Biomimetic Strategies in Vascular Tissue Engineering: Designer Tissues from Designer Materials

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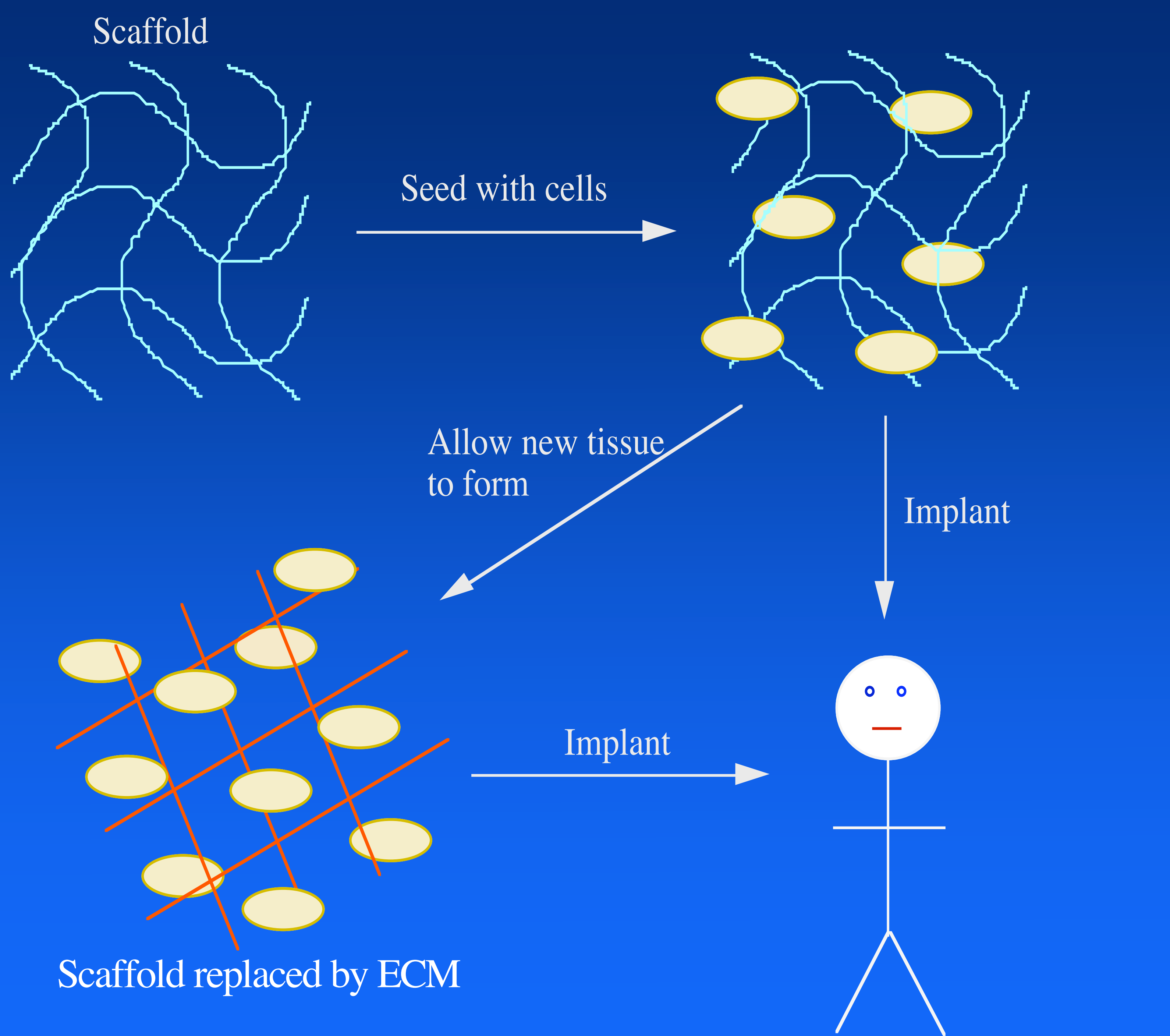
What is Tissue Engineering?

Application of engineering principles to:

- Understand tissue structure/function relationships
- Alter tissue function
- Develop biological tissue substitutes

Tissue Engineered Cartilage and Skin

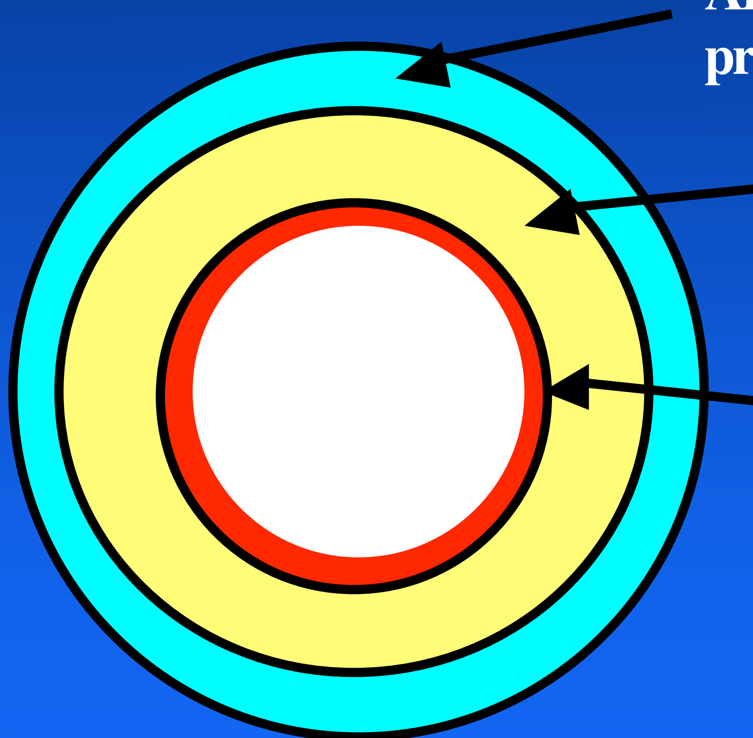

Scaffold
Seed with cells
Allow new tissue
to form
Implant
Implant
Scaffold replaced by ECM

Blood Vessel Replacement

- Needed for treatment of advanced atherosclerosis, aneurysmal disease, dialysis access & trauma.
- Current replacements are usually autologous tissue or synthetic biomaterials.
  - Replacement with current biomaterials is limited to vessel diameters > 6 mm.
  - Large patient population lacks suitable donor tissue.

Recreating the natural structure of the vessel through tissue engineering may provide solution.

Arterial Structure: Requirements for a Functional Substitute



ADVENTITIA: Fibroblasts - mechanical properties

MEDIA: Smooth muscle cells and elastin fibers - mechanical properties, contractility

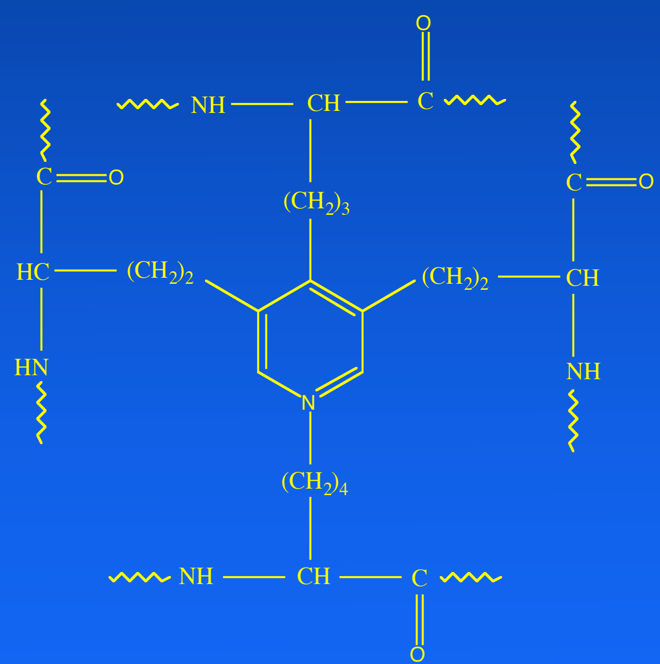
INTIMA: Endothelial cell lining - non-thrombogenic lining, control of SMC proliferation

Tissue Engineer's Toolbox

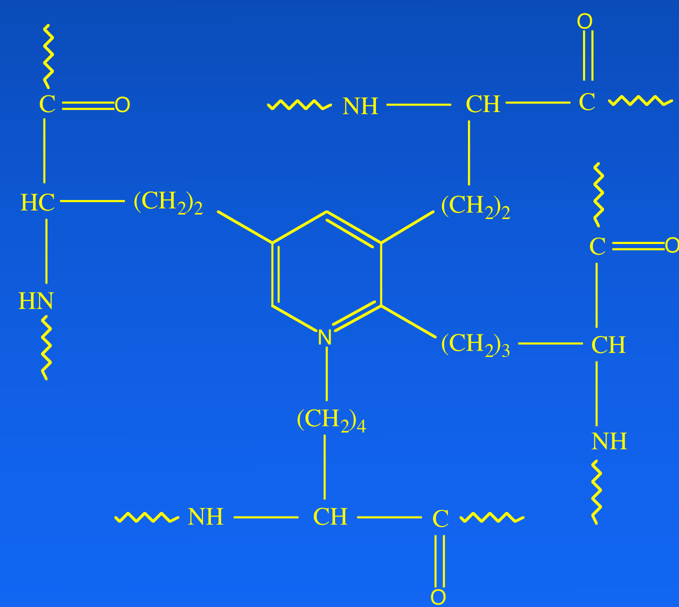
- Cell source
- Genetic manipulation of cells
- Scaffold materials
- Bioactive factors
- Mechanical conditioning

Lysyl Oxidase Crosslinking in the ECM to Improve Mechanical Properties of Engineered Tissues

