Recent Progress in Capturing and Neutralizing Inflammatory Cytokines

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Abstract

Inflammatory cytokines are key players in modulating immune response to mount effective host defense. However, excessive production of inflammatory cytokines contributes to the destructive components responsible for various inflammatory disorders. As a result, treatment strategies have been developed to lower the cytokine levels or block their bioactivity. In particular, therapeutic agents that directly capture and neutralize cytokines have gained significant attention as they bypass the interactions with the host cells and therefore are less likely to induce immunogenic response and clearance. Among them, 'monoplex' platforms such as cytokine-neutralizing antibodies are commonly designed to target a specific cytokine for neutralization. Meanwhile, to address the multiplexity of the cytokine targets in diseases, 'multiplex' platforms such as glycosaminoglycan-containing biomaterials and cell membrane-coated nanoparticles are emerging. Herein, we review recent progress of these cytokine-neutralizing platforms and discuss their applications in treating inflammatory disorders. Overall, the structure-function relationship underlying these cytokine-neutralizing platforms will lead to the design of novel therapeutics toward effective management of inflammatory diseases.

Keywords

Inflammatory disorder, cytokine neutralization, anti-cytokine, monoplex, multiplex

Introduction

Inflammation is a vital and dynamic process of the immune system in response to injury and infection.[1, 2] In this process, inflammatory cytokines secreted by immune cells play vital roles in upregulating immune reactions that drive the immune system to remove harmful stimuli, restore tissue homeostasis, and initiate the healing process.[3] However, when the production of inflammatory cytokines becomes excessive, inflammation remains unresolved and inflammatory disorders arise.[4] For example, pathological 'cytokine storm' occurs in sepsis as a result of uncontrolled inflammatory responses to bacterial infections, which contributes to the high morbidity and mortality of the disease.[5, 6] In the process of wound healing, an uncontrolled cytokine production by the immune cells can lead to destruction of the wound tissue and promote further infiltration of the immune cells, which together perpetuate a vicious circle of chronic inflammation.[7, 8] Moreover, in the case of inflammatory arthritis, an elevated level of various inflammatory cytokines are responsible for aggravating and sustaining joint inflammation, leading to long-standing synovitis, bone destruction, and ultimately joint dysfunction.[9, 10]

The essential roles played by inflammatory cytokines in numerous disorders have motivated the development of therapeutic agents aiming to attenuate their bioactivity through various mechanisms.[11, 12] For instance, some therapeutic agents directly bind with cytokines for neutralization, whereas others bind with cognate receptors on the target cell surface to block their interaction with incoming cytokines.[13] Some agents induce the internalization or downregulation of cytokine receptors of the target cells, therefore restricting cytokine-triggered cell activation.[14] In addition, compounds that induce the clearance of the target cells are also used to reduce the overall cytokine response.[15] In general, anti-cytokine agents that directly capture and neutralize cytokines can avoid interactions with cytokine receptors on the target cells and thus are less likely to be internalized and cleared by the target cells. Moreover, capturing and neutralizing free cytokines instead of targeting cell receptors also reduces the transmission of intracellular signals, which may elicit adverse events such as transient cytokine release and mitogenic activity.[16] Overall, these therapeutic advantages make anti-cytokine agents directly capturing and neutralizing inflammatory cytokines a highly attractive approach for the treatment of various inflammatory disorders.

In this article, we review recent progress in developing cytokine-neutralizing agents with a focus on platform technologies that directly capture and neutralize inflammatory cytokines

(**Figure 1**). Based on their mechanisms of action, some anti-cytokine agents are 'monoplex', which bind with a specific cytokine for neutralization. In this category, cytokine-neutralizing antibodies represent the most dominant and rapidly growing class of anti-cytokine therapeutics. We provide a thorough review of cytokine-neutralizing antibodies currently in clinical use. In addition to free antibodies, biomaterial-conjugated antibodies are emerging with unique applications through their altered pharmacokinetic profiles and biodistribution *in vivo*. In contrast to monoplex platforms, 'multiplex' anti-cytokine agents are able to concurrently neutralize multiple cytokines that reflect the multiplexity of the cytokine targets in diseases. In this category, two emerging technologies include glycosaminoglycan (GAG)-containing biomaterials and cell membrane-coated nanoparticles. The former mimic the intracellular matrix for dynamic cytokine binding and neutralization and the latter harness natural cell membranes as broad-spectrum cytokine-neutralizing agents. Herein, we review recent progress in the rational design of each anti-cytokine platform and discuss its application by highlighting the material structure–function relationship. Overall, we believe that therapeutic platforms featuring potent, dynamic, and safe cytokine neutralization ability are of great importance and potential for effective treatment of inflammatory diseases.

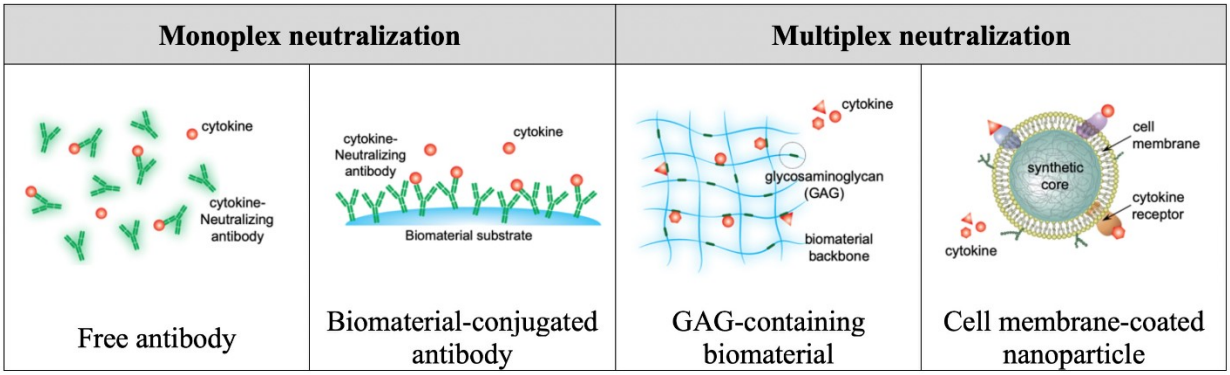

Figure 1. Major therapeutic platforms that directly capture and neutralize inflammatory cytokines. Monoplex platforms such as cytokine-neutralizing antibodies and antibodies conjugated with biomaterials are designed primarily with one specific cytokine as the target. Meanwhile, multiplex platforms such as glycosaminoglycan (GAG)-containing biomaterials and cell membrane-coated nanoparticles are designed for concurrently neutralizing multiple cytokines that reflect the multiplexity of cytokines involved in inflammatory diseases.

