



Surface engineering for lymphocyte programming[☆]



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ABSTRACT

The once nascent field of immunoengineering has recently blossomed to include approaches to deliver and present biomolecules to program diverse populations of lymphocytes to fight disease. Building upon improved understanding of the molecular and physical mechanics of lymphocyte activation, varied strategies for engineering surfaces to activate and deactivate T-Cells, B-Cells and natural killer cells are in preclinical and clinical development. Surfaces have been engineered at the molecular level in terms of the presence of specific biological factors, their arrangement on a surface, and their diffusivity to elicit specific lymphocyte fates. In addition, the physical and mechanical characteristics of the surface including shape, anisotropy, and rigidity of particles for lymphocyte activation have been fine-tuned. Utilizing these strategies, acellular systems have been engineered for the expansion of T-Cells and natural killer cells to clinically relevant levels for cancer therapies as well as engineered to program B-Cells to better combat infectious diseases.

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1. Introduction

The field of drug delivery has in many ways focused on the controlled delivery of soluble biomolecules to tissue types of interest and increasingly to targeted cell types. While this mode of delivery covers many categories of therapeutics, including both small molecule drugs and biologics such as peptides, proteins, and nucleic acids, certain types of biologics require presentation from a surface, rather than soluble presentation, for their desired cellular function. Biomimetic materials, in particular, that aim to mimic the physical, chemical, and biological aspects of natural biological materials for cellular engineering, must take into account this feature of surface presentation.

One of the varied areas of biology where the proper balance of physical, chemical, and biological interactions appears most critical is in the signaling of the immune system. Thus, for the engineering of lymphocytes, surface engineering of biomolecules is key to deliver the proper signals for lymphocyte programming. This review highlights lymphocyte immunoengineering approaches including the components of the necessary chemical and biological signals that engender cellular responses, the required features of the surfaces that best present these signals such as surface fluidity, and the geometric and physical properties of the supporting substrate that also modulate lymphocyte behavior (Fig. 1) (Table 1).

Lymphocytes include T-Cells, B-Cells and natural killer (NK) cells, all of which arise from the common lymphoid progenitor [1]. T-Cells and B-Cells are primarily responsible for the effector functions of the adaptive immune system, while NK cells serve as innate effector cytotoxic lymphocytes. Lymphocyte development begins in the bone marrow during hematopoiesis with lymphocytes migrating to peripheral lymphoid tissue following maturation. In the body, lymphocytes interact with a variety of cell types and signaling molecules that provide the cues necessary to initiate expansion, activation, anergy or cell death. T-Cell interaction with professional antigen presenting cells (pAPC), as well as stromal cells, largely shapes the adaptive immune response to pathogens and plays a role in auto-immunity [1]. pAPCs include dendritic cells, macrophages and to a lesser extent B-Cells that all express the major histocompatibility (MHC) class II molecule to allow presentation of exogenous antigens. B-Cells, in contrast, are capable of interacting with soluble antigen directly, allowing for a varied approach to targeting their response. After the immune system is activated by a specific pathogen and that pathogen is subsequently cleared, some of the pathogen-specific T-Cells and B-Cells become memory cells, ready to respond quickly if the same pathogen is ever seen again in the future. While lymphocytes are the chief cells involved in adaptive immunity and the long-term immunological memory necessary for effective vaccination, this review focuses on lymphocytes in the context of direct programming to elicit primary functions. Much work has been done in the area of vaccine design, although it has often focused on soluble antigen, small molecule

adjuvants and release formulations for optimal temporal stimulation instead of surface engineering, as well as focusing on delivery to dendritic cells rather than to lymphocytes [2,3]. In particular, Purcell et al. provides a good review of the interplay between lymphocytes in response to peptide antigens and their involvement in long term immunity [4] and Irvine et al. provides a good review of nanoparticles for use in vaccines [5].

Modulating the immune system through cellular based systems, particularly for anti-cancer immunotherapies has seen great success in recent trials with therapies targeting the anti-tumor response both through the direct modulation of lymphocytes and through *ex vivo* expansion of dendritic cells. In particular, chimeric antigen receptor (CAR) T-cell therapies for a subset of otherwise non-responsive cancers have seen high levels of efficacy and are in various stages of clinical trials in the USA [6,7]. Most CAR-T-cell therapies rely on adoptive transfer strategies that have certain risks associated with the genetic modulation of T-cells for the purpose and have had adverse events resulting from antigen recognition leading to cytokine storms [8]. In contrast to CAR-T-cell expansion for adoptive transfer, Sipuleucel-T therapy was recently approved for refractory prostate cancer with high efficacy in a subset of patients but carries its own costs and associated risks [9]. For Sipuleucel-T therapy, patient specific *ex vivo* expansion of dendritic cell populations in the presence of immunostimulatory molecules followed by reinfusion had an initial cost-per-patient of \$93,000 in 2010 that has since risen [9]. Many of the challenges associated with these therapies in terms of cost and regulatory hurdles could be overcome with sufficiently effective acellular strategies currently in pre-clinical stages as discussed in this review.

As understanding of these natural systems has advanced, investigators have sought to design artificial systems capable of mimicking and controlling these interactions to shape the lymphocyte response. Moving towards this goal, engineered particle and surface based systems have been designed that can activate a variety of lymphocyte subtypes *in vitro* and *in vivo* for purposes of anti-cancer therapies. Across multiple stages of translation to the clinic, activation of lymphocytes *ex vivo* and *in vivo* have been studied. As the majority of lymphocyte engineering strategies in the past two decades have focused on cancer therapies, engineering of cytotoxic T lymphocytes (CTLs) has arguably advanced the furthest and strategies for genetically engineering T-Cells have already reached the clinic in the form of CAR-T-Cells [10]. Similar in some contexts, cellular based artificial antigen presentation systems have likewise seen significant development [11], but face challenges related to the manufacturing and amplification of dendritic cells or other professional APCs *ex vivo* [12].

Unlike strategies to modify lymphocytes directly such as with CAR T-Cell engineering [13], artificial antigen presenting cells (aAPCs) [14] and surface engineering for lymphocyte modulation function within the domain of activating lymphocytes through their existing molecular

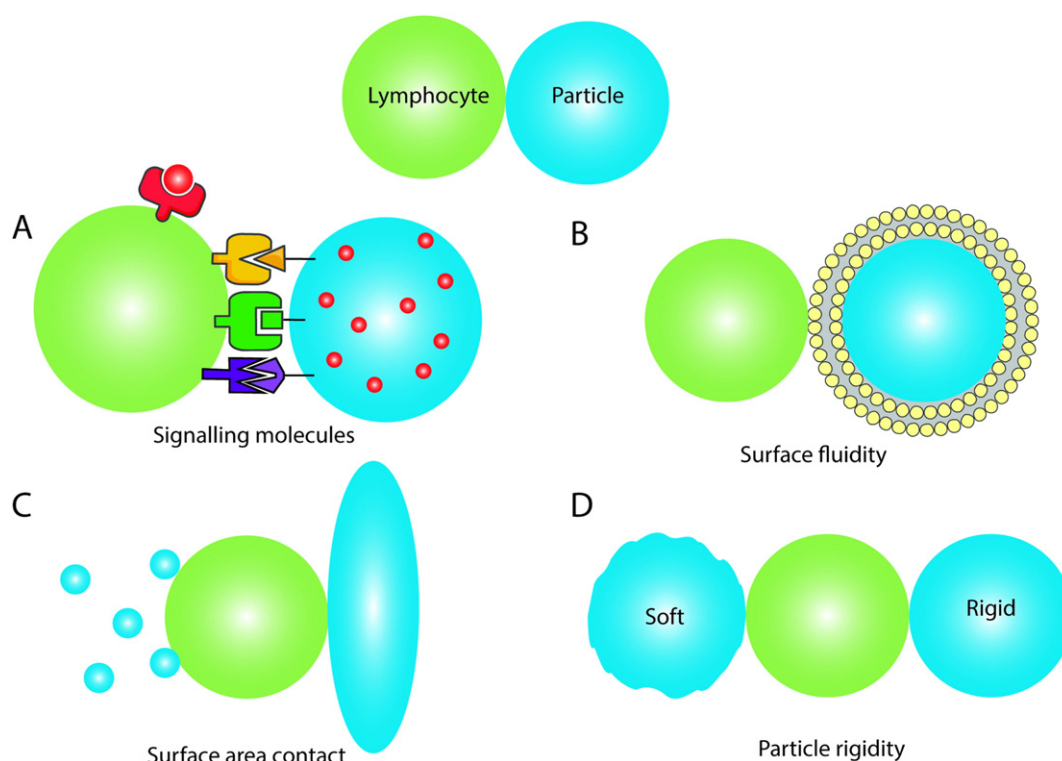


Fig. 1. Lymphocyte interaction with engineered surfaces is affected by particle (A) signaling molecules, (B) surface fluidity, (C) shape and size and (D) particle rigidity.

machinery. This approach of lymphocyte activation has the benefit of being potentially safer in terms of lower risk of run-away activation, induced tumorigenesis, or mutagenesis due to the lack of viral modification of the lymphocytes. Particle and surface based acellular aAPC engineering have fewer of the translational challenges associated with the use of live cellular aAPC systems in terms of cost and regulatory hurdles for translation [15].