- Oxidative deamination of lysine leads to allysine formation
- Condensation of three allysines with one lysine



Desmosine

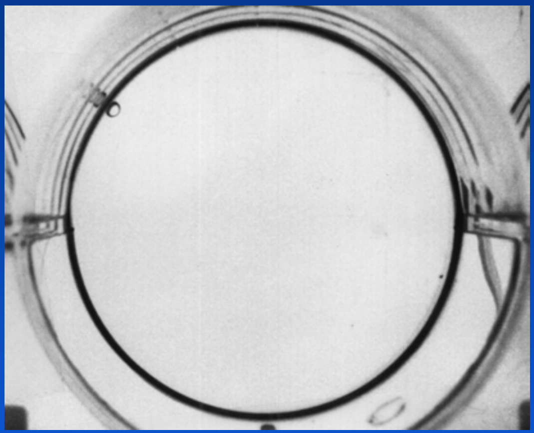


Isodesmosine

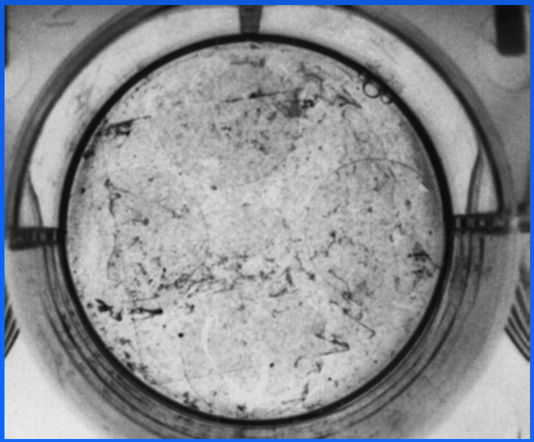
Expression of Active Lysyl Oxidase in Transfected Smooth Muscle Cells

- LO-transfected SMCs show more LO activity as comparerd to mock-transfected SMCs

LOX Mock

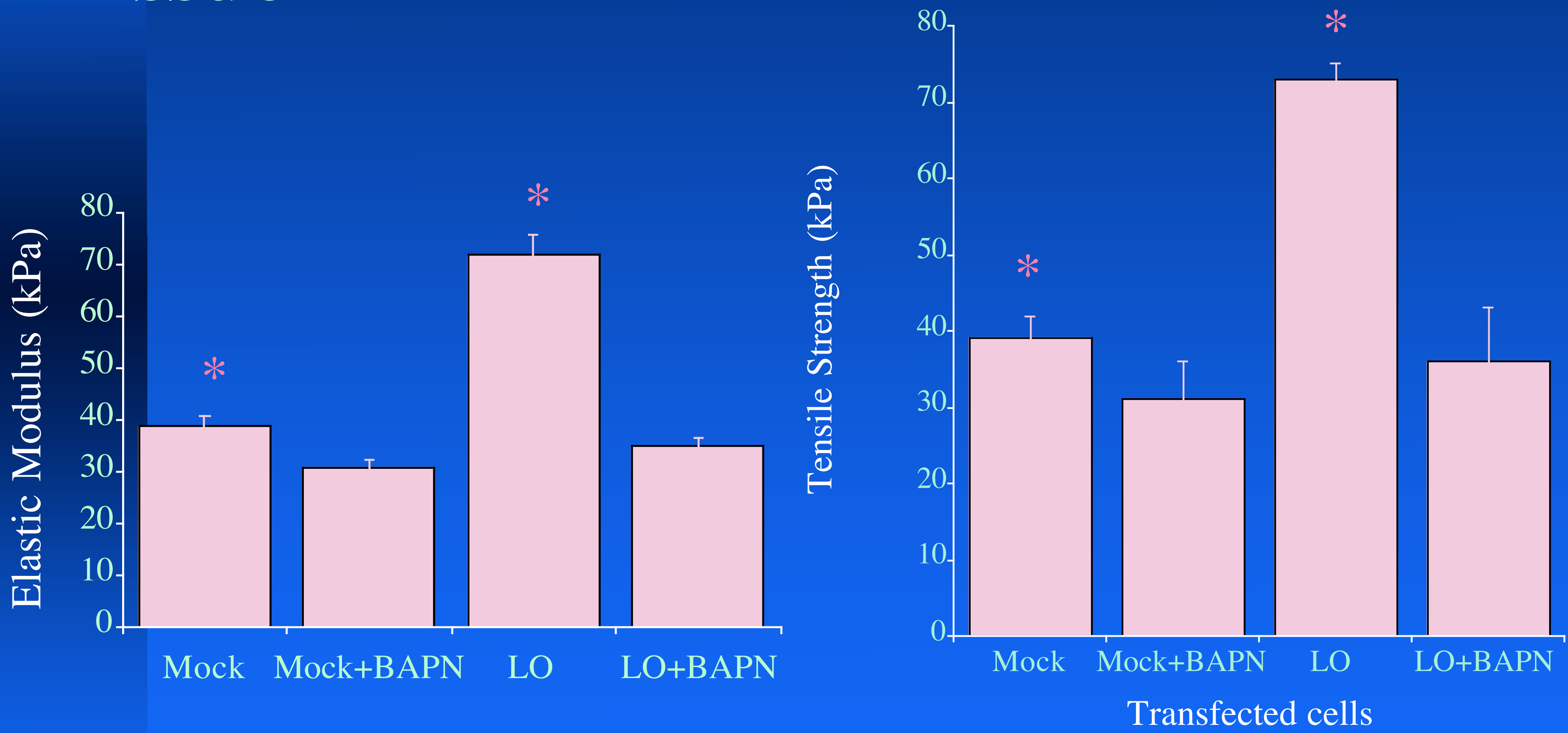


Mock-transfected SMCs



LO-transfected SMCs

Lysyl Oxidase Crosslinking Improves Mechanical Properties of Engineered Tissue



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What to Use as a Scaffold?

- Biodegradable with non-toxic products
- Biocompatible and permeable to nutrients
- Desirable to have mechanical properties that match the natural tissue (strength, flexibility)
- Needs to be fabricated to correct shape and allow facile seeding of cells into the scaffold structure

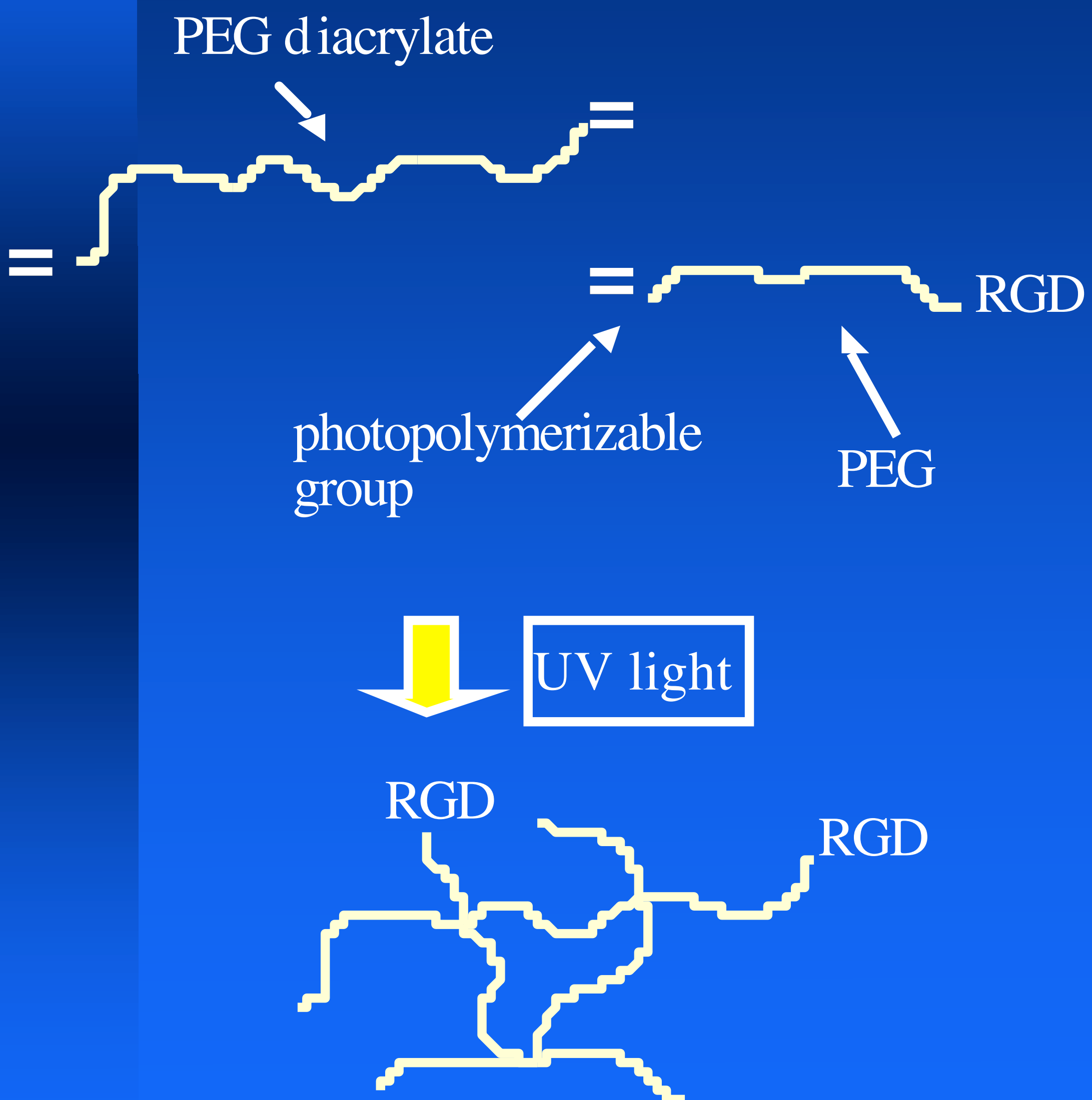
Synthetic Polymers (PLGA)
Natural Polymers (Collagen

