Process Design for Bioactive Hydrogel for Use in Tissue Engineering

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Problem

Tissue transplantation faces risks such as shortage of donor organs, immunogenic responses, donor site morbidity and infection or pain post-surgery.

Bioactive scaffolds are a viable alternative to grow and differentiate cells into the desired organ but processes are complex and time consuming.

Figure 1. Cancer patient with massive humerus defect[1]

Design Approach

Create dynamically alterable environment to allow for stem cell attachment then osteoblast cell differentiation
Overall easy, modular, flexible, reproducible procedure
Process must be biocompatible

Design Applications

Made for researchers and clinicians to create tissue from patient's own cells
Tissue created will replace defects caused by trauma, cancer and disease
Organs created by engineered tissue will eliminate need for donor

Design Criteria

1. Mechanical Tunability
   25-40 kPa[2]
2. Cell Viability
   75-85%
3. Hydrogel Ligand Retention
   > 'simple diffusion'

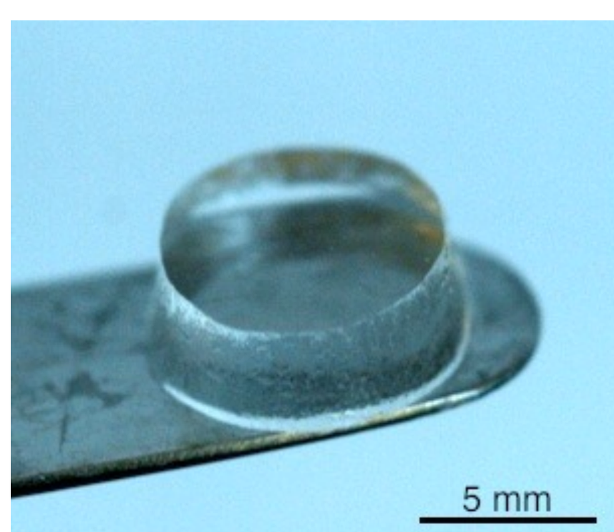
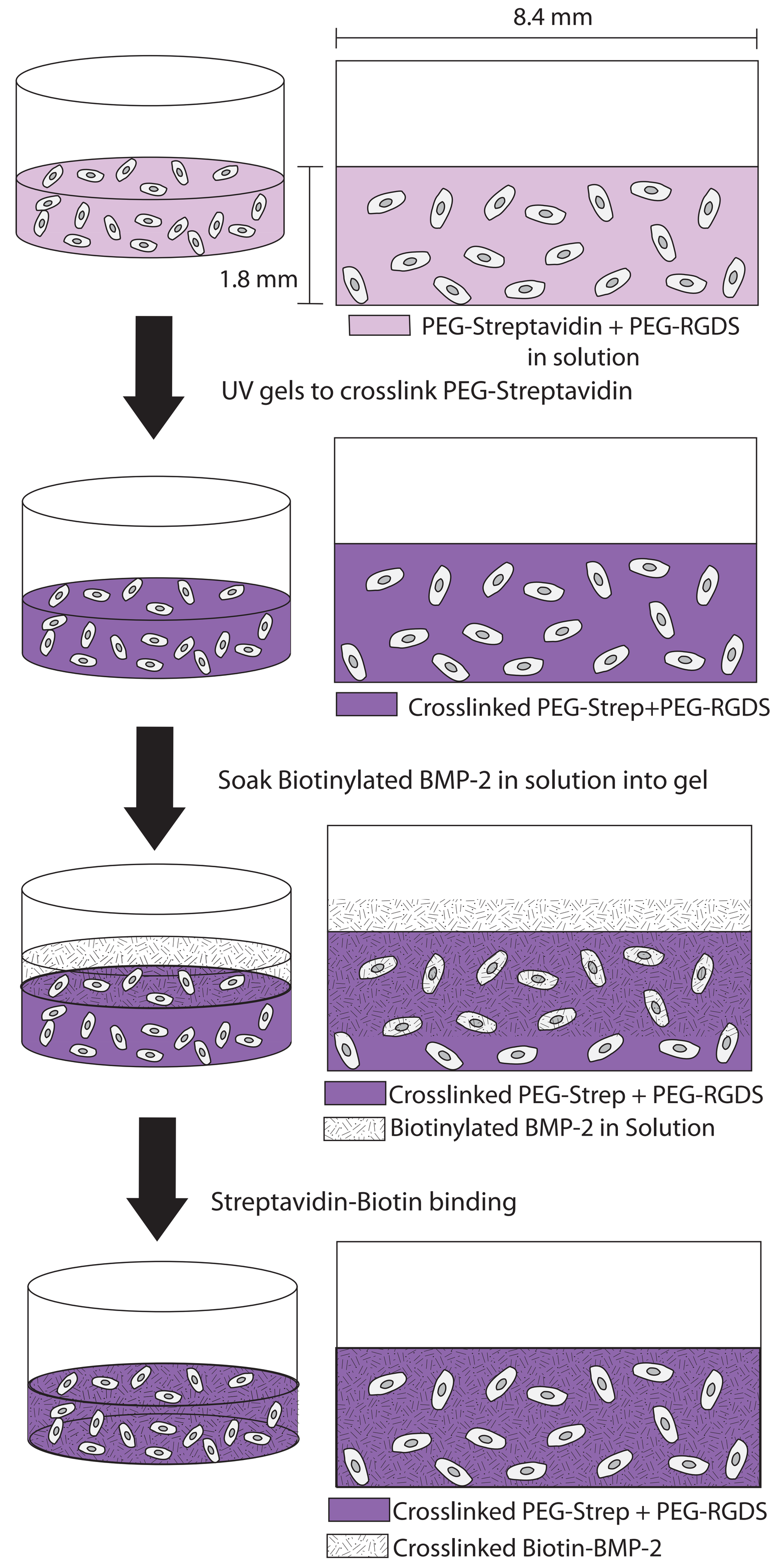


