Apoptotic Cell-Mimetic Polymers for Anti-Inflammatory Therapy

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The field of biomaterials has seen a strong rejuvenation due to the new potential to modulate immune system in our body. This special class of materials is called “immunomodulatory biomaterials”. Generally, three fundamental strategies are followed in the design of immunomodulatory biomaterials: (1) immuno-inert biomaterials, (2) immuno-activating biomaterials, and (3) immuno-tolerant biomaterials. While many applications of immuno-inert biomaterials such as biocompatible medical implants have been already proposed in the past decades, the ability to engineer biological activity into synthetic materials greatly increases the number of their potential uses and improves their performance in more traditional applications. The major focus of researchers is now set on developing immuno-tolerant biomaterials for anti-inflammatory therapies. In this review, we therefore introduce recent developments of immuno-tolerant biomaterials. Especially we introduce an apoptotic cell membrane-inspired polymer and its post-inflamatory effects on immune cells in this article.

Key Words: Apoptosis; Phosphatidylserines; Anti-Inflammatory Agents; Immunomodulation; Biocompatible Materials

INTRODUCTION

1. History of immuno-inert biomaterials

Biomaterials have been historically developed with the aim to transplant then into the human body as substitutes for damaged tissue or a biological function.1,2 Biomaterials may be metals, ceramics, polymers, or even living cells and tissue. They can be used as surface coatings, fibers, films, or particles for use in biomedical products such as heart valves, hip joint replacements, dental implants, or drug delivery carriers. The first generation of biomaterials was developed under the concept of immune evasion because the biggest challenge of “foreign objects” has been the action of the immune system. Therefore, it was preferable that they be ‘inert’ and not interact with the biology of the host organism such as proteins, lipids, nucleic acids, sugars, and amino acids. One of the most successful approaches to produce bio-inert materials has been the used of polyethylene glycol (PEG).3 For example, covalent attachment of PEG to proteins or drugs called “PEGylation” has been shown to improve the safety and efficiency, and many PEGylated pharmaceuticals are currently on the market. This technology can be also used as a means to design anti-fouling surfaces. Grafting PEG to solid surfaces significantly reduces protein adsorption and cell adhesion.4-6 In addition to PEG, zwitterionic polymers have recently been extensively developed for anti-fouling purpose.7,9 Among them, one of the cell membrane lipids, phosphatidylylcholine (PtdCho)-inspired polymers such as 2-methacryloyoxyethyl phosphorylcholine (MPS) have been used in various medical devices.10-12 These polymers have extended the applications of biomaterials from traditional implants to biosensing, prodrug carriers, subcellular bioimaging, and cell manipulation.

2. Challenges of immuno-modulating biomaterials

Since studies of bio-inert biomaterials are already well advanced, many researchers have recently switched their focus from bio-inert to bio-modulating materials that promote or inhibit immune responses. However, controlling the immune system with biomaterials is still a challenge due to their methodological complexity. For example, an-
ti-tumor activity is expected when immune systems are activated, while excess activation can cause unexpected symptoms such as allergies (Fig. 1). On the other hand, suppressing the immune system lowers inflammation, but also leads to diminished resistance against infections. Therefore, to obtain immunotherapy with a biomaterial, it is necessary to perfectly understand the biological reactions induced by implanted materials and to adequately design the shape, physical properties, and chemical properties of the material. Fig. 2 shows three types of biomaterials according to their association with the immune system. The first category is called “immuno-inert biomaterials” as described above. The second type is called “immuno-activating biomaterials” designed to exhibit anti-tumor and drug-responsive properties. The third type is known as “immuno-tolerant biomaterials” which suppress and modulate unnecessary uncontrollable inflammation and inhibit rejection responses.
IMMUNE-ACTIVATING BIOMATERIALS

1. Adjuvant materials

As mentioned above, much effort has been previously made to design biomaterials to minimize the host’s immune responses against implanted materials. However, biomaterials can also be designed to activate the host’s immune responses and/or provide therapeutic effects. The first application of this concept was the use of non-biological adjuvant materials such as \( \gamma \)-polyglutamic acid (\( \gamma \)-PGA), poly (lactic-co-glycolic acid) (PLGA), or poly (\( \varepsilon \)-caprolactone) (PCL). These biodegradable materials can increase the host’s immune response to vaccines. New types of pH-responsive nanoparticles have also been developed because the degradation rate for such biodegradable polymers is not fast enough for effective antigen deliveries. The nanoparticles with pH-cleavable crosslinkers are rapidly hydrolyzed under lysosomal acidic conditions (pH 5) and release antigens into dendritic cells (DCs). By contrast, Stayton and co-workers proposed antigen delivery strategies that mimic virus infection mechanisms. They focused on polypropylic acid (PPAA), which has a pKa around pH 6.0-6.5, making it hydrophilic at a biological pH and dramatically hydrophobic at endosomal pH due to the protonation of carboxyl groups along its backbone. Due to the polymer phase transition, PPAA disrupted lipid bilayer membranes and antigens tethered to PPAA by disulfide bonds were released under endosomal reductive conditions.