Bioactive Materials for Tissue Engineering

SCAFFOLDS SHOULD:

- Provide support while tissue is forming
- Make room for tissue to form
- Support adhesion of desirable cells
- Exclude adhesion of undesirable cells
- Influence cell behavior to optimize tissue formation
- Provide an appropriate mechanical environment

Photopolymerized Scaffolds



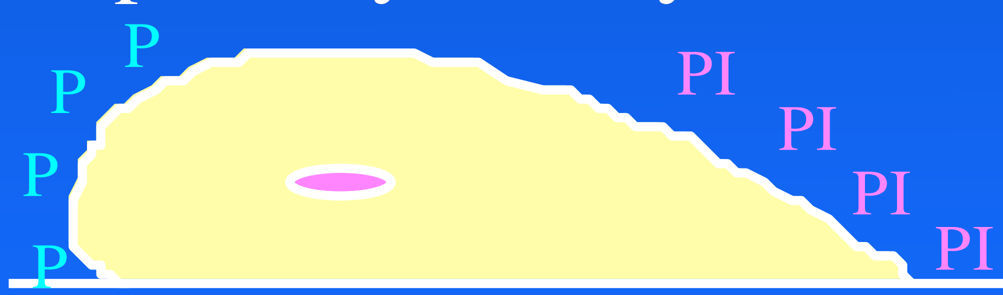
Polymer solution

UV light

PEG hydrogel with bioactive factors

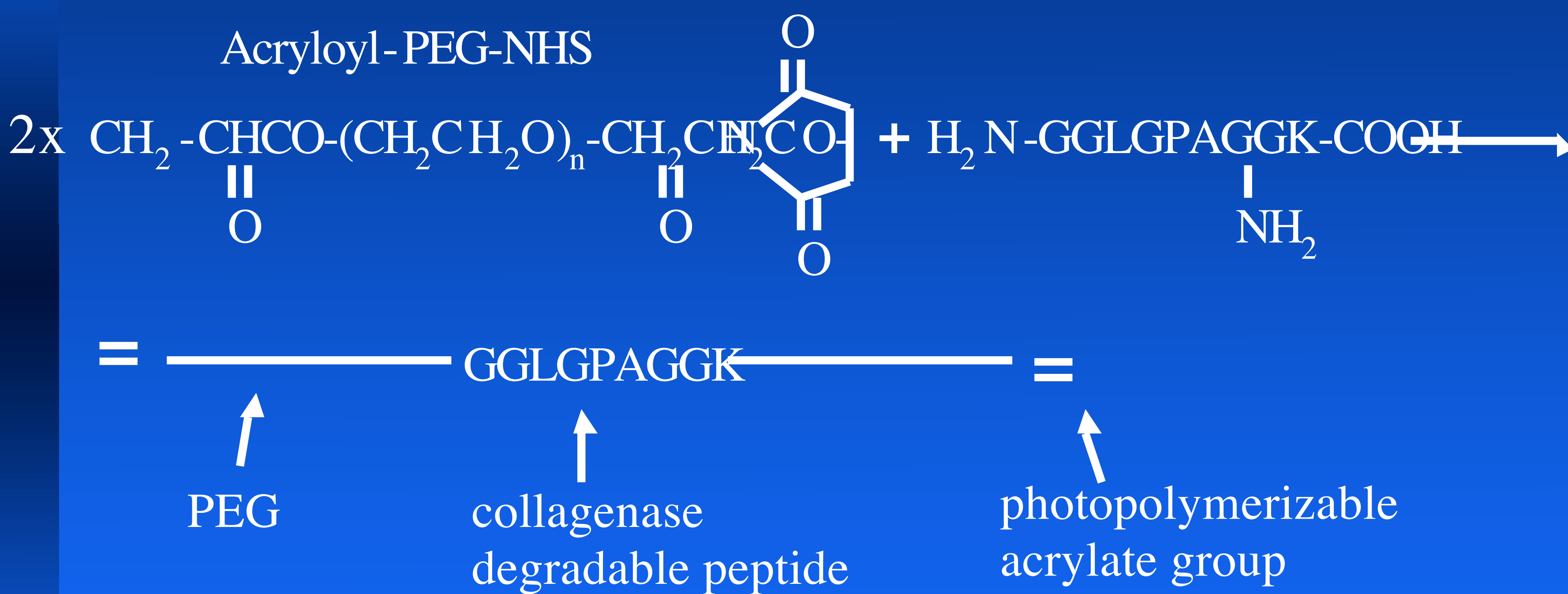
Bio-targeted Degradation

- In order to match scaffold degradation to tissue formation, degradation should be linked to cell migration.
- Cells secrete proteolytic enzymes during migration to degrade the ECM. **GOAL:** Target hydrogel degradation to specific proteolytic enzymes.



Proteolytic enzymes localized to the leading edge of the cell, with protease inhibitors at other locations to strictly localize proteolysis to the cellular pathway. Proteases secreted depend on cell type.

Structure of proteolytically degradable hydrogels



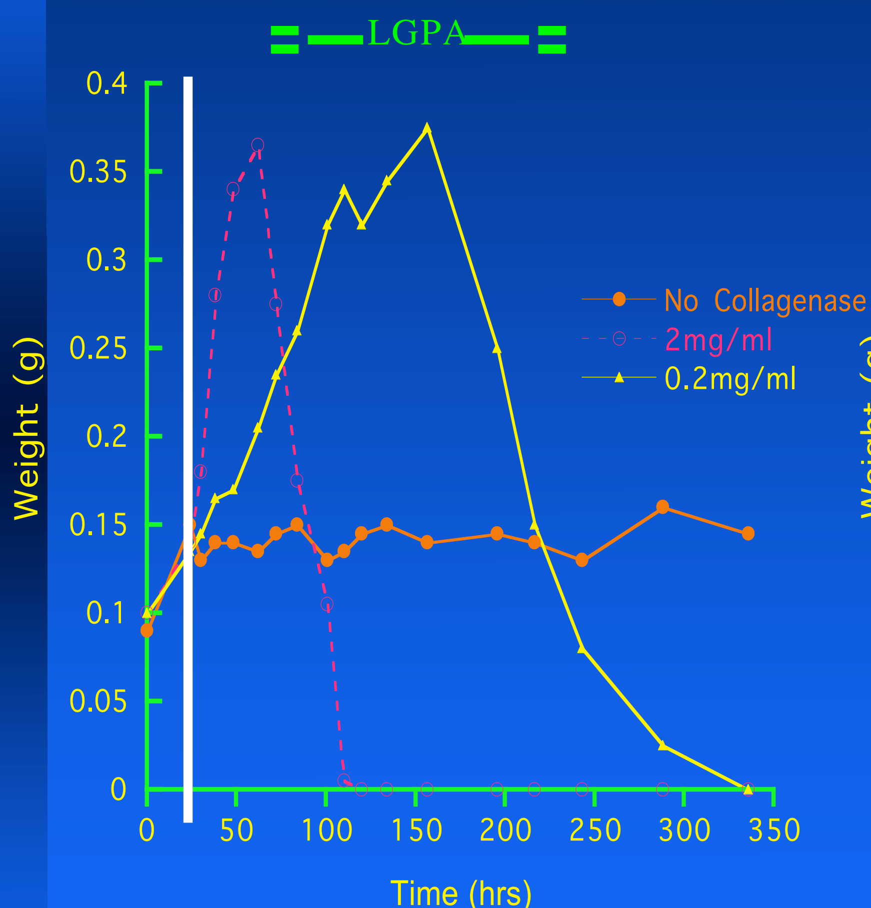
* Other peptides can be used for other proteases