The approach of lymphocyte engineering through outside-in signaling mediated by intact cellular machinery maintains similarities to traditional drug delivery approaches in that it requires determination of the biological factors, dosages and manner of presentation necessary to result in particular cellular responses. Similar to the identification of small drug molecules that mediate specific biological responses, engineered surfaces must possess the specific three-dimensional

Table 1
Summary of various surface engineering features for lymphocyte programming.

Lymphocyte target	Surface feature ^a	Material ^b	Signal protein ^c	Result	Ref
T-Cells	MHC dimer for antigen specific activation	Dynal® microbead	S1: MHC Dimer + S2: Anti-CD28	Micro-aAPC gave 10 ⁶ expansion ex vivo over two months of tumor specific T-Cells	[14]
	Paracrine delivery of IL-2	PLGA	S1: Anti-CD3 + S2: Anti-CD28	Enhanced CD8 + expansion with IL-2 delivery	[36]
	Ellipsoidal shape – micron scale	PLGA	S1: MHC Dimer + S2: Anti-CD28	20-fold increase in T-Cell proliferation in ellipsoidal aAPC compared to spherical	[22]
	Ellipsoidal shape – nano scale	PLGA	S1: MHC Dimer + S2: Anti-CD28	3-fold stronger <i>in vivo</i> induction of T-Cells by ellipsoidal aAPC compared to spherical aAPC	[23]
	MHC dimer with FasL	Dynal® microbead	S1: MHC Dimer + S2: FasL	Killer aAPC induced T-Cell apoptosis with both high efficacy and specificity	[40]
	SLB with variable ligand mobility	DMPC and DPPC	S1: Anti-CD3	T-Cell activation and IS formation preferentially induced by more fluid membranes	[65]
	Paramagnetic nanoparticle	Iron-dextran	S1: MHC Dimer + S2: Anti-CD28	5.5-fold increase in T cell expansion with magnetic clustering of nano aAPC compared to non-clustered nano aAPC	[76]
B-Cells	Planar substrate with variable rigidity	PDMS	S1: Anti-CD3 + S2: Anti-CD28	4-fold increase in T cell expansion on softest substrate compared to stiffest substrate.	[135]
	Patterned antigen with surface fluidity	Liposome	Trimeric HIV-1 spikes	B-Cell activation against HIV-1 antigen ex vivo	[50]
	Combine B-Cell and CD4 + T-Cell stimulation	Copolymer	OVA antigen + B-Cell epitope	Enhanced activation of B-Cells in combination with CD4 + cells	[51]
NK Cells	Planar substrate with variable rigidity	Polyacrylamide	Anti-IgM	Enhanced B-Cell activation on stiffer substrates	[137]
	Membrane bound IL-15	K562 cells	sIL-15 + 4-1BBL	Ex vivo expansion of NK cells	[53]
	Membrane bound IL-15 and IL-21	K562 cells	sIL-15 + sIL-21	Enhanced NK cell stimulation with both IL-15 and IL-21	[54]

^a MHC = major histocompatibility complex, SLB = supported lipid bilayer.

^b PLGA = poly(lactic-co-glycolic acid), DMPC = 1,2 dimyristoyl-sn-glycero-3-phosphocholine, DPPC = dipalmitoylphosphatidylcholine, PDMS = polydimethylsiloxane.

^c S1 = Signal 1 protein, S2 = Signal 2 protein.

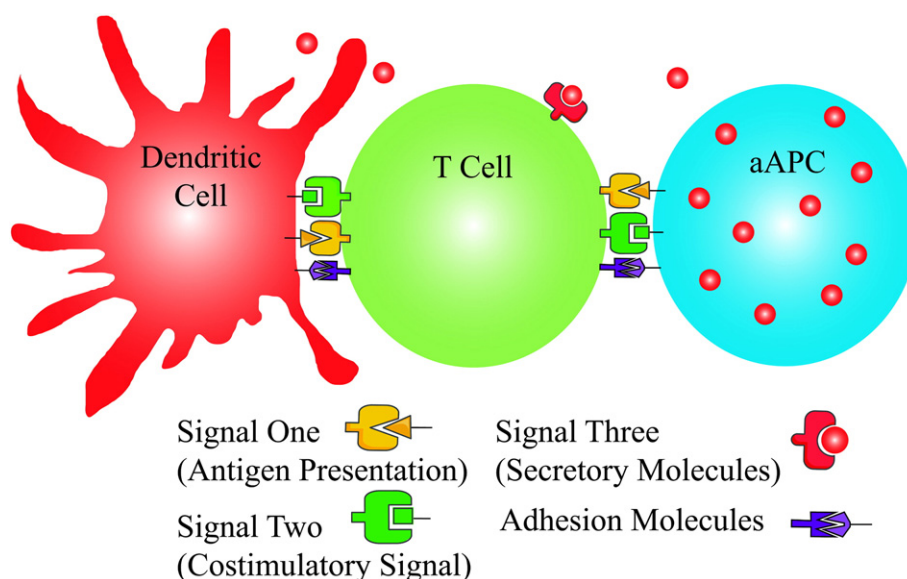


Fig. 2. Surface engineering for T-Cell immunomodulation primarily aims to recapitulate the signaling molecules and context provided by professional APCs *in vivo*. Presentation of Signal 1 for antigen presentation and Signal 2 for costimulation can be achieved with MHC class I and anti-CD28 respectively. Signal 3 has been shown to enhance T-Cell activation the form of soluble secretory molecules such as IL-2 and additional adhesion molecules such as ICAM-1 and LFA-3.

structures needed to bind to key biomolecule targets, including proteins, often along with targeting, such as to immunological tissues. Similar to the formulation of release systems for small molecule drug delivery, engineered systems for the controlled presentation of specific biological factors from a surface may need to also consider spatial and temporal factors relating to local dosages and the avoidance of undesirable clearance. Development of these technologies applies knowledge of chemical engineering, immunology, and materials science. A challenge with this approach is that the requirements for modulating lymphocyte responses have been determined primarily through empirical means and are not fully explored for all cell types or intended cellular responses and can also be different in varying model systems. Despite this limitation, lymphocyte engineering through the rational design of acellular materials has the potential to enable unique safe and specific *in vivo* cellular therapies without the challenges, risks, and expense of *ex vivo* cellular engineering. The presentation of chemical and biological factors from defined biomaterial surfaces could significantly impact many areas of medicine.

2. Chemical and biological factors (signal proteins)

This area of lymphocyte engineering best matches the traditional approach of the drug delivery field as it involves the selection of bioactive molecules as well as their dosages to elicit particular cellular responses. Lymphocytes primarily interact with their environment through intramembrane protein complexes that allow for outside-in signaling to occur when they bind other protein complexes or biomacromolecules. It is important to design and select both the specific biological factors to be included in each system, as well as the ratios between the signals involved.

Particle and surface based strategies for engineering lymphocyte responses have been primarily focused on engineering T-Cells for expansion to cytotoxic T lymphocyte populations as cancer therapies [10]. For therapeutic cancer applications, activation of T-Cells with MHC class I is of particular interest for its ability to induce expansion of cytotoxic T lymphocytes that can infiltrate the tumor and trigger apoptosis of cells presenting the same signal. Surface engineering for B-Cell programming has progressed more slowly due to additional challenges including recapitulation of all the signals present in the spleen and peripheral lymphoid tissues, but progress has been made in recapitulation of specific signaling molecules using acellular systems. Natural killer cell surface

engineering using specific biological factors has likewise presented challenges as it has advanced.

