Immune-instructive materials as new tools for immunotherapy

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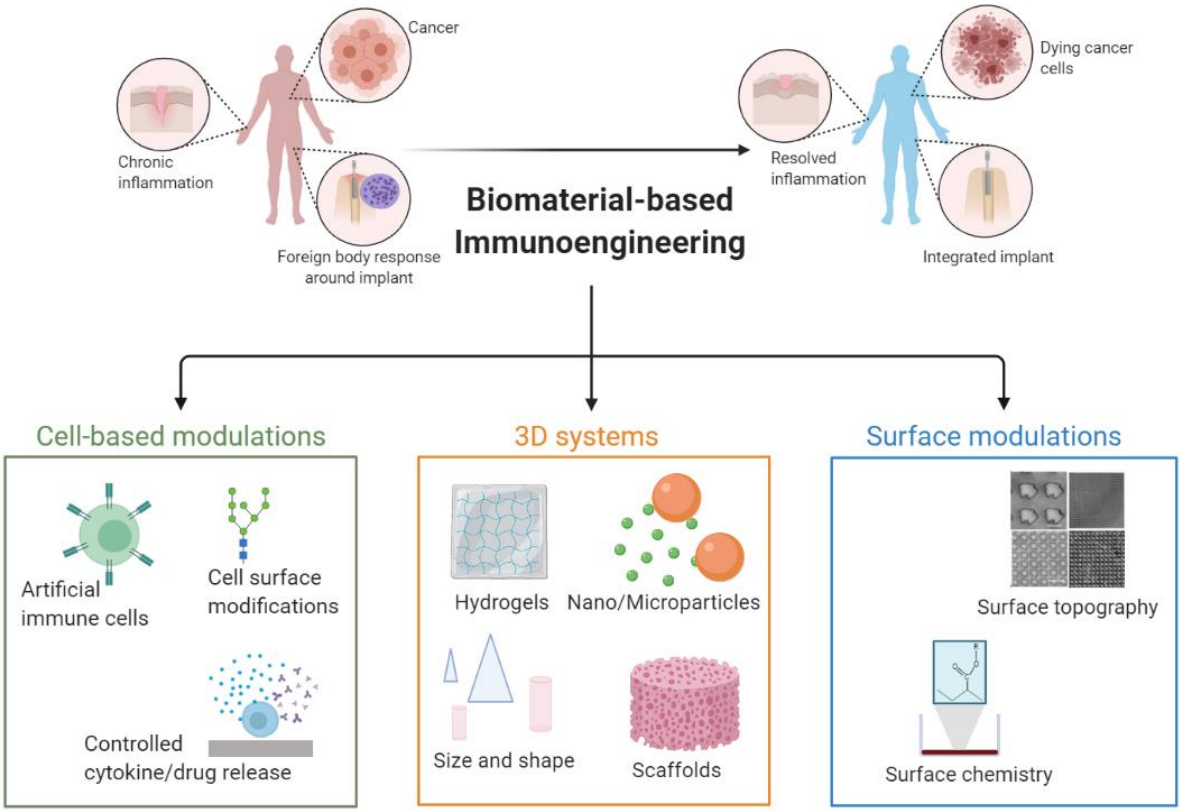
Abstract

Immune instructive materials, materials with ability to modulate or mimic the function of immune cells, provide exciting opportunities for developing new therapies in many areas including medical devices, chronic inflammation, cancer, and autoimmune diseases. In this review we highlight some of the latest research involving material-based strategies for modulating macrophage phenotype and dendritic cell function, as well as a brief description on biomaterial use in T cell and natural killer cell engineering. We highlight studies on material topography, size, shape and surface chemistry to reduce inflammation, along with scaffold and hydrogel delivery systems that are used for modulating DC phenotype and influencing T cell polarization. Artificial antigen presenting cells are also reviewed as a promising approach to cancer immunotherapy.

Graphical Abstract



Introduction

The immune system is composed of various components including lymphoid organs, immune cells and immunoactive mediators [1] that collectively play a major role in maintaining tissue homeostasis and protecting the body by recognizing and removing pathogens, dying cells and tumor cells. The immune system can distinguish between healthy and unhealthy cells by recognizing intracellular molecules released from dying cells and fragments of damaged tissues known as damage-associated molecular patterns (DAMPs). Pathogens such as bacteria and viruses are endowed by molecular patterns recognized by the immune system called pathogen-associated molecular patterns (PAMPs). Once the immune system is alerted to PAMPS and danger signals are discharged into the extracellular space, an inflammatory response occurs through binding to toll-like receptors (TLRs) and other pattern recognition receptors (PRRs) expressed by the immune cells as well as some stromal cells. Neutrophils are the first of the innate immune cells to arrive and respond to tissue injury and/or infection in the body. Their primary role is to eliminate the foreign substance, which they do via three main mechanisms; phagocytosis, degranulation, and the release of nuclear material in the form of neutrophil extracellular traps (NETs) [2,3]. Closely followed is the infiltration of circulating monocytes which mature into macrophages that also act to phagocytose foreign materials and clear apoptotic neutrophils, cell derbies and necrotic cells. Macrophages are highly plastic cells that can respond to different microenvironmental signals and acquire distinct functional phenotypes exemplified by the so called M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophages. During initial stages of tissue damage, macrophages adopt a pro-inflammatory phenotype and secrete pro-inflammatory cytokines (e.g. TNFα, IL1β, MCP-1) that encourage phagocytosis. Pro-inflammatory macrophages gradually skew towards anti-inflammatory phenotypes which are associated with healing and the down regulation of inflammatory factors. Prolonged activation of M2-like macrophages can however, lead to fibrosis, such as in wound healing [4].

