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Biomimetic Delivery with Micro- and Nanoparticles

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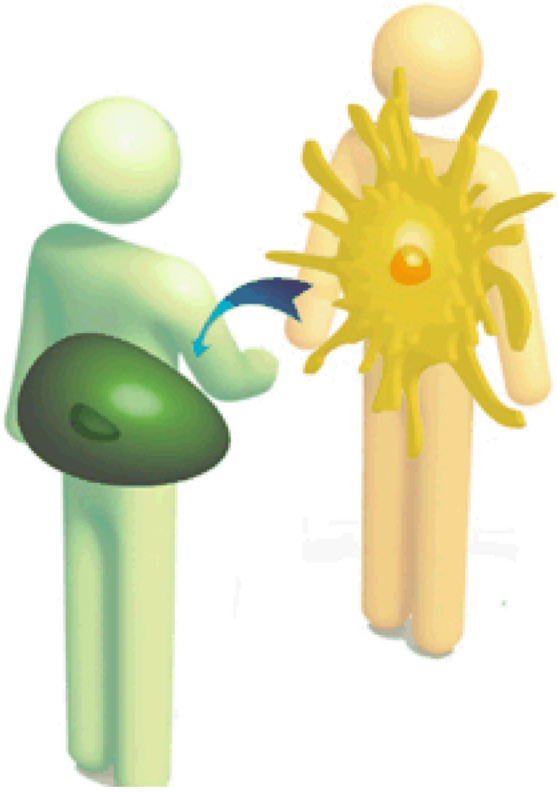
Abstract



The nascent field of biomimetic delivery with micro- and nanoparticles (MNP) has advanced considerably in recent years. Drawing inspiration from the ways that cells communicate in the body, several different modes of “delivery” (i.e. temporospatial presentation of biological signals) have been investigated in a number of therapeutic contexts. In particular, this review focuses on (1) controlled release formulations that deliver natural soluble factors with physiologically relevant temporal context, (2) presentation of surface-bound ligands to cells, with spatial organization of ligands ranging from isotropic to dynamically anisotropic, and (3) physical properties of particles, including size, shape and mechanical stiffness, which mimic those of natural cells. Importantly, the context provided by multimodal, or multifactor delivery represents a key element of most biomimetic MNP systems, a concept illustrated by an analogy to human interpersonal communication. Regulatory implications of increasingly sophisticated and “cell-like” biomimetic MNP systems are also discussed.

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Keywords

Biomimetic; Particulates; Controlled Release; Anisotropy; Biological Communication

1. Introduction

The body is a complex staging area in which various cell-based interactions contribute to the viability and function of its parts. These interactions (e.g. between a cell and an endogenous biomolecule, the surrounding extracellular matrix, or another cell) each facilitate a tremendous amount of information exchange, often leading to complex and even orchestrated actions from a target cell. Classic examples of such cell-based information exchange in the body include (as just a few examples): 1) interactions between dendritic cells and lymphocytes that prime a specific immune response to an identified pathogen, 2) interactions between leukocytes and endothelial cells lining blood vessels that direct leukocytes to extravasate into surrounding tissue, and 3) interactions between osteoblasts and osteoclasts that regulate bone homeostasis and serum levels of various minerals. Common to each of these instances of cell-associated information exchange is the systematic delivery or presentation of biomolecules (both secreted factors and surface-bound ligands) with precise temporal and spatial context. Importantly, this overall context can be as much a part of the overall “information” exchange as the identity of the biomolecules themselves. While the extracellular matrix may afford some level of spatial control over the orientation of biomolecular signals, individual cells are considerably more complex sources from which different types of signals can originate simultaneously or sequentially with directionality. Further, even the size, shape, and mechanical properties of a cell may be essential to the proper presentation of these signals to elicit the appropriate response.