2.1. T-cells

Biochemical activation or deactivation of T-Cells specifically requires two signals, which have been recapitulated with a variety of approaches to guide activation or deactivation [16]. T-Cell activation *in vivo* occurs primarily in lymph nodes, spleen or Peyer's patches, where T-Cells interact with professional APCs. pAPCs interact with T-Cells primarily through two protein complexes termed Signal 1 and Signal 2 for simplicity. Signal 1 provides antigen specificity for T-Cell expansion *via* interaction of the T-Cell receptor (TCR) with MHC class I or II with bound cognate antigen for activation of CD8 + or CD4 + T-Cells, respectively. Signal 2, in contrast, provides the context for Signal 1 to allow for a variety of T-Cell responses, among them expansion, anergy and apoptosis. In addition to these two primary signals, soluble Signal 3 and adhesion molecules assist in providing guiding cues to lymphocytes as shown in Fig. 2. Signals 1 and 2 have historically been used in a 1:1 mol ratio, but this factor has not specifically been studied.

2.1.1. Signal 1: cognate antigen presentation

Recapitulation of Signal 1 in acellular T-Cell engineering approaches have traditionally been achieved *in vitro* using a constitutively active, agonist anti-CD3 antibody to trigger T-Cell expansion [17–20], but this approach is not suitable for *in vivo* activation due to the lack of specificity for TCRs of expanded T-Cells [16,21]. Furthermore, expansion of T-cells with agonist anti-CD3 antibodies has been shown to lead to dwindling CD8 + T-cell expansion in successive generations of cells, as cells become exhausted [14]. As an alternative, MHC class I or II have been bound directly to particles [14,22–26] or surfaces for both antigen specific as well as CD4 + or CD8 + TCR activation; for this reason, recombinant forms of MHC class I or class II are much more promising for antigen specific CTL expansion either *in vivo* or *ex vivo* for clinical applications. To further increase avidity of the MHC molecule for specific TCRs, dimers [25] and tetramers [27] of MHC have been engineered at the molecular level that have been shown to be highly potent for antigen specific T-Cell expansion. In particular, the use of a dimerized MHC class I peptide complex on microbeads was shown to give a 10^6 expansion of CTLs *ex vivo* with the estimated possibility of generating 10^{11} antigen specific CTLs from a 500 mL leuopak in under two months

[14]. This level of CTL expansion is sufficient to be clinically relevant for human cancer cases [28,29], but requires substantial investment for *ex vivo* expansion. In contrast, activation *in vivo* with an acellular material could be the gold-standard for therapy, as it would not necessitate the manufacturing and regulatory hurdles associated with cellular therapies.

Approaching the goal of fully acellular systems that can stimulate robust T-cell expansion *in vivo* following administration, degradable particle systems using dimerized MHC IgG pre-loaded with selected antigen and co-injected with T-cells intravenously in an adoptive transfer murine model have demonstrated improved survival in aggressive B16-F10 melanoma model [26]. These particles were shown to be capable of stimulating antigen specific T-Cells to proliferate up to 30-fold over a span of seven days, however, the cells showed high levels of PD-1 expression [26]; treatment of the expanded T-Cells with anti-PD-1, however, protected the T-Cells and allowed them to remain effective *in vivo*.

2.1.2. Signal 2: costimulation

Whereas Signal 1 provides antigen specificity for TCR selection, costimulatory Signal 2 provides context to trigger a specific fate for T-Cells interacting with the engineered system. Most aAPCs are engineered to interact with T-Cells through at least two costimulatory signals, targeting the CD28 receptor on T-Cells with either B7.1/B7.2 (CD80/86) or anti-CD28 to provide Signal 2, while simultaneously providing activation of the TCR with MHC or anti-CD3 [30]. The use of anti-CD28 presents benefits over B7.1/7.2 due to the fact that it can be engineered to specifically bind only to CD28 and not the inhibitory CTLA-4, thus avoiding T-Cell deactivation [30]. This is particularly important in cases of tumor models that have upregulated deactivating signals, as the binding affinity of B7.1/7.2 to CTLA-4 is higher than to CD28, making it a less suitable costimulatory molecule than alternatives such as anti-CD28 [31]. As an additional alternative Signal 2, 4-1BBL with affinity for T-Cell expressed 4-1BB (CD137) has been identified as a potent costimulatory molecule that works synergistically when combined with anti-CD28 for CTL expansion [27,32–35].

2.1.3. Signal 3: soluble factors

Tertiary signals for T-Cell engineering includes soluble signals such as IL-2, which has been included in biodegradable particle formations for controlled release with strong evidence of improved activation. Incorporation of IL-2 for local paracrine delivery to T-Cells from biodegradable microparticles improved activation and expansion of CD8 + T-Cells compared to exogenous IL-2 delivery in the bulk solution, though the local particle based release of IL-2 was noted to result in CD4 + cell apoptosis [36]. In this specific study, particle degradation and release rates were shown to be important for IL-2 release, with sustained release over multiple days being necessary for benefits in expansion [36]. In addition to IL-2, IL-21 has been demonstrated to result in expansion of highly potent CD8 + CTLs when using a cellular aAPC system [37]. For more specific lymphocyte engineering, other soluble signals including TGF- β 1, TNF- α , IL-12, IL-1 can be utilized to induce specific T-Cell subtype responses including Treg, TH17, TH1 and TH2 CD4 + T-Cells [38]. Incorporation of these soluble protein molecules in degradable polymeric microparticles or nanoparticles for acellular aAPCs can be achieved using water-in-oil-in-water double emulsion methods, similarly to published approaches for IL-2 release [36].

2.1.4. Adhesion molecules

Interaction between professional APCs and T-Cells *in vivo* is facilitated by binding of T-Cell expressed LFA-2 (CD2) and LFA-1 with APC expressed ICAM-1 and LFA-3. Utilization of ICAM-1 and LFA-3 adhesion molecules has been shown to greatly assist in the artificial recapitulation of immunological synapse formation on micropatterned surfaces as well as liposome and particle based systems [17,19,20,39]. Utilization of adhesion molecules on microparticles and nanoparticles for

activation of T-Cells *in vivo* may improve the ability of these particles to stay adhered to T-Cells encountered in the blood stream long enough for activation to be initiated or for the conjugates to be trafficked to a lymph node.

2.1.5. Killer aAPCs: T-cell deactivation

In contrast to most T-Cell engineering approaches, another strategy for lymphocyte engineering includes “killer aAPCs” designed to eliminate T-Cells possessing a highly specific TCR for a certain antigen [40]. Killer aAPCs have been designed as microparticles coated with a combination of MHC dimer for TCR specificity and FasL (CD95L) as the Signal 2 molecule to deliver an apoptotic command to T-Cells in an antigen specific manner. These particles were shown to mediate 80% antigen specific killing of cognate CD8 + T-Cells only whereas the non-cognate T-Cells were not affected [40]. This strategy of selective T-Cell elimination may prove to be highly useful in cases of autoimmunity where T-Cells are erroneously attacking a patients' own cells or in cases of host versus graft disease in allograft organ rejection [24,40–42]. Later generations of these killer aAPCs have been designed with anti-Fas antibodies as an alternative to FasL [42].

2.2. B-cells

Although not as frequently investigated as T-Cells, B-Cells are another class of lymphocytes that have been engineered with the intent to elicit humoral immunity as opposed to cellular immunity. While B-Cells are capable of responding to soluble antigen, the manner of antigen presentation to the B-Cells can alter the subsequent response.

2.2.1. Requirements of B-cell/antigen interaction

B-Cell activation has classically been viewed as the result of crosslinking of surface bound immunoglobulin (sIg) receptors by repeated antigenic patterns on a target. Upon crosslinking of these sIg molecules, a phosphate kinase cascade is initialized which results in maturation of the B-Cell into a form that can produce soluble antibodies [43]. Further interactions with cognate helper T-Cells results in further maturation and differentiation into plasma cells for rapid synthesis and secretion of high affinity antibodies [44].