NRV -> plasmin

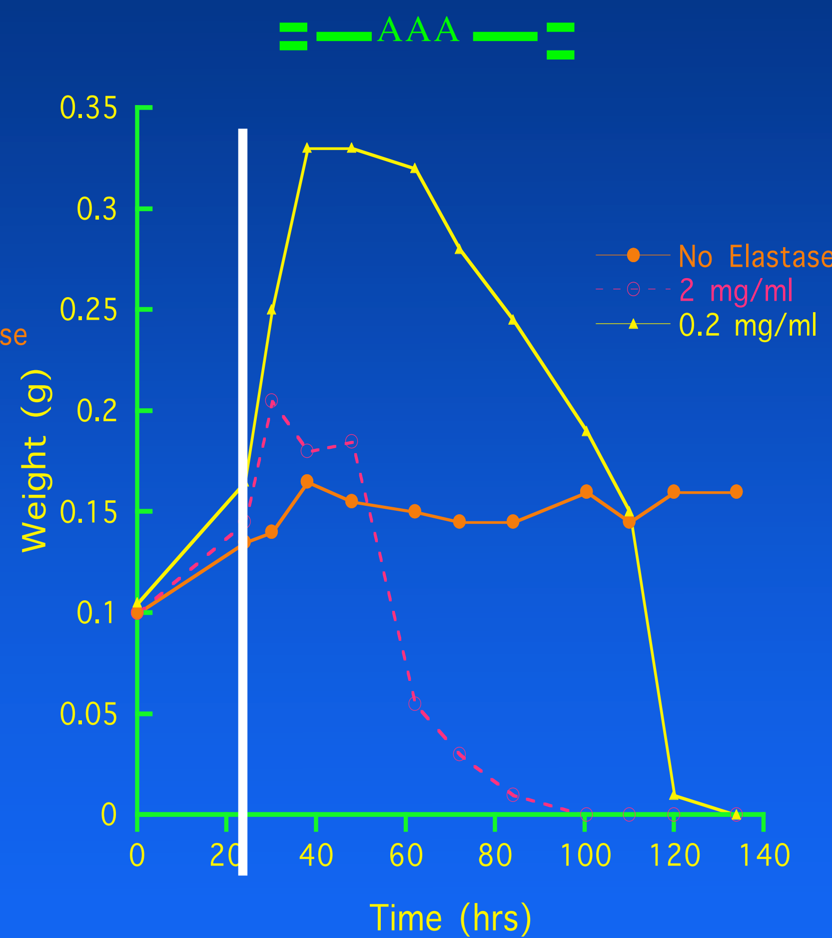
poly-alanine -> elastase

AAPF -> cathepsin G

Proteolytic Degradation Study



Collagenase-Sensitive Hydrogel

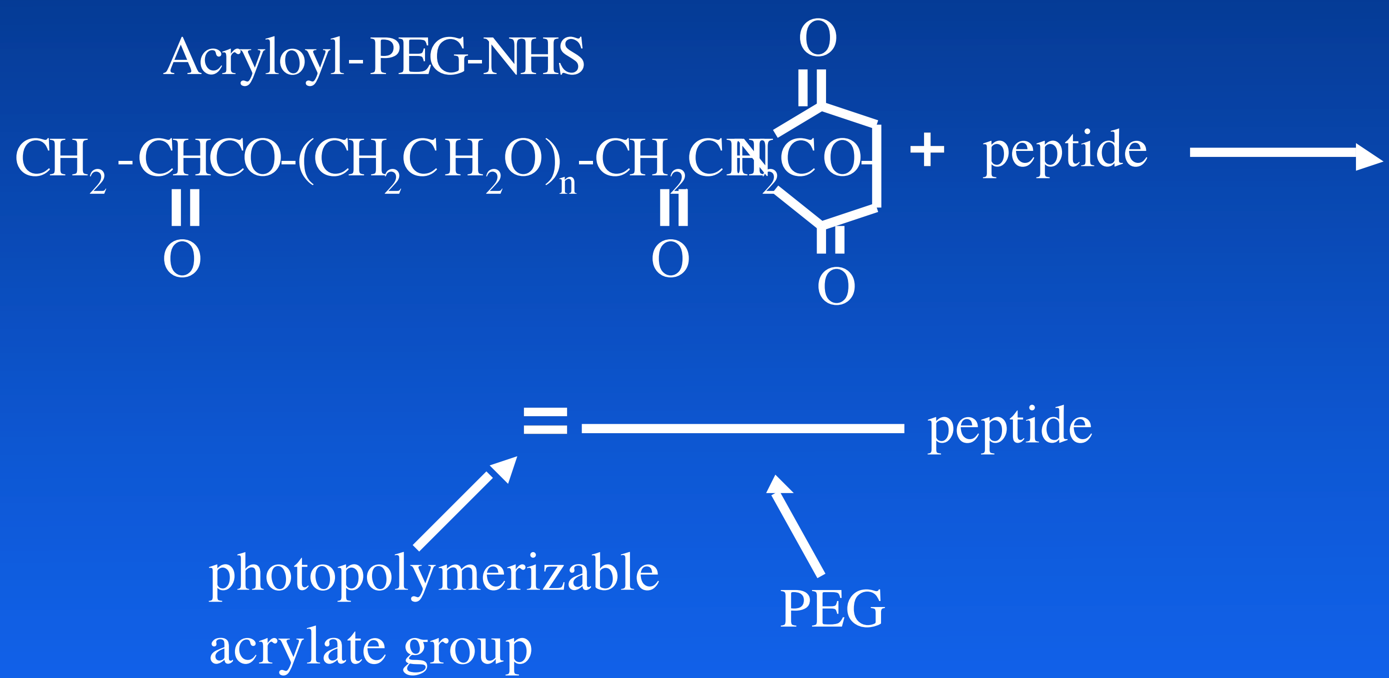


Elastase-Sensitive Hydrogel

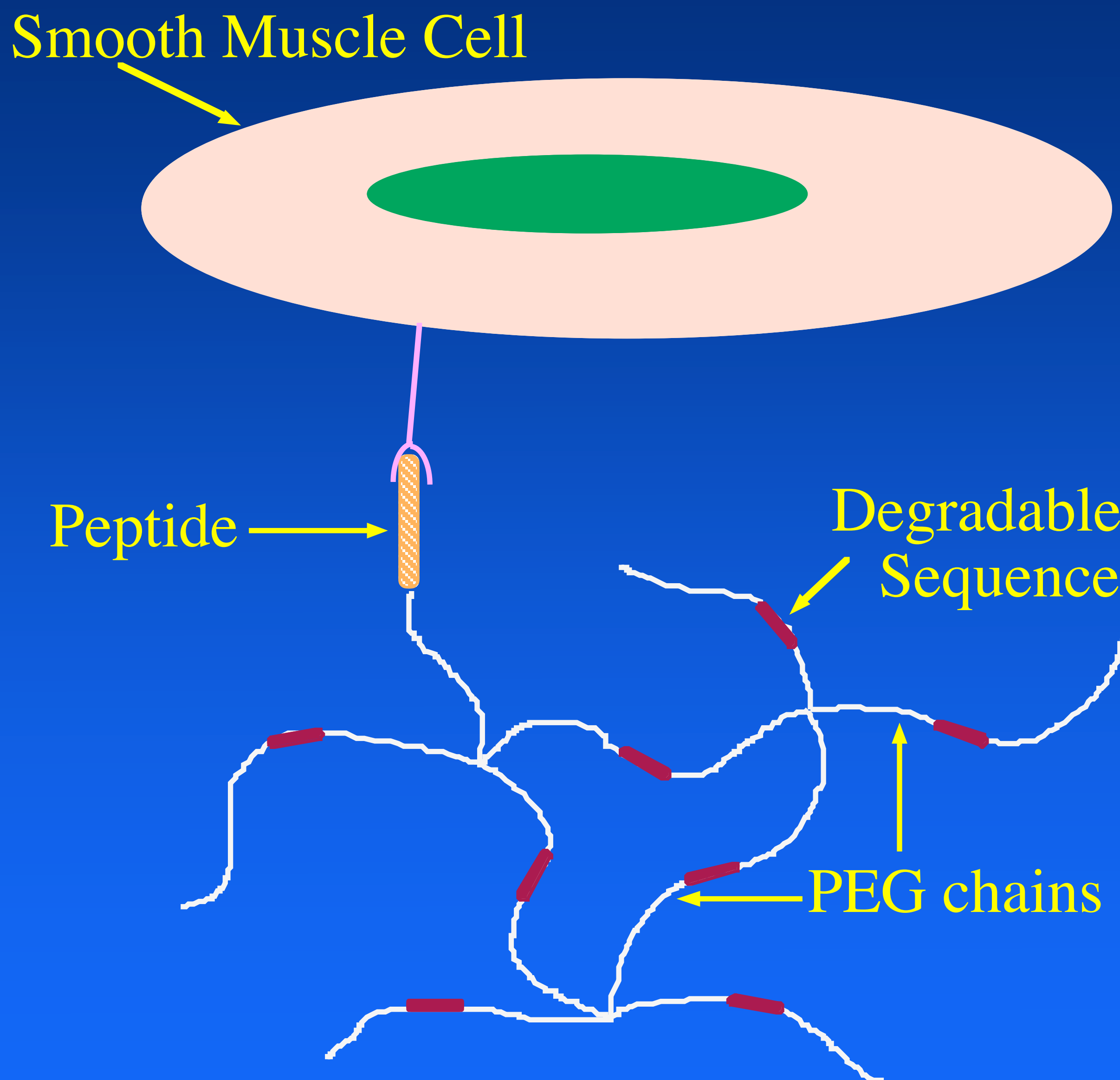
Biospecific Cell Adhesion

- Mediate adhesion via specific receptors on the cell surface
- Incorporate specific receptor ligands into non-adhesive scaffold material (PEG)
- Cell selectivity can be achieved by incorporating ligands specific for one cell type
  - REDV → endothelial cells
  - RGDS → ubiquitous