The fast acting, first line responder cells of the innate immune system make a crucial contribution to the activation of the adaptive immune response. The adaptive immune response is a much slower process than the innate arm, but has specificity and an immunological memory. Natural killer (NK) cells are both effectors of innate immunity and play a role in the adaptive immune response. They are important cells of the immune system in that they have the ability to recognize and kill unhealthy cells in the absence of antibodies and major histocompatibility complex (MHC) presented on infected cells, allowing for a much faster immune reaction. The role of NK cells in both the innate and adaptive immune responses is becoming increasingly important in research especially in the area of cancer therapy [5].

Immune responses to self and foreign antigens are tightly regulated processes and dysregulated activation of immune cells could lead to local or systemic pathologies. Typically, lack of optimal immune activation or immune suppression could prone the hots to infection whereas sustained or exaggerated immune activation could cause tissue damage, chronic inflammation and fibrosis. Immune modulation has gained significant attraction as the treatment of choice for many pathologies such as chronic inflammation, cancer, and autoimmune diseases. In parallel to advances in conventional methods of immunomodulation (e.g. use of monoclonal antibodies and cytokines) [6,7], there has also been a significant push towards the use of natural and synthetic materials for immune engineering [8]. The use of biomaterials for immune response engineering spans across many areas including medical devices, regenerative medicine, vaccination, and even immunotherapies where materials are used as artificial cells or for delivering cytokines [7,9]. Both innate and adaptive arms of the immune system have been considered for developing immune engineering approaches using materials. Within the innate immune system, different strategies to modulate the function of macrophages, dendritic cells (DCs) and NK cells have received significant attention due to their key

role in immune regulation. Macrophages play a central role in maintaining tissue homeostasis, defence against pathogens, wound healing and regulating post-injury inflammatory responses [10]. DCs on the other hand are professional antigen presenting cells that act as sentinels of the immune system with an essential role in shaping both protective (e.g. in infection and vaccination) and pathological (e.g. autoimmune diseases and allergy) adaptive immune responses to antigens [11]. NK cells enhance the maturation of dendritic cells (DCs) and thus stimulate cytotoxic T lymphocytes (CTLs) to attack and kill tumor cells [4], whilst at the same time are key components of defence against cancerous cells themselves [12]. While many approaches focus on modulating adaptive immune responses (e.g. T cell polarisation) via targeting DCs, different strategies to either mimic or directly modulate T cell activation have also been described in recent years.

This review will focus on some of the latest research highlighting the use of biomaterials for modulating the immune response with focus on interaction with macrophages, DCs, T cells and NK cells in different host scenarios.

Material based strategies for modulating macrophage functional phenotype

Macrophages have the ability to change their phenotype according to their surrounding environment and needs, therefore, selectively targeting macrophage subpopulations for pro-healing therapy may provide an attractive strategy in regenerative processes. Such plasticity provides opportunities for modulating their phenotype using 'bio-instructive' cues. This is why a large number of studies have focused on identifying physicochemical attributes such as topography, size, shape and surface chemistry of biomaterials that can control macrophage phenotype [13-15] (Figure 1). The importance of macrophages in orchestrating adverse immune responses against foreign materials (the so-called foreign body response of FBR) has been demonstrated over many decades (extensively reviewed elsewhere) [16,17]. In a seminal study, Doloff *et al* [18], examined the role of different innate and adaptive immune cells in FBR to 500 µm alginate spheres using C57BL/6 mice with different immune mutations and varying levels of immunodeficiency affecting B cells, T cells, neutrophils, NK cells and macrophages. They discovered that B cell loss (*IghM*^{null}, B KO) resulted in a partial loss of fibrosis whereas, T cell loss (*Rag2*^{null}, T & B KO) made fibrosis worse. This may be due to the loss of the regulatory T cells that are important for supressing immune reactions. Macrophage dysfunction (Rag2/γ KO) resulted in a complete loss of fibrosis upon implantation of the alginate spheres as did macrophage depletion using clodrosome treatment. The macrophage receptor colony stimulating factor-1 receptor (CSF1R) has been shown to selectively polarize and modulate macrophage phenotypes. To test if this receptor could inhibit fibrosis development on the alginate micropspheres, the authors examined the effect of the inhibitory small molecule GW2580 to inhibit the kinase domain thus preventing cross-phosphorylation and activation of CSF1R. CSF1R inhibition by GW2580 was as effective as clodrosome-based macrophage depletion. Additionally, the chemokine CXCL13 expressed by monocytes/macrophages was lost following macrophage depletion, and it has also been reported to be responsible for B cell recruitment [19]. CXCL13 neutralization resulted in loss of B cell recruitment and reduced fibrosis thus these findings suggest B cells play a role in fibrosis potentially due to their ability to regulate macrophage phenotype and response, although their exact role needs further investigation. These experiments highlight the fundamental role of macrophages in driving FBR to implanted microspheres and a partial contribution from B cells whereas depletion of other immune cells such as neutrophils, T cells and NK cells did not have a positive impact on reducing fibrosis. These findings consolidate the central role of macrophages in FBR and provide insight into the immunological basis of the FBR and potential therapeutic targets to mitigate inflammatory and fibrotic events.