Figure 2. PEGDA Hydrogel Scaffold

Design Processes

Method 1- Avidin-Biotin Binding



Step 1

Initial solution also includes:
- Cells
- Buffer
- Photoinitiator

Step 2

- Gel solidifies, and encapsulates cells
- Commence 7 day cell culture

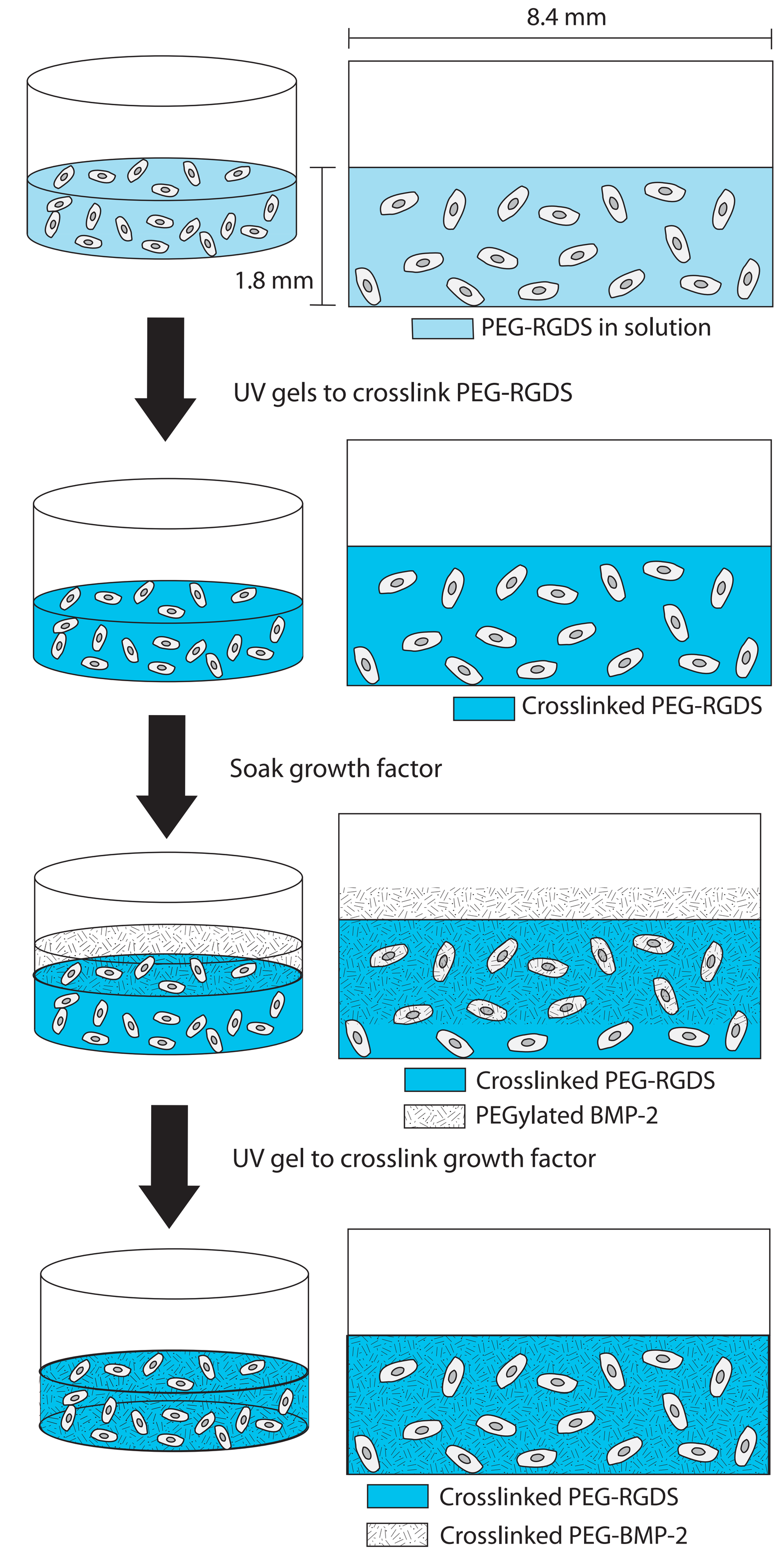
Step 3

- Growth factor diffusion for 24 hours

Step 4

- Growth factor attachment
- Commence differentiation period

Method 2-PEG-BSA Diffusion



Microscale Diagram of Growth Factor Incorporation

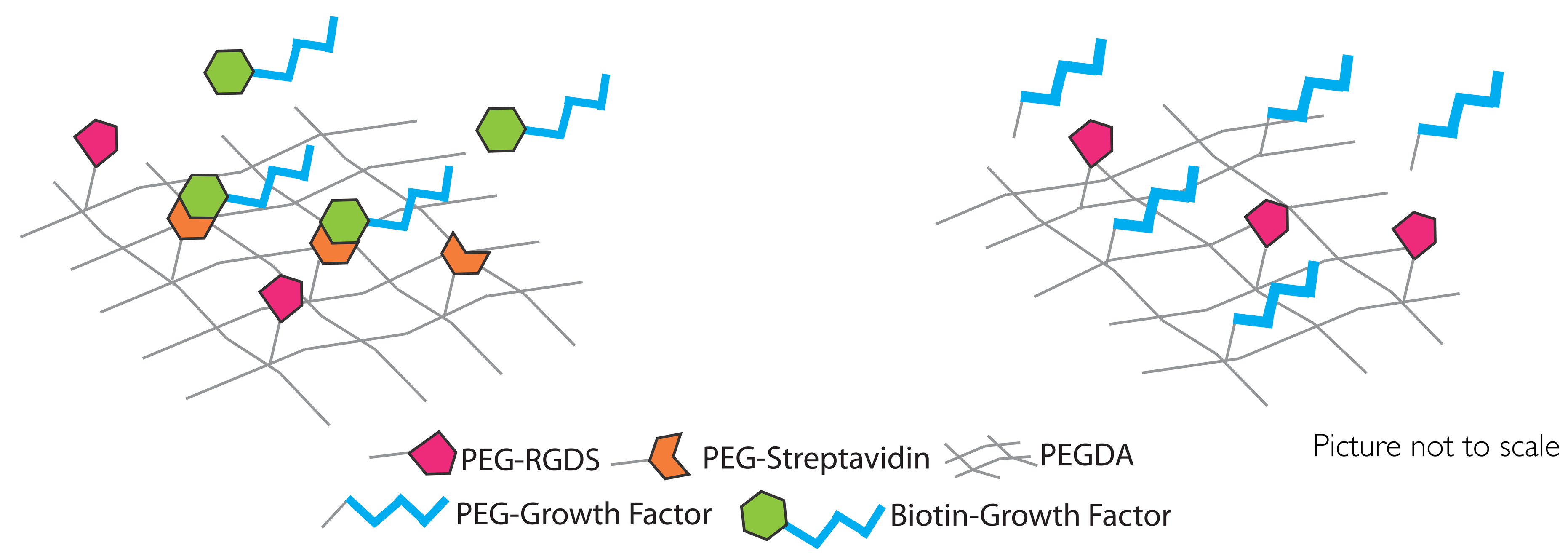


Figure 3. Microscopic schematic of hydrogel scaffold post crosslinking modification. Attachment and differentiation factors are attached to the hydrogel using PEG spacer