Synthetic, biomimetic delivery systems made from micro- and nanoparticles (MNP) attempt to recreate one or more of the complex naturally occurring interactions between cells with varying degrees of complexity. Accordingly, a particle that intends to imitate a cell may “deliver” (either release or presentation) a biomolecule (soluble or surface-bound) in a way that resembles how these signals are presented naturally in situ. However, more recent biomimetic delivery systems have also included artificial mechanisms to present biomolecules in a temporal and spatial context that mimics the actual context of biomolecule presentation in nature. Furthermore, biomimetic MNP delivery systems whose size, shape, and/or surface properties mimic natural delivery vehicles, such as pathogens or erythrocytes, have been used to enhance delivery to specific cell populations, pass through physiological barriers, and even avoid the body’s natural surveillance and clearance mechanisms.

This review highlights the relatively nascent field of *biomimetic delivery* using MNP. This topic is distinct from the field of *biomimetic materials* (mimicking the properties of natural materials using synthetic materials), which has been a hot topic of discussion over the past two decades.[1, 2] In contrast, biomimetic delivery intends to mimic the prose and context of signal presentation that is interpreted by cells in order to generate a desired outcome. We focus specifically on biomimetic MNP-based systems that deliver soluble factors, present surface-bound ligands, and/or utilize physiologically relevant sizes, shapes, or mechanical properties. This review will not address synthetic particles deigned for intracellular delivery (which can, in one way, be considered as mimicking viruses, bacteria, or apoptotic bodies) as this topic has been reviewed in great detail elsewhere.[3–6] Biomimetic MNP systems with varying temporospatial complexity are presented, and the motivation for more complex systems is discussed. Finally, we present an analogy between various modes of cell-based information exchange and interpersonal communication as a novel way of thinking about biomimetic delivery systems.

2. Biomimetic Delivery of Soluble Factors

2.1. Paracrine Signaling in Nature

Paracrine signaling (i.e. the secretion of biomolecules which diffuse into local tissues and elicit responses in nearby cells) is responsible for many aspects of biological development,[7] tissue regeneration,[8] and immunity.[9] Growth factors and cytokines are two major classes of natural paracrine signaling biomolecules, which can have various effects on target cells depending on the responding cell phenotype, timing of delivery, and integration with other factors. For instance, wound healing involves tightly orchestrated interactions between several distinct cell populations, largely facilitated by paracrine signaling. The complex sequence of cell migration, proliferation, differentiation, and protein synthesis during wound healing occurs in response to secretion of various growth factors in a defined temporal pattern, as depicted schematically in Figure 1a, and reviewed extensively elsewhere.[8, 10] Osteogenesis (bone repair) is another example of a physiological process that depends on the coordinated activity of multiple cell types by precise, multi-factor paracrine signaling. At least six different classes of growth factors, secreted by several distinct types of cells with a specific temporal pattern in response to bone tissue injury, direct specific responding cells to proliferate and differentiate.[11, 12] Finally, paracrine signaling between immune cells through various cytokines controls their proliferation and differentiation. For example, upon activation by antigen presenting cells (APC), naïve T cells can differentiate into at least five distinct lineages in response to specific cytokines secreted by APCs and other cells in the local microenvironment.[13] Once again, integration of multiple paracrine signals by responding T cells will ultimately determine their response. The importance of signal integration is seen with transforming growth factor-β (TGF-β), which will induce differentiation of immunosuppressive regulatory T cells (Treg) in the presence of IL-2, compared to differentiation to an inflammatory phenotype (Th17) when combined with IL-6.[13] Ultimately, as will be discussed, it is now becoming possible to mimic the natural temporal patterns of soluble factor secretion by encapsulating factors in MNP with controllable release kinetics (Figure 1b). The following sections describe such biomimetic approaches to soluble factor delivery.