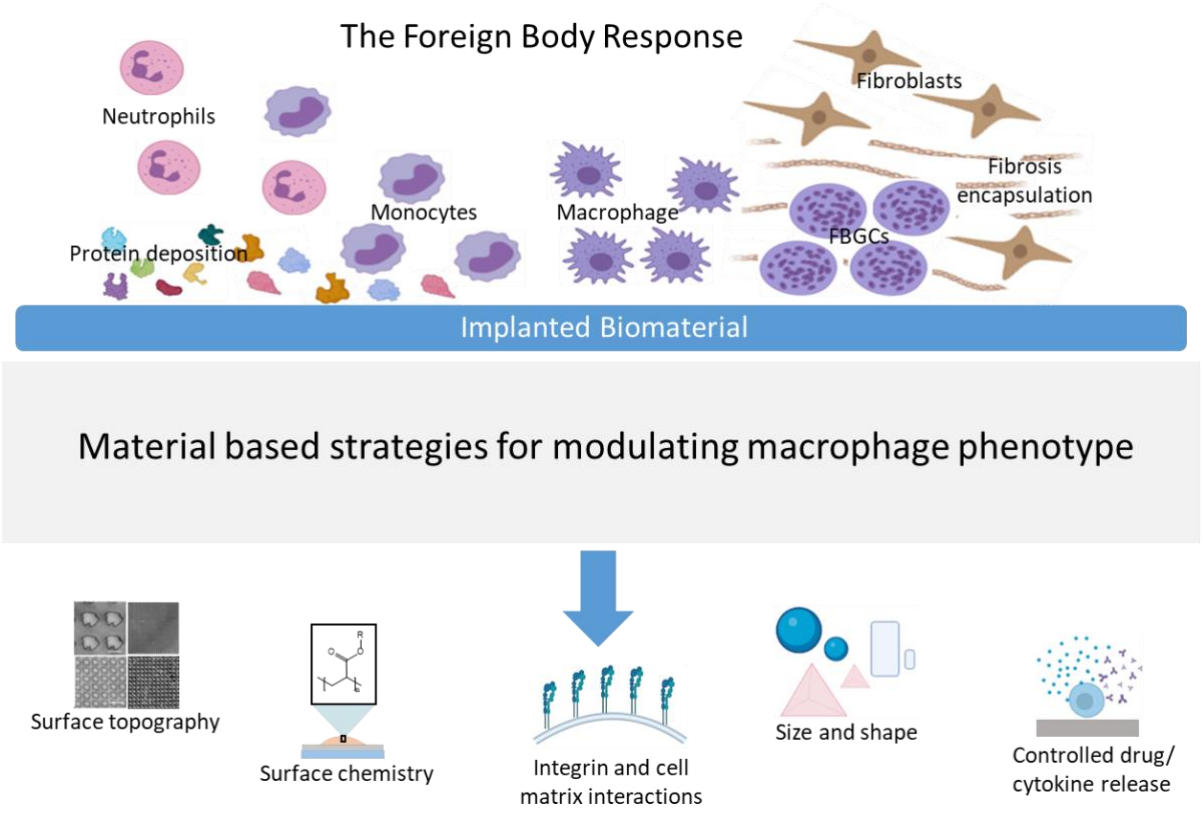


Figure 1. Example material-based strategies for modulating macrophage phenotype in response to materials placed within the body. The implantation of biomaterials can result in a foreign body reaction, in which macrophage play a dominant role. This can lead to chronic inflammation and fibrosis rendering the implanted biomaterial non-functional. Material based strategies can help to mitigate these unwanted host effects.

As a means of steering macrophage polarization towards pro-healing phenotypes, Vassey *et al* [14], used a high throughput microarray platform to screen thousands of different surface topographies (TopoUnits) in order to identify topographies that could control macrophage attachment and phenotype. Since there is little in the way of understanding the correlation between topography and cell response, here authors used machine learning to predict a composite dependent variable that incorporated both macrophage phenotype and attachment data (log(M2/M1) x cell attachment). In this approach TopoUnits with high attachment and a specific phenotype (M2 or M1) were given high values whereas TopoUnits with low attachment and specific phenotype were given low values for. The study revealed macrophages strongly adhered to micropillars in general, with 5 - 10 µm diameter pillars showing highest macrophage attachment. Smaller, denser micropillars drove macrophage polarization towards a M2-like phenotype, whereas M1 phenotypes were driven by larger, more disperse surface features. Taken together, the authors were able to screen thousands of topographies for their impact on macrophage attachment and phenotype and using machine learning could decipher the importance of specific topography descriptors in driving particular cell responses. These data revealed the importance of micropillars of certain diameter compared to a wide variety of other shapes in controlling macrophage behaviour. This illustrates that surface topographies can potentially be rationally designed to fight against the likes of foreign body response against implanted medical devices. In another comprehensive study, Veiseh *et al* [15], examined the role of spherical biomaterial geometry on biocompatibility in rodent and non-human primate (NHP) animal models. The study showed that implanted alginate hydrogel, ceramic, metal and plastic spheres of 1.5 mm diameter, significantly abrogated foreign body reactions and fibrosis compared with smaller sized spheres (0.5