More recent studies suggest that the primary means of activation of B-Cells *in vivo* is not by soluble antigen, but rather by APCs presenting membrane bound antigen [45]. This membrane bound antigen is not presented in the context of an MHC protein as would be required for T-Cell activation. The primary means of B-Cell signaling in this context is the microcluster [46] which contains 50–100 sIg receptors clustered together [45]. It was recently discovered by dSTORM imaging that these receptor microclusters initially exist as smaller clusters ranging from 1 to 10 receptors but upon activation by simulated surface bound antigen, they would aggregate into larger microclusters accompanied by and increased contact area with the antigen presenting surface [47]. Microcluster formation was recently mechanistically linked to a decrease in the lateral mobility of the BCR upon antigenic activation, thus restricting the normally diffusive single receptor to a relatively rigid microstructure [48]. Interestingly, upon initial BCR engagement, the B-Cell will undergo a morphological change to flatten out along the antigen presenting surface, similar to the related T-Cell [46]. The resulting prolonged contact allows for the formation of an immunological synapse similar to the T-Cell/APC interaction [45].

2.2.2. Particulate systems for B-cell activation

Numerous particulate systems have been created to activate B-Cells. A repeated antigenic pattern on the surface of calcium phosphate nanoparticles resulted in a significantly stronger B-Cell response to soluble cognate antigens. This activation was determined to be 100-fold stronger than the soluble antigen case [49]. Similarly, a lipid nanoparticle system was developed with HIV antigens loaded onto the surface with a regular spacing of 12–14 nm [50]. This regular spacing of B-Cell antigens

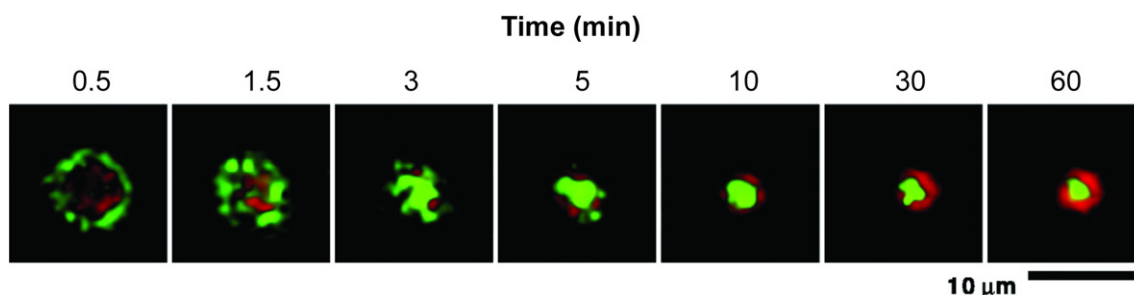


Fig. 3. Immunological synapse formation between T cells and a supported lipid bilayer. Images over time of MHC-peptide (green) and ICAM-1 (red) at area of contact show eventual formation of immunological synapse with outer pSMAC and inner cSMAC. Reproduced with permission from *Science* [63].

was linked to a 12-fold increase in B-Cell stimulation as measured by cytokine secretion compared to a soluble control [50].

For B-Cells, it has also been shown that a solid particle surface is not necessary for efficient stimulation as polymerized antigen was found to be capable of eliciting an effective B-Cell response. This reaction was found to be correlated to the number of repeat units in the construct with an approximately 10-fold increase in B-Cell mediated T-Cell activation for a 6-fold increase in the number of repeating units [51].

2.3. Natural killer cells

Natural killer (NK) cells are a lymphocyte population with crucial involvement in anti-cancer activity [52]. NKs are difficult to expand *in vivo*, but have been expanded *ex vivo* using a variety of strategies [53]. In particular, NK cell expansion *ex vivo* has primarily utilized cellular based aAPC systems with membrane bound IL-15 in combination with 4-1BBL to generate clinically relevant numbers of NK cells for anti-cancer applications [53]. This strategy, while thus far has only been implemented using K562 cellular aAPC systems, could be readily adapted to acellular particle aAPC or surface based systems in the future to alleviate concerns over the use of another human cell line. In addition to the use of membrane bound IL-15, membrane bound IL-21 has been shown to be very potent for NK cell expansion *ex vivo* using the same K562 cellular aAPC system [54]. Over the course of 42 days it was shown that proliferation of natural killer cells was 10,000 times higher in the presence of membrane bound IL-21 compared to comparable aAPC without membrane bound IL-21.

3. Surface patterning and fluidity

Interactions between lymphocytes and APCs or soluble antigens are highly complex in spatial arrangement. An immunological synapse (IS) is formed at the interface between lymphocytes and APCs. The IS is characterized by dynamic rearrangements of proteins into specific clusters. Randomly distributed, immobilized proteins cannot recapitulate this organization and clustering [55]. As a result, patterned and fluid surfaces have been engineered to replicate the IS and enhance lymphocyte modulation.

3.1. T-cells

When a biological APC engages with a T-Cell, peptide antigen-MHC (pMHC) on the APC is recognized by the TCR, leading to the formation of the immunological synapse. The IS is composed of concentric rings containing clusters of specific proteins (Fig. 3). In order for the IS to form, relevant molecules must migrate towards the interaction site and form clusters. The IS can be recapitulated and studied by patterning relevant proteins in a certain spatial arrangement [20,56,57] or by engineering surface fluidity so that the proteins have lateral mobility to form the IS when engaged with a T-Cell [17,58,59]. The IS has been studied in depth using a variety of techniques including supported lipid bilayers,

microfabricated surfaces and super-resolution optical microscopy to ascertain the identity of the signaling molecules involved and their precise spatial arrangement for maximal activation [60].

3.1.1. Spatial arrangement of immunological synapse

The small size of the immunological synapse of approximately 20 nm in diameter for individual receptor clusters [60,61] and the Abbe diffraction limit of approximately 250 nm for light microscopy necessitates the use of single molecule localization super-resolution microscopy techniques including PALM or STORM to study individual immunological synapses [60]. Single molecule localization microscopy techniques have allowed individual supramolecular activation complexes (SMAC) of the immunological synapse to be probed in live cells, showing evidence of a bullseye-like structure of a central SMAC (cSMAC) with distinct composition compared to the surrounding peripheral SMAC (pSMAC) and exterior distal SMAC (dSMAC) of the immunological synapse (Fig. 3) [60,62,63]. The cSMAC, where initial activation occurs, contains the TCR/CD3 complex, as well as signaling molecules LAT and LCK. The pSMAC, in an annulus around the cSMAC, is composed almost entirely of LFA-1 [60,62]. Interestingly, TCR signaling occurs in the dSMAC following initial activation at the cSMAC, which has implications for the design of aAPCs as individual focal spots of anti-CD3 or pre-clustered MHC are unlikely to be optimal [60].

3.1.2. Surface patterning

Microfabrication allows for deposition of proteins in a site-specific manner on surfaces at resolutions down to approximately 1 μm to allow for effective pre-clustering of signal molecules to study lymphocyte activation [56]. Using microfabrication with biotinylated photoreactive to pattern anti-CD3 onto a glass substrate, solid focal spots were shown to be more effective for T-Cell activation than annulus shaped protein deposition, demonstrating the importance of receptor clustering for T-Cell activation [20]. This study further concluded that differences in T-Cell activation were due specifically to the shape of the protein deposition spots and not due to density of ligand or surface area of the receptor, which has implications for particle systems that are capable of only displaying a certain surface area to T-Cells based on their geometry [20,22].

In contrast to microfabricated surfaces that allow for receptor localization at the micron level, nanoarrays of gold nanoparticles to specifically localize ligands at defined intervals between 35 and 150 nm have been used to study the importance of inter-receptor spacing for T-Cell activation [57]. Using anti-CD3 conjugated to gold nanoparticles, a spacing of 60 nm was shown to be optimal for maximal threshold stimulation of T-Cells [57], which has implications for the design of particle-based aAPC systems with surface conjugated ligands where ligand localization is more challenging to define.

3.1.3. Surface fluidity

The fluidity of proteins on a synthetic surface of a particle is an important component of mimicking the membrane fluidity of a cell.

