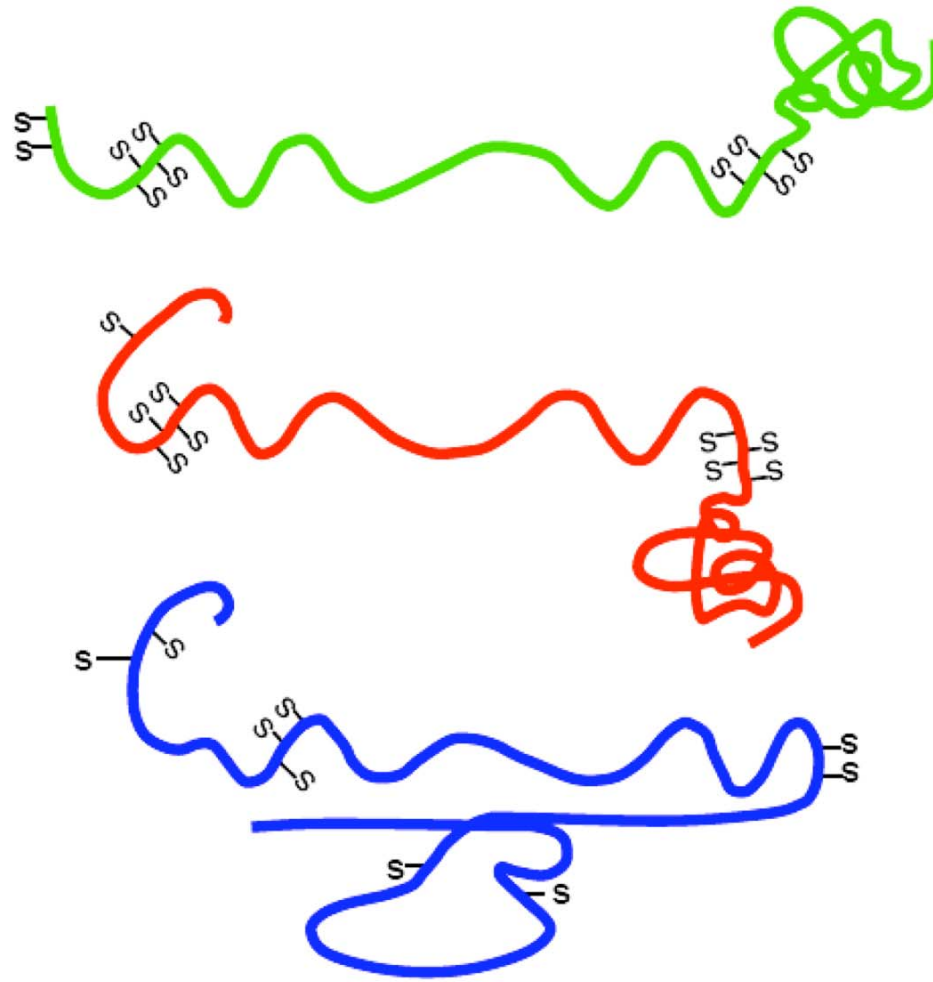


Seliktar D., *Ann. N.Y. Acad. Sci.*, 2005; 1047: 386–394.

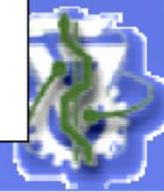


# Making a Biosynthetic Material

## Part 2: Denatured Fibrinogen Molecule

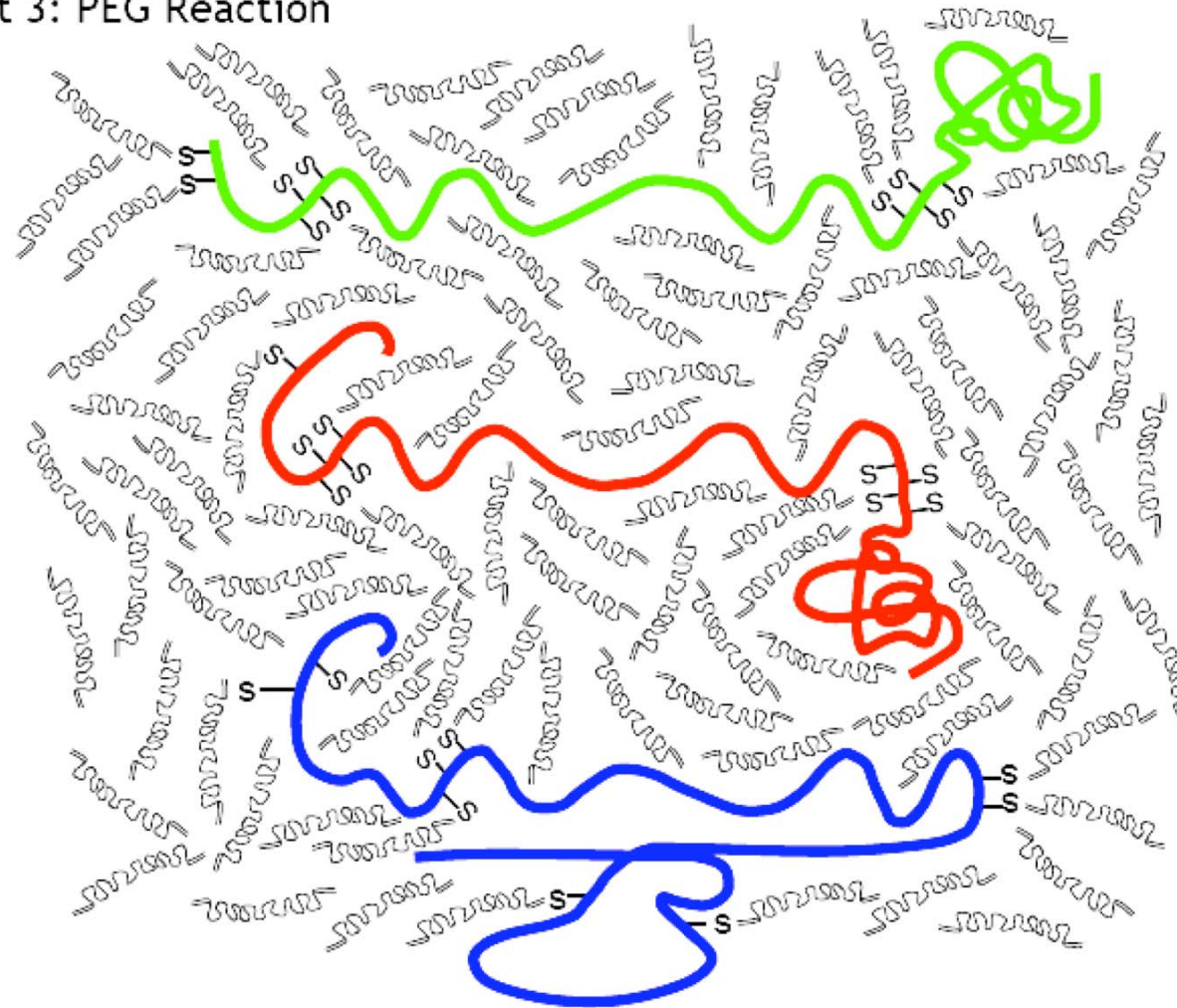


Seliktar D., *Ann. N.Y. Acad. Sci.*, 2005; 1047: 386–394.