Therapeutic Antibodies for Monoplex Cytokine Neutralization

Free antibodies

In 1998, infliximab (Remicade) became the first antibody approved for the treatment of inflammatory disorders. Infliximab is a chimeric antibody consisting of human constant domains and mouse variable domains and it specifically binds with tumor necrosis factor (TNF) and thus blocks its bioactivity.[17] Over the next two decades following infliximab approval, the market of cytokine-neutralizing antibodies has grown significantly, with 18 monoclonal antibody products targeting various inflammatory cytokines currently approved by the US Food and Drug Administration (FDA) (**Table 1**).

Among these approved products, chimeric antibodies were produced in genetically modified mouse hybridoma cells that secreted antibodies carrying the human constant domains.[18] The resulting antibodies displayed improved half-life and reduced immune response compared to unmodified antibodies with the mouse constant domain.[19] Later, humanized antibodies were generated by fusing the complementarity-determining regions of mouse antibody onto a human IgG framework and subsequently expressed in hybridoma cells.[20] This engineering approach reduced the incidences of anti-antibody response in patients compared to those receiving the chimeric antibodies.[21] More recently, fully human antibodies consisting of entirely human sequences were produced from transgenic mice expressing the human IgG germlines as opposed to the normal murine germlines. Alternatively, fully human antibodies were also generated through a library of bacteriophages each expressing a fragment of the human antibody to transfect bacterial cells.[22] The improved half-life and reduced percentage of immune response in human patients led to wide clinical success of fully human antibodies.[17] Meanwhile, a few antibody-like molecules have been developed for cytokine neutralization. For example, etanercept was engineered by fusion of a human tumor necrosis factor receptor 2 (TNFR2) immune adhesin onto the Fc domain of human IgG framework. The TNF receptor domain could neutralize both TNF-α and TNF-β, while the human IgG Fc domain reduced immunogenicity of the molecule.[23] In addition, certolizumab pegol adopted a chemical conjugation approach to functionalizing a humanized Fab fragment with polyethylene glycol (PEG) to improve the half-life of the antibody fragment.[24] Overall, cytokine-neutralizing antibodies constitute a flourishing family of compounds for the treatment of various inflammatory diseases.

Table 1. Cytokine-neutralizing antibodies in clinical use

Target	Name	Trade name	Antibody format	Approved indications	Year of approval
TNF-α	Infliximab	Remicade	Chimeric TNF-α-specific antibody	Crohn's disease, rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, ulcerative colitis, plaque psoriasis	1998
	Etanercept	Enbrel	Human TNFR2-Fc fusion protein	Rheumatoid arthritis, juvenile idiopathic arthritis, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis	1998
	Adalimumab	Humira	Human TNF-α-specific antibody	Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, chronic plaque psoriasis, juvenile idiopathic arthritis, ulcerative colitis, hidradenitis suppurativa, uveitis	2002
	Certolizumab pegol	Cimzia	PEGylated Fab domain of humanized TNF-α-specific antibody	Crohn's disease, rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, plaque psoriasis	2006
	Golimumab	Simponi	Human TNF-α-specific antibody	Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, ulcerative colitis	2009
IL-6	Tocilizumab	Actemra / RoActemra	Humanized IL-6R-specific antibody	Rheumatoid arthritis, juvenile idiopathic arthritis, giant cell arteritis, CAR T cell-induced cytokine release syndrome	2010
	Siltuximab	Sylvant	Chimeric IL-6-specific antibody	Multicentric Castleman's disease	2014
IL-17	Secukinumab	Cosentyx	Human IL-17-specific antibody	Plaque psoriasis, ankylosing spondylitis, psoriatic arthritis	2015
	Ixekizumab	Taltz	Humanized IL-17-specific antibody	Plaque psoriasis, psoriatic arthritis, ankylosing spondylitis	2016
	Broadalumab	Siliq	Human IL-17R-specific antibody	Plaque psoriasis	2017
IL-23	Ustekinumab	Stelara	Human IL-12/23-specific antibody	Psoriasis, psoriatic arthritis, Crohn's disease, plaque psoriasis, ulcerative colitis	2009
	Guselkumab	Tremfya	Human IL-23-specific antibody	Plaque psoriasis	2017
	Tildrakizumab	Ilumya	Humanized IL-23-specific antibody	Plaque psoriasis	2018
	Risankizumab	Skyrizi	Humanized IL-23-specific antibody	Plaque psoriasis	2019
IL-1	Canakinumab	Ilaris	Human IL-1β-specific antibody	Cryopyrin-associated periodic syndrome (CAPS), juvenile idiopathic arthritis, periodic fever syndrome	2009
IFN-γ	Emapalumab	Gamifant	Human IFN-γ-specific antibody	Primary hemophagocytic lymphohistiocytosis	2018

IL-5	Mepolizumab	Nucala	Humanized IL-5-specific antibody	Severe asthma, eosinophilic granulomatosis with polyangiitis (Churg-Strauss Syndrome)	2015
	Reslizumab	Cinqair	Humanized IL-5-specific antibody	Severe asthma	2016