mm). This effect continued even after six months. Notably, they demonstrated that stiffness of the material did not play a critical role in biocompatibility. Authors also reported that increasing sphere size led to a significant reduction of innate immune cell accumulation in peripheral tissue. This could be because the low numbers of macrophage surrounding the larger spheres (1.5 mm) are not becoming activated, resulting in reduced recruitment and extravasation of additional macrophages. Kinetic profiling also revealed negligible numbers of neutrophils and a limited number of other myeloid cells over time on larger spheres. To examine the activation states of macrophages, a range of spheres were implanted into the intraperitoneal space and omental fat pad tissue for up to 7 days. Gene expression analysis showed increased expression of wound healing markers for both 0.5 mm and 1.5 mm spheres in the intraperitoneal and peripheral omentum fat compartments at day 4, but this decreased for the larger spheres by day 7. Authors followed these observations by investigating the survival of islets of Langerhans (islets) encapsulated within 0.5 mm and 1.5 mm alginate microcapsules using a xenogeneic treatment model of transplanting rat pancreatic islets into STZ-induced diabetic C57BL/6 mice. Cellular deposition covered smaller capsules but not larger capsules, high expression of PDX-1 (islet viability marker) and minimal α-SMA were detected in the 1.5 mm capsule group and blood-glucose used as an indicator to cell graft function over time failed in the 0.5 mm capsule group but not in the 1.5 mm group even until 175 days. Modulating the spherical dimensions of a range of materials and its translation to a clinical application significantly mitigated foreign reactions and fibrosis. While the mechanisms of the observed size-dependent control of FBR is yet to be fully elucidated, these data could have significant implications for the design of implanted biomedical devices for a range of applications.

Others have focused on identifying chemical cues that can control macrophage attachment and polarization into distinct functional phenotypes. Vegas *et al* [20], developed a large combinatorial library of alginate-based hydrogels with a variety of amines, alcohols, azides and alkynes to identify materials with reduced immune recognition. Three triazole-containing analogues fabricated into microcapsules were shown to reduce foreign body reactions in rodent and NHP models. Specifically, microcapsules showed little presence of macrophages (CD68+ cells), myofibroblasts (αSMA) and general cellular deposition with no cellular toxicity evident. The lack of immune cell recruitment and/or activation to the surface of the three triazole microcapsule groups indicated that the chemical modification of the polymer chains may be creating distinctive surfaces. Two of the three triazole materials displayed enriched surface localization of their modification and a slight improvement in reducing FBR in NHPs over the other triazole material which had a more uniform modification distribution. The chemical modification of these materials proved crucial, as mechanical stability, surface roughness and protein adsorption, could not explain *in vivo* performance. Furthermore, Rostam *et al* [13], discovered a selection of acrylates and methacrylates which promoted macrophage attachment and differentiation towards pro- or anti-inflammatory phenotypes *in vitro* and in a murine model of FBR. The authors report that while the molecular basis of macrophage polarization in these studies is yet to be fully understood, it is likely to be driven by differential adsorption of biomolecules from biological fluids (e.g. serum) on different polymer surfaces [13]. This was evidenced by the protein layer on M1-like inducing polymers being 2-fold thicker than the protein layer on the naive and M2-like inducing polymers and suggests that the total amount of adsorbed protein may play a role in macrophage polarization. To investigate this mechanism, mass spectroscopy was used to identify the proteins on different polymer surfaces. These experiments revealed a number of unique proteins on different polymer surfaces that may play a role in macrophage polarization, which is a point for future studies.

In another study Xie *et al* [21], used zwitterionic polymers as coatings onto continuous glucose monitors (CGM). Following implantation into healthy and diabetic SKH1 mice and NHPs, inflammatory profiles were evaluated with an *in vivo* fluorescence imaging system (IVIS). Compared with uncoated CGM, inflammatory profiles of the coated CGM were acute at day 1 to 3 however, decreased over

time (up to 8 days). Similar kinetics were displayed for decreasing signal noise over time following CGM implantation. The authors conclude that the zwitterionic coating could eliminate numerous inflammatory responses leading to decrease in sensor-associated noise. However, the molecular basis of such effects is not fully understood yet.

