MALEIMIDE-FUNCTIONALIZED POLYETHYLENE GLYCOL HYDROGELS FOR HUMAN CELL CULTURE

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Matrigel organoids are too variable for consistent organoid

- **Pros**
  - Used to mimic the *in vivo* environment
  - Easy to make in large quantities (thermoresponsive polymer)
  - Large number of growth factors for cell growth

- **Cons**
  - Matrigel has batch to batch variability
  - Derived from Mouse-Embryonic fibroblasts (nonhuman source)
  - Unknown components prevent full understanding of underlying biology

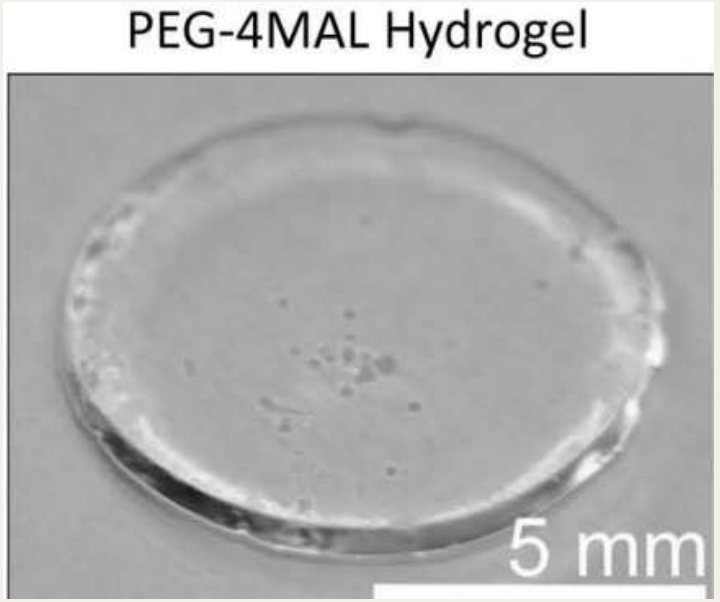
Polyethylene Glycol (PEG) Hydrogels are biocompatible and can be modified for cell adhesion

