

**Tissue Engineering**  
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**Lecture - 27**  
**Host integration and immune responses- Part 2**

Hello everyone in the previous lecture, we studied some basics of immunology. In today's lecture, we will look at immune responses to biomaterials and how we can design immunomodulatory biomaterials to elicit desirable responses from the host system to your biomaterial. This lecture is presented by me, Vasudha, an MS scholar in Doctor Vignesh Muthuvijayan's lab.

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**Inflammatory response to bio-materials**

- The duration and the intensity of the inflammation or the wound healing response is dependent on the size, shape, chemical and physical property of the implant
- It is a measure of biocompatibility
- Chemicals released from plasma, cells, and injured tissue mediate the inflammatory response



In inflammatory responses to biomaterials, it has been observed that the duration and the intensity of the inflammation or the wound healing response are dependent on various properties of the materials. Such as the material's physical and chemical properties; its size, shape and other biological aspects.

The inflammatory response to biomaterials is a measure of the material's biocompatibility. In response to the material's placement in the host system, various chemicals are released from the plasma, the cells of the injured tissue and these chemicals mediate the inflammatory response.

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**Host responses toward biomaterials:**

1. Biomaterial implantation-

-accompanied by injury through the surgical procedure  
-initiates an inflammatory response to the biomaterial-  
starts with formation of a provisional matrix.

2. Implantation of engineered cell-material hybrids

elicits an adaptive immune reaction toward the cellular  
component - influences the host response to the material  
component.

3. degradable devices

immune response is additionally affected--by degradation  
products and surface changes of the biomaterial that occur  
due to the degradation process.



These are the different host responses towards biomaterials. Upon biomaterial implantation, which is done through a surgical procedure, there is an injury accompanied by the implantation. In response to the injury, an inflammatory response is initiated in the host system, and this starts with the formation of a provisional matrix, Usually, which is collagen deposition. If you are implanting engineered cell-material hybrids, the cellular component of the material elicits an adaptive immune response from the host. If you are implanting degradable materials, other than the material itself, it's degradation products and the surface changes on the material can elicit various immune responses.

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**Onset of the inflammatory response: blood proteins and alarmins induce cellular (granulocyte / monocyte) activation**

Nanoseconds after the first contact with tissue, proteins from blood and interstitial fluids adsorb to the biomaterial surface.

This layer of proteins determines the activation of

coagulation cascade  
complement system  
platelets and immune cells

The protein layer guides their interplay which results in the onset of the inflammatory response.



So, within a few nanoseconds of the contact of the material with the tissue, various blood proteins and also interstitial fluids get absorbed onto the material surface. This layer of proteins determines the activation of various pathways, such as the coagulation cascade, the complement system, and also causes activation of platelets and various immune cells. And this protein layer also guides the interplay between these pathways, which results in the onset of the inflammatory response.

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**Onset of the inflammatory response:**

(i) Fibrinogen adsorbed on surface of biomaterial---

Undergoes adhesion related conformational change--exposure of integrin binding domains --binds to phagocytes and can activate them-- thus initiating inflammatory response

(ii) complement---series of proteins in serum involved in immune response.

--activated upon binding to bio materials-- in inflammatory conditions-- activated via 3 pathways: classical, alternative and lectin pathway---BM surfaces--first 2 pathways..

Activation is always associated with the biomaterial adsorbed protein layer--

which can attach to IgG  
Spontaneously adsorb to BM surface

--- pathway activation is initiated when the other members of the pathway are cleaved by coagulation cascade members---onset of inflammatory responses at the implant sites.



At the onset of the inflammatory response, fibrinogen can get absorbed to the surface of the biomaterial. Adhesion of fibrinogen onto the biomaterial surface can induce some conformational change in fibrinogen, hence exposing some integrin-binding domains. These domains can then bind to various phagocytes and causing their activation and thus initiating an inflammatory response.

Another class of proteins is serum proteins, the complement proteins which are involved in the immune response. They get activated upon binding to materials via three different pathways, the classical, alternative, and the lectin pathway. In the case of biomaterials, it is the classical and the alternative pathway, that are generally activated.