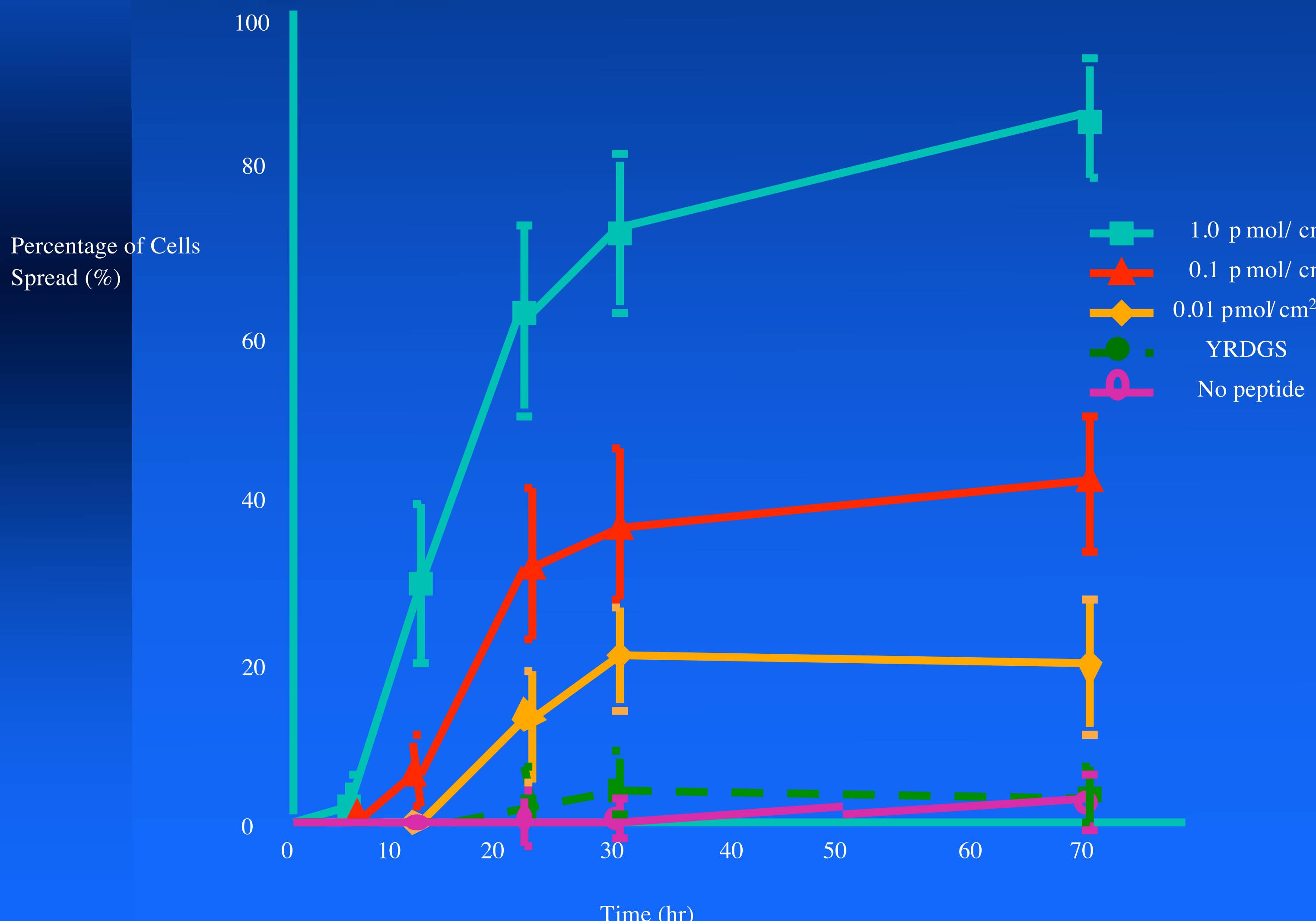
Design of cell adhesive materials



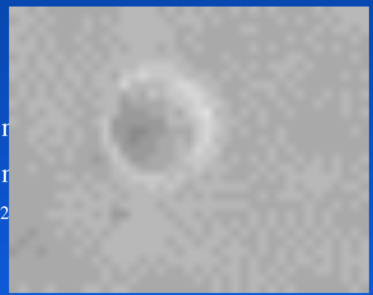
Upon photopolymerization with a PEG-diacrylate derivative, the >97% of the peptide is grafted into the hydrogel matrix. Numerous ligands can be grafted into a single hydrogel material using this scheme.


Smooth Muscle Cell
Peptide
Degradable Sequence
PEG chains

Adhesion of Smooth Muscle Cells to Hydrogels Containing RGDS



RDGS

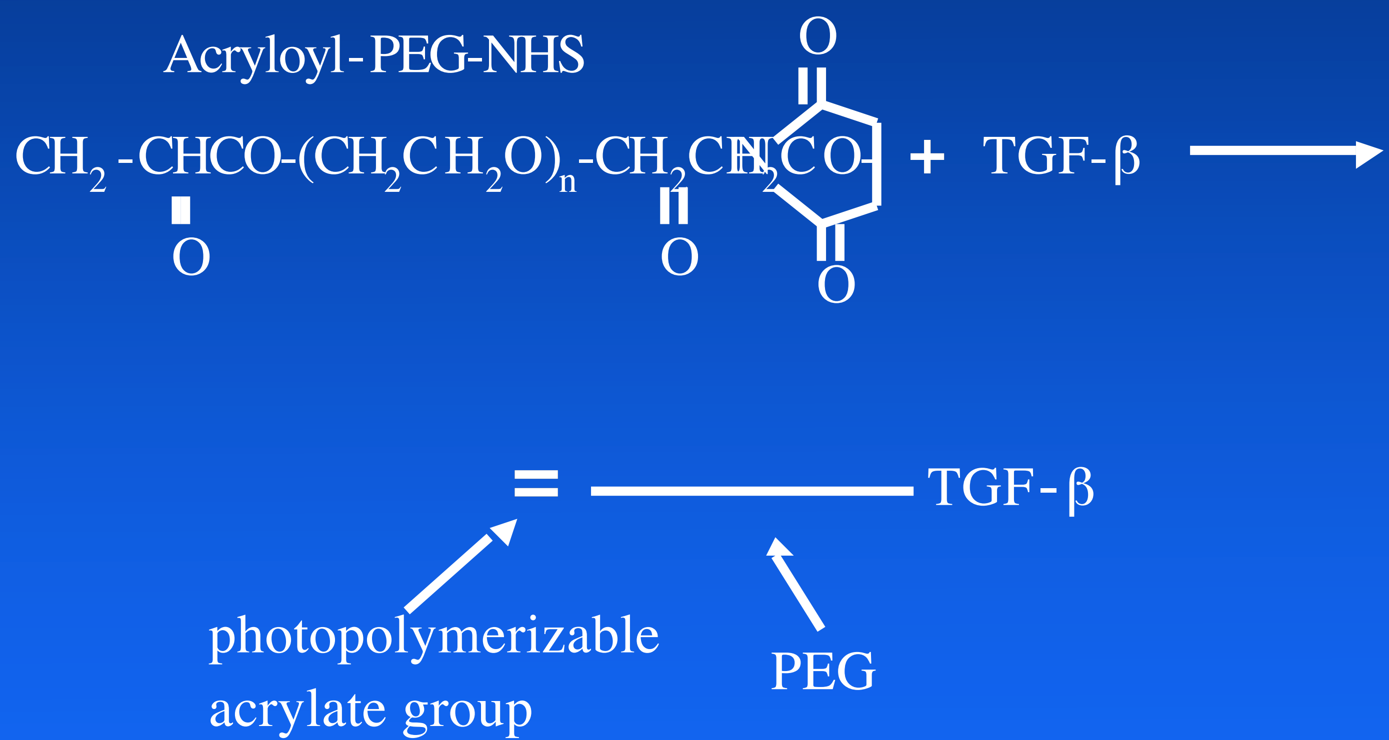


RGDS

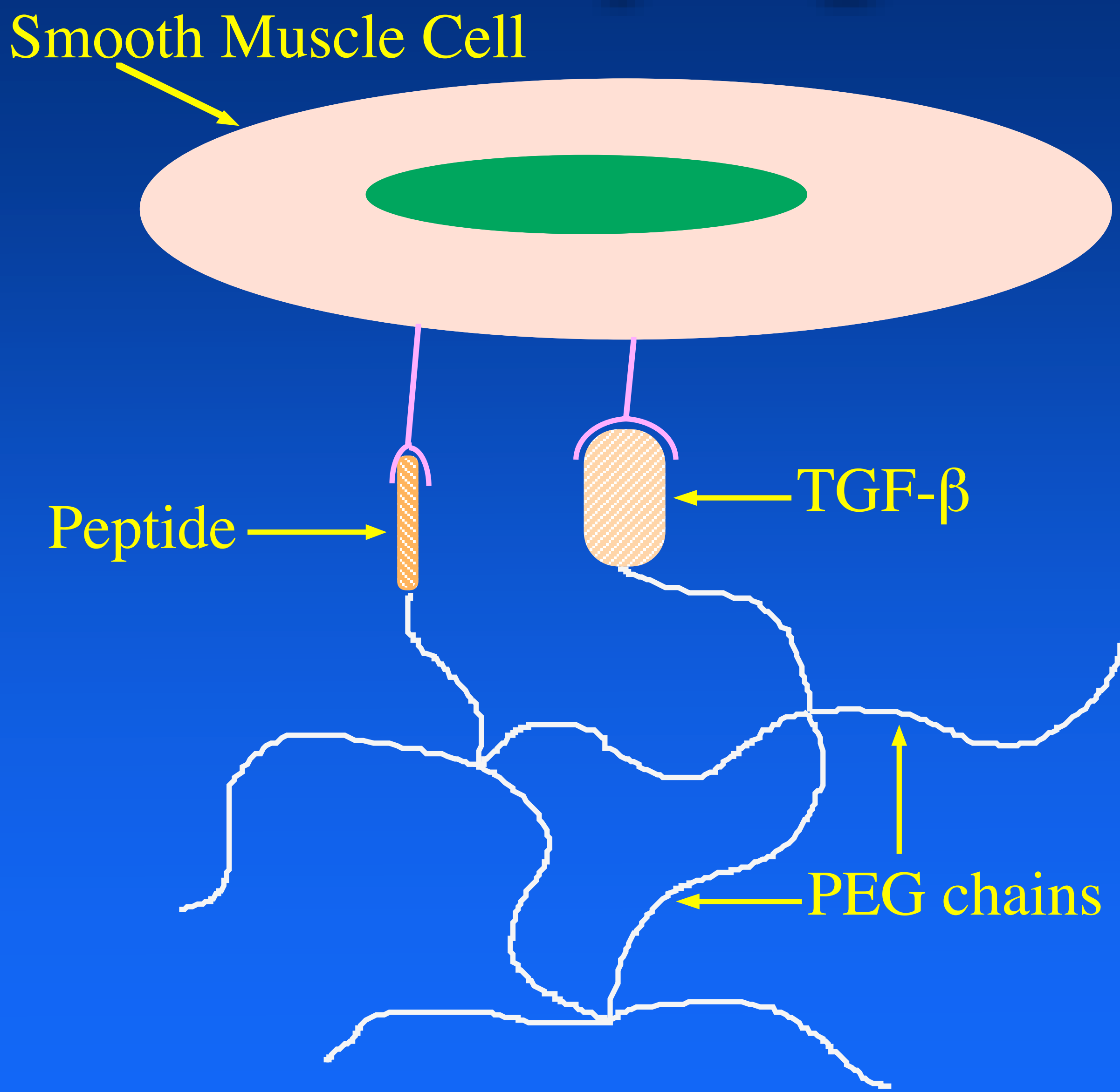
Bioactive Factors to Alter Tissue Formation: Transforming Growth Factor-β

- TGF-β has been shown to increase matrix protein production of many cell types.
- TGF-β does not increase proliferation of smooth muscle cells.