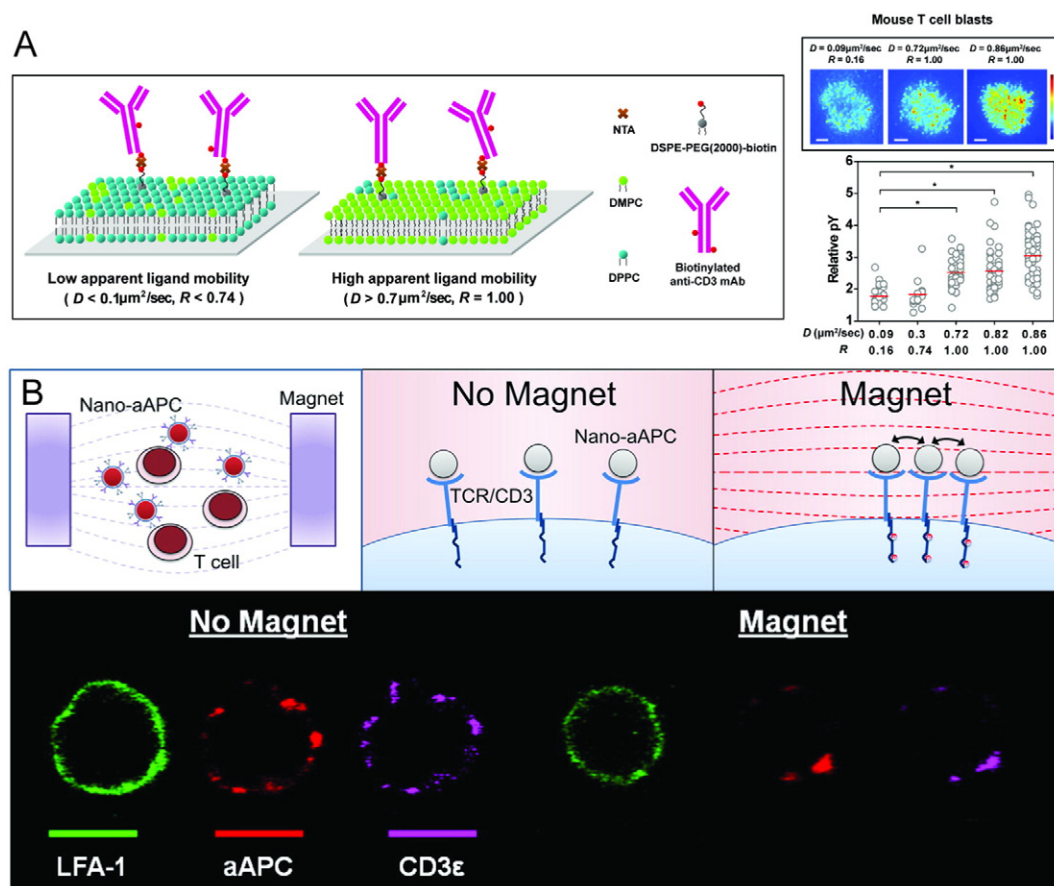


Fig. 4. Receptor clustering is critical for T cell activation. (A) Supported lipid bilayers conjugated with biotinylated anti-CD3 mAb via NTA (neutravidin) were synthesized with different compositions of DMPC (1,2-dimyristoyl-sn-glycero-3-phosphocholine) and DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine) to produce ligand mobility. SLBs with a higher diffusion coefficient (D), which corresponds to greater ligand mobility, induced higher levels of TCR signaling [44]. (B) Paramagnetic nano-aAPC induce clustering of the TCR/CD3 complex on T cells under the influence of a magnetic field. Reproduced and modified with permission from ACS Nano [76].

Surface fluidity allows particles to behave in a more biomimetic manner by enabling receptor clustering, which is an important aspect of the physiological interaction between T cells and APCs.

Supported lipid bilayers (SLB) on planar substrates have long been utilized to study receptor interactions between cells, in particular as they enable membrane fluidity and facilitate receptor clustering. Artificial APC surfaces have been engineered to mimic the lateral diffusivity of the IS by coating a support substrate with an SLB containing Signal 1, Signal 2, and adhesion molecules. SLBs with immobilized ligands separated by chromium strips for precise localization have been used to demonstrate the importance of receptor clustering for T-Cell activation [18]. This study was one of the first to demonstrate the necessity of ligand positioning and fluidity for the formation an immunological synapse required for robust T-Cell activation [18]. SLBs have also been used to study the requirements for TCR triggering, which were found to include surface-anchoring of pMHC, T-Cell surface adhesion, and subsequent ability of the T-Cell to move, suggesting that a fluid aAPC/T-Cell contact area is optimal [64]. Ligand mobility, specifically, has been shown to be an important parameter in modulating T-Cell response [65]. SLBs of different lipid compositions were engineered to vary ligand mobility, and those with greater ligand mobility had increased CD3 accumulation at the IS and increased phosphotyrosine (pY) signaling at the TCR microclusters (Fig. 4).

Knowledge of the importance of membrane fluidity for immunological synapse formation has implications for lymphocyte surface engineering that have been investigated through the use of liposomes with conjugated signal molecules that allow for free movement and clustering of the biomolecules [17,59]. As planar SLBs are only relevant for *ex vivo* T cell expansion, 3D aAPCs with membrane fluidity have

been engineered by incorporating Signal 1, Signal 2, and adhesion molecules into liposomes [17,58,59]. TCR molecules on T-Cells engaging with liposome-based aAPCs formed clusters over time that co-localized with the aAPCs, suggesting the formation of an IS [58]. In the first generation of liposome-based aAPCs, the pMHC molecules were randomly distributed throughout the membrane [58]. However, pre-clustering MHC class II on lipid rafts for highly concentrated spots of signaling molecules has been shown to increase activation of T-Cells [66]. As a result, liposome-based aAPCs were enhanced by pre-clustering the T-Cell ligands in microdomains on the aAPC surface, which significantly increased antigen-specific T cell stimulation [59]. Microdomains composed of anti-CD3, anti-CD28, and anti-LFA-1 were anchored to GM-1-enriched liposomes by neutravidin bound cholera toxin. This liposome-based aAPC generated almost a 1.5-fold increase in the expansion of T-Cells compared to bead-based aAPCs with immobilized ligands, with an overall expansion of over 150-fold over the course of 14 days. Liposome-based aAPCs allow receptor clustering but are limited by shape, size, and the number of signaling molecules that can interact with the T-Cell. SLBs atop a particle core would allow for control over these parameters, in addition to surface fluidity. Particles coated with lipid bilayers have been successfully engineered for targeted drug delivery applications [67,68], and are an interesting approach for next generation aAPCs.

Coating particles with cell membranes is an additional technique for generating surface fluidity that provides a biomimetic surface in terms of both fluidity and protein content and enables particles to evade clearance. Particles have been coated with membranes of many cell types, such as red blood cells [69], platelets [70], and macrophages [71], and cell membrane coated nanoparticles have been shown to induce

receptor clustering [72]. Nanoporous silicon (NPS) particles coated with leukocyte membranes bound to endothelial cells and led to clustering of ICAM-1 [72], suggesting that this technique could be applied to aAPCs to drive receptor clustering on T-Cells.

3.1.4. Alternative methods to enable receptor clustering

Alternative engineering methods allow for receptor clustering on T-Cells without patterning the particle surface or using a fluid lipid bilayer. One way to accomplish this is through the use of a semi-flexible polymer based on poly(isocyanate peptides) conjugated with anti-CD3 and anti-CD28 to allow for receptor clustering, as well as efficient multivalent binding [68,73]. The polymers were able to activate T-Cells at significantly lower concentrations *in vitro* than rigid spherical poly(lactic-co-glycolic acid) (PLGA) counterparts, but their efficacy and biodistribution *in vivo* has yet to be studied.

With the knowledge of receptor clustering necessary for TCR activation, nanoparticle systems capable of self-clustering in response to paramagnetic activation have been designed that allow for larger focal spot formation, with a length scale similar to that of micron-sized particles [74,75]. Perica et al. engineered paramagnetic iron-dextran nano-aAPC to drive receptor clustering [74,76]. Under the influence of an external magnetic field, nanoparticles bound to T-Cells aggregate, leading to receptor clustering on the T-Cell and an increase in TCR cluster size (Fig. 4). T-Cells activated by the nano-aAPC in a magnetic field mediated tumor rejection in a melanoma adoptive immunotherapy model and were more effective in activating naïve T-Cells than nano-aAPC without magnetic field counterparts. In addition, magnetic preclustering of these nano aAPC resulted in 80% long term survival of mice compared to unclustered controls [74,76].

3.2. B-cells

Similar to T-Cells, B-Cells form an immunological synapse when engaged with APCs, and receptor clustering is an important component of B-cell activation [77,78]. Antigen binding to the BCR triggers BCR crosslinking and the formation of microclusters, which initiate downstream signaling that leads to B-Cell activation [77,79]. BCR/antigen clusters in the cSMAC, while LFA-1/ICAM-1 migrate to the pSMAC [78, 80]. However, unlike T-Cells, adhesion molecules are not required for mature synapse formation if the affinity of BCR-antigen binding is high enough [78]. SLBs and liposomes have been used to recapitulate this spatial organization for B-Cell modulation.