Activation is always associated with the biomaterial adsorbed protein layers. So, immunoglobulins such as IgG can bind to the protein layer, which is adsorbed onto the biomaterial surface and helps in the attachment of various complement molecules. And the pathway activation is initiated when other members of the pathway are cleaved by

coagulation cascade members and hence causing the onset of the inflammatory response at the site of the implant.

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**Onset of the inflammatory response:**

Results in

- Mast cell degranulation
- increasing vascular permeability
- attracting and activating of granulocytes and monocytes
- inducing granulocyte ROS (reactive oxygen species) release
- platelet adhesion and activation on biomaterial surfaces-- propagate the coagulation cascade

coagulation cascade and complement system closely interact on the biomaterial surface and modulate each other's activity.

The cross-talk between both systems operates synergistically in inflammatory cell activation.



The onset of the response, results in mast cell degranulation, increase in vascular permeability, which causes extravasation of immune cells into the tissue, attraction, and activation of granulocytes and monocytes, inducing the release of reactive oxygen species and platelet adhesion and activation on the biomaterial surface, which can propagate the coagulation cascade.

The coagulation cascade and the complement system closely interact with each other at the biomaterial surface and modulate each other's activity. The crosstalk between the two systems operates synergistically in inflammatory cell activation.

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**Onset of the inflammatory response:**

(iii) ECM adhesion proteins:

Fibronectin and vitronectin---also attach to bio material surface

Critical in regulating inflammatory response to bio material

- promote fusion of macrophages--forming FBGC on bio material surface



Also, ECM adhesion proteins such as fibronectin and vitronectin, can attach to the biomaterial surface and are critical in regulating the inflammatory response to biomaterials. These proteins can also promote the fusion of macrophages to form something called foreign body giant cells, FBGC, which we will look at later. These ECM adhesion proteins can help in the fusion of macrophages to form FBGC on the biomaterial surface.

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**Onset of the inflammatory response:**

Basically cell adhesion and activation at the bio material surfaces occurs via interaction of adhesion receptors and adsorbed proteins at bio material surfaces...

Integrins---major adhesion receptors on leucocytes

Ligands for integrins on bio material surfaces---fibrinogen, factor X, iC3b, fibronectin, vitronectin - all of which have been shown to attach to biomaterial surfaces

*Induce phagocytosis, degranulation, release of ROS and cytokines---events which play important role in the inflammatory response toward biomaterials*



Basically, cell adhesion and activation at the biomaterial surface occurs via the interaction of adhesion receptors on the leukocytes which are usually integrins, and the adsorbed proteins which are present on the biomaterial surface, and the ligands for the integrins which are present on the leukocytes.

On the biomaterials surface, the ligands for the integrins can be fibrinogen, factor X or the ECM components such as fibronectin and vitronectin. The interaction between the integrins and the ligands can induce phagocytosis, degranulation, the release of reactive oxygen species, and cytokines. All these events play an important role in the inflammatory response to biomaterials.

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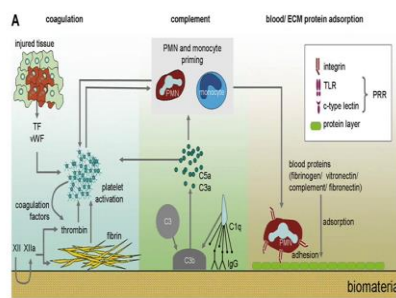


Fig. Adsorption of blood proteins and activation of the coagulation cascade, complement and platelets result in the priming and activation of PMNs, monocytes and resident macrophages.

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In this image, you can see the protein layer, to which molecules, blood proteins such as fibrinogen or ECM components such as fibronectin, vitronectin can attach. Here, you can see the different systems, the coagulation system, the complement system.

Here, the injured tissue is releasing tissue factor, von Willebrand factor, which can cause platelet activation. Also, upon attachment to the biomaterial surface, factors such as factor VII can undergo auto activation causing the release of thrombin. The release of thrombin can further cause platelet activation; this can cause clot formation. Platelet activation, as you can see, can also cause a polymorphonuclear leukocyte and monocyte priming.