Cell adhesion and communication between cells and their microenvironment through interaction between integrins and extracellular matrix components are known to affect cell proliferation, migration and differentiation. A study by Cha *et al* [22], revealed that IL-4 incorporated into GelMA hydrogels with THP-1 or primary monocytes induced an anti-inflammatory (M2) macrophage phenotype, whereas IL-4 incorporated PEGDA hydrogels induced pro-inflammatory (M1) macrophage phenotype. A key difference between the hydrogels is the presence of cell adhesive sequences in GelMA and their absence in PEGDA gels. Authors therefore hypothesized that macrophage phenotype differences might be regulated through attachment and downstream signalling, which is mediated via integrin subunits and focal adhesions. PEGDA hydrogels inability to provide binding sites resulted in low expression levels of focal adhesion kinase *PTK2* compared to GelMA hydrogels whereby *PTK2* and Vinculin (*VCL*) were enhanced. Changes in integrin expression effectively translated to marked changes in cell behavior and cytoskeletal organization as determined by Vinculin and F-Actin staining. Further to this, low levels of integrin αD and β2 were expressed when monocytes were encapsulated in GelMA hydrogels and high levels of integrin α2 and β1 which remained unchanged in PEGDA gels. When THP-1 cells encapsulated in GelMA were exposed to a neutralizing antibody for integrin α2β1 in the presence or absence of IL-4, high levels of *PTK2* and *VCL* expression were mitigated and decreased in expression of M2-related *STAT6* and *IL10* was seen. This study shows potential to steer the host's immune response towards pro-healing by incorporating cell binding domains into biomaterials to facilitate integrin interactions, such as those with α2β1. This clearly highlights the importance of considering cell-matrix interaction in designing immune-instructive matrices and represents an exciting and novel opportunity to control and improve the clinical outcomes of biomaterial-based implants and cell therapies [22].

More recently materials have also been used to develop artificial M2 macrophage (AM2M) with anti-inflammatory function. Ma *et al* [23], fabricated AM2M cells using nanogels composed of gelatin (GC) and chondroitin sulfate (CS|)cloaked by a macrophage RAW 264.7 cell membrane. The purpose of the study was to enhance the M2-macrophage therapeutic effect in osteoarthritis. To investigate this, papain was injected into knee joint cavities of mice to simulate osteoarthritis. The AM2M cells were found to decrease joint surface erosion, chondrocyte apoptosis and downregulate the expression of ROS, as well as down-regulate the secretion of pro-inflammatory cytokines due to targeting and adhesion of AM2M to inflamed sites. These studies highlight the potential application of materials based artificial cells for immune modulation however there is more to be done in this area and advances in microengineering, biofabrication and new materials will enable more intricate design and functionalities for artificial immune cells [23].

Material based strategies for controlling dendritic cell function

DCs act as a bridge between innate and adaptive arms of the immune system. Modulating DC phenotype can directly influence T cell polarization, with therapeutic applications ranging from vaccine adjuvants to immunotherapy for cancers and autoimmune diseases.

There are many examples where biomaterials in different formats (scaffolds, nano/microparticles, or hydrogels) have been successfully used to control DC phenotype and function as platforms for delivering immune modulatory cytokines or drugs (Figure 2). A recent study by Srinivasan *et al* [24],

showed that delivering and slow-termed release of growth factor granulocyte monocyte colony-stimulating factor (GM-CSF), anti-inflammatory dexamethosome and maturation stimulus peptidoglycan via glutaraldehyde-crosslinked gelatin microparticles within an agarose scaffold could support *in vitro* the generation of tolerogenic DCs. Tolerogenic DCs showed lower levels of maturation markers and increased levels of tolerogenic markers, such as ILT-3, leading to suppression of allogeneic T cell proliferation. Another scaffold-like system to suppress DC activation was fabricated as a biodegradable elastic cryogel. This needle-injectable micro-composite cryogel were hybridized with calcium peroxidase microparticles to controllably produce bactericidal hydrogen peroxide and was shown to maintain immature DC phenotype both *in vitro* and *in vivo,* even when mice were purposely contaminated with pathogenic bacteria [25]. DCs treated with IL-10, a potent tolerogenic cytokine, can be locally delivered within hydrogels to attenuate disease progression of multiple sclerosis (MS) in a murine model by suppressing B and T cell proliferation. This effect was dependent on DC injection site where delivery of DCs to the neck local to MS-associated central nervous system-draining cervical lymph nodes did significantly ameliorate paralysis whereas injection to the flank had no effect [26]. These observations highlight that migratory behaviour of DCs is likely to be as important as their functional phenotype. This DC transplantation model could be adapted for other diseases where aberrant T cell activation underpins the pathology. Macroporous scaffolds loaded with GM-CSF (a potent inducer of DC differentiation/activation) and tumour antigens, with or without addition of other immune-stimulatory compounds (e.g., TLR ligands), have shown promise in promoting *in situ* DC activation and inducing robust anti-tumour T cell activation after *in vivo* implantation [27,28]. This is an attractive alternative and potentially more efficient than the current practice of *ex-vivo* differentiation/stimulation of DCs and their injection to patients.

A recent development is the engineering of DC surfaces. Polydopamine nanostructures (calcium mediated) can be engineered *in situ* on DC surfaces, which within 10 minutes leads to unique bidirectional control of maturation. The polydopamine structures allow an effective suppression of DC maturation by acting as an efficient scavenger of ROS, and irradiation with a 808 nm laser relieves the suppressed state, thus activating DCs via the photo-heat effect of the polydopamine [29]. Additionally, the modification of a synthetic glycopolymer onto DC surface has been shown to improve DC interaction with T cells. Image tracking revealed both increased frequency and duration of contact between DCs and T cells, hence generating higher T cell activation and increased tumour toxicity [30]. Comparatively the effect of free glycopolymer in the medium did not promote any obvious interactions between DCs and T cells, highlighting the importance of glycopolymer-adhesion on DC surfaces and further revealed its effect on T cells is mainly through mannose receptors.