2.2. Sustained Release of Individual Paracrine Factors

MNP delivery systems that provide sustained release of natural biomolecules (e.g. proteins and peptides) have been explored widely since the development of encapsulation techniques by Langer and Folkman in 1976.[14] However, beyond sustaining relevant plasma concentrations of drug, MNP delivery systems may also mimic the local paracrine release of growth factors and cytokines by cells in the body, and signal other nearby cells to proliferate, differentiate, or alter their patterns of protein expression. Sustained release MNP systems address two key limitations associated with injecting soluble paracrine factors: short half-life and widespread tissue distribution, or lack of acute localization.[15] More specifically, releasing growth factors from MNP in a sustained fashion effectively extends their therapeutic activity from minutes to days (or even months) and restricts the effects of such factors to defined local environments. Over the past three decades, MNP systems have been used broadly for local (paracrine) delivery of a host of individual growth factors and cytokines, with broad therapeutic applications. A few examples include delivery of vascular endothelial growth factor (VEGF) to promote angiogenesis and treat ischemia,[16, 17] bone morphogenetic proteins (BMPs) to promote osteogenesis for bone repair,[18, 19] glial cell-line derived neurotrophic factor (GDNF) to promote nerve regeneration and treat spinal cord injuries or peripheral nerve defects,[20, 21] epidermal growth factor (EGF) to promote reepithelialization and treat burns or chronic skin wounds,[22] and IL-10 to suppress aberrant inflammation and treat inflammatory bowel disease.[23] Though such MNP delivery systems may present physiologically relevant concentrations of natural factors in a “paracrine”

fashion, a general lack of clinical efficacy (i.e. suboptimal physiological responses)[24] may stem from an over-simplification of the delivered "message" when compared to the rich context of multiple signals that would be observed in a natural in vivo milieu. Thus, subsequent sections will focus on MNP systems that demonstrate a higher level of biomimesis by delivering multiple factors with appropriate, physiologically relevant temporal patterns (e.g. simultaneous or sequential multifactor delivery).

2.3. Simultaneous and Sequential Release of Multiple Soluble Factors

Two general approaches to biomimetic delivery of multiple soluble factors may be considered. In nature, different populations of cells within a local area may secrete different signals (simultaneously or at different times) to coordinate a response. For example, at different stages in wound healing, endothelial cells and activated macrophages may secrete VEGF into the local microenviroment, while platelets and fibroblasts provide platelet derived growth factor (PDGF).[10] Alternatively, a single cell may secrete multiple factors simultaneously or at different times. For example, dendritic cells (a particularly potent type of APC) commonly secrete a multiple cytokines (e.g. IL-2 and TGF-β, or IFN-γ and IL-12, or IL-6 and TGF-β, etc.) to promote T cell differentiation toward a particular lineage.[13] For biomimetic MNP delivery, these natural modes of multifactor delivery translate into systems with either multiple sets of particles (or particles and scaffolds) that each release specific factors, or alternatively composite particles that release multiple factors from distinct compartments. These two biomimetic strategies are illustrated in Figure 2.

2.3.1. Delivery of Multiple Factors from Different Particles—In the past decade, recognition of the clinical limitations of single growth factor delivery has led to considerable efforts toward more biomimetic delivery of multiple soluble factors, with specific applications in angiogenesis, bone regeneration, and immunotherapeutics. In each of these examples, not only is the identity of the released signal important to providing instructions to direct the process, but also the timing and rate of release. One of the chief advantages of using multi-particle systems for multifactor delivery is that they allow precise control over the release kinetics of each individual factor, thereby enabling recreation of natural temporal patterns of secretion (Figure 1b). The timing and release kinetics from biodegradable polymeric MNP can be precisely controlled by a variety of design parameters, including but not limited to particle size and porosity, polymer molecular weight and degradation rate, polymer coatings, protein molecular weight, and ionic or hydrophobic interactions between protein and polymer.[25–28] For hydrogel MNP, crosslinking may also provide control over the rate of protein release.[29] Furthermore, recent mathematical models[28, 30, 31] which predict release kinetics based on MNP design parameters, may be used to guide the design of controlled release formulations to generate desired temporal release profiles (i.e. in silico rational design).

A number of examples highlight the importance of integrating multiple paracrine signals, delivered with specific temporal patterns / distinct kinetics, for eliciting enhanced cellular/tissue responses. For instance, efforts to improve pro-angiogenic growth factor therapies to treat ischemia, or promote vascularization in wound healing, have led to the development of several MNP systems for sequential release of VEGF and PDGF.[24, 32, 33] In nature, VEGF has been shown to be important for vascular permeability and endothelial cell proliferation and migration (early stages of angiogenesis); whereas, PDGF promotes vascular stability by pericytes and smooth muscle cells (later stages of angiogenesis).[34] Based on these observations, Richardson et al. hypothesized that dual delivery of VEGF and PDGF would enhance formation of mature vasculature, compared to individual delivery of either factor.[24] By mixing lyophilized VEGF with PDGF-containing poly(lactic acid-co-glycolic acid) (PLGA) microparticles, and processing and mixing those particles with lyophilized