As the cytokine-neutralizing antibodies continue to be a mainstay of therapeutic options, antibody technologies are evolving.[17, 25] There are tremendous research efforts ongoing to further improve the performance of existing antibodies or to develop new antibodies for disease treatment. One active research area is to develop engineering approaches to prolonging the circulation half-lives of the antibodies.[26] Among various factors that affect circulation, the primary determinant is antibody binding with neonatal Fc receptor (FcRn), which rescues the antibody from lysosomal degradation and therefore promotes their recycling.[27] As a result, molecular characteristics such as pH-dependence, isoelectric property, and glycosylation have been used as design cues to generate novel antibody variants so that IgG-FcRn binding can be modulated to enhance half-life.[28] Such approaches can potentially reduce the dosage, dosing frequency, and eventually the cost of anti-cytokine treatments. Another research area lies in new antibody designs to enhance or diversify antigen-binding activity. In this perspective, bispecific antibodies that can simultaneously bind to two distinct antigens or epitopes have gained significant interests.[29] Such dual specificity has allowed antibodies to target specific tissues of inflammation for onsite cytokine neutralization.[30] The dual specificity has also been used to concurrently neutralize two cytokines, therefore inhibiting nonoverlapping proinflammatory functions for enhanced efficacy.[31] The third active research area is on product development of antibody, which increasingly emphasizes risk assessment at early stage instead of late stage.[32] In this perspective, high throughput experimental screening such as phage display have been combined with computational algorithms to predict and enhance 'developable' properties of the antibody candidates regarding manufacturing feasibility, stability in storage, and absence of off-target reactivity.[33] Overall, cytokine-neutralizing antibodies offer exciting opportunities for treating inflammatory diseases and are expected to generate long-lasting therapeutic impact.

Biomaterial-conjugated antibodies

One limiting factor of free antibodies stems from their dose-limiting side effects associated with the non-selective biodistribution. To address this limitation, antibodies have been increasingly conjugated with biomaterials to alter their *in vivo* pharmacokinetics and

biodistribution for favorable therapeutic index. In addition, the biomaterial conjugation could also expand the use of antibodies to areas where free antibodies were unable to access. These benefits have led to cytokine-neutralizing antibodies conjugated with various biomaterial platforms including polymers, hydrogels, and nanoparticles for a wide range of biomedical applications (**Figure 2**).

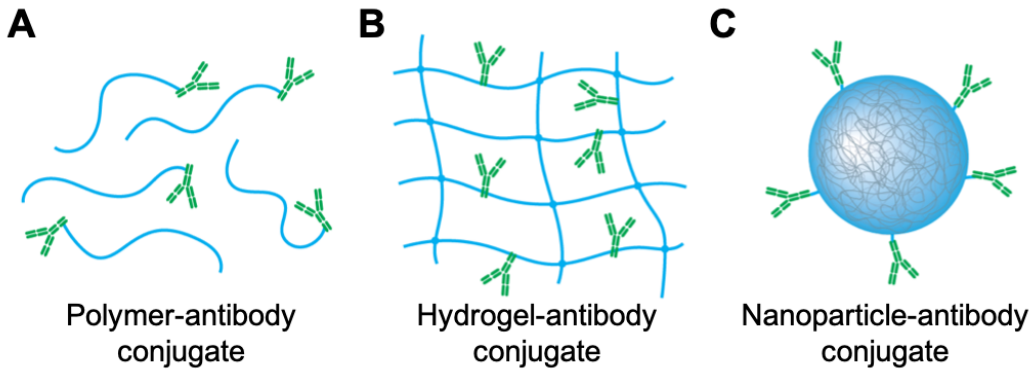

Figure 2. Schematic illustration of biomaterial platforms made from (A) polymers, (B) hydrogels, and (C) nanoparticles in conjugation with antibodies for cytokine capture and neutralization.

Conjugation with polymers can increase the molecular weight of the antibodies and limit their diffusion rate when administered to tissues, making the conjugates ideal for localized treatment of inflammatory conditions.[34] For example, anti-TNF-α and anti-IL-1β were conjugated with high molecular weight hyaluronic acid (HA) and used as a topical treatment for burn injury.[35] Compared to free antibodies, HA significantly increased the antibody residence time in the superficial region following the burn injury. In a rodent model of deep partial-thickness burns, the conjugates were effective in attenuating the acute inflammation and reducing the secondary necrosis. This was further validated by the fact that much fewer immune cells infiltrated into the region where the polymer-antibody conjugates were retained. Notably, in polymer-antibody conjugates, the polymer backbone could affect the antibody-cytokine binding affinity depending on the use of polymers. For example, anti-IL-1β conjugated with HA or carboxymethylcellulose (CMC) showed an association kinetics comparable to that of the free antibody. However, the captured cytokines dissociated three times more slowly from the HA conjugates than from the CMC conjugates.[36] Such differential dissociation was likely due to the

conformational changes of the polysaccharide as a function of antigen binding. In addition to polymer backbone, the size of cytokine targets would also affect the binding kinetics. For example, anti-TNF-α conjugated to CMC or HA both showed reduced adsorption and desorption of TNF-α when compared to free antibody. TNF-α has a molecular weight three times larger than IL-1β, which might become a determining factor in binding events and outperform the effect from the polymer backbone.

Conjugation of cytokine-neutralizing antibodies with hydrogels is another approach of modulating local inflammation while minimizing the systemic side effects associated with free antibodies. One popular application of such conjugates is to treat burn injury, where the injury progression is driven by local inflammatory cytokines through complex cascades.[37] HA hydrogel conjugated with anti-TNF-α significantly reduced the development of necrotic tissue in a rat partial-thickness burn model.[38] With the same model, the free antibody was shown to inhibit macrophage infiltration in the periphery but not at the surface, while the conjugated antibody was able to hinder macrophage infiltration at both the periphery and the surface.[39] Measurements of local antibody concentration showed that the increased antibody residence time in the superficial region strongly correlated with the pattern of inflammatory cell infiltrate in the tissue.[40] These results together demonstrated the benefit of antibody-hydrogel conjugates for localized treatment. To study the effect of hydrogel crosslinking, anti-IL-1β and anti-TNF-α were conjugated with HA hydrogels and applied for the treatment of burn wound.[41] Intriguingly, the hydrogels were shown to bind with and neutralize cytokines *in vitro*. However, they were unable to reduce the inflammation *in vivo*. The lack of efficacy *in vivo* was attributed to the high density of the hydrogel cross-linking, which limited cytokine diffusion into the gel matrix and diminished the neutralization efficacy. This result suggests that when designing the conjugates, hydrogel crosslinking density needs to be optimized to maintain gel-like properties while maximizing cytokine influx for effective neutralization. Meanwhile, tuning residence time of cytokine-neutralizing antibodies can be an effective strategy in regulating the inflammatory response associated with acute injuries.