Advances in the fabrication of artificial APCs (aAPCs) have opened up new avenues of usages, mimicking different aspects of DCs such as cell size, peptide-MHC combinations as well as costimulatory molecules. aAPCs have been used to shorten and simplify the T cell expansion process by portraying a controlled and well-defined system that is not being influenced through the cellular environment. While they are able to give strong and direct signals for T cell expansion, aAPCs are unable to migrate into tissues highlighting the need for localised delivery [31,32]. Synthetic aAPCs can be fabricated in different types and can vary in size, surface ligand distributions, ligand mobilities as well as shapes – all variables have shown to have an effect on T cell activation. However, the majority of aAPCs attempt to reflect size and ligands of natural DCs, using for example immortalized cell-lines, microbeads or polymeric PLGA-based particles with co-stimulatory molecules attached [33-36]. In an interesting study, PLGA-nanoparticles with varying aspect ratios were shown to be superior to spherical PLGA-aAPCs in inducing T cell activation and proliferation [37], indicating that particle geometry is another critical consideration in the generation of aAPCs. Mini-PLGA-aAPCs that have the ability to present antigens and stimulate T cells were shown to be effective both in *in vitro* and *in vivo* studies leading to inhibition of growth and metastasis of ovarian cancer [38]. Most artificial APCs have

been used to simulate activated DCs and hence stimulate a T cell response, but a recent study by Rhodes *et al* [39], has successfully explored the function of biomimetic tolerogenic PLGA/PBAE-blend-based aAPCs to induce regulatory T cells and consequently tolerance (Figure 2). PLGA/PBAE aAPCs bound more frequently to naïve CD4+ T cells than control aAPCs and were found to induce significantly higher levels of CD25+FOXP3+ cells than PLGA aAPCs. Induced CD25+FOXP3+ cells exhibited a suppressive phenotype with high levels of co-expression of CD39 and CD73, produced low amounts of pro-inflammatory cytokines and suppressed the proliferation of CD4+ effector T cells both *in vitro* and *in vivo*.