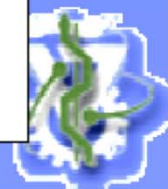


# Making a Biosynthetic Material

## Part 3: PEG Reaction

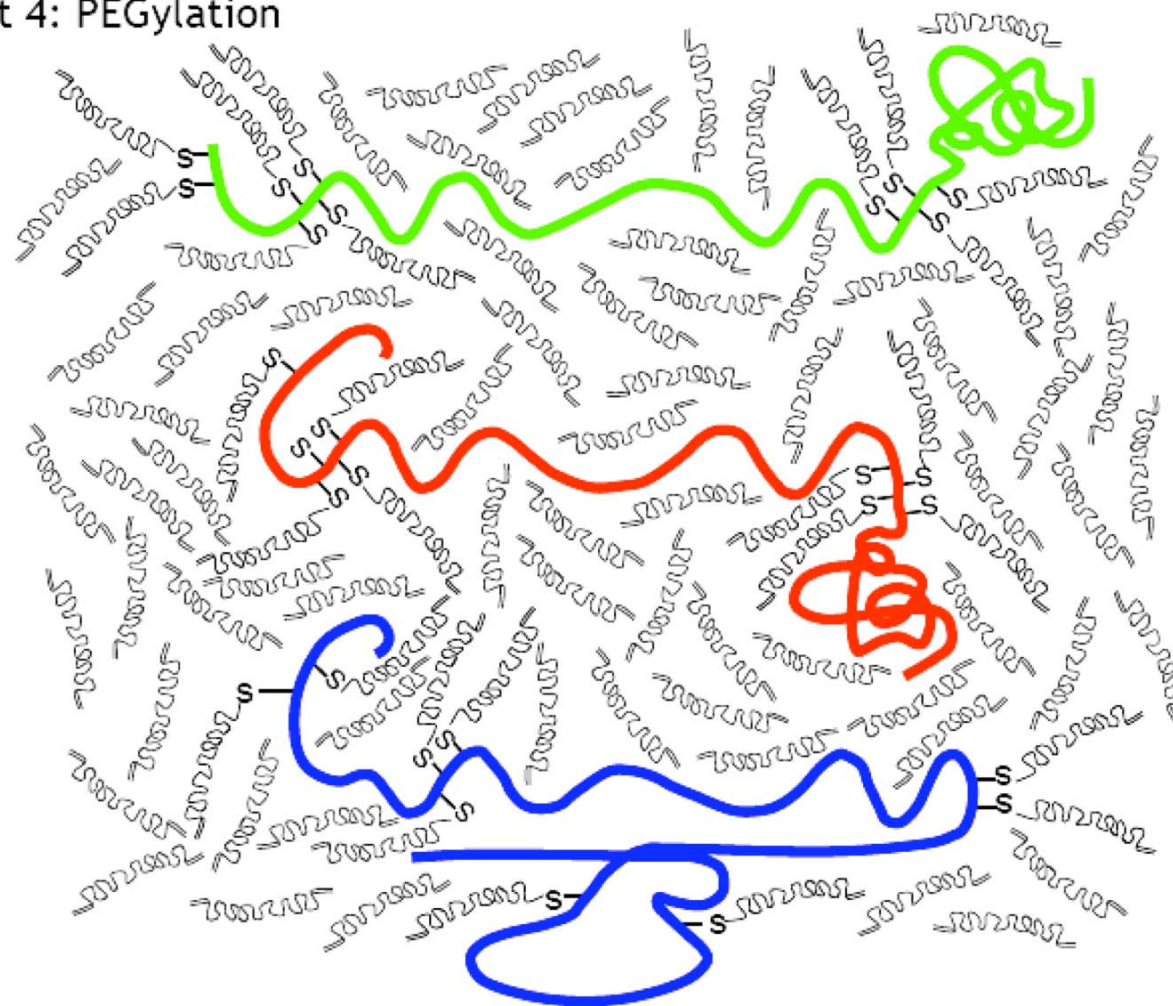


Seliktar D., *Ann. N.Y. Acad. Sci.*, 2005; 1047: 386–394.



# Making a Biosynthetic Material

## Part 4: PEGylation

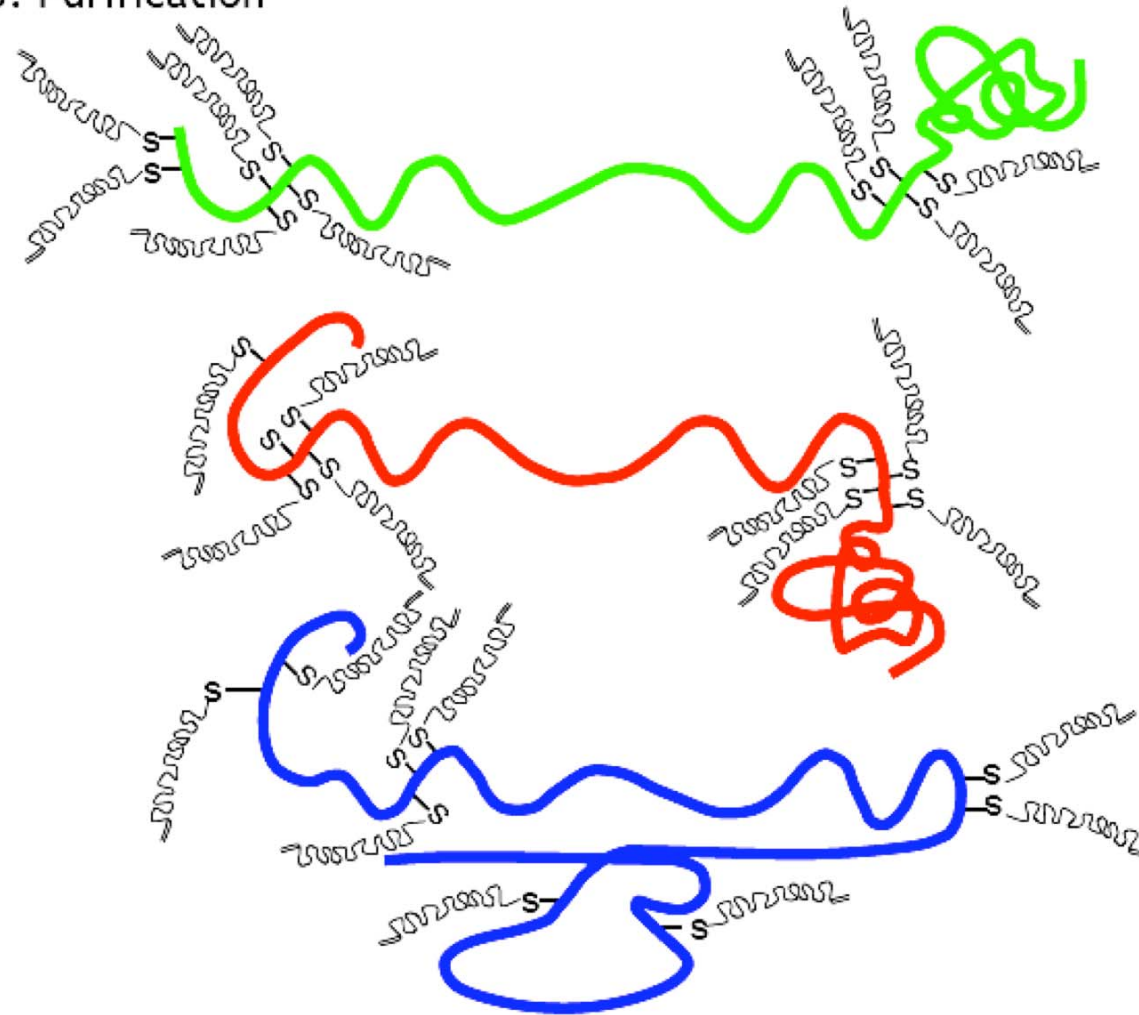


Seliktar D., *Ann. N.Y. Acad. Sci.*, 2005; 1047: 386–394.