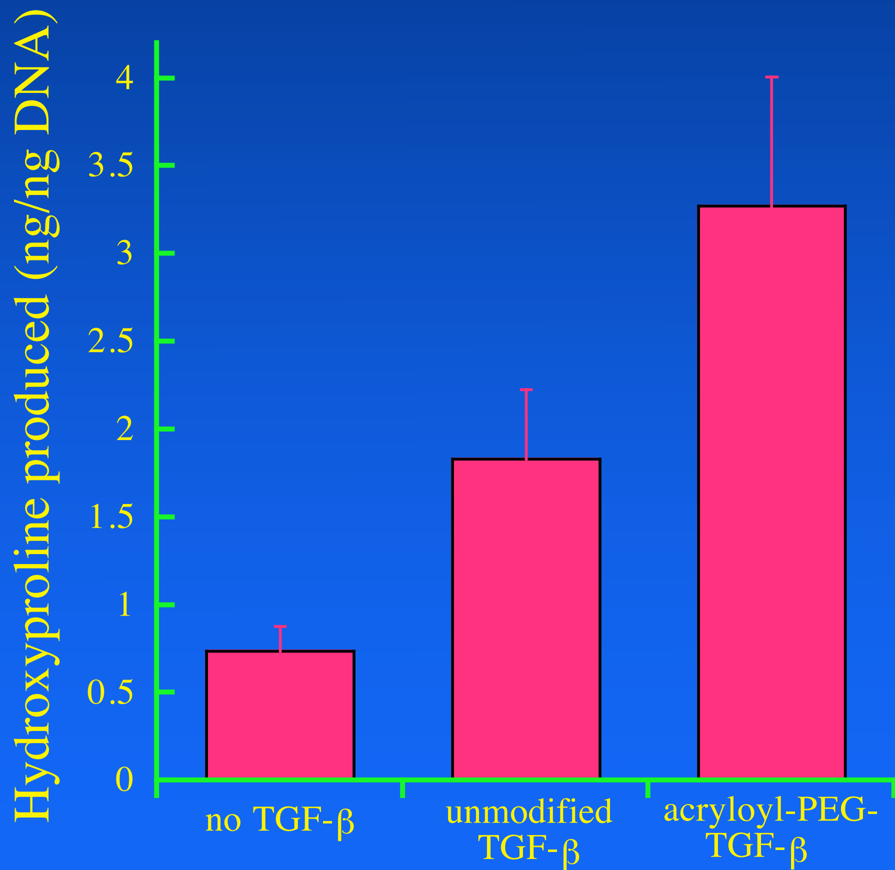
Synthesis of acryloyl-PEG-TGF-β



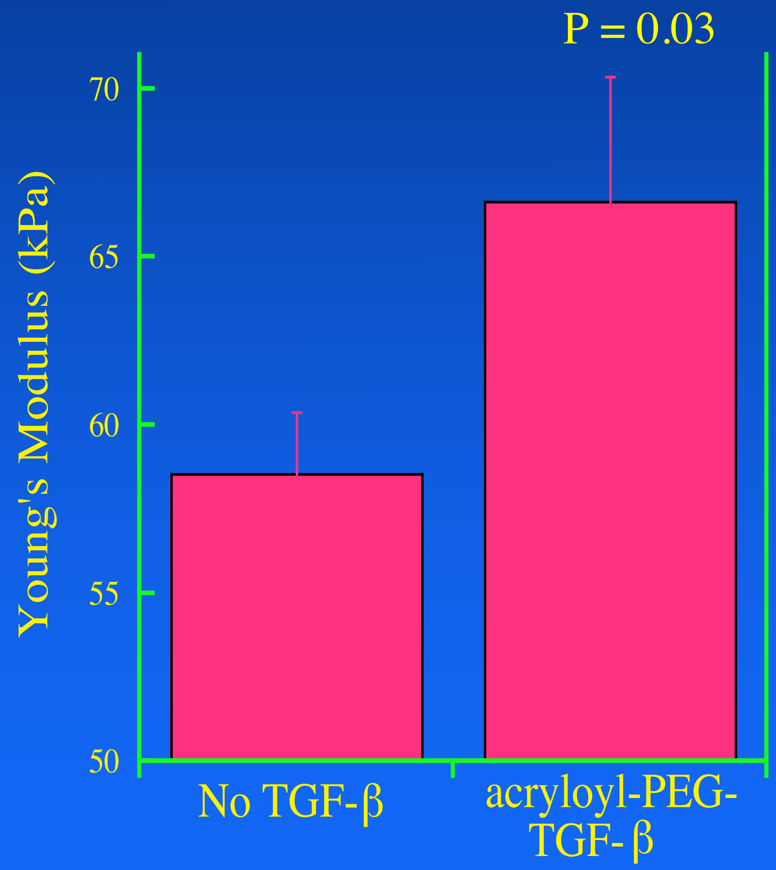
Schematic of Hydrogel Scaffold



Effect of tethered-TGF-β on matrix production of SMCs in RGDS-modified PEG hydrogels



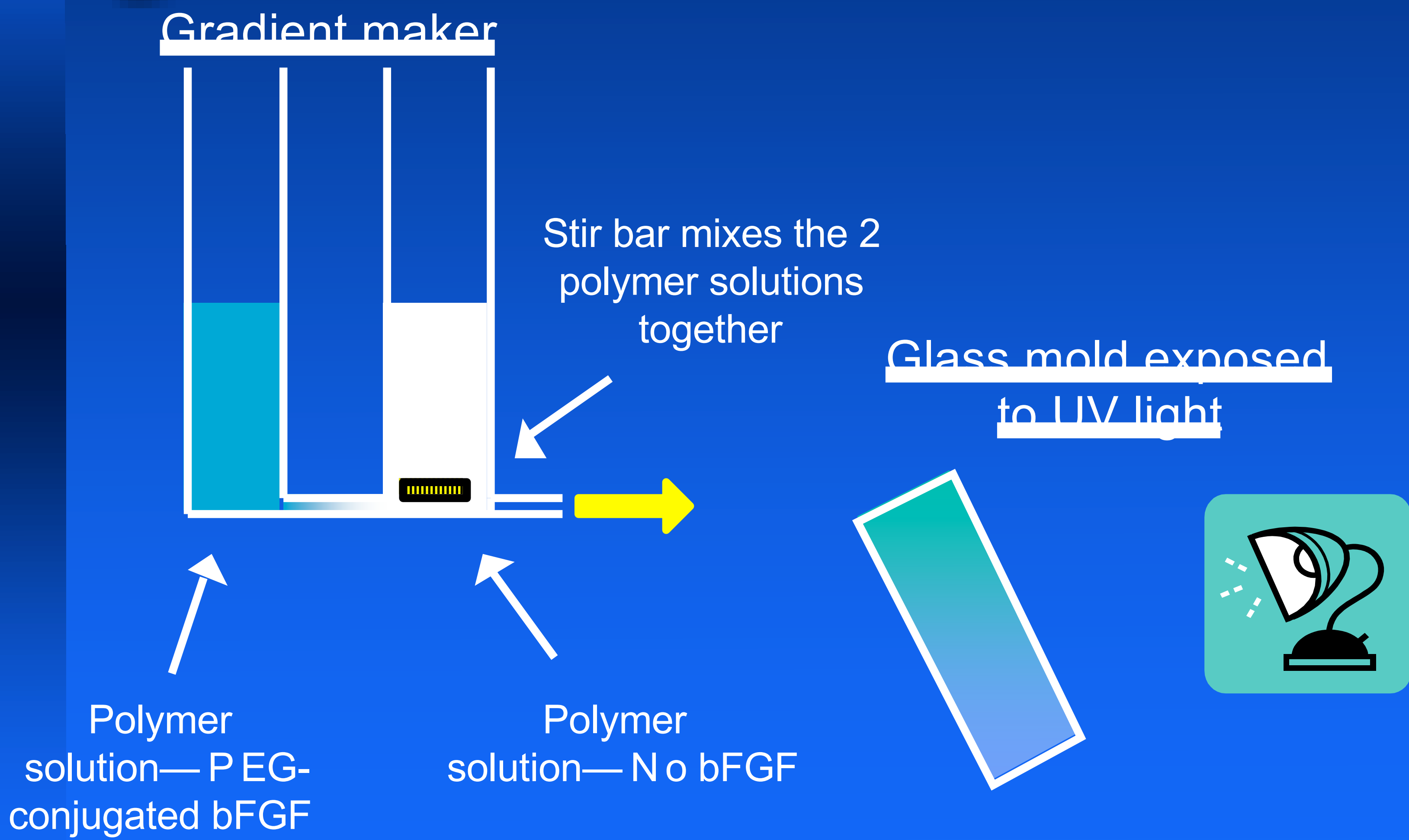
Effect of Tethered-TGF-β on Young's modulus of hydrogel with SMCs



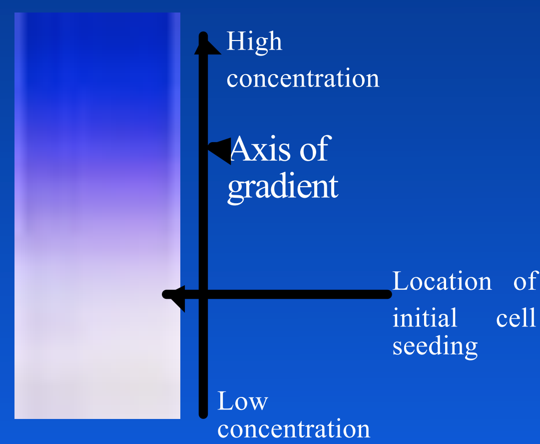
* 14% increase when acryloyl-PEG-TGF-β added