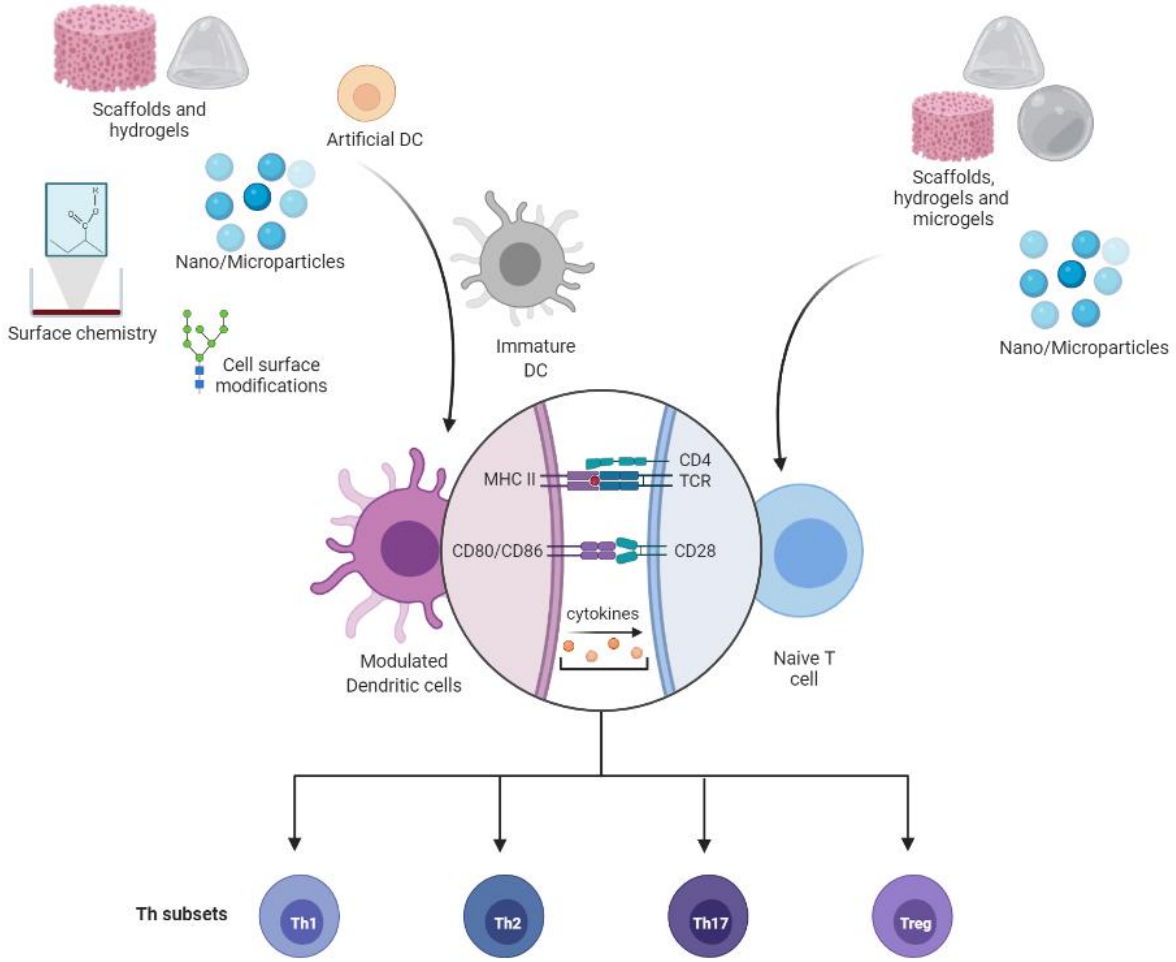


Figure 2. Examples of material-based strategies used to modulate DC and T cell activation status to drive the adaptive immune response. DCs can be modulated in their activation status by particulates, surface chemistry, cell surface modifications or scaffolds, hydrogels, or even replaced by aAPCs. DC modulation is linked to T cell response, which itself can be modulated by scaffolds, particulates and micro/hydrogels leading to a diverse adaptive immune response.

Biomaterial use in T cell engineering

T cells are one of the main effector cell types of the adaptive immune response and as such a prime target for immune system modulation. T cell activation is guided by interaction with DCs, and mainly by DC surface markers, as well as cytokines secreted by DCs. DC maturation status therefore usually determines T cell polarisation towards one of many distinct functional phenotypes such as Th1, Th2, Th17 or Treg [40]. The ability to control T cell polarization offers opportunities for treating

autoimmune/inflammatory diseases, infectious diseases, and tissue injury by promoting Treg, Th1 and Th2 polarization respectively. While materials-based strategies in this area may be less advanced, several biomaterials have been developed to mimic DC interaction with T cells in order to stimulate or reprogram T cell function [41-44] (Figure 2). For example, Coronel *et al* [45], used a synthetic PD-L1 presenting microgel that interacts with PD-1 on T cells and reprograms local immune responses to transplanted pancreatic islets, leading to a significant increase of regulatory T cells. In a seminal study Sadtler *et al* [46], showed that ECM based scaffolds derived from bone- and cardiac muscle can drive a Th2 response as well as IL-4-dependent macrophage polarization, a critical element for functional muscle recovery. This effect is mediated by mTOR/Rictor-dependent Th2 pathway activation through IL-4. In another study it was shown that Th2 cell polarization in response to biological scaffolds can synergistically improve the efficacy of immune checkpoint inhibition and decreased tumor formation [47]. The scaffold induced an immune microenvironment which is distinct from the tumor microenvironment and specifically, macrophages and CD4+ T cells were driven towards a type 2-like immune response. Additionally increased immune cell infiltration and infiltration of eosinophils angiogenic factors and complement was observed. These observations could inform design of novel ECM-based materials to induce Th2 polarization to promote healing as well as synergising with conventional checkpoint inhibitors in anti-tumor therapies, whereas before much focus was put on inducing pro-inflammatory responses in tumor therapies.

Artificial cell development has also been attempted in the context of T cells. Polymeric microparticles with size similar to naïve and activated T cells but with variable elasticities were capable of penetrating 3D scaffolds while also able to release cytokines with or without external stimulation. Altogether they can perform most T cell functions and are thought to be advantageous in cancer immunotherapies e.g., for delivering essential therapeutics or immune signalling molecules [48]. Hickey *et al* [49], engineered a hyaluronic acid-based hydrogel with tunable stiffness that presents two stimulatory signals required for T cell activation (anti-CD3 and anti-CD28) and termed them an artificial T cell stimulating matrix. The stiffness tunability allows effective mechanotransduction required for effective T cell receptor signalling. The matrix skews T cell phenotype by changing its biophysical properties (stimulatory ligand density, stiffness and ECM proteins), and adoptive transfer of modulated T cells has been shown to significantly suppress tumour growth and improve animal survival compared to T cells stimulated with traditional methods [49].

Biomaterial use in natural killer cell engineering

NK cells are an essential part of tumor immunosurveillance and have been found to be less active in animal and human studies when cancer and metastasis susceptibility is high [50]. They act against malignant cells without prior sensitisation by releasing cytotoxic granules containing perforin and granzymes and through death receptor-mediated pathways (e.g., FasL/Fas) [51]. NK cells also produce chemokines and cytokines such as IFN-γ, TNF-α and RANTES making them important cells with immunomodulatory function [51]. For several years now NK cells have emerged as potential candidates for improved cell-based immunotherapies, in particular immunotherapies against cancer. Current biological pharmaceuticals that affect the function of NK cells are based on individual molecules that do not replicate the nanoscale organization of proteins [52]. The majority of interactions in immunological synapses involve clusters of cell surface molecules and the size of these clusters has been correlated with effects on cell signalling and such nanostructural changes may be key drivers of immune cell activation [53]. A study by Loftus *et al* [52], used artificial clusters of leukocyte-stimulating ligands that mimic immunoreceptor nanoclusters, mounted on a scaffold of

graphene oxide as a template and functionalized with CD16 antibodies. These artificial nanoclusters bound to NK cells and stimulated them to undergo degranulation of their cytolytic granules and secretion of IFN-γ [52].