2. Hemagglutinating virus of Japan-envelope (HVJ-E)

Anti-tumor immunity can be also induced by viral envelopes such as Hemagglutinating Virus of Japan-envelope (HVJ-E). HVJ-E is a purified product prepared through the complete inactivation of the genome in HVJ by UV irradiation. HVJ-E was originally developed as a novel vector for plasmid DNA, peptides, and drugs because of the fusogenic properties such as Hemagglutinating Virus of Japan-envelope (HVJ-E) for DCs and T cells successfully inhibited immune responses. To induce immune tolerance, several strategies have been developed by using various biomaterials for the delivery of adjuvants, antigens, and drugs. For example, PLGA particles that contained costimulator anti-sense oligonucleotides for DCs and T cells successfully inhibited immune responses. In another example, PLGA particles modified with anti-DEC205 antibodies, anti-CD1c antibodies, and P-2 peptides were also developed. These examples show the potential application of targeting PLGA particles to maintain immunological tolerance.

However, previous immune-suppressive biomaterials as mentioned above simply operate as delivery carriers. In other words, the materials themselves do not have any anti-inflammatory properties. However, in recent years, material-based immuno-suppressive systems have been developed, and a few specific examples will be introduced here. Stable, organic free radicals such as 2, 2, 6, 6-tetramethylpiperidine 1-oxyl (TEMPO) are often used as catalysts for oxidation reactions in organic synthesis and can act as radical scavengers that detect radical generation reacting systems. Yoshitomi et al. focused on the radical scavenging ability of TEMPO for removing reactive oxygen species (ROS) at inflammatory regions. Normally, large amounts of ROS are produced during inflammation, and exposure to this oxidative stress can cause various diseases such as...
arteriosclerosis. However, reactive oxygen must be selectively removed as it plays an important role during intracellular aerobic respiration in mitochondria. They succeeded in improving the cognitive function of mice that model Alzheimer disease by orally administering TEMPO-functionalized PEG.26

2. Apoptotic cell-inspired biomaterials

Ideally, biomaterials should mimic the living tissue or biomolecules from a mechanical, chemical, biological, and functional point of view. As mentioned in the introduction, one of the most successful biocompatible polymers, MPC polymer, was developed by learning from mammalian cell membrane lipid PtdCho. MPC polymers have been extensively applied to various medical devices, including an artificial joint (Aquala®, Japan Medical Materials), cardiovascular stent (Endeavor®: Medtronic), left ventricular assist device (EVAHEART®, Sun Medical), oxygenator (PrimO2x®: Sorin), and soft contact lenses (Proclear®: Cooper Vision). Therefore, we discuss the potential to create a novel immune-suppressing biomaterial by focusing on the lipid membrane in the last section.

In general, cell membranes have a lipid bilayer constructed by phospholipids such as electrically neutral sphingomyelin (Sph) and PtdCho that occupy the outside membrane and anionically charged phosphatidylethanolamine (PtdEA), PtdSer, and phosphatidylinositol (PtdIno) that are located on the cytosol side.27 Cell membranes keep this heterogeneous structure by active transportation at inner sites by scramblase, and the cytoskeleton is therefore stabilized by anionic phospholipids. However, apoptosis induces the destruction of these highly maintained asymmetrical structures, forcing PtdSer to be exposed on the surface of apoptotic cell membranes, which are then recognized by immune cells (Fig. 3).28 Apoptosis is a genetically programmed cell death mechanism and is necessary to regulate embolic development and maintain homeostasis. Many studies also revealed that PtdSer acts as a trigger molecule for these anti-inflammatory effects.29 Voll et al.30 reported that apoptosis induced leukocytes to downregulate inflammatory cytokine production and upregulate anti-inflammatory cytokines for the production of immune cells. Several therapeutic effects of PtdSer liposomes have been also reported for chronic heart failure,31 Alzheimer’s disease32 and osteoporosis.33 Table 1 summarizes the recent progress of anti-inflammatory therapies using apoptotic cells and PtdSer liposomes.30-37

