

# Module 10 Assignment

## 585.751 Immunoengineering

**There was a mix-up, as per agreement with Dr. Ben-Akiva. I can submit this slightly different version of Assignment 10.**

1. (50 points) PEG is extensively used in designing nanoparticles and larger biomaterials to prevent immune cell recognition. Answer the following questions about PEG:

- a. (20 points) What is the mechanism by which PEG reduces immune recognition of and response to a nanoparticle or implanted biomaterial?

PEGylation is the process that involves conjugating hydrophilic polyethylene glycol (PEG) polymer chains to a molecule, such as a drug, therapeutic protein, or the surface of nanoparticles and biomaterials. It produces changes in conformation, electrostatic binding, hydrophobicity etc. PEG chains are hydrophilic, which contribute to create a “water cloud” around the conjugated material. The PEG layer sterically hinders nanoparticles from interacting with other particles and proteins in the blood. By preventing opsonization and reducing protein adsorption to the nanoparticles, macrophages do not bind and recognize the nanoparticles, and the nanoparticles can evade immune recognition and engulfment by the MPS.

- b. (15 points) What are the advantages and disadvantages of PEGylation?

PEGylation increases the circulation time of the nanoparticles or therapeutics agents in the blood and avoid them to be quickly cleared from the blood. By masking the therapeutic agent or nanoparticle from the immune system, PEGylation decreases the likelihood of an immune response against the nanoparticle, potentially reducing adverse events. The hydrophilic nature of PEG can improve the solubility of hydrophobic drugs, facilitating their absorption. PEGylation also, allows the nanoparticles to circulate and extravasate to the tumors or target tissues.

There are a variety of disadvantages to PEGylation:

- **Limited efficacy:** 50% of injected dose end up in the liver and spleen after 48h.
- **Liver or Spleen Accumulation:** a significant portion of PEGylated substances may end up in the liver of spleen, which can lead to off-target effects.
- **Can reduce uptake by target cells:** PEG prevents protein bindings.
- **May induce immune response:** people develop anti-PEG antibodies, anti-PEG IgM, which leads to accelerated blood clearance (ABC) upon subsequent injections. After second injection, association of anti-PEG IgM with the PEG particles may allow the immune cells to bind to the particles and clear them but also can lead to IgM mediated complement activation immune response.

- c. (15 points) Describe one alternative approach to PEGylation in engineering materials with “stealth” properties.

**Shape modulation:** Engineered material shape can be modulated to control MPS recognition and uptake. In vitro, it has been shown that particle shape affects macrophage uptake, and in vivo their distribution within the body with the ellipsoid particles being dispersed throughout the animal.



Spherical particles compared to elongated ellipsoidal or cylindrical particles are more rapidly phagocytosed by macrophages and cleared by the MPS organs. Ellipsoidal or cylindrical particles, have a different biodistribution pattern compared to spherical particles, leading to longer circulation in the bloodstream.

2. (50 points) The immune system plays a key role in tissue engineering and regenerative medicine that is still being elucidated. List 3 ways in which the immune system has been shown to be involved in tissue regeneration (either from the lecture videos or your own research). Additionally, describe one way in which a biomaterial for tissue engineering can be designed to modulate the immune system in order to improve regeneration.

### **Macrophage mediated tissue regeneration[1]**

Macrophages produce a variety of factors stimulating the production and activation of fibroblasts. Fibroblast and epithelial cells reconstitute a fibrous tissue which replaces the wounded tissue. Another important aspect of macrophage activity in tissue regeneration, is the activation of myofibroblasts, critical in the formation or remodeling of the ECM. Macrophages also participate in the regeneration of peripheral nerves.

### **Angiogenesis and Revascularization [1]**

Creation of new blood vessels after tissue injury and vascular remodeling are essential to provide O<sub>2</sub> and nutrients to repair damaged tissue and removing waste. Immune cells play an important role in vascular remodeling and promoting angiogenesis. A variety of immune cells, including M1 and M2c macrophages, dendritic cells, mast cells, eosinophils, and neutrophils, secrete pro- angiogenic mediators that stimulate the formation of new blood vessels from existing ones. M2a, M2c macrophages, NK cells, and CD4+ T-cells, induce arteriole genesis and vascular remodeling via secreted mediators.

### **Treg Functions in Tissue Regeneration**

Treg participate into tissue regeneration by modulating the inflammatory response. They help neutralize inflammatory cytokines and inhibit neutrophil extravasation. Tregs promote apoptosis of neutrophils and enhance the phagocytosis of dead neutrophils, they induce macrophage polarization towards the M2 phenotype, which is more conducive to healing. They also help tissue repair by dampening inflammation in suppressing CD4, CD8 T -cell, and effector T-cells. They can enhance ECM growth by activating myoblast production.

One way to design a biomaterial to mitigate the immune system is either making biomaterials out of ECM components or coating biomaterials with ECM: using such surgical meshes can have a direct effect on M1 activation, preventing it and furthermore, these biomaterials can induce IL-10 a cytokine essential in tissue repair [2] [3].

Another approach is the de-cellularization of tissues as scaffolds which enables the removal of most immunogenic components and additionally, the de-cellularized ECM can contain immunomodulatory cytokines and growth factors.

[1] P. Abnave and E. Ghigo, "Role of the immune system in regeneration and its dynamic interplay with adult stem cells," *Semin. Cell Dev. Biol.*, vol. 87, pp. 160–168, 2019, doi: 10.1016/j.semcdb.2018.04.002



[2] A. Vishwakarma *et al.*, “Engineering Immunomodulatory Biomaterials To Tune the Inflammatory Response,” *Trends Biotechnol.*, vol. 34, no. 6, pp. 470–482, 2016, doi: 10.1016/j.tibtech.2016.03.009

[3] J. Kajahn *et al.*, “Artificial extracellular matrices composed of collagen I and high sulfated hyaluronan modulate monocyte to macrophage differentiation under conditions of sterile inflammation,” *Biomatter*, vol. 2, no. 4, pp. 226–273, 2012, doi: 10.4161/biom.22855