Acknowledgements

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Design Criteria Goals are Met

1. Mechanical Tunability

PEG w/v	Young's Modulus (kPa)
3%	2
5%	23
6%	**46**
10%	140

Table 1. Results from mechanical testing study. Results show that Young's Modulus of 6% PEGDA yields favorable results for osteoblast differentiation

2. Cell Viability

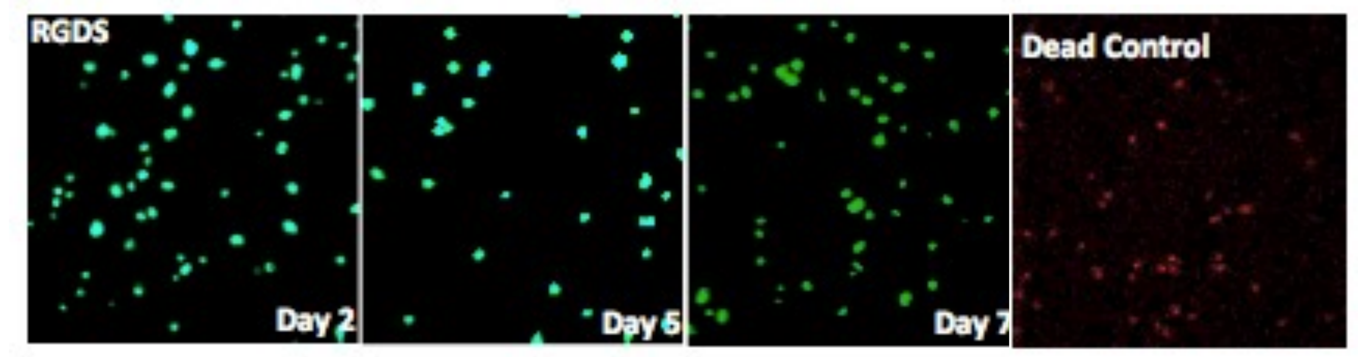

Figure 4. Representative images from Live/Dead assay for days 2, 5 and 7 (from left to right). The images are a representation of hydrogels made w/RGDS. Dead control is a hydrogel treated with 70% ethanol. Cell viability at Day 7 was found to be ~90%.