# Making a Biosynthetic Material

## Part 5: Purification

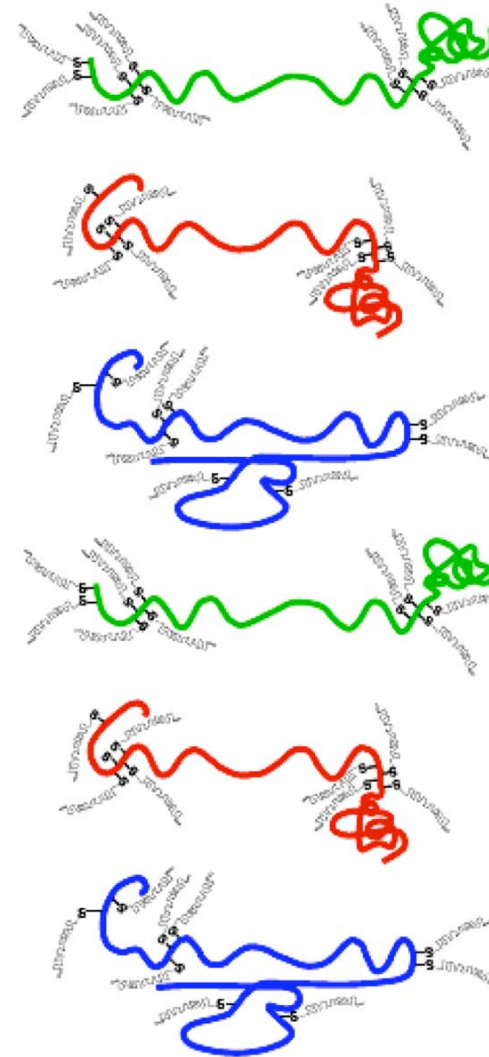
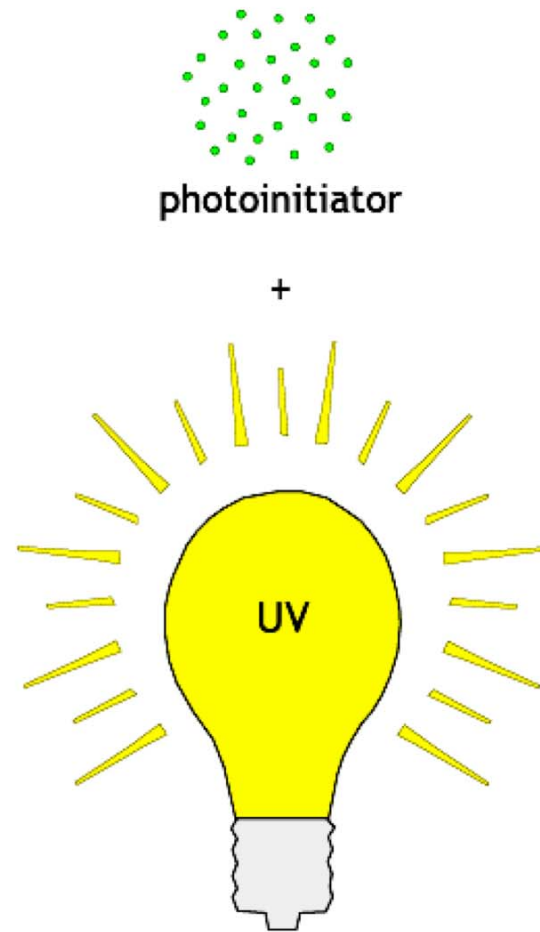


Seliktar D., *Ann. N.Y. Acad. Sci.*, 2005; 1047: 386–394.



# Making a Biosynthetic Material

## Part 6: Photoinitiation

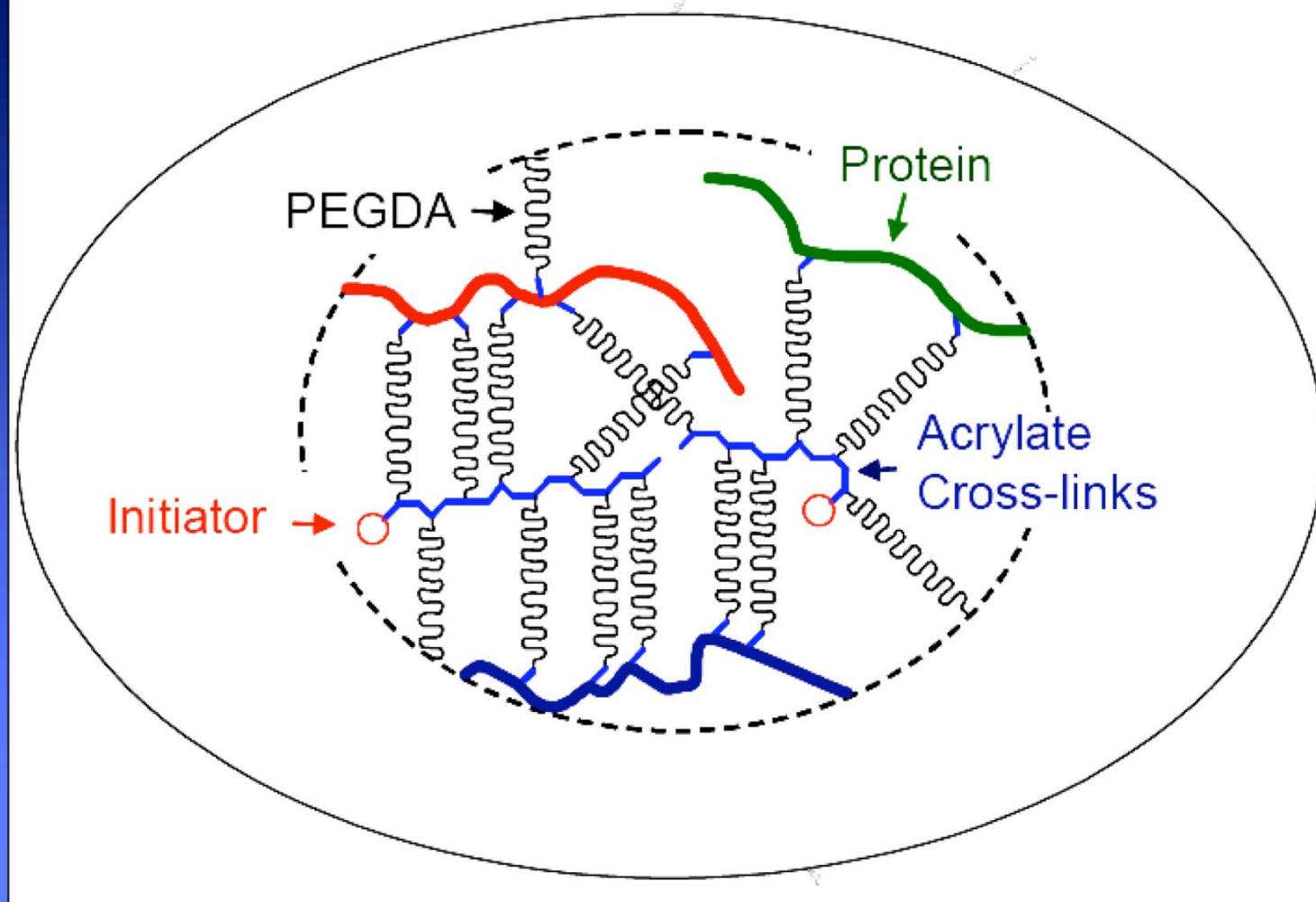


Seliktar D., *Ann. N.Y. Acad. Sci.*, 2005; 1047: 386–394.