3.2.1. Surface fluidity

Supported lipid bilayers have been used to study B-Cell activation by membrane-anchored antigen *in vitro* [46,48,78–84]. Typically, SLBs contain biotinylated anti-IgM, anti-IgD, or anti-IgG as surrogate antigen tethered to biotinylated lipids with a streptavidin linker [85]. Anti-IgG SLBs with lateral mobility result in enhanced BCR microcluster formation and signaling compared to immobilized IgG, which form small and unstable microclusters that lead to inefficient signaling [79]. Although adhesion molecules are not required to induce BCR clustering and synapse formation, incorporating ICAM-1 into SLBs can enhance contact formation for lower-avidity antigens [78,82].

Although biotinylation is widely used to conjugate IgG to SLBs, it is difficult to control the location of biotin binding on the antibody, which can lead to inaccessibility of the antigen to BCRs, and multiple IgG molecules can bind to one streptavidin molecule [85]. Zhang et al. addressed these issues by using a modified D domain from staphylococcal protein A molecule fused with a polyhistidine tag, which binds to nickel-containing SLBs [85]. Protein A binds IgG with high affinity only in the constant region. This technique enhanced lateral mobility compared to tethering IgG with streptavidin. However, this approach cannot be used to target B-Cells expressing IgG BCRs since the linker protein will bind the BCRs. Others have similarly utilized nickel-containing SLBs to conjugate histidine-tagged antigen and adhesion molecules [86–89], as well as the inhibitory receptor FcγRIIB, which blocks BCR microcluster formation and prevents downstream signaling [90].

Results using planar lipid bilayers suggest that a liposome-based design for B-Cell therapies may be more effective than particles with immobilized antigen [91]. Researchers have used liposomes presenting peptide antigen to stimulate a B-Cell IgG response by including a co-stimulatory TLR ligand [91] and to induce tolerance by incorporating the ligand for CD22, an inhibitory co-receptor [92]. Studies have focused to a greater extent on delivering free antigen encapsulated in liposomes to B-Cells [93]. However, given that membrane-anchored antigen stimulates B-Cells more strongly, surface presentation on fluid particles is a promising new direction [80].

4. Surface area contact

The surface topography and area of contact with substrates has been shown to dramatically influence cellular behavior and aspects of cellular phenotypes [94]. Lymphocytes are one cell type where the physical surface topography has been shown to be highly influential on cellular programming. Topographical designs have been engineered to replicate the

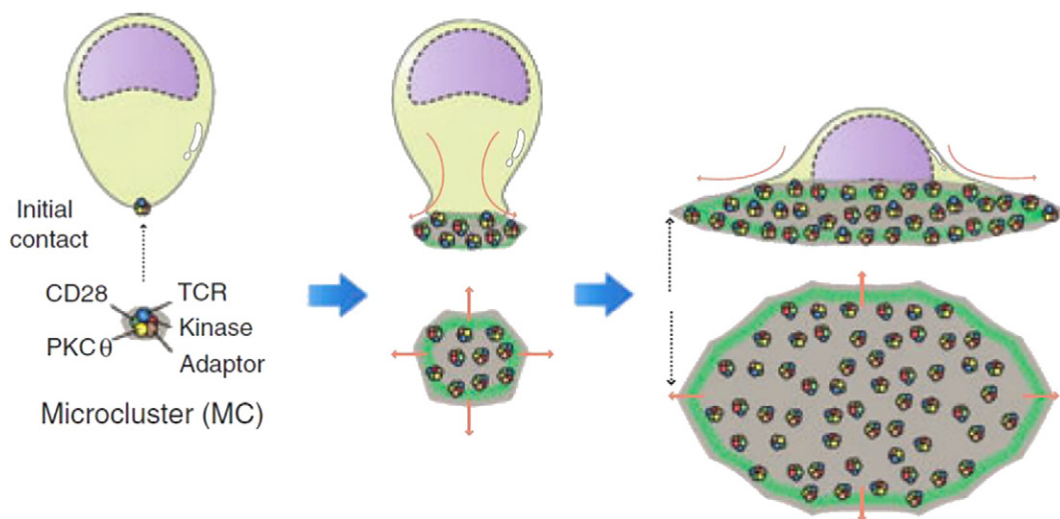


Fig. 5. Diagram of the changes in cellular morphology during the formation of the immune synapse. The microcluster forms the initial point of contact and then leads to the initial spreading of the T-Cell membrane on the surface of the APC. As more microclusters are engaged, the T-Cell spreads out along the flat surface of the aAPC. Reproduced and modified with permission from *Immunology* [101].

primary agents with which lymphocytes, specifically T-Cells and B-Cells, interact for increased influence on cellular activity. The interactions of T-Cells with particulate systems have frequently been investigated and have been demonstrated more effective at T-Cell stimulation than soluble or flat surface bound immune signal proteins [95].

4.1. T-cells

The primary mimetic target for topographical design in the context of T-Cells is the APC. Taking cues from the natural biology of a T-Cell/APC interaction, several particulate and high surface area structures have been designed for T-Cell activation.

4.1.1. Requirements of APC/T-cell surface area interactions

The APC can be thought of as a critical bridge between the innate and adaptive immune responses. The dendritic cell is the cell subtype in the body that has been designated as the most effective APC to direct T-Cell activity [96]. Thus, the dendritic cell/T-Cell interaction is likely the most important phenomenon to consider when designing a surface topography for T-Cell programming.

Immature dendritic cells in the periphery undergo rapid micropinocytosis and act as sentinels for the immune system [97]. Upon encountering a danger signal such as a pathogen associated molecular pattern or an inflammatory cytokine, they will detach from the periphery and migrate to the lymph nodes. The dendritic cell will then undergo a maturation process to halt rapid micropinocytosis, and up-regulate expression of T-Cell stimulatory proteins on the surface. Following lymph node infiltration, resident T-Cells will begin to rapidly scan the antigen presenting cell surface. If a cognate interaction is formed between a TCR and the associated MHC complex, a conjugate between the T-Cell and APC will be formed. Typically the area of this contact is on the order of microns [98].

Following the initial interaction of T-Cell and APC, dramatic morphological changes have been observed in both cell types during the formation of the immunological synapse. During this interaction, the dendritic cell has been shown to increase the area of contact for the T-Cell to engage by flattening the cell surface through cytoskeletal remodeling. Interruption of this morphological change is associated with significant reduction in T-Cell stimulatory capacity [99]. During the formation of a mature immunological synapse, the T-Cell will also undergo morphological change by flattening its surface to allow for maximal cellular contact. Within 2–15 min of a T-Cell contacting a cognate dendritic cell, it has been observed that T-Cells will flatten out over the surface of the APC (Fig. 5) [100,101]. This morphological change observed in T-Cells has been attributed to intracellular calcium signaling which subsequently controls several aspects of T-Cell polarization and migration [102].

4.1.2. Particle size as a parameter to control surface area interaction

The simplest way to control the available area for an artificial T-Cell/particle interaction is through the use of isotropic particles and the modulation of particle size. For a sphere, the surface area increases proportional to the square of the radius. It has been generally accepted that the more area available for a T-Cell to interact with on a particle, the better the subsequent T-Cell stimulation will be [55]. This has been exhibited using biodegradable polymeric particles. In one study, particles that were approximately 8 μm in size (similar order of magnitude of T-Cell size) and 130 nm in size were compared for T-Cell stimulation capacity [103]. It was found the microparticles could elicit a three-fold better immune response than the nanoparticles as evidenced by IL-2 secretion [103]. In addition to particle size, Janus particles have been appropriated to illustrate this point as well. By patterning the surface of polymeric microparticles using particle lithography, Chen et al. demonstrated that particles with higher surface areas coated with stimulatory molecules resulted in approximately 1.5-fold increased T-Cell proliferation [104].

Despite the drawback exhibited *in vitro* by nanoparticles as T-Cell stimulatory agents, other modifications have been made in particle synthesis that enable them to be more potent potentiators of T-Cell action. Steenblock et al. demonstrated that biodegradable nanoparticles encapsulating IL-2 could result in 3–4 fold higher T-Cell activation than equivalent amounts of soluble IL-2, suggesting a high local concentration at T-Cell mediated by the proximity of the nanoparticles when engaged with cognate T-Cells [36]. A similar effect was exploited in the local release of TGF- β from biodegradable nanoparticles for the induction of T-Reg cells from naïve T-Cells [105].