Cytokine-neutralizing antibodies can also be conjugated to nanoparticles for benefits including enhanced stability, specific targeting, and prolonged retention after local injection. For example, to neutralize inflammatory cytokines in arthritic joints, nanoparticles made from crosslinked chitosan and HA were conjugated with anti-IL-6 antibodies (**Figure 3**).[42] In this

study, carbocyclic groups of the antibodies reacted specifically with the amine groups at the nanoparticle surface. The nanoparticle-antibody conjugates exhibited a stronger inhibition of macrophage activation and the effect lasted longer when compared to free anti-IL-6 antibody. The benefit was attributed to the antibody immobilization on the nanoparticles, which reduced their degradation. In another study for the treatment of acute temporal lobe epilepsy (TLE), superparamagnetic iron oxide nanoparticles were coated with polyethylene glycol (PEG) and then conjugated with anti- IL-1β antibody.[43] The resulting nanoparticle-antibody conjugates not only enhanced the neuroprotective effect in an acute rat model of TLE through IL-1β neutralization, but also targeted the magnetic nanoparticles to the astrocytes and neurons in epileptogenic tissues, leading to a higher T2 sensitivity in magnetic resonance imaging than nanoparticles without antibody conjugation.

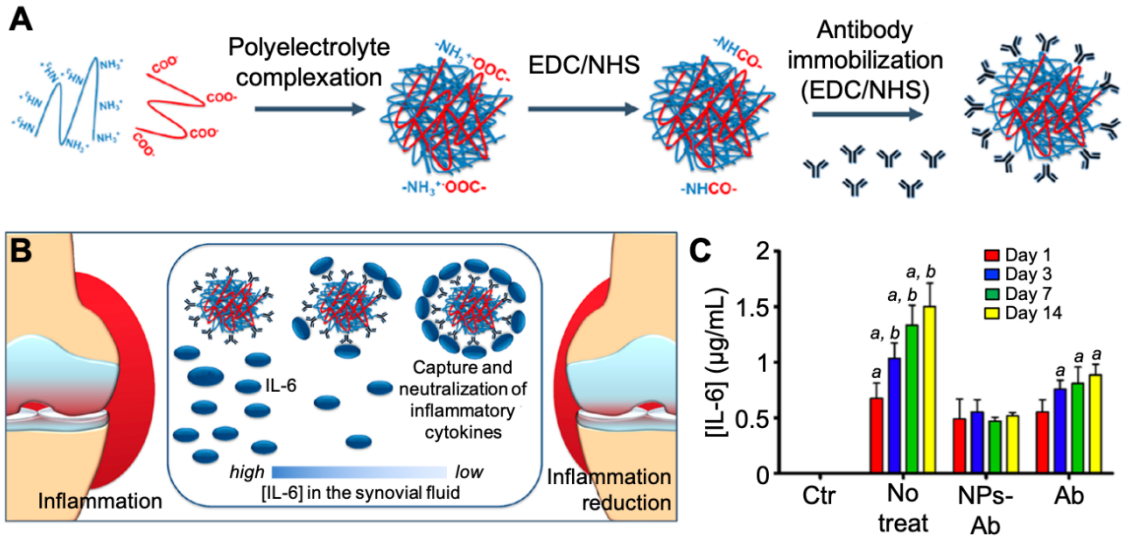

Figure 3. (A) Schematics of nanoparticles made from crosslinked chitosan and hyaluronic acid, followed by conjugation with anti-IL-6 antibodies. (B) Schematic functions of the nanoparticles in neutralizing IL-6 and reducing the inflammation in the synovial fluid. (C) *In vitro* efficacy of the nanoparticles in reducing IL-6 production from human articular chondrocytes stimulated with macrophage conditioned medium. Ctr: cells without stimulation nor treatment; No treat: stimulated cells but without treatment; NPs-Ab: stimulated cells treated with nanoparticles; Ab: stimulated cells treated with soluble antibodies. Reproduced with permission from ref 42. Copyright 2018 American Chemical Society.

Biomimetic Platforms for Multiplex Cytokine Neutralization

Glycosaminoglycan-functionalized hydrogels and nanoparticles

Extracellular matrix glycosaminoglycans (GAGs), such as heparan sulfate and heparin, are known to bind with a diverse range of cytokines primarily through electrostatic interactions between the positively charged amino acid residues of the cytokines and the negatively charged sulfate groups on the GAGs.[44] By varying the GAG composition, concentration, and sulfation degree, the extracellular matrix is able to modulate cytokine transport within the matrix and influence the bioactivity of the cytokines.[45] Such dynamic binding interactions have recently inspired the use of GAGs as cytokine-scavenging component to construct biomaterials for capturing and neutralizing inflammatory cytokines. With the capability of modulating complex binding events and inhibiting multiple cytokines, these biomimetic materials have become a unique multiplex cytokine neutralizing platform.

GAG-based hydrogels have been synthesized to trap cytokines within tissues and thus to attenuate inflammation in chronic wounds. In one design, hydrogels were made with various desulfated heparin derivatives and cross-linked with star-shaped PEG (starPEG, **Figure 4**).[46] These hydrogels were able to neutralize the chemoattractant function of monocyte chemoattractant protein-1 (MCP-1) and IL-8 *in vitro* and *in vivo* when applied onto excisional wounds. This sequestration effect resulted in significantly reduced influx of immune cells into the wound. Mechanistic studies showed no binding of the less heparin-affine cytokines TNF-α, IL-1β, and IL-6 by the hydrogels, whereas chemotactic factors such as macrophage inflammatory protein 1 (MIP-1)α, MIP-1β, and epithelial neutrophil-activating protein 78 (ENA-78) interacted with the GAG-based hydrogels. Nevertheless, as a secondary effect of the reduced influx of immune cells into the wound, the overall expression of inflammatory cytokines and chemokines such as TNF-α, IL-1β, growth-regulated oncogene-α (GRO-α), and MCP-1 was also diminished. Furthermore, the capacity of the hydrogels to capture MCP-1 and IL-8 decreased as the sulfation degree of the matrix decreased. The results from this study are consistent with another study showing that the binding of strongly charged cytokines correlated with the integral space charge density of the hydrogel, while the binding of weakly charged cytokines was governed by the GAG sulfation pattern.[47] Overall, the GAG-based hydrogel was beneficial for wound healing by decreasing inflammatory signaling, leading ultimately to enhanced granulation tissue maturation, vascularization, and re-epithelialization.