# Making a Biosynthetic Material

## Part 7: Hydrogel Formation



Seliktar D., *Ann. N.Y. Acad. Sci.*, 2005; 1047: 386–394.

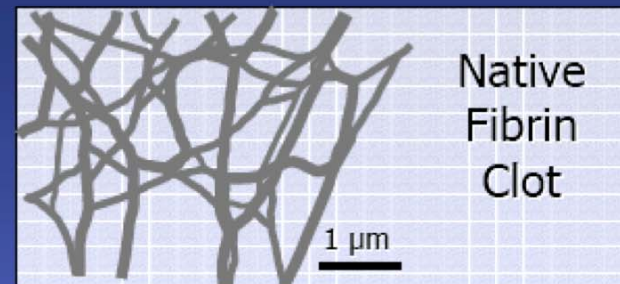


# PEGylated Fibrinogen Hydrogels

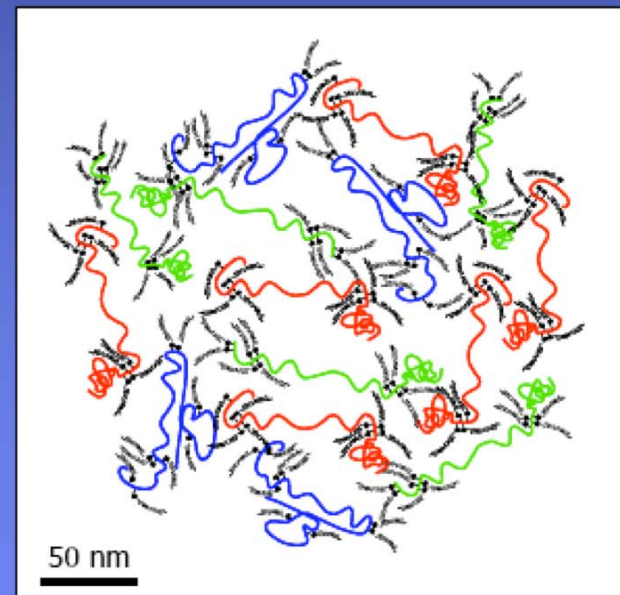


## Hydrogels Advantages

- PEG-fb Pore Size: 10-50 nm
- PEGylated protein less degradable
- Limited enzyme diffusion
- Surface erosion degradation



## PEG-Fb Molecular Structure

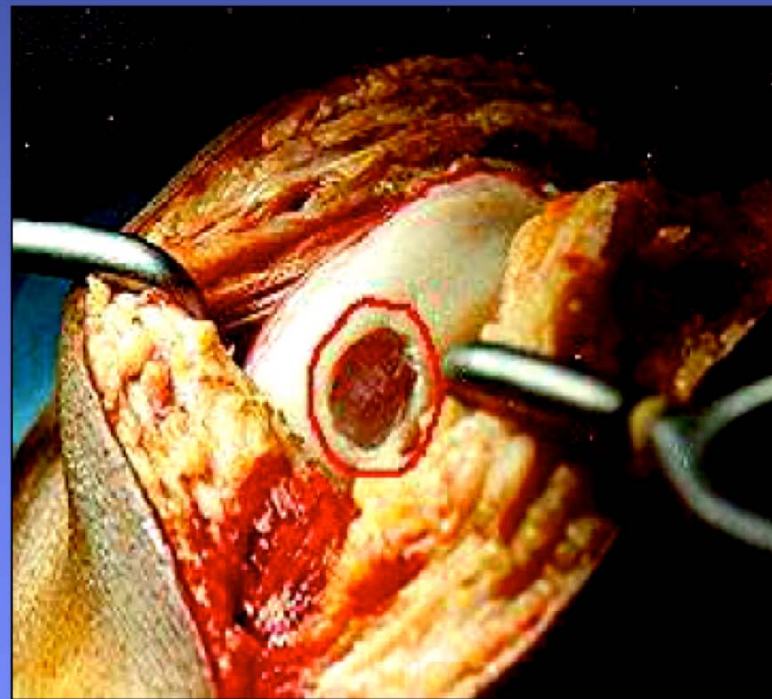
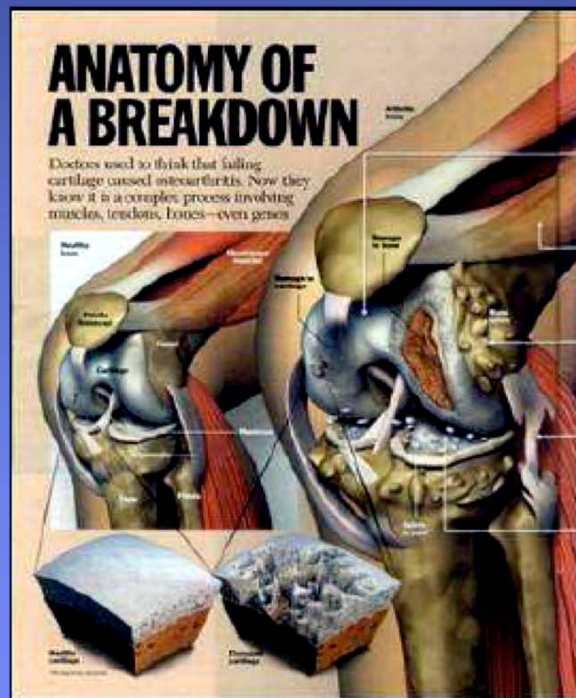


Dikovsky D, Bianco-Peled H, Seliktar D., *Biomaterials*. 2006 Mar;27(8):1496-506.