In the complement system, here, you can see that cleaved components of the complement system, such as C3b can directly attach to the biomaterial surface. Components such as C1q, they bind to the IgG molecules, immunoglobulins, which can be attached to the biomaterial surface. Whether the complements are being activated by the classical or alternative pathway. They all converge at C3 convertase, which can then release anaphylatoxins such as C5a and C3a, which can cause a PMN and monocyte priming.

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#### Danger signals and pattern recognition receptors

**Alarmins**- endogenous equivalent of pathogen-associated molecular patterns (PAMPs)

Examples of alarmins:

- Heat shock proteins
- ATP
- Uric acid

Alarmins -

- released by cells dying in a non-programmed way (necrosis) to signal tissue damage.
- Recognized by macrophages and dendritic cells via pattern recognition receptors (PRRs)

↓  
Scavenger receptors

Toll-like receptors

C-type lectins

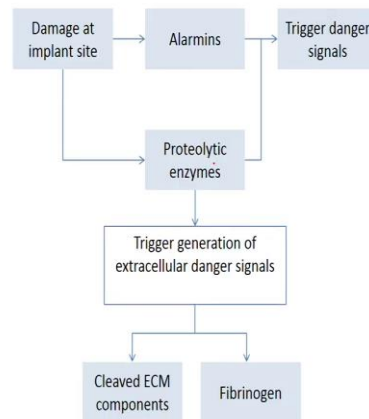


Now, we will look at different danger signals and pattern recognition receptors that are involved in causing the inflammatory response. Alarmins are the endogenous equivalence of pathogen-associated molecular patterns, examples of some of them are heat shock proteins, ATP, uric acid. Alarmins are released by cells that are dying in a non-programmed way such as necrosis, as opposed to programmed cell death, which is apoptosis.

So, these alarmins can be recognized by macrophages and dendritic cells, which have pattern recognition receptors on them. Examples of such receptors include scavenger receptors or toll-like receptors and C type lectins.

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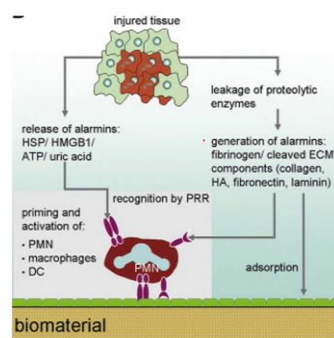
#### Danger signals and pattern recognition receptors



When there is damage at the implant site, alarmins are released. The release of alarmins can trigger danger signals, or the damage at the implant site can cause the release of various proteolytic enzymes, which can again trigger danger signals. The release of proteolytic enzymes can trigger a generation of extracellular danger signals, such as cleaved ECM components such as collagen peptides, fibronectin and also causes the release of fibrinogen.

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#### Danger signals and pattern recognition receptors



Basically, induced danger signals are capable of immune cell activation at biomaterial surfaces





Here, you can see that, upon injury to the tissue caused by implantation, there is a release of alarmins and leakage of proteolytic enzymes. So, these alarmins are being recognized by the pattern recognition receptors, which are present on the polymorphonuclear leukocytes, causing their activation.

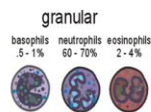
The leakage of proteolytic enzymes also causes the generation of alarmins, which can be fibrinogen or cleaved ECM components. And these cleaved ECM components can also get absorbed into the protein layer on the biomaterial, which can cause further immune reactions.

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#### PMN activation—effects

Other than DCs and macrophages, PMNs engage with protein coated BM surface

Triggers phagocytic response and degranulation

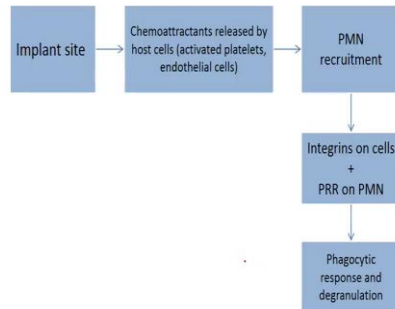


Also, as we saw previously, other than dendritic cells and macrophages, polymorphonuclear leukocytes can engage with the protein-coated biomaterial surface. They can trigger phagocytic response and degranulation. These are what we define as granular leukocytes.

