Module 10 Assignment

585.751 Immunoenginnering

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 they 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 decrease 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.

Inflammatory Response and Resolution [1]
Immune cells such as neutrophils and macrophages are among the first cells to the site of injury. These cells help to clear debris and pathogens from the site on infection. Neutrophils help create a microenvironment conducive to the recruitment and activation of other immune cells, including monocytes. Monocytes can then differentiate into macrophages, which play a variety of roles in the immune response, including phagocytosis of pathogens and debris, secretion of cytokines and modulation of the inflammation.
M1-like pro-inflammatory macrophages maintain inflammation and initiate the first steps of tissue healing, and M2-like anti-inflammatory macrophages contribute to resolve inflammation and promote tissue remodeling. Pro-inflammatory macrophages can activate effector T cells, contributing to the amplification of the immune response against pathogens; stimulate T cells to produce and release pro-inflammatory cytokines (Il-2, IFN-γ, TNF-α); and recruit additional immune cells, such as neutrophils and monocytes.
In contrast, regulatory T cells (Tregs) help maintaining an anti-inflammatory environment, supporting the resolution of inflammation.
Immune cells regulate stem/progenitor cell proliferation, differentiation, and even dedifferentiation [1]
The immune cells release a variety cytokines, chemokines, and growth factors, that can either promote or inhibit the regenerative capacity of stem cells.
For instance, mesenchymal stem cells (MSCs) are multipotent cells responsible for the regeneration of adult tissue: extracellular vehicles (EVs) secreted by MSCs can promote tissue regeneration by creating a pro-regenerative environment, enabling endogenous stem and progenitor cells to repair affected tissues.
Angiogenesis and Revascularization [1]
Immune cells, including macrophages and specific subtypes of T cells, produce angiogenic factors, such as VEGF ad FGF that promote the growth of new blood vessels, crucial for providing oxygen and nutrients to regenerating tissues and removing waste products.

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 even can induce M2-related cytokine IL-10 [2] [3].
Another approach is the de-cellularization of tissues as scaffolds which enables the removal of most immunogenic components and 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