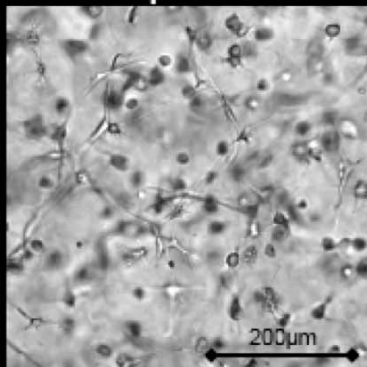


# The Clinical Problem

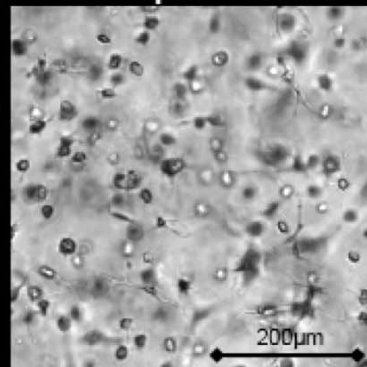
- Cartilage lesions and major cartilage loss from trauma and injury
- Engineered biomaterials can serve as an orthobiologic matrix (acellular) for articular cartilage regeneration



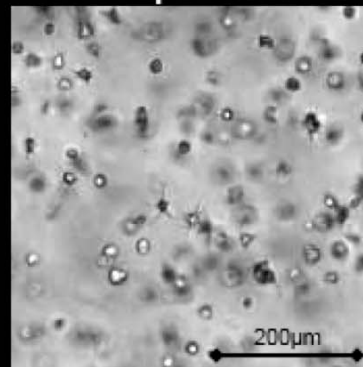
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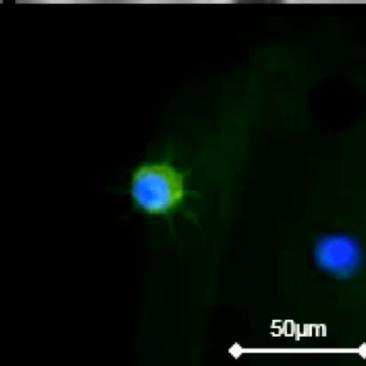
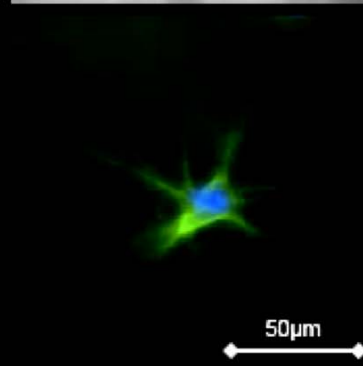
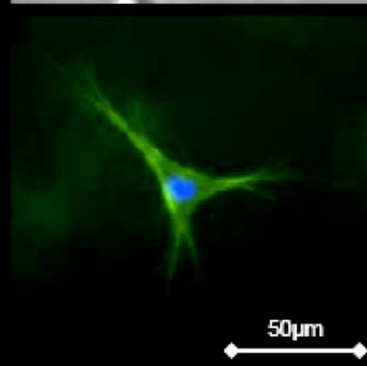
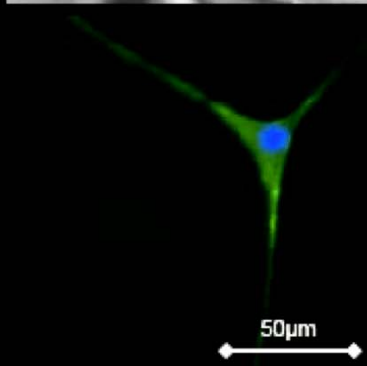
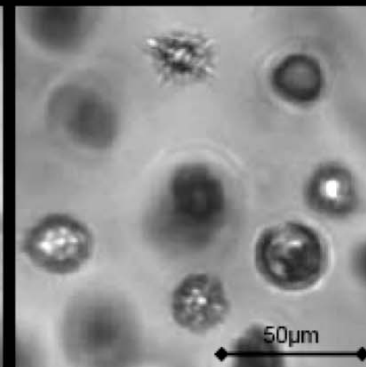
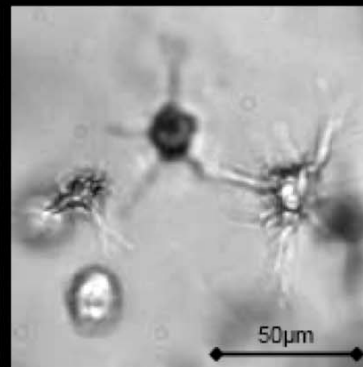
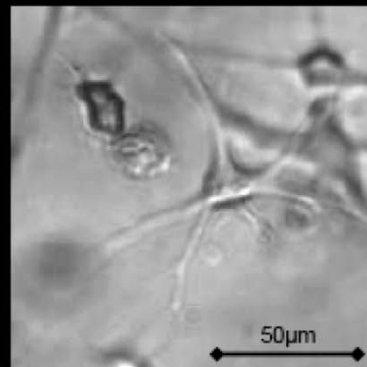
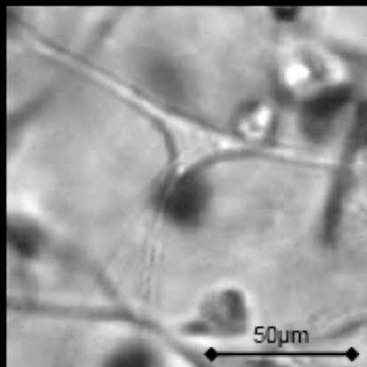
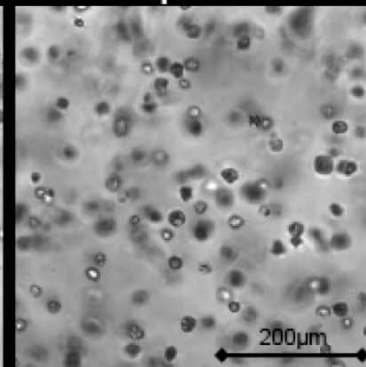
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C: shape index=2

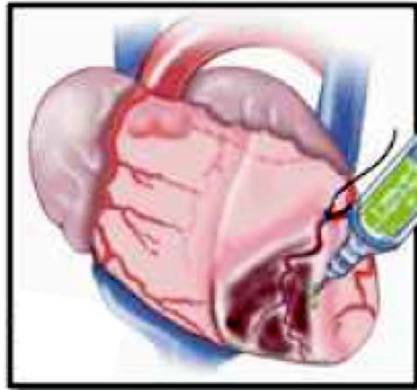


D: shape index=1





## EMBRYONIC STEM CELL THERAPY



Adult stem cells

Stem cells help  
regenerate  
damaged  
heart muscle.