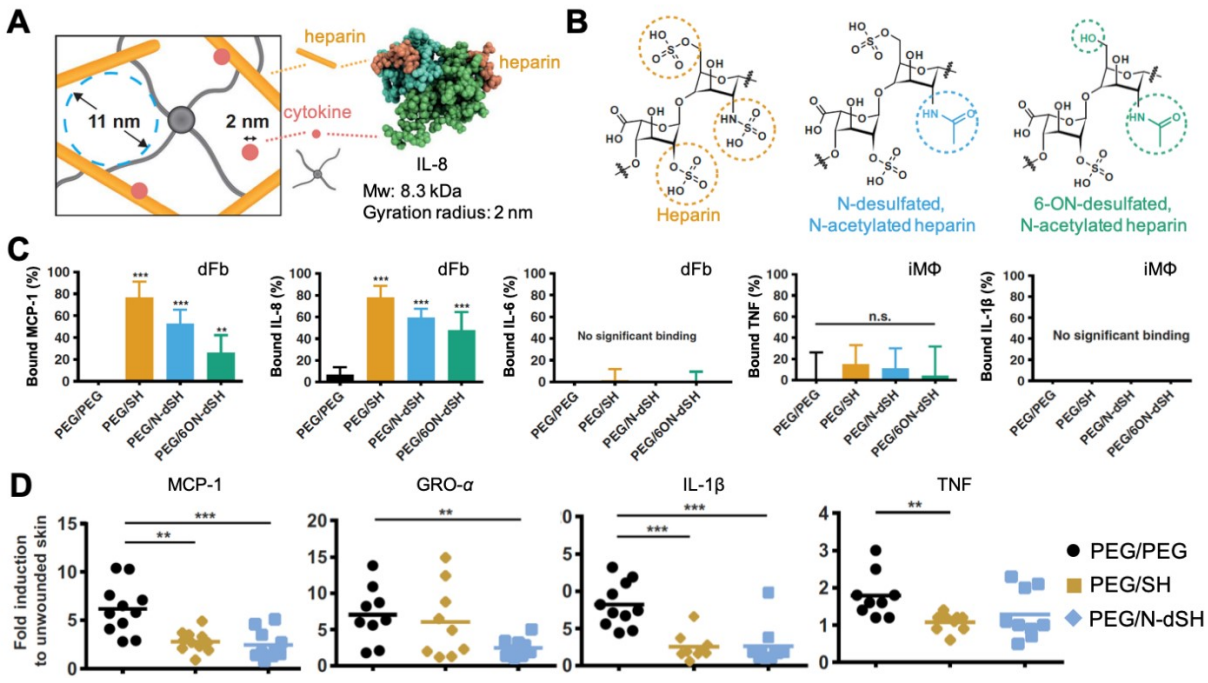

Figure 4. (A) Schematics of hydrogel scaffolds formed by cross-linking of starPEG and different heparin derivatives (starPEG-GAG hydrogels). The hydrogel mesh size (11 nm) and gyration radius (2 nm) of IL-8 are also depicted. (B) Structures of native heparin (SH) and selectively desulfated heparin derivatives including N-desulfated, N-acetylated heparin (N-dSH), and 6-ON-desulfated N-acetylated heparin (6ON-dSH). (C) Characterization of starPEG-GAG hydrogels binding with cell-derived MCP-1 and IL-8. Conditioned medium derived from human activated dermal fibroblast (dFb) or inflammatory macrophage (iMΦ) was incubated with the different hydrogels. After 24 hours, the remaining cytokines were quantified by ELISA, and the amounts of hydrogel-bound cytokines were calculated. Bars represent mean ± SD of data from four dFb and seven iMΦ donors. (D) Characterization of starPEG-GAG hydrogels in inhibiting inflammation during wound healing in mice. Wounds on the backs of C57BL/6 wild-type mice were inflicted by 6-mm punch biopsy and treated with hydrogel discs for 5 days. RNA was isolated from whole wound tissue, gene expression was analyzed, and expression was calculated and compared to unwounded skin. Each symbol represents one wound. Bars represent means ± SD. ANOVA with multiple comparisons versus PEG/PEG using Bonferroni *t* test or Dunnett's method: ***P ≤ 0.001, **P ≤ 0.01, *P ≤ 0.05 (B and C). Unpaired t test: **P ≤ 0.01, *P ≤ 0.05. Reproduced with permission from ref 46. Copyright 2017 American Association for the Advancement of Science.

Various nanoparticle formulations using GAGs as building blocks have also been developed for cytokine neutralization. For example, heparins conjugated with D-erythro-sphingosine showed a lipid-like structure and self-assembled into stable nanoparticles that suppressed the production of inflammatory cytokines including TNF-α, IL-6, and IL-1β in lipopolysaccharides (LPS)-stimulated macrophages much more strongly than native heparin.[48] The initial success also motivated the development of conjugates with a series of GAG derivatives including chondroitin sulfate (CS), HA, and low-molecular-weight heparin (LH). Such rationale design allowed for studies on the relationship of conjugate structure and their anti-inflammatory activity, which revealed a critical role played by the degree of sulfation in determining the anti-inflammatory activity. [49] In another study, heparin-based nanogels were embedded in HA hydrogel, which exerted dual functions for the repair of brain tissue following stroke; loading VEGF for delivery to the brain followed by binding and decreasing brain levels of stroke-induced TNF-α directly at the lesion site (**Figure 5**).[50, 51] Heparin nanoparticles sequestered cytokines, reduced astrocytic scar formation, and ultimately promoted tissue repair after the stroke. Heparin was also mixed with oppositely charged chitosan oligosaccharide to form stable nanoparticles with diameters in the range of 100 to 200 nm.[52] These nanoparticles were able to bind with cytokines such as the stromal cell-derived factor 1α (SDF-1α) and VEGF while preserving their bioactivity. In another design, nanoparticles were constructed with a layer-by-layer approach; first depositing polylysine onto negatively charged polylactic acid cores, followed by depositing of a heparin shell onto the positively charged core.[53] The nanoparticles were further conjugated with fragments of C-C chemokine receptor type 5 (CCR5) responsible for inhibiting the CCR5 ligand-mediated leukocyte adhesion. In the study, only the combination in one "nano-trap" of heparin and CCR5 fragments imparted the strongest anti-adhesion effect in blocking the adhesion of monocyte to human umbilical vein endothelial cells (HUVECs).