Polyethylene glycol (PEG) hydrogels

-Have intrinsic low-protein adsorption

-History of safe in vivo use

-Free radical addition created by chemical activation or UV radiation

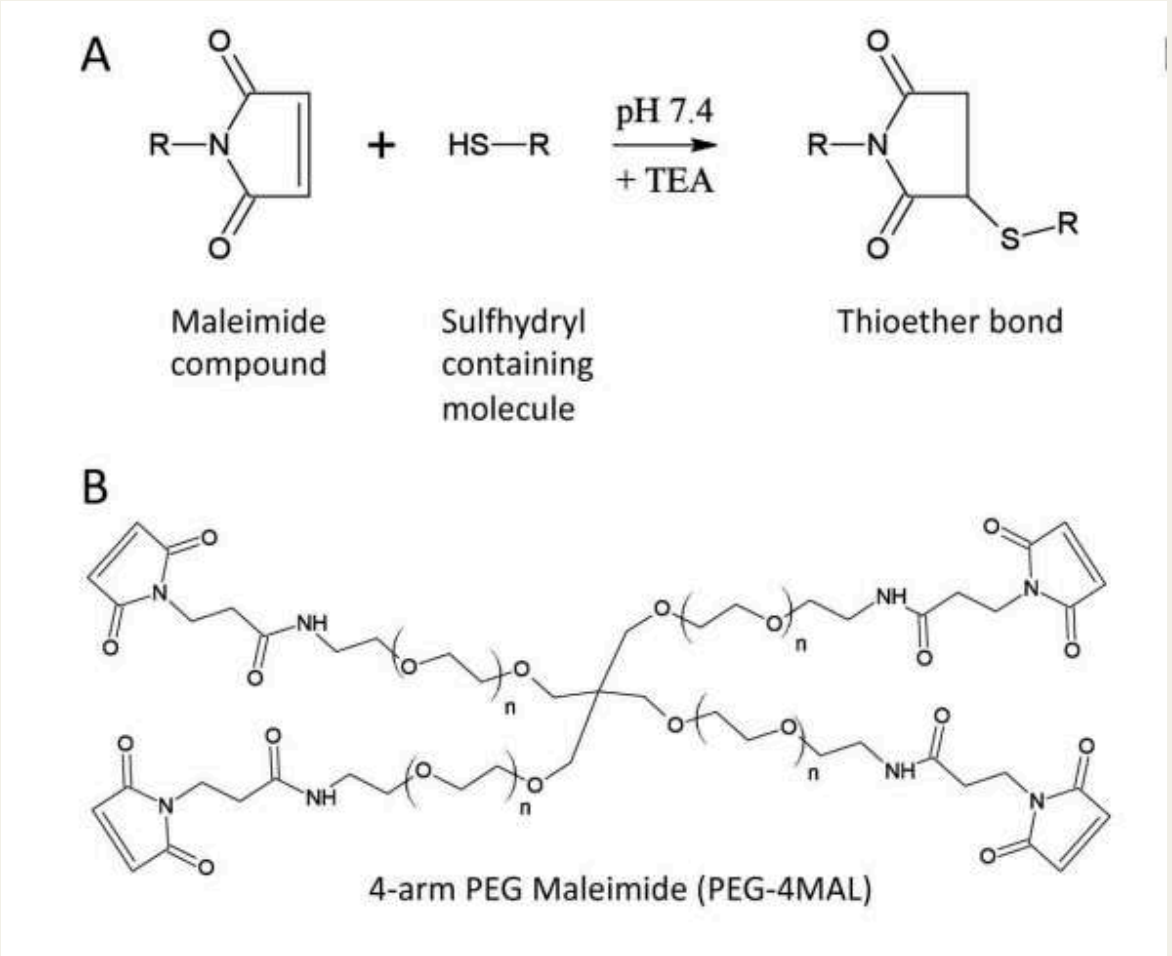


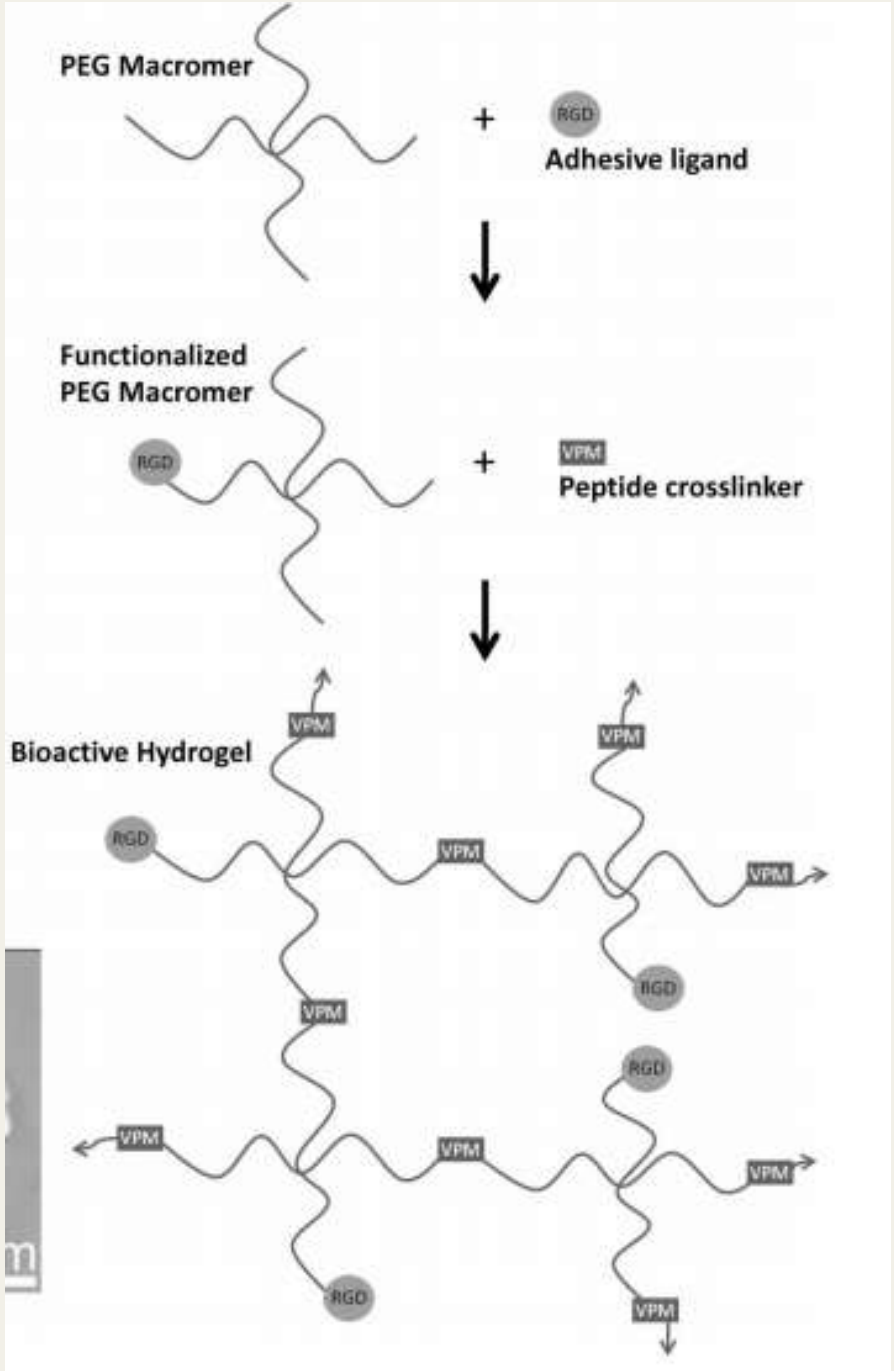
PEG- maleimide hydrogels

- Stoichiometric hydrogels with improved cross-linking efficiency
- Bio-ligand incorporation
- High specificity for thiols (S-H group)
- Michael Addition: avoid use of UV light but instead requires HEPES buffer