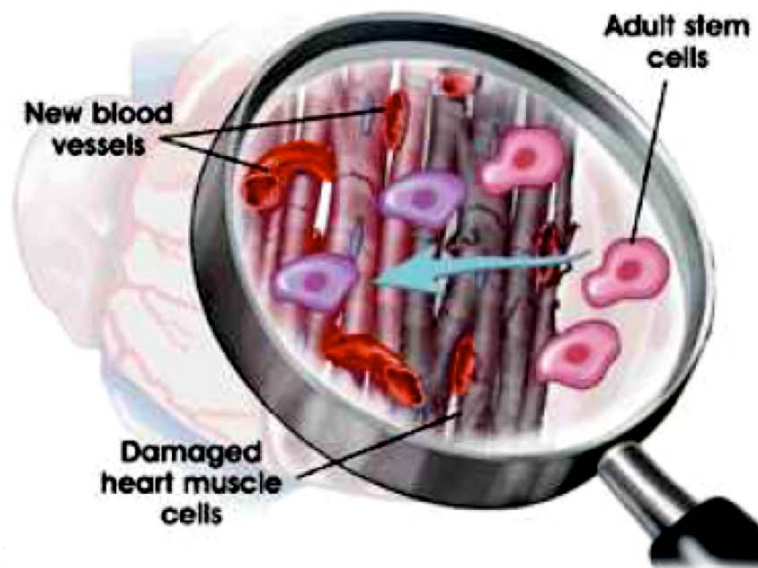
Damaged  
heart muscle  
cells

rese Winslow, Lydia Kibiuk

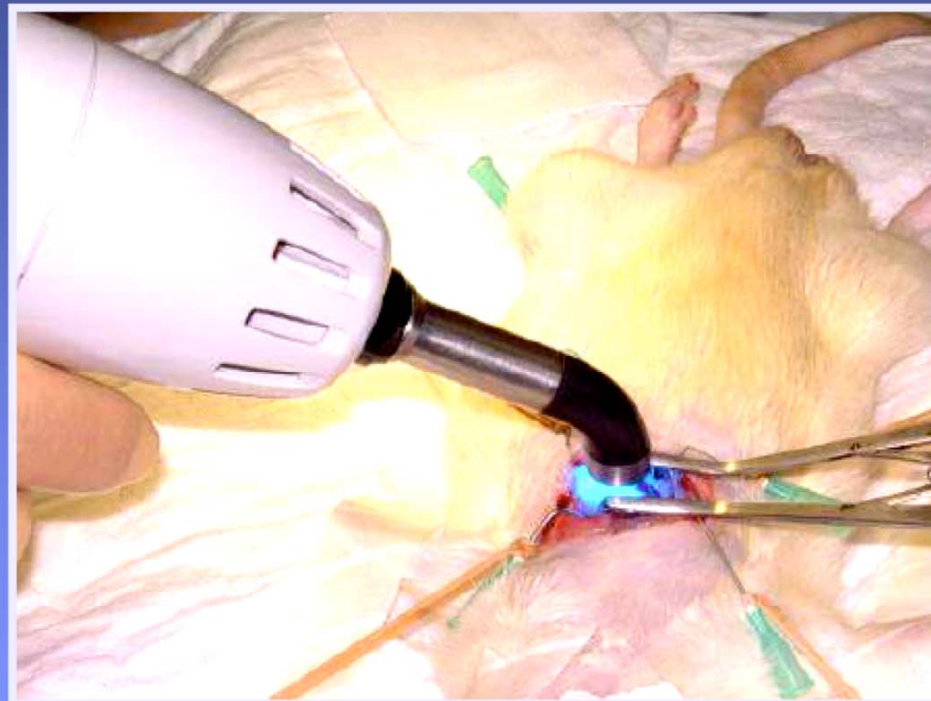
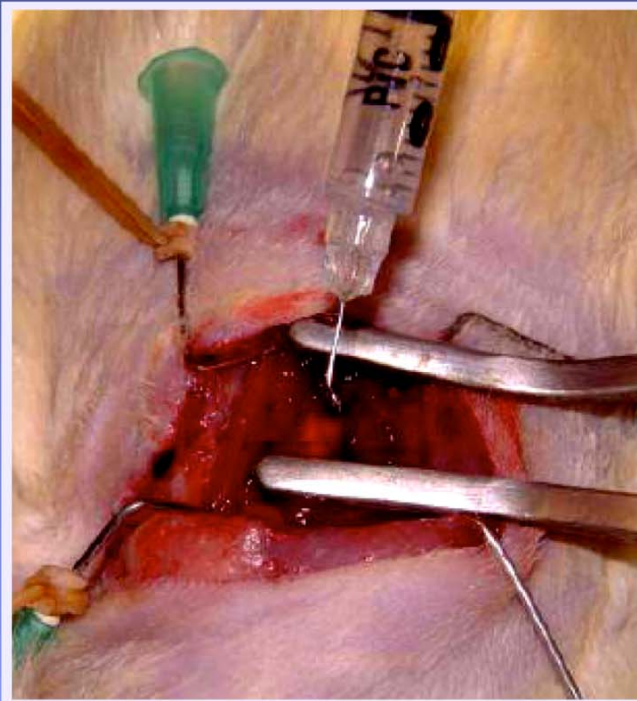
## ADULT STEM CELL THERAPY



The stem cells induce new blood vessel  
formation in the damaged heart muscle  
and proliferation of existing vasculature.



# In vivo experiment Cardiac Applications



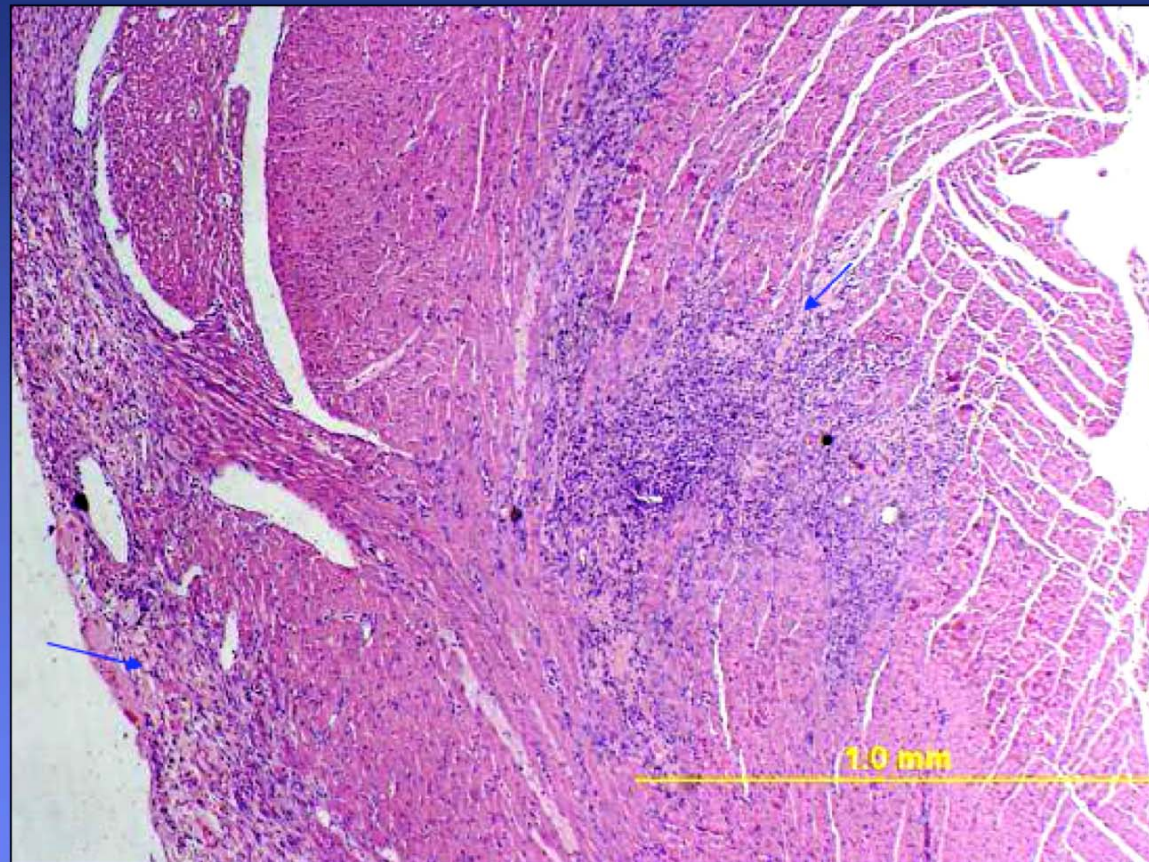
Photoinitiation is used to polymerize the gel inside the heart muscle

In collaboration with Prof. Lior Gepstein, Dr. Manhal Habib



# *In vivo* study: Cell Graft

- Implanted rat heart 5 days after injection using PEGylated fibrinogen (injection sites are indicated with arrows).