We have recently designed apoptotic cell membrane-inspired polymers which have a phosphoryl serine (PS) group because PtdSer mimetic polymers are very promising materials for anti-inflammatory therapies (Fig. 4).38,39 However, the synthesis of PtdSer-mimetic polymers has been diff-

![Table 1. Anti-inflammatory effects using apoptotic cells and PtdSer liposomes](image-url)

| Samples          | Anti-inflammatory effects | Ref. |
|------------------|--------------------------|------|
| Apoptotic cells  | IL-10 ↑, TNF-α ↓         | 30   |
| Apoptotic cells  | TGF-β1 ↑                 | 34   |
| Apoptotic cells  | TGF-β1 ↑, IL-10 ↑         | 35   |
| Apoptotic cells  | IL-12 ↓, TNF-α ↓, IFN-γ ↓| 36   |
| Liposomes        | IL-10 ↑, TNF-α ↓         | 31   |
| Liposomes        | TNF-α ↓, NO ↓            | 32   |
| Liposomes        | TGF-β1 ↑, PEG2 ↑         | 33   |
| Liposomes        | IL-10 ↑, IL-1b ↓         | 37   |

![Fig. 3. Major phospholipids constituting the cell membrane and collapse of asymmetrical distribution of the phospholipid bilayer due to the progression of apoptosis. Sph: sphingomyelin, PtdCho: phosphatidylcholine, PtdEA: phosphatidylethanolamine, PtdSer: phosphatidylserine, PtdIno: phosphatidylinositol.](image-url)
cult as serine has two reactive groups (the amino- and carboxy-groups) that limit the effective and selective modification routes. Therefore, we used a phosphoramidite method which is commonly used in solid phase syntheses of DNA. The advantage of using this reaction is its high selectivity under mild conditions when forming a phosphate ester between two hydroxyl groups.39 First, we synthesized the methacryloyloxyethyl phosphorylserine (MPS) monomer using 2-hydroxyethyl methacrylate, N-α-(t-butoxycarbonyl)-L-serine t-butyl ester, and t-butyl tetraisopropylphosphorodiamidite. Next, poly (MPS) was obtained by free radical polymerization and then deprotection and oxidation of the PtdSer using t-butyl peroxide and trifluoroacetic acid. We applied poly (MPS) to lipopolysaccharide (LPS)-activated RAW 264.7 macrophages to investigate the immune-suppressive effects. Amoeboid cell extensions of macrophages in LPS stimulated conditions were inhibited in the poly (MPS)-treated groups. In addition, the effective suppression of NF-κB in macrophages was observed over 10 mM of PS groups (Fig. 5).38 To investigate how macrophages recognize poly (MPS), the localization of rhodamine-modified poly (MPS) on macrophages was studied. A homogeneous distribution of the poly (MPS) in the cytoplasm was observed. By calculating the up-taken amount of poly (MPS) in the cell, we found that 50 pg or more of poly (MPS) within each macrophage is necessary to inhibit inflammatory behavior (Fig. 6). Furthermore, we investigated the time-dependent immunosuppression induction of poly (MPS). The NF-κB expression was inhibited after 3 h incubating, indicating that a minimum time for immunosuppression is 3 h. The findings in this study are expected to open new doors in developing advanced immuno-suppressive agents and materials for anti-inflammatory therapies such as autoimmune diseases.

CONCLUSION

This review article summarizes recent research examples of immuno-modulating biomaterials and systems. These materials are classified into three categories: (1) immuno-inert, (2) immuno-activating, and (3) immune-tolerant biomaterials. Particularly, we mainly focused on the immune-tolerant biomaterial systems. In previous immune-tolerant biomaterial systems, the materials themselves did not have any anti-inflammatory properties. However, the ability to engineer biological activity into synthetic materials has enabled the design of biomaterials which harness the host’s immune responses and provide therapeutic effects. One of the examples is apoptotic cell-membrane mimetic polymers. Advantages of using such polymeric materials for anti-inflammatory therapy is cost-effectiveness as it is inexpensive compared to molecular targeted drugs. However, controlling the immune system is still a challenge due to its methodological complexity. For example, excess activation of the immune system may cause unexpected symptoms such as allergies, while suppressing of the immune system also leads to diminished re-
sistance against infections. Therefore, to obtain immuno-
therapy with a biomaterial, it is necessary to design the
shape, physical properties, and chemical properties of the
material adequately. In the future, biomaterials will as-
sume an even greater role in anti-inflammatory therapies
through biologically inspired design.

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CONFLICT OF INTEREST STATEMENT

None declared.

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