VEGF prior to processing into a fused particulate scaffold, significant release of VEGF within the first week and extended release of considerable PDGF for at least four weeks was achieved. Notably, subcutaneous delivery of only VEGF from the particle-based scaffolds resulted in a significant increase in density of immature blood vessels. On the other hand, sole delivery of PDGF led to maturation of existing vasculature, without an increase in vascular density. However, delivery of both factors significantly increased density of mature vasculature, suggesting synergistic effects of biomimetic VEGF and PDGF co-delivery.[24] Similar synergistic results were seen with sequential delivery of VEGF and PDGF from an alginate-PLGA microsphere mixture in a hind-limb ischemia model. In this case, VEGF and PDGF levels peaked at week 2 and 4, respectively, and dual delivery resulted in significantly greater vascularity and mean vessel diameter, with well-formed smooth muscle-lined arterioles.[32] Finally, other growth factor combinations in sequence, including VEGF and sphingosine 1-phosphate (S1P),[35] or basic fibroblast growth factor (bFGF) and PDGF,[35, 36] also appear to be viable for exploration with sequential MNP delivery.

In addition to enhancing angiogenesis, sequential delivery of factors from nanoparticles has been explored by Yilgor et al. to enhance bone regeneration. Specifically, two bone morphogenetic proteins (BMPs), BMP-2 and BMP-7, were released in a temporal fashion from PLGA and poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) nanoparticles, respectively.[37–39] While BMP-2 delivery alone suppressed mesenchymal stem cell proliferation and enhanced alkaline phosphatase (ALP) activity more than BMP-7 alone, sequential delivery of BMP-2 followed by BMP-7 resulted in significantly greater ALP activity, indicating a synergistic effect on osteogenic differentiation. Furthermore, the temporal delivery of the factors was important, as less natural simultaneous delivery resulted in lower ALP activity than BMP-2 alone.[38] These results stress the value of using a biomimetic approach to deliver multiple factors in a physiologically relevant temporal fashion. Recognizing bone healing as a complex process involving both vascularization and osteogenesis, Kempen et al. looked at combining delivery of angiogenic and osteogenic factors (i.e. VEGF and BMP-2).[40] Drawing inspiration from natural bone healing, where establishment of a vascular bed precedes bone formation and VEGF expression peaks days following injury,[12] VEGF and BMP-2 were released sequentially from gelatin hydrogel and PLGA microspheres, respectively. When implanted ectopically in rats, the dual delivery system released a large burst of VEGF within the first three days, while BMP-2 release was sustained for eight weeks. Ultimately, the combination of VEGF and BMP-2 promoted development of a supportive vascular network and significantly enhanced ectopic bone formation compared to BMP-2 alone.[40] While the studies with BMP-2, BMP-7, and VEGF demonstrate the value in taking a multifactor approach to growth factor delivery, future therapies will likely involve delivery of even more factors to better mimic the body’s natural healing mechanisms and enhance therapeutic outcomes.

Besides the aforementioned applications for regenerative medicine, and some of those further reviewed by Chen et al.,[41] biomimetic multifactor delivery from MNP may be used for immunotherapies. Several groups have used MNP to deliver individual cytokines for various immunosuppressive or anti-tumor therapies including IL-10,[23] IL-2,[42] GM-CSF,[43] TNF-α,[44] and others.[45] Such localized cytokine delivery marks an important step toward directing desired immune responses; however, especially for local immunosuppression, multiple factors will probably be required for optimal responses. As one example of this, a combination of IL-2 and TGF-β1 were utilized for the *in vitro* induction of Treg from naïve CD4+ T cells (in the presence of surface-bound activation signals, as described in section 3.1.5).[46] This combination of cytokines mimics that secreted by peripheral tolerogenic dendritic cells (DCs), which naturally induce Treg differentiation.[47] Importantly, delivery of IL-2 or TGF-β alone is insufficient for Treg differentiation and survival, while Treg induction efficiency with IL-2 and TGF-β1 may be

further enhanced by additional release of rapamycin (an immunosuppressive drug, perhaps not biomimetic).[46] Ultimately, biomimetic local delivery of this combination of factors may have potential to induce Treg differentiation *in vivo* to treat aberrant or undesired inflammation in a number of autoimmune and inflammatory diseases, or even suppress allograft rejection by inducing tolerance at the site of a transplanted tissue or organ.