Phelps, E. A., (2012), Advanced Materials Adv. Mater., 24: 64-70.

Michael-Addition reaction forms a gel





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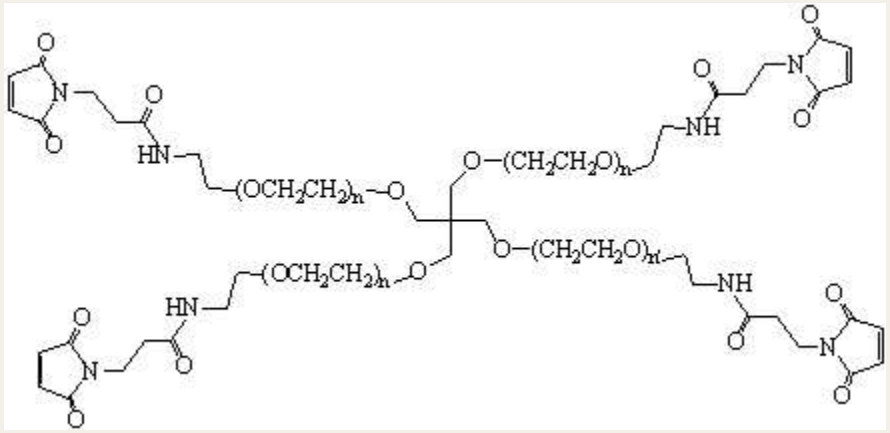
Organoid Chemistry

4- (Reactive) Arms PEG-Maleimide



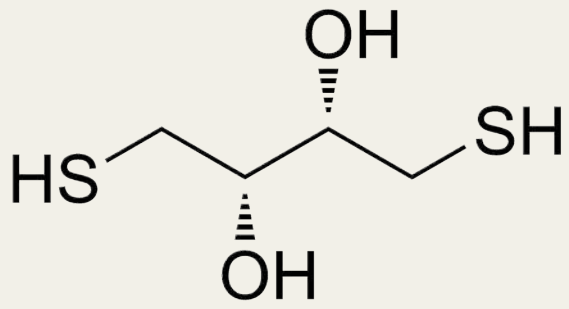
Step 1
→

GRGDSPC (RGD)

Peptide that adheres the cells to the matrix. It tricks the cells into thinking that they are inside the body.