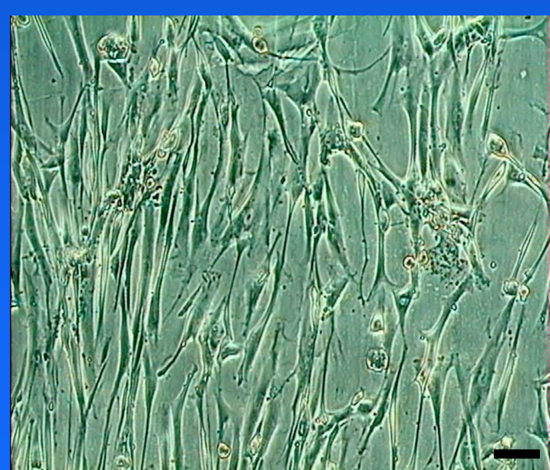
* No significant difference was seen between unmodified TGF-β and no TGF-β

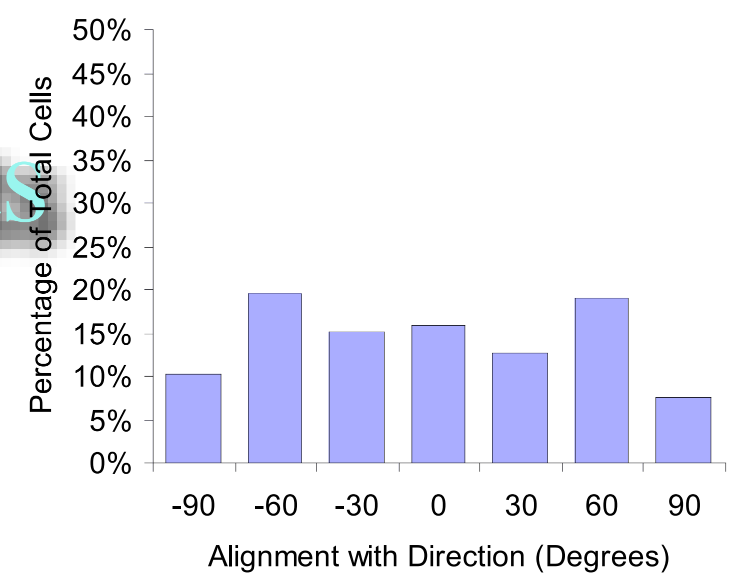
Hydrogels formed with a EGF-gradient

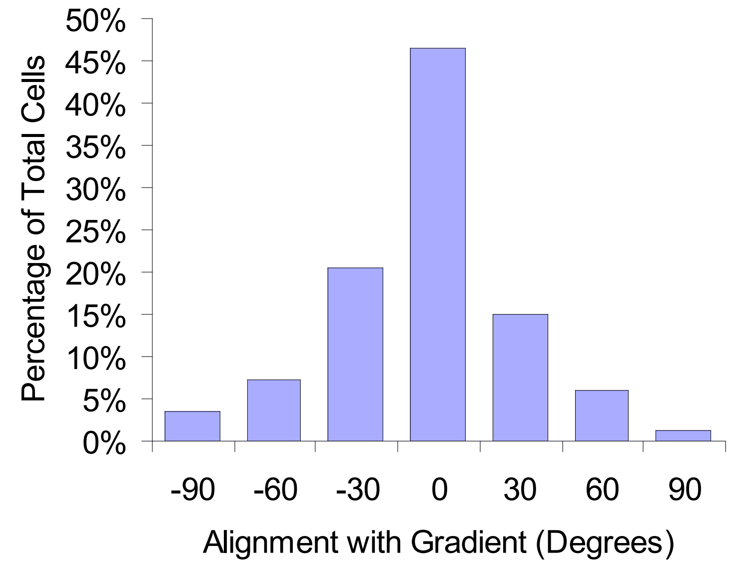


Tethered Gradients

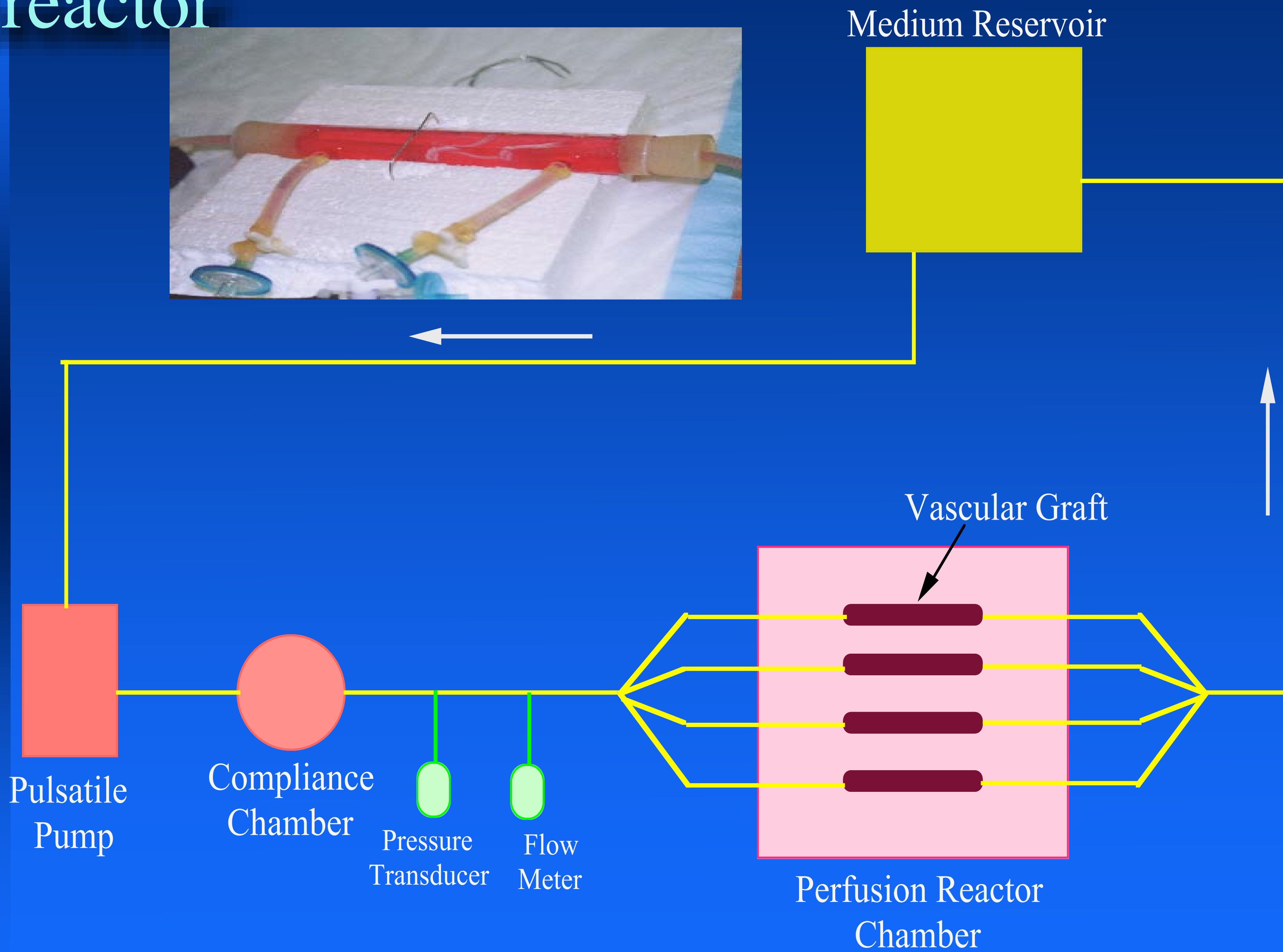






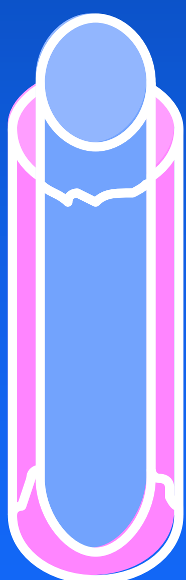


Mechanical Conditioning: Vascular Graft Bioreactor



Fabrication of Tissue Engineered Vascular Grafts via Hydrogel Photopolymerization

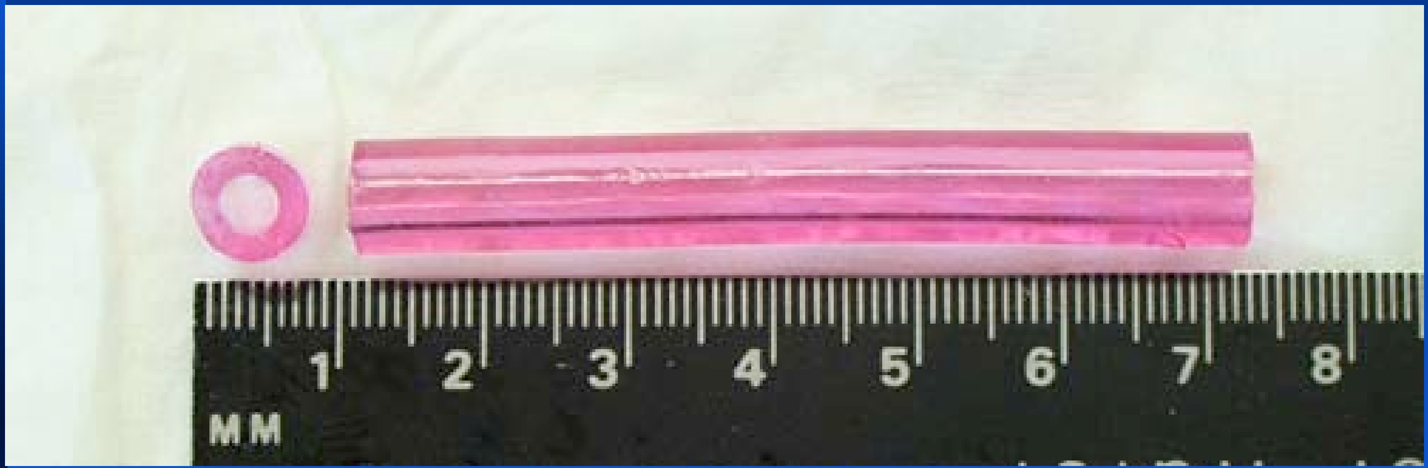
1. Fill annular mold with SMC suspension in aqueous polymer solution. Expose to light.