Interestingly, despite the poor performance of nanoparticles *in vitro* for T-Cell activation, *in vivo* effects of stimulatory nanoparticles on T-Cells can be more pronounced. For example, Fife et al. examined antigen loaded particles of sizes ranging from 20 nm to 2 μm in terms of *in vivo* T-Cell stimulatory capacity [106]. It was found that 40 nm was the optimal size for targeting the particles to the lymph nodes. It was found that this advantage enabled superior T-Cell stimulation *in vivo* as evidenced by a 10-fold increase in IFN- γ secretion by T-Cells in response to antigen stimulation for the 40 nm particles compared to the 1 and 2 μm particles [106].

4.1.3. Particle shape as a parameter to control surface area interaction

Another way to modulate the available area for particle/lymphocyte interaction is by alteration of particle shape. There have been several protocols developed for synthesis of non-spherical particles [107], however a popular and widely accepted method is the thin film stretching method. Pioneered by Ho et al., this protocol involves casting the particles into a thin plastic film, heating the particles above their glass transition temperature, stretching the film, cooling the film, and releasing the particles from the film [108]. Although this method was originally developed for the synthesis of 1D-stretched prolate ellipsoidal particles, it has been extended to the synthesis of particles of a wide variety of shapes [109]. Furthermore, this method has recently been automated to allow for scaled up production of anisotropic particles [110].

The impact of shape on the particle's interaction with cells has been extensively studied in the past decade [107]. With respect to anisotropic particles as a drug delivery vector, they have been determined to have two key advantages compared to equivalent spherical particles. The first is reduced non-specific cellular uptake by phagocytosis. This effect has been shown in anisotropic particles of various shapes [111] and sizes [112] resulting in reduced phagocytic uptake. Through a series of experimental and modeling studies, this effect has been linked to the orientation of the particle as it approaches the cell membrane [113]. Due to anisotropy, an ellipsoidal particle was shown to prefer to bind non-specifically to a cell along its longer axis. However, the cell prefers to engulf the particle along a shorter axis. Combined, these two phenomenon result in the reduction in non-specific phagocytosis. The second and more crucial feature of the anisotropic particle for lymphocyte programming is the increased frequency of targeted binding. Antibody bound anisotropic ellipsoidal particles have been shown to bind to target cells two-fold more compared to spherical particles [114]. This has been attributed to the larger radius of curvature and the flatter surface afforded by particle anisotropy. This effect was extended *in vivo* where rod-shaped particles were shown to have 7.5 times more accumulation to brain endothelium when targeted to that location with transferrin [115].

Due to these advantageous properties of anisotropic particles, they have recently been investigated as platforms for T-Cell stimulation. Sunshine et al. constructed aAPCs from spherical and prolate ellipsoidal microparticles of approximately 4 μm in size [22]. Despite similar levels of protein and protein density for the two shapes, the ellipsoidal particles elicited a much stronger 20-fold higher T-Cell proliferation response compared to spherical particles. Furthermore, it was observed under confocal microscopy that the T-Cell preferentially translocated to the long axis of the ellipsoid with the flatter surface (Fig. 6) [22]. In addition, the increased T-Cell stimulatory capacity of ellipsoidal particles was

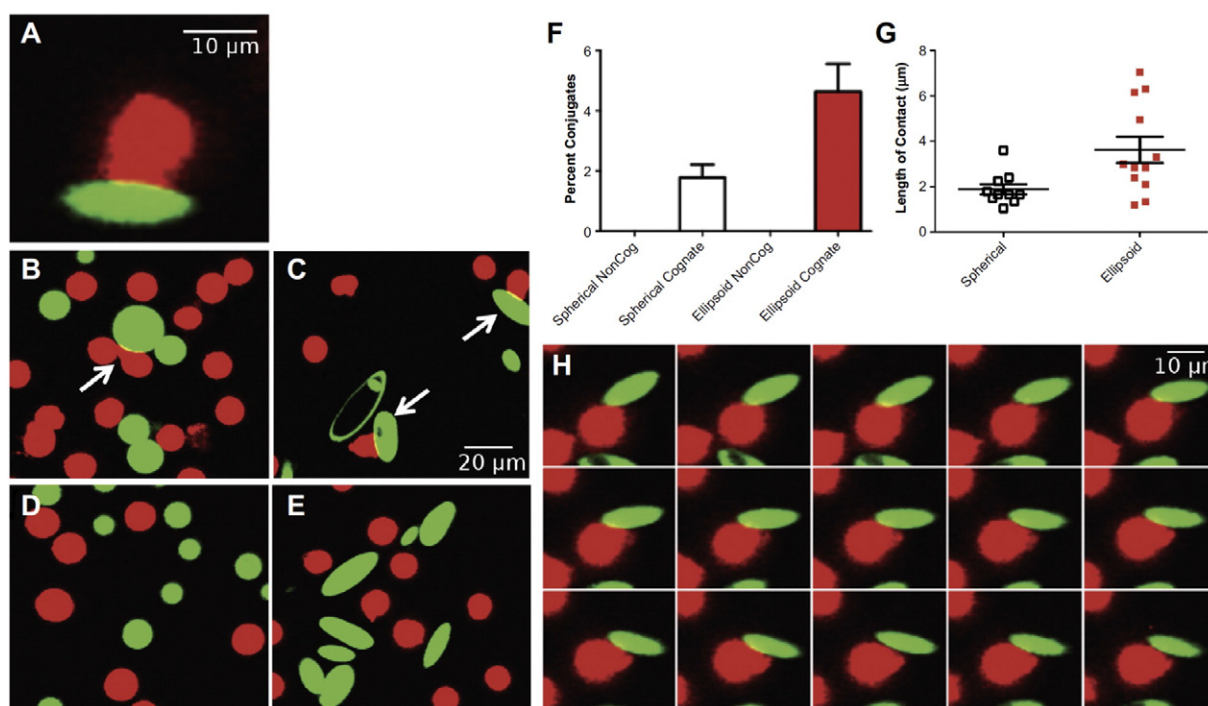


Fig. 6. Non-spherical ellipsoidal particles allow for more surface area of contact between the T-Cell and the aAPC. (A) Image of the T-Cell (red) and the aAPC (green) interacting in a conjugate. Cognate (B) spherical and (C) non-spherical aAPC interacting with T-Cells. Non-cognate (D) spherical and (E) non-spherical aAPC show minimal interaction with T-Cells. (F) Ellipsoidal shape leads to a higher percent of aAPC/T-Cell conjugates. (G) The measured length of contact between T-Cells and aAPC is higher with ellipsoidal aAPC. (H) A time lapse image of a non-spherical aAPC interacting with the T-Cell demonstrates preference for the flat surface of the aAPC over time. Reproduced with permission from *Biomaterials* [12].

observed to mediate a significantly better anti-tumor T-Cell response *in vivo* compared to spherical particles, with 20% long term survivors compared to 0% for spherical [22]. Particle shape has also been determined to be an important design parameter in the construction of nanodimensional aAPC [23]. Comparing prolate ellipsoidal and spherical nanoparticles of size 200 nm, it was shown that the non-spherical particles could trigger a 15-fold stronger T-Cell proliferative response compared to their spherical equivalents. This effect was also observed *in vivo* with non-spherical particles resulting in three-fold higher concentrations of antigen specific T-Cells in the periphery compared to spherical particles [23].

4.1.4. Nanotopography as a parameter to control surface area interaction

Nanotopographical features have also been shown to influence the efficiency of T-Cell programming by artificial constructs. This has recently been investigated with the use of single walled carbon nanotubes (SWNTs). Fadel et al. was able to synthesize these constructs with varying degrees of surface roughness as mediated by treatment with different acids [116]. Increased surface roughness at the nanodimensional level was shown to increase available surface area for protein adsorption. Subsequently this increased surface roughness was correlated to an increase of 2–6 fold IL-2 secretion by anti CD-3 based stimulation of T-Cells [116]. The impact of surface roughness was partly attributed to the presence of protein clusters that allowed for rapid rebinding of the T-Cell following dissociation of the T-Cell [117]. This technology was extended to antigen specific stimulation of T-Cells through the immobilization of MHC Class I proteins for CD8 + T-Cell stimulation [118].