Step 2
→

Dithiothreitol (DTT)



+

GCRDVPMSMRGGDRCG (VPM)

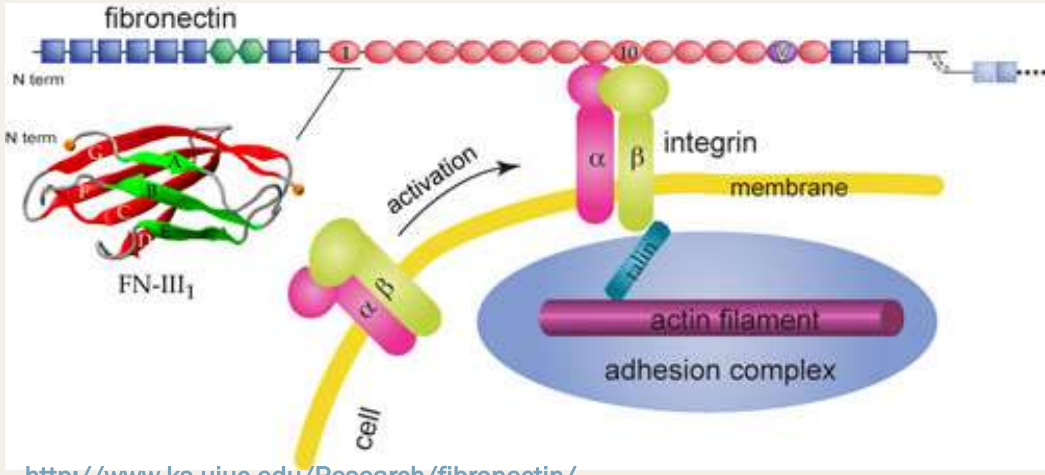
Crosslinker to maximize cell encapsulation

+

Cells

Cells required adhesive regions to attach to ECM

- **Integrins**
  - Main cell receptor protein that is used to bind to the extracellular matrix
  - Are principle receptors on animal cells
  - Consists of two transmembrane subunits, α and β



http://www.ks.uiuc.edu/Research/fibronectin/

- **Cells**
  - Cells have proteins that are located on the surface of them that allows it to bind to the extracellular matrix
  - They also have specific identifications i.e. $\alpha_5\beta_1$ of their integrin's that bind them to a specific ligand
  - Ligand binding is carried out through integrin receptor recognition of small peptide sequences such as RGD
- **Arg-Gly- Asp Acid (RGD)**
  - Sequence of Amino Acids that are linear
  - Functionalizes the system
  - Mimics tissue in the body

There are several tunable parameters to engineer designer organoids

- Weight %
- Pore Size
- Crosslinker
  - *GPQ is a slower process but allows organoids to be studied for months at a time*
- Ratio of degradable to non
  - *i.e 100% DTT*
  - *50;50 DTT, VPM*

Challenges

- Inability of cells to adapt quickly from tissue to the culture conditions which causes cell death
- It is difficult to create an organoid model, that can be used and tested on for patient generalization due to the heterogeneity within the complicated disease
- While there have been many research advances in the treatment of patients, it can be difficult to study cells that are not in the body for a long period of time

Conclusions

- Michael Addition Chemistry promoted by multi-armed PEG MAL allows for efficient reaction and decreases cell loss to senescence
- Better cross linking chemistry due to stoichiometric ratio flexibility
- This is an engineered hydrogel to replace matrigel models because it's bioadhesive, modular, and not cytotoxic.

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