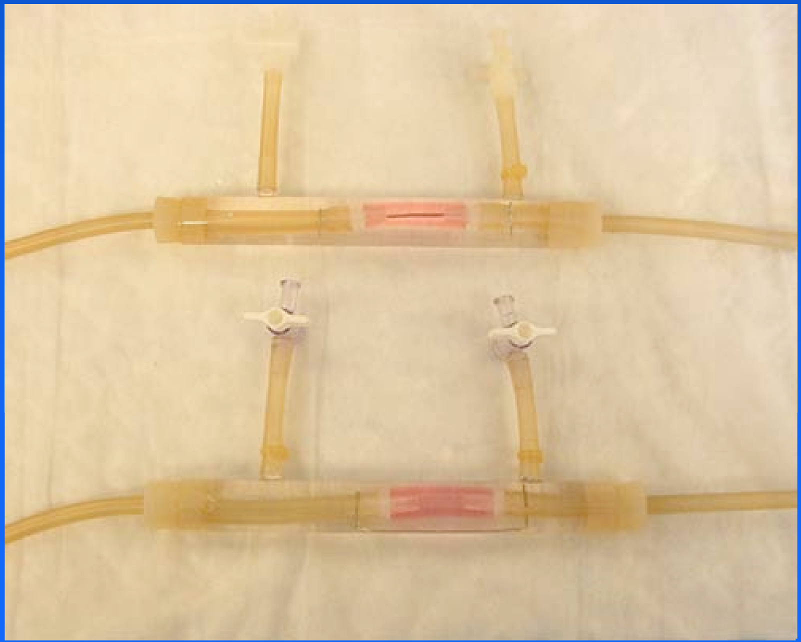


2. Add intimal layer by interfacial photopolymerization of an EC suspension in polymer solution.

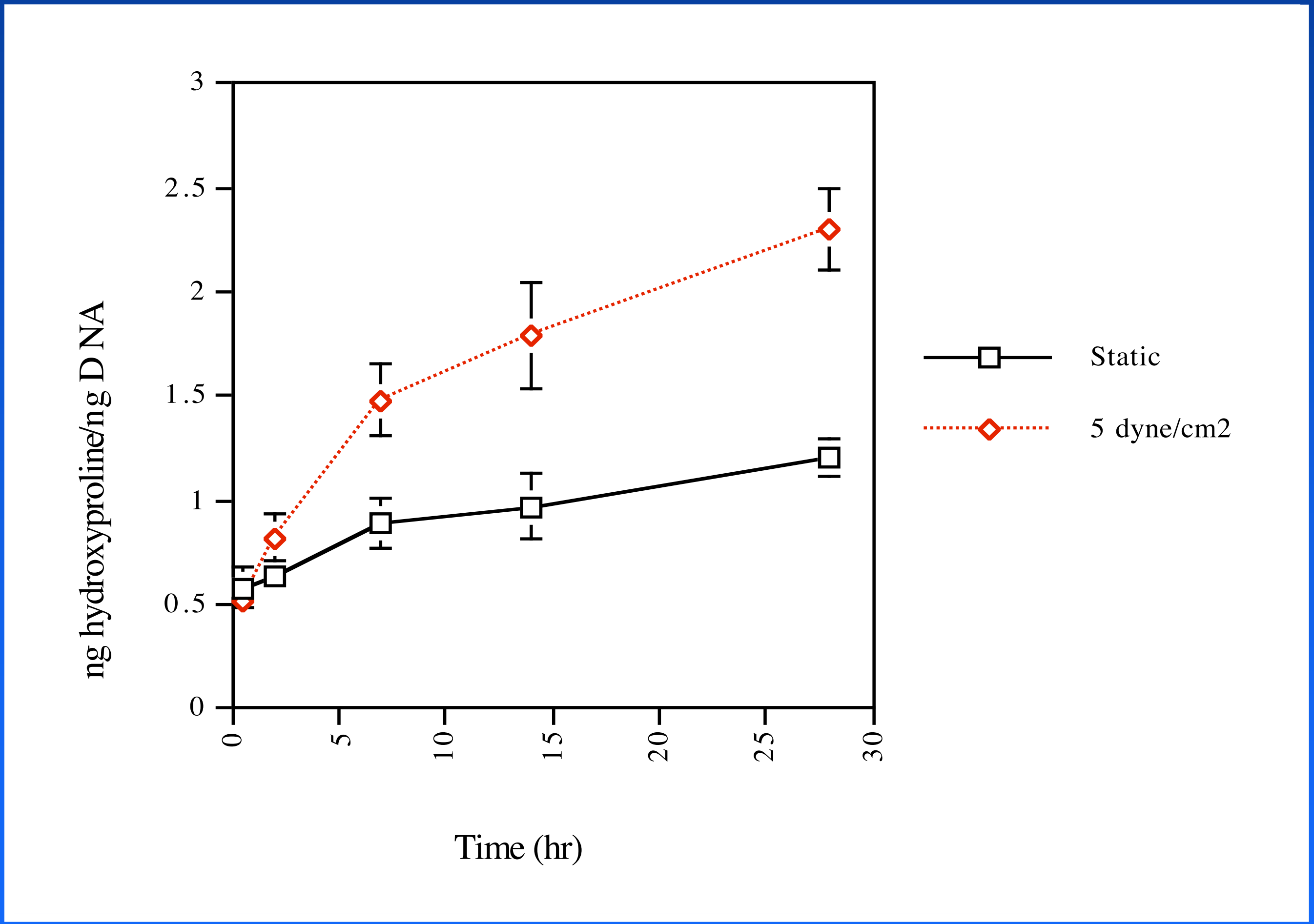
3. Add adventitial layer by interfaci photopolymerization of a fibroblast Suspension in polymer solution.

Tissue Engineered Vascular Grafts

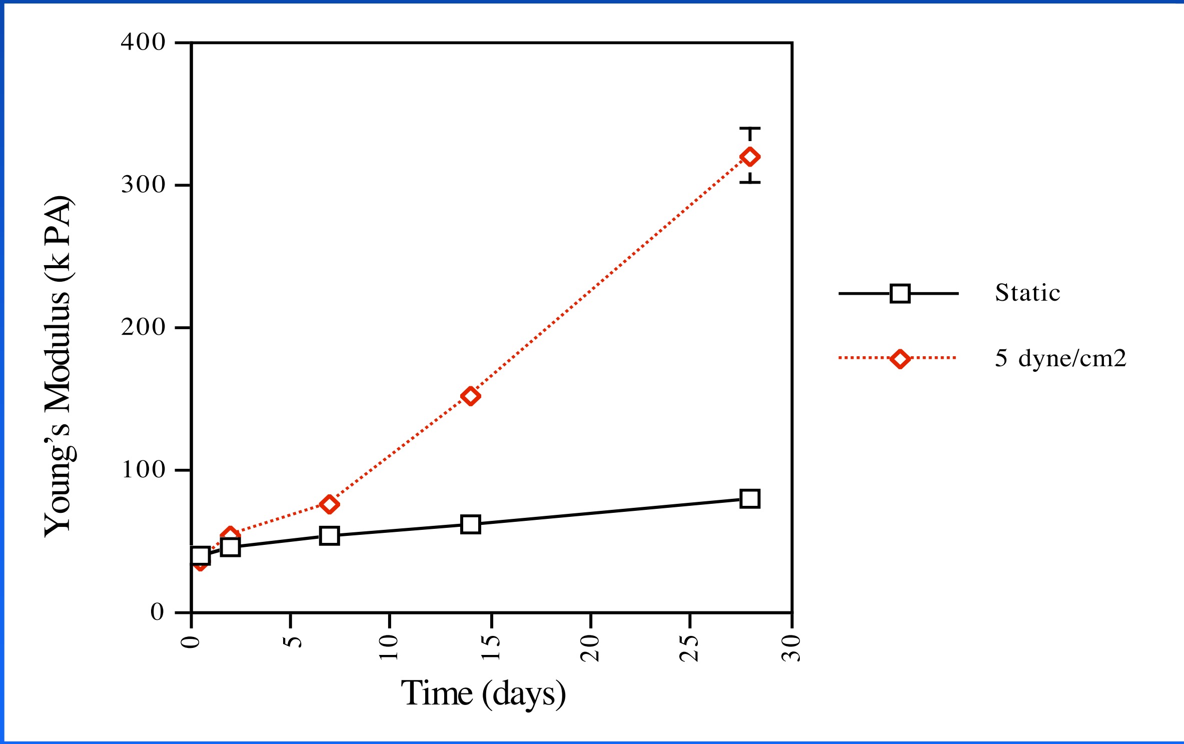




Mechanical Conditioning Increases Collagen Secretion



Mechanical Conditioning Improves Tissue Mechanical Properties



Conclusions

- Tissue engineering should be able to provide options for vascular grafts
- Complex tissue engineering problems, like vascular grafts, will likely require utilization of the full "tool box" for clinical success

http://westlab.rice.edu

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