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#### PMN activation--effects

Following injury and protein deposition, inflammatory cells predominantly polymorphonuclear leukocytes (PMNs, granulocytes) migrate from the blood toward the implant site.



Here, you can see that when there is damage at the implant site, chemoattractants are released by host cells, such as activated platelets or endothelial cells. This causes the recruitment of polymorphonuclear leukocytes. And the PRR, which is the pattern recognition receptors present on the PMNs will interact with the integrins present on the cells and this causes phagocytic response and degranulation.

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#### PMN activation--effects

PMNs secrete proteolytic enzymes and ROS

- these destructive agents may corrode material surfaces Ex. polyurethane
- damage surrounding tissue, prolonging the inflammatory response
- metabolic exhaustion and depletion of the granulocytes' oxidative resources.

Due to the continuous release of ROS, the microbial killing capacity of PMNs is dramatically reduced, which has been related to severe biomaterial-centred infections



PMNs can secrete various proteolytic enzymes and reactive oxygen species. These are highly destructive agents and can corrode material surfaces; this has been seen in the

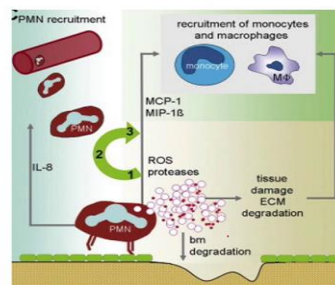
case of polyurethane. They can also damage the surrounding tissue, hence prolonging the inflammatory response. They also cause metabolic exhaustion and depletion of the granulocytes oxidative resources. Due to the continuous release of ROS, the microbial killing capacity of PMNs gets reduced, hence making the biomaterials more susceptible to infections.

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#### PMN activation—effects

PMN- significant source of immunoregulatory signals like IL-8

Autocrine PMN attraction by IL-8 -----> prolonged presence of granulocytes within chitosan materials



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PMNs are also a significant source of immunoregulatory signals such as IL-8. Autocrine attraction by IL-8, shown prolonged presence of granulocytes within chitosan materials.

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#### Chronic inflammation: dual role of macrophages as inflammatory mediators and wound healing regulators in the foreign body reaction

Chronic inflammation- persistent inflammation at implant site



Macrophages- critical role in wound healing & Tissue regeneration

- Role of macrophages in wound healing:
- Phagocytosis of wound debris
- Release of enzymes important for tissue reorganization
- Release of cytokines and growth factors to induce fibroblast migration and proliferation



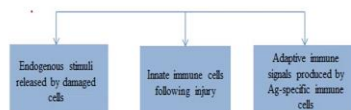
As we know, chronic inflammation is the persistent inflammation present at the implant site. At the site of implantation, monocytes will arrive. These monocytes will differentiate into tissue macrophages. These macrophages will foster invasion of additional inflammatory cells. Now we will look at the dual role of macrophages, which can act as inflammatory mediators and also wound healing regulators in foreign body reaction.

Macrophages have been found to be critical in wound healing and tissue regeneration. The role of macrophages in wound healing includes phagocytosis of the wound debris, the release of various enzymes important for tissue reorganization, and the release of cytokines and growth factors to induce fibroblasts migration and proliferation.