SWNTs with high surface roughness and antigen specific T-Cell stimulatory capacity were synthesized with immobilized PLGA particles encapsulating IL-2 and hydrophobic magnetite [119]. It was found that encapsulation of the IL-2 at the SWNT/T-Cell binding site resulted in comparable levels of T-Cell stimulation compared to 1000-fold greater soluble IL-2. Furthermore, the stimulation was noted to be stronger than

spherical Dynabeads with soluble IL-2. T-Cells activated and produced using this method were then used therapeutically in a melanoma treatment model where it was found that the T-Cells stimulated by the SWNTs with encapsulated IL-2 had near identical tumor treatment capabilities to Dynabeads with 1000-fold greater concentration of soluble IL-2 [119].

4.2. B-cells

B-Cells have the ability to respond to soluble antigens with repeated patterns. In this context, designing particle based systems capable of reaching B-Cells *in vivo* following administration is the primary criterion for designing the size and shape of systems designed to stimulate B-Cells [120,121]. Nanoparticles <200 nm in diameter are known to reach lymph nodes following intravenous injection [122] and the use of ellipsoidal, rod-shaped [107] or biomimetic nanoparticles [123] that demonstrate enhanced circulation time may further enhance the fraction of nanoparticles that reach lymphatic tissue as opposed to accumulating in the liver and spleen, although further research is needed [124].

More recent work has demonstrated that the B-Cell is most likely *in vivo* to encounter antigen sequestered on the surface of an antigen presenting cell and engage the APC in a manner similar to the immunological synapse of the T-Cell [125]. It has been shown that the B-Cell will initially spread out along a flat surface of the APC to engage as many of the cognate antigens presented on the surface as possible. Following this phase, the B-Cell/APC membrane contact will contract resulting in a mature synapse between the two cells [80]. During this time, the B-Cell can continue to collect antigen from the surface and move along the surface of the APC [78]. Existing particle based platforms for B-Cell activation are designed for the delivery of soluble antigen to B-Cells. In order to accurately mimic this interaction, future systems for B-Cell stimulation could consider antigen presentation from a surface that is biomimetic. For example, such a surface could contain a radius of

curvature and a particle size of comparable character to a dendritic cell and also follow T-Cell aAPC technology.

5. Surface rigidity and mechanical properties

The effect of substrate stiffness on lymphocyte modulation has been relatively unexplored but has been shown to have an important role in the differentiation and activation of other cell types, such as mesenchymal stem cells (MSCs) [126–129] and endothelial cells [130–132]. For example, Engler et al. showed that substrate stiffness alone can drive MSC differentiation fate [127]. A limited number of studies have shown that lymphocyte activation and differentiation may be affected by planar substrate rigidity [133–138]. This is an interesting area for further investigation of particle-based systems, as particle stiffness is an important component of cellular biomimicry.

5.1. T-cells

It has been shown that mechanical forces can mediate T-Cell signaling, through both the TCR/CD3 complex and the costimulatory receptor, CD28 [139,140]. T-Cells cultured on elastomer pillar arrays presenting CD3 and CD28 activating antibodies generated traction forces on the pillars [133]. Traction forces were also generated through the TCR on pillars presenting antigen-loaded MHC. Additionally, inhibition of actin polymerization [18,64,141] and myosin [142] significantly inhibits TCR signaling. This evidence suggests that cytoskeletal and cellular forces play a role in T-Cell activation at the immunological synapse. Optimizing particle rigidity may allow T-Cells to perform mechanosensing during the formation of the immune synapse as they do with biological APCs.

While particle stiffness has not been explored, it has been shown that the rigidity of planar surfaces plays a role in T-Cell activation, proliferation, and differentiation. Kam and colleagues have investigated the effect of substrate stiffness on *ex vivo* T-Cell activation and expansion [135,136]. Human T-Cells were cultured on PDMS substrates of varying elastic modulus coated with anti-CD3 and anti-CD28, and softer substrates ($E < 100$ kPa) exhibited enhanced polyclonal expansion compared to stiffer substrates ($E > 2$ MPa) [135]. Specifically, softer substrates yielded a higher level of CD4+ and CD8+ T-Cell stimulation, as evidenced by increased proliferation and IL-2 production. Additionally, softer substrates generated a greater proportion of IFN- γ producing Th1-differentiated cells. In a different study, mouse T-Cells expanded on polyacrylamide gels of elastic modulus varying from 10 to 200 kPa showed increased IL-2 secretion on stiffer substrates [136]. The seemingly opposite trends in the two studies are likely due to the different ranges of elastic moduli tested—the mouse T-Cells were cultured on polyacrylamide gels ranging in elastic modulus from 10 to 200 kPa [136], while the human T-Cell study tested PDMS substrates ranging from 50 to 100 kPa to >2 MPa [135]. Both papers show that the optimal elastic modulus for T-Cell stimulation in the ranges tested is approximately 100 kPa. A different group looked at a larger range of elastic moduli—Jurkat T-Cells were cultured on polyacrylamide gels of elastic modulus (E) ranging from 200 Pa to ~ 6 kPa [134]. T-Cell signaling persisted for a longer period of time on softer substrates, but the efficiency of T-Cell activation and expansion on the different substrates needs to be further investigated.

Additional research needs to be performed to determine the effect of surface rigidity of particles, in addition to planar substrates, on T-Cell activation and differentiation. In engineering particle-based aAPCs, mimicking the low compressive modulus of biological APCs may optimize the T-Cell-aAPC interaction. Dendritic cells have been measured to have an elastic modulus of ~ 800 Pa when activated [143], so T-Cell activation may be optimal on surfaces with lower elastic moduli. The effect of particle stiffness on T-Cells needs to be explored to better understand the mechanics of lymphocyte signaling and to optimize particle therapies designed to modulate lymphocytes.

5.2. B-Cells

There has been a limited amount of research on the effect of substrate stiffness on B-Cell stimulation. Unlike T-Cells, B-Cells encounter antigen *in vivo* on substrates of varying stiffness—for example, B-Cells sense antigen on the surface of stiff viral capsids, on the membranes of infected host cells, and in soluble form. Liu and colleagues found that B-Cells cultured on antigen-coated polyacrylamide gels of elastic moduli varying from 2.6 to 22.1 kPa were more strongly activated and were better able to perform antigen affinity discrimination on the stiffest substrate [137]. B-Cells were even more efficiently activated on a PDMS surface with an elastic modulus of 1100 kPa compared to a 20 kPa substrate, as measured by formation of the immune synapse [138]. However, the softer substrate showed enhanced B-Cell expansion and antibody response. Further evaluation with a larger number of substrate elastic moduli is necessary. Nonetheless, these studies suggest that B-Cells perform mechanosensing and that optimizing particle rigidity based on the desired response could be beneficial for therapies designed to modulate B-Cells, such as particle-based vaccines and B-Cell lymphoma treatments.

6. Conclusion

Recent advances in the field of immunoengineering have enabled the development of technologies designed to activate and deactivate lymphocytes, including T-Cells, B-Cells, and NK cells. Particle-based systems that program lymphocytes aim to mimic biological cells in terms of physical, chemical, and biological properties in order to improve the particle-lymphocyte interaction. Surfaces have been engineered to present various chemical and biological factors designed to induce a specific lymphocyte response, and properties, such as shape, size, surface area, and rigidity, have been optimized. Researchers have attempted to recapitulate the complex spatial arrangement of proteins and membrane reorganization that occurs during the formation of the immunological synapse by engineering surfaces with patterned proteins and surface fluidity. While these features have all been individually implemented, in the future, enhanced biological factors, surface diffusivity, surface area, and mechanical properties will likely be combined to create highly effective advanced biomimetic materials for immunotherapy.

Abbreviations

TCR	T-Cell receptor
APC	antigen presenting cell
aAPC	artificial antigen presenting cell
CTL	cytotoxic T lymphocytes
MHC	major histocompatibility complex
BCR	B-Cell receptor
NK	natural killer
IS	immunological synapse
Ig	immunoglobulin
IL	interleukin
SMAC	supramolecular activation cluster
SLB	supported lipid bilayer
PLGA	poly(lactic-co-glycolic acid)
NPS	nanoporous silicon
SWNTs	single walled carbon nanotubes
MSCs	mesenchymal stem cells
PDMS	polydimethylsiloxane
E	Young's elastic modulus

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