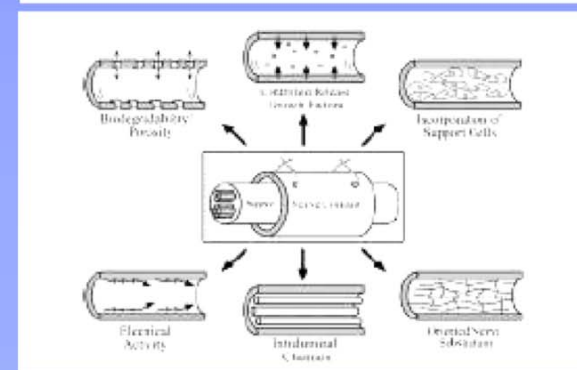
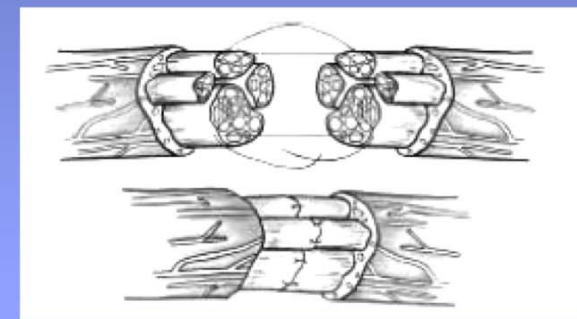
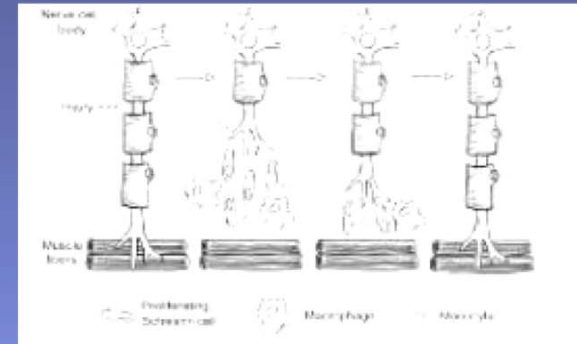
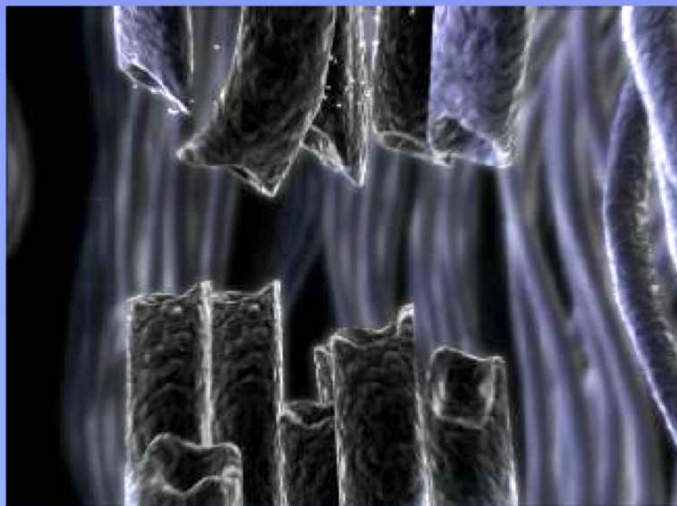