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#### Chronic inflammation: dual role of macrophages as inflammatory mediators and wound healing regulators in the foreign body reaction

- Different functions are promoted by different macrophage subsets
- Based on macrophage functions involved in maintaining homeostasis:
  - Classically activated macrophages
  - Regulatory
  - Wound healing macrophages
- Different macrophage populations are generated in response to either:

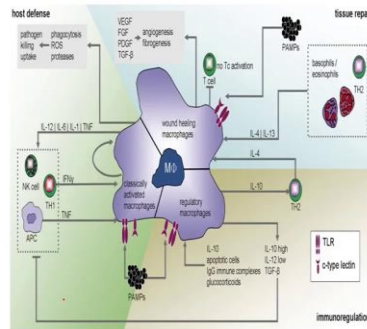


Different functions are promoted by different macrophage subsets. Based on the macrophage functions involved in maintaining homeostasis, they are classified as classically activated macrophages, regulatory macrophages and wound healing macrophages.

Different macrophage populations are generated in response to different types of stimuli, which can be endogenous stimuli released by damaged cells, or stimuli from innate immune cells following injury, or adaptive immune signals produced by antigen-specific immune cells.

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**Chronic inflammation: dual role of macrophages as inflammatory mediators and wound healing regulators in the foreign body reaction**



Macrophages---heterogeneous population of cells with different phenotypic profiles performing distinct functions in host defense, wound healing, and immune regulation.

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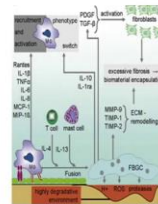
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Here, you can see the different subsets of macrophages, wound healing, regulatory and the classical classically activated macrophages. So, therefore, macrophages are a heterogeneous population of cells with different phenotypic profiles. We can design immunomodulatory biomaterials, that can activate a particular phenotypic profile of macrophages.

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**Foreign body giant cell formation (FBGC)**

- Macrophages that attach and recognize a foreign material show classically activated phenotype (phagocytic, inflamm. cytokines)
- Single macrophages are able to phagocytose particles up to a size of 5 μm.
- If the particle size is larger, macrophages attempt to coalesce to FBGCs.
- IL-4, IL-13, CCL-2 - help in formation of FBGCs
- properties of the biomaterial surface are important for FBGC formation;
- Since biomaterials are immediately covered, it is the adsorbed protein layer that renders the surface fusogenic.



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*Implant surface properties dictate macrophage responses*

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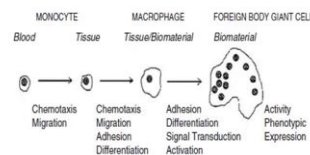
Now, we will look at foreign body giant cell formation by macrophages. Macrophages that attach to a foreign material show a classically activated phenotype, where you can see heightened phagocytic activity and release of inflammatory cytokines.

Usually, like single macrophages can phagocytose a foreign material of about 5 micrometers in size. If the particle size is larger than that, then the macrophages attempt to coalesce to form FBGC that is the Foreign Body Giant Cells. As you can also see in the figure, IL-4 and IL-13 help in the fusion of macrophages to form FBGC. The properties of the biomaterial surface are important for FBGC formation. And the protein layer which is observed on the biomaterial's surface causes the fusion of macrophages to FBGC.

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#### Foreign body giant cell formation (FBGC)

- If the FBGCs do not succeed in phagocytosing the foreign material, they remain at the biomaterial-tissue interface
- display a reduced phagocytic activity and enhanced degradative capacity
- In an attempt to resorb the non-phagocytosable biomaterial, FBGCs secrete protons, enzymes, and ROS



If the FBGCs do not succeed in phagocytosing the foreign material, they remain at the biomaterial-tissue interface. Then, over time, they display reduced phagocytic activity and enhanced degradative capacity. In an attempt to resorb the material, which it was not able to phagocytose, the FBGC will secrete protons, enzymes and reactive oxygen species.

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### Matrix Metalloproteinases (MMPs)

- Macrophage-derived proteolytic enzymes



- MMP-9
  - Key enzyme in degradation of collagen implants with combined action of other gelatinases and collagenases
  - Increased levels indicative of inflammation and associated with poor wound healing

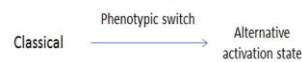


Another class of enzymes is matrix metalloproteinases. They are macrophage-derived proteolytic enzymes. They can be collagenases or gelatinases; MMP-8, MMP-13 here are collagenases, MMP-2, MMP-9 are gelatinases. MMP-9 is a key enzyme involved in the degradation of the collagen implant with the combined action of other gelatinases and collagenases. Increased levels of MMP-9 are indicative of inflammation at poor wound healing.