Park *et al* [54], developed immunomodulatory microspheres (IMM-MS) composed of PLGA and iron oxide nanocubes (IONC) for MRI image guided cancer immunotherapy to which INF-γ was encapsulated. The chemokine CXCL10 is important for NK cell recruitment through the IFN-γ triggered signaling pathway. In this study, when two carcinoma cell lines were exposed to IFN-γ released from IMM-MS, there was an increase in CXCL10. To test if the mechanism of CXCL10 release resulted in the recruitment of NK cells to the tumor microenvionment, IMM-MS were tested in a VX2 rabbit liver tumor model. The microspheres were successfully delivered to the tumor site and an increase in NK cell recruitment and infiltration into the tumor was seen, allowing the cells to exert their cytotoxic effects [54].

Glucose oxidase (GOX) can consume intracellular glucose by decomposing glucose to gluconic acid and H_2O_2 [13]. Thus, the generation of H_2O_2 can induce tumor starvation and death. A study by Zou *et al* [55], generated artificial NK cells (aNK) by mixing red blood cell membranes RBCM without immune checkpoints as the outer membrane, with perfluorohexane and GOX which served as cytoskeleton to construct the aNK. Murine mammary tumor cells (4T1) became apoptotic and necrotic following treatment with aNK as opposed to a macrophage cell line (RAW264.7). This was due to the higher uptake of aNK and lower resistance to GOX. The aNK cell construct not only showed to selectively kill tumor cells *in vitro* and *in vivo* but also recruited immune cells to protect the host and re-educated macrophages to switch from M2 to M1 phenotypes, which resulted in phagocytosed antigen fragments and presentation of the antigens to T cells. This was evidenced by the upregulation of M1 macrophage markers (CD80, CD86, MHC II) and the down regulation of M2 macrophage markers (Arg, CD206). The re-education of macrophages was attributed to the generation of H2O2. This study opens up a new concept in the development of aNK cells, as well as a new application for graphene-based nanomaterials. Nanomaterial-based phototherapeutic strategies also continue to attract attention for treatment of solid tumors however, incomplete tumor removal by photothermal therapy (PTT) can results in tumor relapse. Zhang *et al* [56], developed a strategy for immunotherapy of hepatocellular carcinoma (HCC) that involved a photothermal agent (2D coordination nanosheets (CONASHs)), DNAzymes, and aNK cells decorated with a HCC specific targeting aptamer (TLS11a) to kill residual or resistant tumor cells after PTT. After laser irradiation, the DNAzyme@Mn-CONASHs damaged tumors and subsequently released Mn^{2+} by tumor acidic-cleaving as a cofactor to DNAzyme for HSP70 gene silencing in order to overcome heat resistance of tumor cells during PTT [56]. DNAzyme@Mn-CONASHs also served as TME acid-sensitive T_1-weighted contrast agents for enhancing MRI signals. This combined PTT treatment significantly improved anti-tumor efficiency and it will be interested to see it taken further.

Conclusion

In this review, we provide a concise overview of recent advances in the field of immune-bioengineering, and research specifically around macrophages, DCs, T cells and NK cells. While in the past biomaterials were often engineered to be 'inert' and ignored by the immune system, in recent years a shift towards 'bio-instructive' biomaterials has been made in the hope of establishing biomaterials as powerful tools for immune modulation. While better understanding of complex

immune cell-cell and matrix communications and cell-biomaterials interactions have paved the way for exciting new research to develope novel biomaterial-based immunotherapies, there are still major gaps in our understanding of the mechanisms by which biomaterials can drive distinct immune cell phenotypes. This remains an active area of research where there is emerging evidence that highlights the importance of surface chemical and topographical attributes as well as focal adhesion point receptors and ligands in promoting certain cell phenotypes. Ultimately, more detailed understanding of structure-function relationship will underpin our capability to design biomaterials with improved immunomodulatory properties. Moreover, using biomaterial composition alone (e.g. specific chemistries and/or topologies), it may be possible to program the immune system to either pro- or anti-inflammatory responses suitable for applications in tumor environments and the FBR respectively. NK cells can also re-educate macrophages to have powerful effects in a tumor microenvironment. Using biomaterials to guide T cell activation by interaction with DCs and control T cell polarization also offer opportunities for treating autoimmune/inflammatory diseases, infectious diseases, and tissue injury, and advances in the fabrication of artificial immune cells using biomaterials have also opened new forms of immunotherapy.

In summary, recent advances in biofabrication and microengineering, together with the growing number of new biomaterials with cell-instructive characteristics, and an increasing understanding of biology behind the interaction between materials and the immune system, have started to provide better understandings on biomaterial-cell relationship. Moving forward, it is important to gain detailed insight on how immune cells respond to specific biomaterials, and probe underlying mechanisms, and how to translate this new knowledge into actual therapies. In this context, elucidating the structure-function relationship between material physicochemical attributes and immune cells responses could enable rational design of more efficient 'immune-instructive' materials.

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Author contributions

LEF and LK contributed equally to this manuscript. LEF, LK and AMG wrote the manuscript. MRA edited the article. AMG supervised and edited the article.

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