Cell membrane-coated nanoparticles

Recently, the extremely rich biological functions of cellular membranes have inspired the development of cell membrane-coated nanoparticles by wrapping natural cell membranes onto synthetic nanoparticle cores. Such top-down biomimicry allows these nanoparticles to harness cell-like functions for multifaceted biointerfacing.[54, 55] Among their emerging applications, mimicking the source cells to bind with inflammatory cytokines for neutralization has attracted

much attention. By displaying the exact antigenic profile as the source cell, these cell-like nanoparticles neutralize cytokines without the need of identifying individual targets. More importantly, by acting as the decoys of the target cells, these cell membrane-coated nanoparticles capture cytokines by precisely mapping the complexity and multiplicity of cytokine-cell receptor binding in disease pathology. With these advantages, cell membrane-coated nanoparticles have emerged as a unique function-driven and multiplex cytokine neutralizing platform.

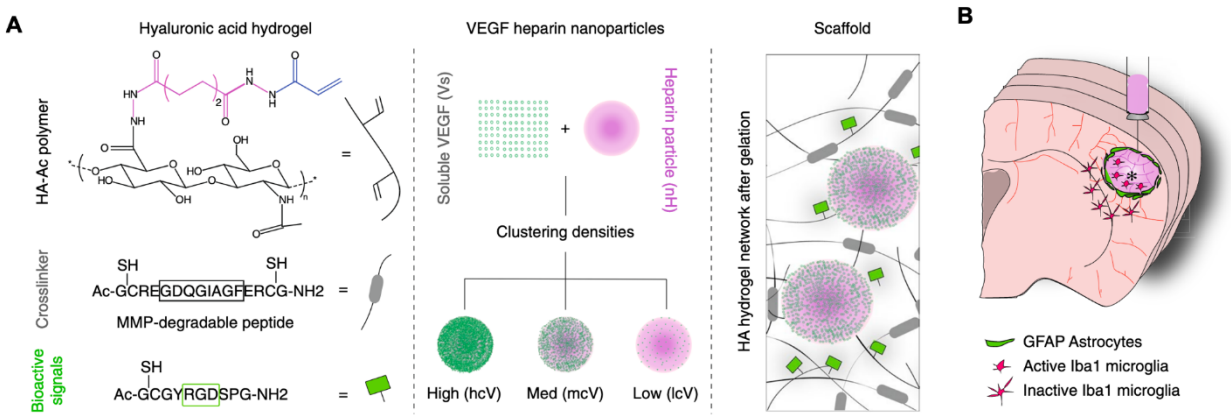

Figure 5. The development of an injectable hydrogel for tissue repair after stroke. (A) The hydrogel was composed of hyaluronic acid and heparin nanoparticles with VEGF clusters of varying densities. (B) Illustration of a stroke cavity within the brain, indicating the presence of astrocytes and microglia. The hydrogel containing the therapeutics was injected directly into the brain tissue cavity. Reproduced with permission from ref 51. Copyright 2018 Springer Nature.

Macrophage membrane-coated nanoparticles (denoted 'MΦ-NPs') was first developed and tested for the management of sepsis (**Figure 6**).[56] MΦ-NPs possess an antigenic exterior identical to the source macrophage cells, thus inheriting their capability of capturing endotoxins through the pattern recognition receptor CD14 present on the macrophage membrane. In addition, MΦ-NPs act as decoys to bind with inflammatory cytokines, hence inhibiting their ability to potentiate downstream pathological cytokine storm largely responsible for sepsis-induced lethality. These two functionalities together enable effective intervention during uncontrolled immune activation, providing a powerful therapeutic intervention for the management of sepsis. *In vitro* studies showed that MΦ-NPs neutralized not only endotoxins but also inflammatory cytokines that otherwise potentiating the sepsis cascade. In a mouse