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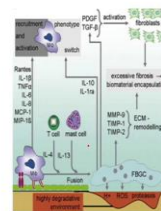
### Fusion-induced macrophage phenotype switch

- Fusion of macrophage to form FBGC:



- IL-4 and IL-13 -----> Fusion inducing cytokines

- After biomaterial recognition, macrophage releases
  - IL-8
  - IL-1β
  - TNF-α
  - IL-8
  - MCP-1
  - RANTES
  - ENA-78

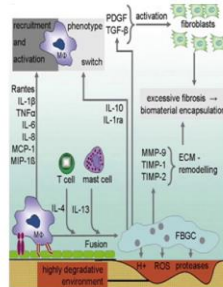


Here, we are looking at the fusion induced macrophage phenotype switch. Upon the fusion of macrophage to form FBGCs, it undergoes a change from the classical to alternative activation state. Here, as we saw before, IL-4 and IL-13 help in the fusion, they induce the fusion of macrophages. And after biomaterial recognition, the macrophage releases various cytokines. These are the various cytokines that are released, and this causes the phenotypic switch.

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#### Impaired wound healing and excessive fibrosis

- At implantation site --> FBGCs produce anti-inflammatory cytokines (IL-10, IL-1ra)
- This immunosuppressive activity is counter-regulated by proteolytic and pro-oxidant microenvironment due to release of ROS and degradative enzymes



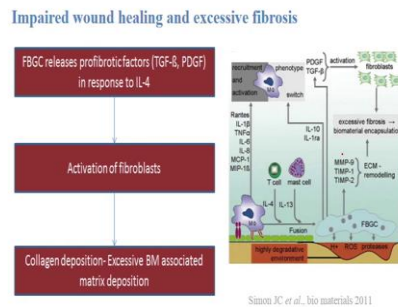
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At the site of implantation, FBGCs produce anti-inflammatory cytokines also, such as IL-10 and IL-1ra. This immunosuppressive activity is counter regulated by the proteolytic and pro-oxidant microenvironment, which is caused due to the release of reactive oxygen species and degradative enzymes.



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The formation of the FBGCs in the foreign body giant cell releases profibrotic factors such as transforming growth factor-beta and platelet-derived growth factors in response to IL-4, and this causes the activation of fibroblasts. Activation of fibroblasts causes excessive collagen deposition causing fibrosis.

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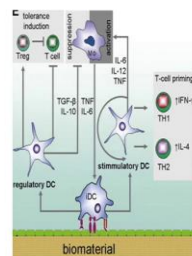
#### Dendritic cell responses to biomaterials

Biomaterials activate DCs by triggering receptors and signaling cascades of the pathogen recognition system

DCs may sense materials using PRRs including toll-like receptors and C-type lectins

Receptor-activating ligands ---> danger signals from adsorbed protein layer

Different polymers ---> different effects on DC maturation and activation



Now, we will look at dendritic cell responses to biomaterials. Biomaterials activate dendritic cells by triggering receptors and signaling cascades of the pathogen recognition

system. Dendritic cells may sense materials using the pattern recognition receptors present on them such as the toll-like receptors and C type lectins.

The ligands for these receptors are the danger signals coming from the adsorbed protein layer on the biomaterial surface. Different polymers show different effects on dendritic cell maturation and activation.

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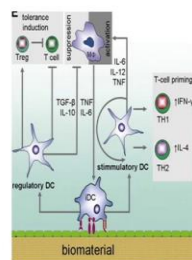
#### Dendritic cell responses to biomaterials

Depending on which PRR is engaged, DC maturation can be promoted or inhibited leading to immunity or tolerance

Immunogenic DCs ---prolong the immune response to biomaterials and delay wound healing

tolerogenic DCs---down-regulate the immune cells and resolve inflammation.