**9 mg/ml PEGylated fibrinogen with 25:1 PEG/Fibrinogen ratio**

In collaboration with Prof. Lior Gepstein, Dr. Manhal Habib



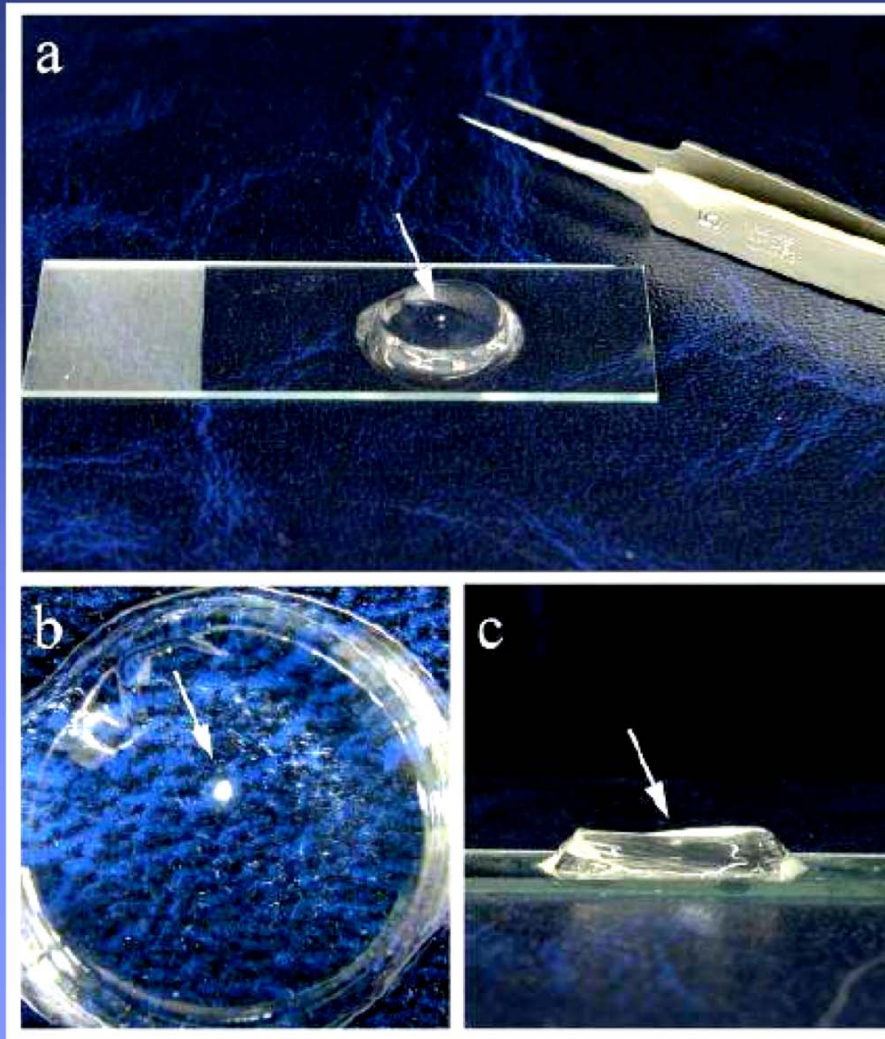
# PNS Injury and Treatment



. Adopted from Schmidt CE and Leach JB



# PEG-Fb: DRG Outgrowth



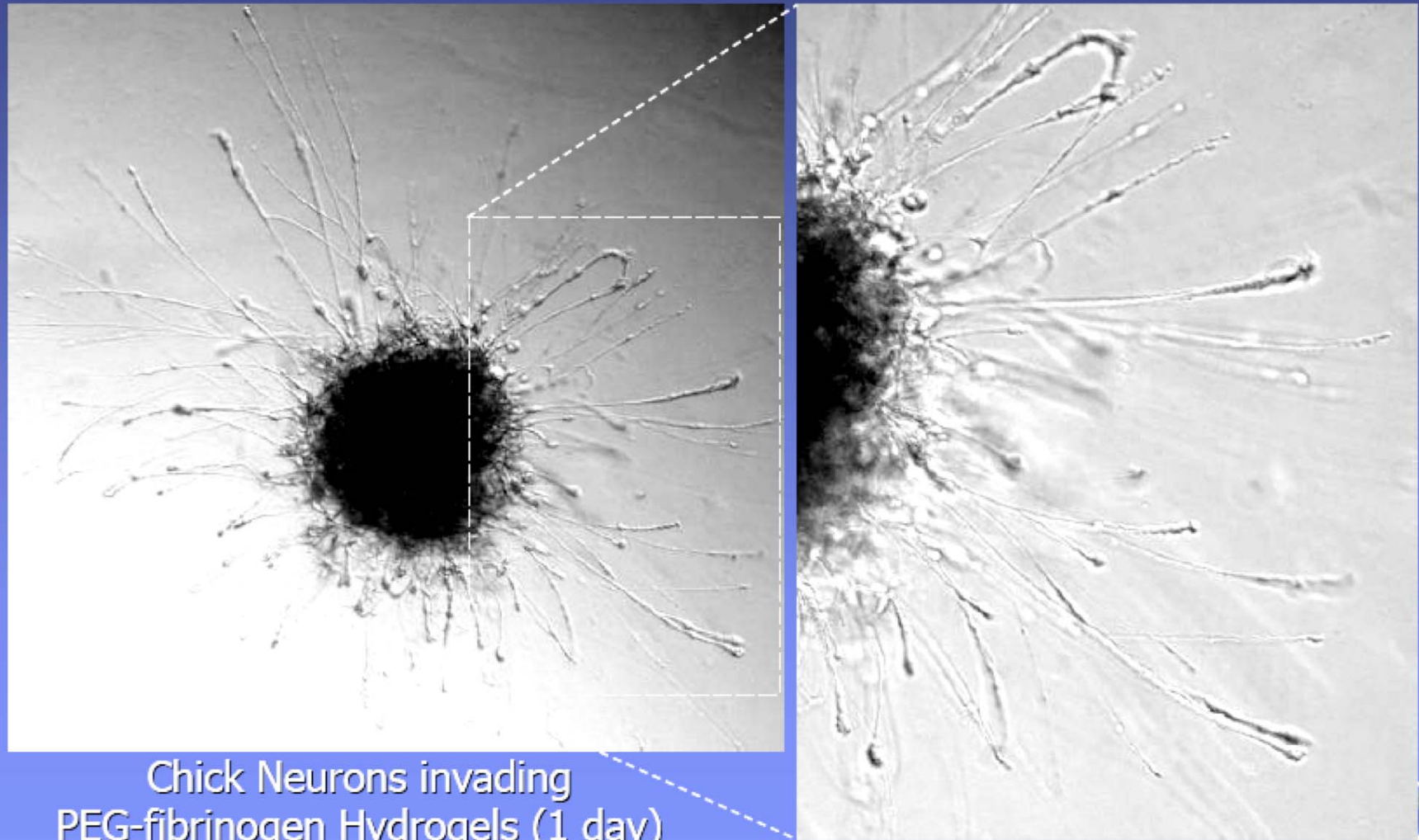
## DRG Encapsulation Assay

- Neonatal chick DRG immobilized into PEG-Fb.
- Hydrogels are polymerized, cultured with NGF.
- Neuronal outgrowth only with NGF.
- Outgrowth kinetics is controlled through hydrogel properties.

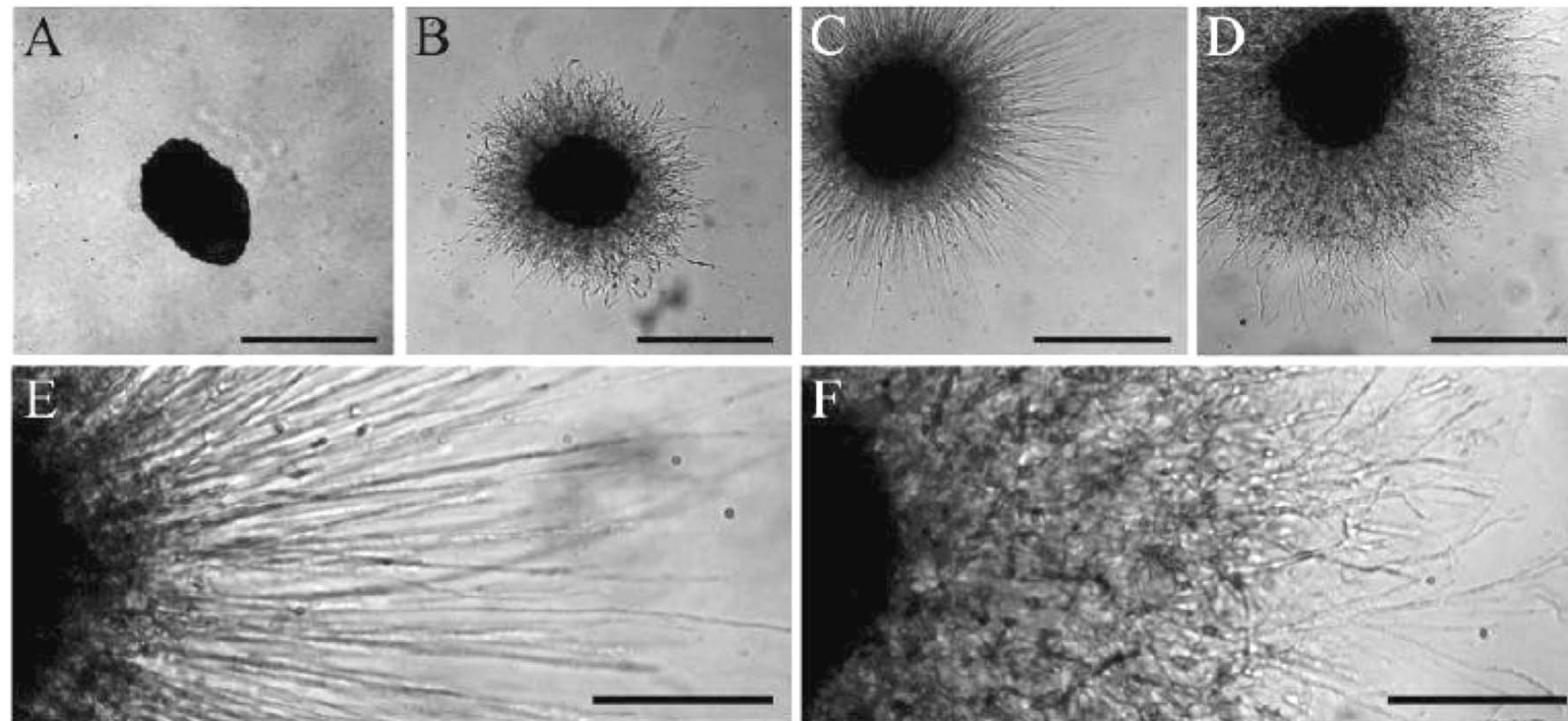


# NGF-mediated 3-D Neuronal Invasion

(chick DRGs in PEG-Fibrinogen)



# DRG Outgrowth in PEGylated Protein Hydrogels



PEG-Albumin (A)

PEG-Fibrinogen (B, D)

PEG-Collagen (C, E)

Sarig-Nadir O. and Seliktar D., *Tissue Eng Part A*. 2008 Mar;14(3):401-11.