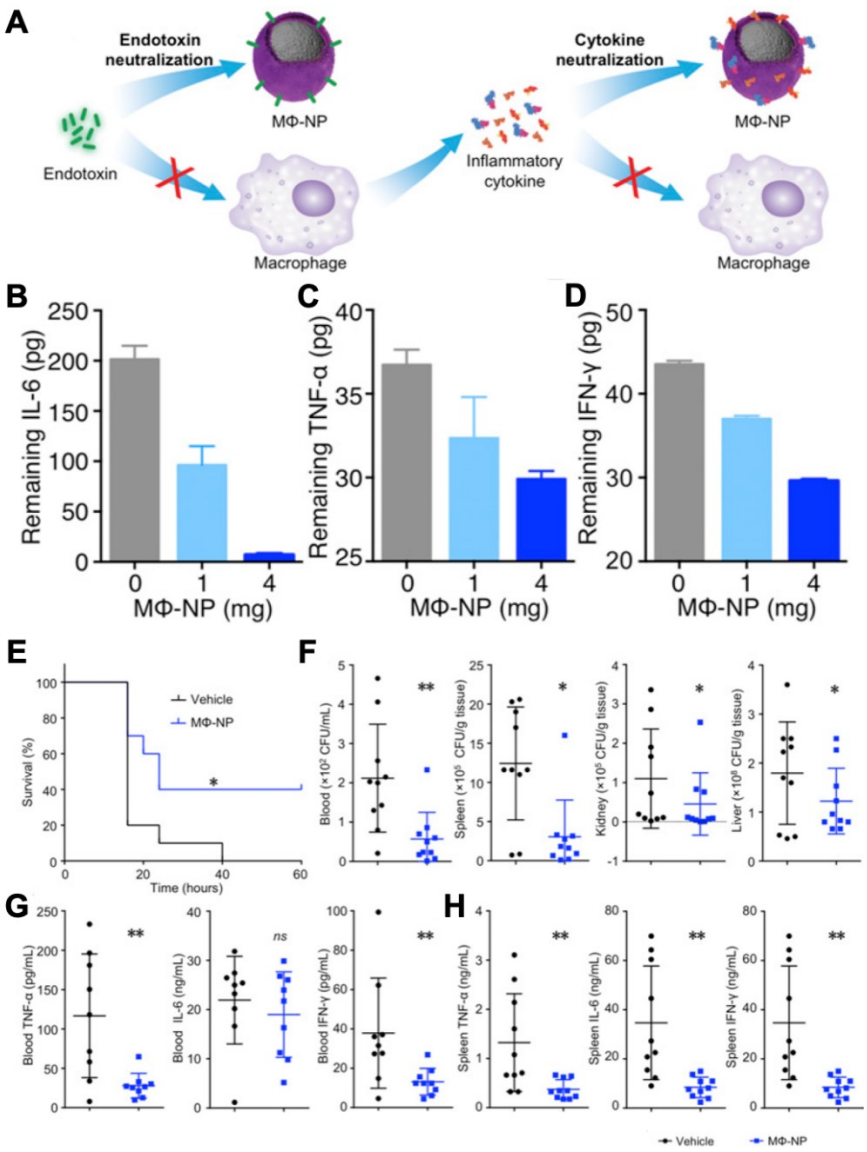

Figure 6. (A) Schematic illustration of using MΦ-NPs to neutralize endotoxins and inflammatory cytokines as a two-step process for sepsis management. (B-D) *In vitro* removal of inflammatory cytokines by MΦ-NPs, including (B) IL-6, (C) TNF-α, and (D) IFN-γ. (E-H) *In vivo* therapeutic efficacy of MΦ-NPs evaluated with a mouse bacteremia model. (E) Survival curve of mice with bacteremia after treatment with MΦ-NPs (n = 10). (F) Bacterial enumeration in blood, spleen, kidney, and liver at 4 h after MΦ-NPs were intraperitoneally injected. (G and H) Inflammatory cytokines, including IL-6, TNF-α, and IFN-γ, from the blood and spleen were quantified with a cytometric bead array (*ns*, not significant; $*P \leq 0.05$, $**P \leq 0.01$). Reproduced with permission from ref 56. Copyright 2017 National Academy of Sciences.

Escherichia coli bacteremia model, treatment with MΦ-NPs reduced inflammatory cytokine levels, inhibited bacterial dissemination, and ultimately conferred a significant survival advantage to infected mice. Overall, MΦ-NPs take advantage of the common functionality of endotoxin binding to macrophage cells, allowing for a universal neutralization approach across different Gram-negative bacterial genus, species, and strains. The top-down fabrication of MΦ-NPs effectively replicates endotoxin-binding motifs on the target cells that are otherwise difficult to identify, purify, and conjugate. Coating macrophage membranes onto nanoparticle surfaces significantly increases the surface-to-volume ratio of given membrane materials, which is critical for efficient endotoxin neutralization.

As another example, neutrophil membrane-coated nanoparticles (denoted 'neutrophil-NPs') were developed as an anti-inflammatory strategy to treat rheumatoid arthritis (RA) (**Figure 7**).[57] Inflammation and damage in RA are mediated by the influx of an immune cell mixture into the synovial joint space.[58] Among them, neutrophils play central roles in initiating and perpetuating RA progression. Neutrophil-NPs inherit the antigenic exterior and associated membrane functions of the source cells. They were shown to neutralize hallmark cytokines including IL-1β and TNF-α that would otherwise activate and recruit neutrophils to potentiate RA progression. Through the neutralization, neutrophil-NPs effectively suppressed synovial inflammation and inhibited chondrocyte activation and apoptosis. Furthermore, neutrophil-NPs also mimicked the natural adhesion between neutrophils and chondrocytes, which subsequently enhanced their penetration into the cartilage matrix for chondrocyte targeting. Neutrophil-NPs injected into mice with collagen-induced arthritis and a human transgenic mouse model of arthritis showed significant therapeutic efficacy by ameliorating joint damage and suppressing overall arthritis severity. The promising results of using neutrophil-NPs for the treatment of RA suggest that coating cell membrane for biomaterial functionalization can lead to effective broad-spectrum cytokine neutralization for the treatment of inflammatory disorders.

Since its initial development, cell membrane coating technology in general has made tremendous progress. A diverse range of cell-like functions are now available on-demand by choosing membranes from appropriate source cells. Additional functions can be achieved by modifying the substrates with different materials. Cell membranes can be coated onto substrates with higher dimensions for a larger contacting area that may facilitate cytokine capture.[59] Cell membrane-coated nanoparticles are also increasingly combined with other biomaterials for