With respect to biomaterial application induction of tolerogenic DCs at the implant site would provide a powerful means to limit the immune response and to promote wound healing and biomaterial integration.



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So, therefore, depending on which pattern recognition receptor is engaged, DC maturation can be promoted, which leads to immunity or can be inhibited which leads to tolerance. Immunogenic dendritic cells prolong the immune response to biomaterials and delay wound healing. Tolerogenic dendritic cells down-regulate the immune cells and resolve inflammation. So, what we can do here is, if we are able to induce the tolerogenic dendritic cells at the implant site, then we can limit the immune response and promote wound healing and integration of the biomaterial into the host.

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#### T lymphocyte responses to biomaterials

T lymphocytes-adhere to synthetic biomaterials *in vitro*.

-promote macrophage adhesion and fusion via paracrine effects.

During the initial response to biomaterials, lymphocytes and macrophages release inflammatory mediators:

cytokines IL-1 $\beta$ , IL-6, TNF $\alpha$   
chemokines IL-8, MCP, MIP-1 $\beta$ , ENA-78

attract and activate inflammatory effector cells such as neutrophils, monocytes, T lymphocytes and natural killer cells.

Interestingly, release of IL-1 $\beta$  and TNF $\alpha$  decline over time in favor of IL-10 and MMP-9, tissue inhibitor of MMPs (TIMP)-1 and TIMP-2 – important mediators for ECM remodeling in wound healing.



Now, we look at T lymphocytes and their responses to biomaterials. T lymphocytes adhere to synthetic biomaterials *in vitro*, it has been shown. They promote macrophage adhesion and fusion via paracrine effects. During the initial response to biomaterials, the lymphocytes, and the macrophages, they release various inflammatory mediators such as various cytokines and chemokines. The release of IL-1 $\beta$  and TNF- $\alpha$  decline over time, and they release tissue inhibitor of MMPs, that is, TIMP-1 and TIMP-2, which are mediators for ECM remodeling and in wound healing.

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#### T lymphocyte responses to biomaterials

activated T cells were identified in response to synthetic materials.

'Synthetic biomaterials do not serve as an antigen'

Given that the biomaterial is not degradable and that no bacteria transiently attach to its surface, T cell activation via antigen presentation does not occur.

the question remains how T cell activation is mediated during foreign body reaction.

?????

It has been suggested, that synthetic biomaterials may present functional groups on their surfaces that act as mitogens.

Mitogens are lectins that can trigger lymphocytes by cross-linking of glycoproteins on the lymphocyte surface.



The question here is synthetic biomaterials do not serve as an antigen. So, how does T cell activation occur during a foreign body reaction? It has been suggested that synthetic biomaterials may present functional groups on their surfaces that can act as mitogens. Mitogens are lectins that can trigger lymphocytes by crosslinking of glycoproteins on the lymphocytes surface.

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#### Implications

A key for long-term survival and function of biomaterials is that they do not elicit a detrimental immune response.

How does one avoid, control, or exploit such responses?

Understanding the complex interactions of host response and material implants reveals the need for and also the potential of "immunomodulating" biomaterials.

Ex. minimally inflammatory scaffolds for tissue repair and immunotherapies eliciting desired B cell (antibody) responses, T cell responses.

biomaterials are being developed to direct specific immunological processes; Ex. bio materials containing proteins, peptides

Major properties and processes that influence biomaterials-directed immunity --> physical dimensions of a material, its epitope content, and its multi valency.



What are the implications? A key for long term survival and function of biomaterials is to reduce the detrimental immune response to biomaterials. How do we avoid or control such responses? For that, a start would be to understand the complex reactions that occur between the host and the material. So that we can design immunomodulating materials that can elicit desirable responses from the host system.

An example would be minimally inflammatory scaffolds, which can elicit desirable B cell and T cell responses. Biomaterials are being developed to direct specific immunological processes. Major properties of biomaterials to direct immunity are physical dimensions of the material, its epitope content, and its multivalency.

Thank you.