3. Hydrogel Ligand Retention

- Model ligands were used in place of the growth factors due to availability and cost effectiveness. The models were on the same order of magnitude in size as the growth factors.

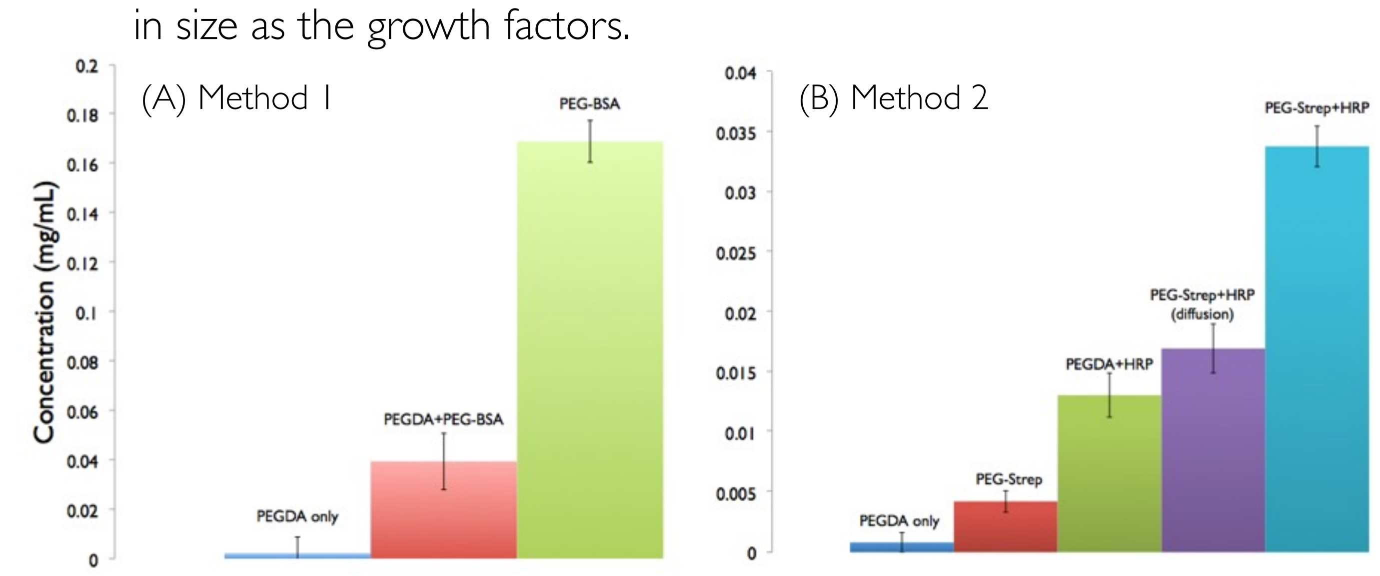

Figure 5. PEGDA only is (-) control. PEG-BSA and PEG-Strep+HRP represent (+) controls with proteins cross-linked initially. (A) Ninhydrin results. PEGDA+PEG-BSA was 24hr PEG-BSA diffusion. (B) Calorimetric assay with TMB results. PEG-Strep has protein cross-linked initially. PEGDA+HRP and PEG-Strep+HRP were 24hr HRP diffusion.

Conclusions

- PEGDA hydrogel mechanically tunable based on initial conditions of formation
- Hydrogel is biocompatible because of choice of hydrogel material, inorganic solvents and UV time.
- Incorporation of ligands using design processes showed modest improvement over simple diffusion
- Method of incorporation can be adapted to different ligands
- Components can be easily replaced for modularity

References

1. http://www.livestrong.com/article/24765-bone-cancer/
2. Engler, A.J., Sen, S., Sweeney, H.L., Discher, D.E., 2006. Matrix