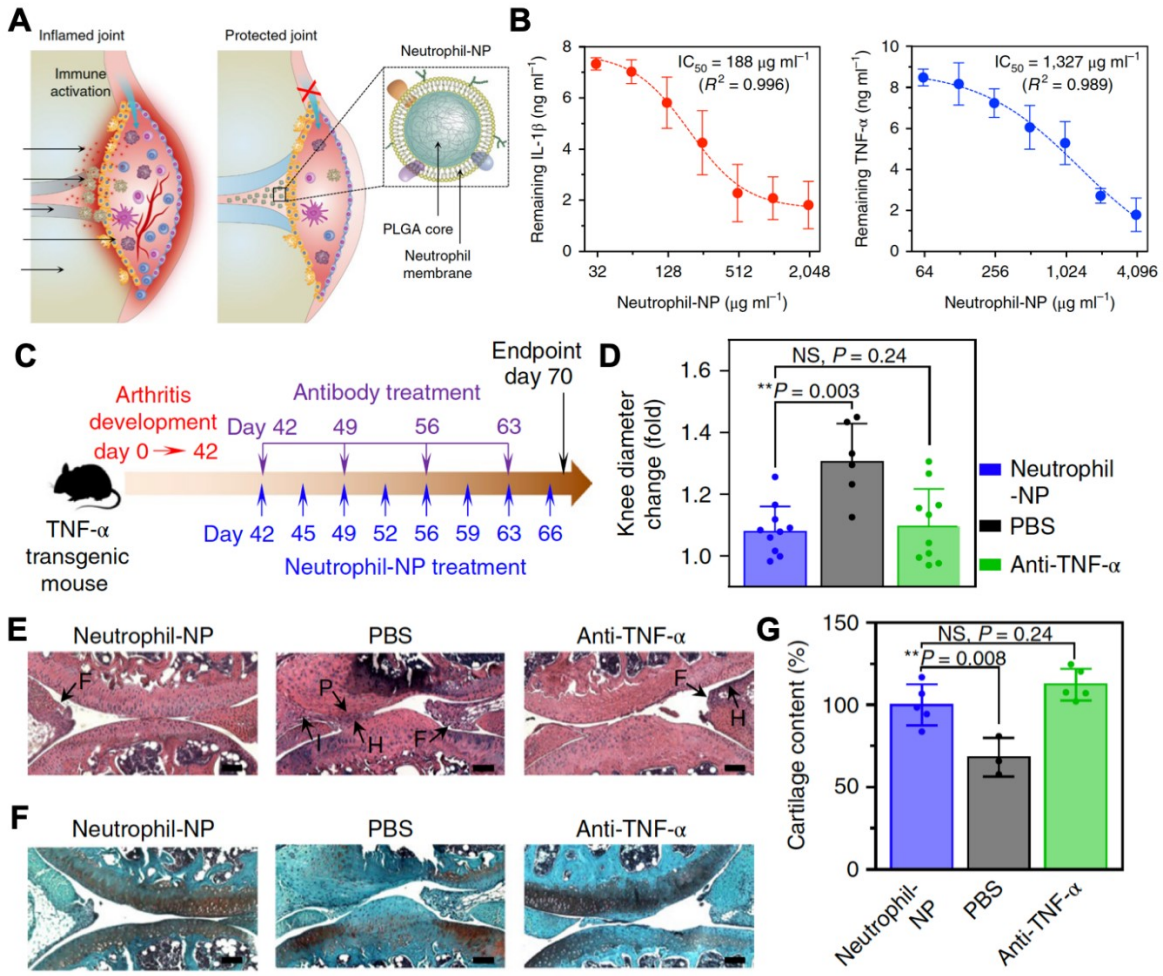

Figure 7. (A) Schematics of neutrophil-NPs designed for suppressing synovial inflammation and ameliorating joint destruction in inflammatory arthritis. Neutrophil-NPs were constructed by wrapping polymeric cores in natural human neutrophil membranes. (B) Binding capacity of human neutrophil-NPs with IL-1β (left) and TNF-α (right). (C) The study protocol of using neutrophil-NPs to treat inflammatory arthritis in a human TNF-α transgenic mouse model. (D) Change of hind knee diameter on day 70 compared to that on day 0. (E, F) Representative images of H&E staining (E) and safranin-O staining (F) of knee sections from mice treated with neutrophil-NPs, PBS or anti-TNF-α antibody. Scale bars, 100 μm. F, synovial membrane fibrillation; H, synovium hyperplasia; I, immune cell infiltration; P, pannus formation. (G) Cartilage content was quantified from safranin-O-stained sections of mice treated in different groups. Statistical analysis was performed using one-way ANOVA with Dunnett's post hoc analysis. Data presented as means ± s.d. $*P \leq 0.05$, $**P \leq 0.01$, $***P \leq 0.001$. Reproduced with permission from ref 57. Copyright 2018 Springer Nature.

synergistic functions. However, one should carefully choose substrates for membrane coating as the substrate materials themselves may produce oxidative stress, thereby triggering inflammation responses instead of curbing it.[60] To minimize such risk, studies aimed at understanding nanoparticle toxicity mechanisms have played an essential role in proper material design and selection.[61] Overall, the cell membrane coating technology holds great promise for innovative therapeutics including anti-cytokine therapy.

Summary and outlook

The capture and neutralization of inflammatory cytokines have been shown effective in treating various inflammatory disorders. In this review, we first summarized recent advances in developing anti-cytokine antibodies. We then reviewed biomaterials that have been increasingly explored to enhance the efficacy of anti-cytokine therapy. Specifically, we highlighted three distinct approaches to functionalizing synthetic biomaterials toward potent and safe cytokine neutralization: (1) conjugation of cytokine-neutralizing antibodies to biomaterials, (2) integration of glycosaminoglycan building blocks to biomaterial networks, and (3) use of natural cell membranes for broad-spectrum cytokine neutralization. Significant progress has been made in these areas, generating a variety of anti-cytokine platforms including polymer conjugates, hydrogels, and nanoparticles. The key advantages and disadvantages of each approach are summarized in **Table 2**. These research outcomes have offered promising opportunities in advancing anti-cytokine therapeutics.

Despite the progress, drug discovery to treat various inflammatory disorders has been challenged by the complexity of inflammation resulted from built-in redundancy, compensation, and necessity.[4] For example, network redundancy makes inhibition against one or few targets inadequate to reduce the inflammation. Meanwhile, due to the inherent sensors and feedback pathways, inhibition of one inflammation pathway may trigger another compensatory proinflammatory response. Furthermore, the vital role of inflammatory response for host defense and survival requires anti-inflammatory drugs to carefully balance the risk-to-benefit relationship. To address these challenges, new strategies are emerging to improve biomaterial-based cytokine capture and neutralization. For example, synthetic polymeric nanoparticles have been engineered with binding affinity for specific protein targets, offering potential alternatives to biological binding ligands such as antibodies. These nanoparticles have been used to capture

animal venoms, bacterial toxins, and recently cytokines for suppressing tumor angiogenesis.[62-65] Other affinity moieties such as aptamers have also been integrated with biomaterials as robust and cost-effective alternatives to antibodies for cytokine capture.[66, 67] Overall, we anticipate that combining biomaterials with cytokine capture and neutralization functionality will become a versatile approach for the treatment of various diseases including inflammatory disorders.

Table 2. Key advantages and disadvantages of major anti-cytokine platforms

	Pros	**Cons**
Free antibody	• Dedicated target • Long circulation • Well established drug development pathways	• Limited local retention • Require antigen identification
Biomaterial-conjugated antibody	• Local administration • Tunable pharmacokinetics through controlled biomaterial degradation	• Require conjugation with possibility of denaturing the protein
GAG-containing biomaterial	• Widely available and low cost • Versatile building blocks to construct various materials	• Cytokine binding profile are poorly defined
Cell membrane-coated nanoparticle	• Function and disease-driven design that bypasses antigen identification • Versatile coating materials for various substrates	• Need to test and verify Immune compatibility

Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of this article.

Acknowledgements

This work is supported by the National Science Foundation Grant DMR-1904702 and the Defense Threat Reduction Agency Joint Science and Technology Office for Chemical and Biological Defense under Grant Number HDTRA1-18-1-0014.

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