For diverse therapies, including those mentioned above, future improvements in clinical efficacy will likely result from more complete and more natural temporal delivery of growth factors and cytokines. Further studies to examine temporal patterns of growth factor expression during natural healing processes in various tissues should provide insight into new combinations of factors and provide inspiration for increasingly biomimetic multifactor delivery with sets of MNP. Yet, despite the apparent advantages of a multi-particle system for biomimetic multifactor delivery, it is conceivable that inhomogeneous spatial distributions of different particles at an injection site (Figure 2a) could potentially have an adverse, or suboptimal effect on local cells, which would effectively "see" factors coming from different sources or directions (Figure 2a). Thus, multifactor release from composite, multi-compartmental MNP may be advantageous in such situations, and accordingly, a limited number of approaches have been explored to date.

2.3.2. Delivery of Multiple Factors from Composite, Multi-compartmental Particles—In terms of multifactor delivery from a homogenous population of MNP (Figure 2b), simply co-encapsulating a mixture of factors rather than a single factor would seem to be the simplest approach. Unfortunately, in this case, the lack of independent control of release kinetics for each individual factor could be a considerable disadvantage. Even in cases where simultaneous release would be desired, differences in growth factor molecular weight and dissimilar interactions with the polymer could likely result in unexpected and often undesired release patterns. However, research in the nascent field of compartmentalized particles (reviewed in [48] and [49]) may pave the way for multifactor delivery from a single particle with independent control of release profiles for each compartment. To date, there have been only a few isolated examples of multifactor delivery from such composite particles. In particular, Choi et al. demonstrated dual delivery of BMP-2 and dexamethasone from core-shell microcapsules fabricated by a coaxial electro-dropping method.[50] As expected, early release occurred from alginate shells, with more sustained release from the PLGA cores. Such trends were seen regardless of which factors were loaded in the various compartments, suggesting that such core-shell microcapsules could be used for sequential delivery of a variety of different pairs of growth factors. Furthermore, the dual delivery of factors to bone marrow stromal cells significantly enhanced their expression of osteogenic markers relative to those cultured with control osteogenic media, and the order of delivery affected osteogenic marker expression at two weeks.[50]

Besides the core-shell microcapsules, Roh et al. developed polymeric microparticles with two distinct phases by simultaneous electrohydrodynamic jetting of parallel polymer solutions. Importantly for dual factor delivery, each phase of these biphasic Janus particles can be independently loaded with different biomolecules.[51] Thus, while not specifically used for biomimetic dual growth factor or cytokine delivery to date, such Janus particles conceivably could be used to deliver two factors, with release kinetics independently controlled by the polymers and microstructure chosen for each phase. Finally, layer-by-layer (LbL) MNP may have potential as biomimetic multifactor delivery vehicles. In recent years, LbL systems, including stimuli responsive LbL capsules that release their contents in response to physical, chemical, or biological stimuli,[52] have been extensively studied for controlled drug delivery.[53, 54] Such LbL systems may also be used for dual delivery of growth factors, as demonstrated in a recent study by Shah et al., where VEGF and BMP-2

were sequestered in multilayer films with repeating tetralayer structure (i.e. degradable poly(b-amino ester) / polyanion / growth factor / polyanion). When multiple tetralayers loaded with VEGF were deposited on top of those containing BMP-2, pseudo-temporal release was attained, with sustained BMP-2 release lasting two weeks and VEGF release during the first week only.[55] Though this particular study used planar multilayer films, LbL deposition on MNP has also been demonstrated.[56] Furthermore, methods to covalently crosslink the layers separating factors may block diffusion between layers[57] preventing mixing of the various encapsulated agents. Ultimately, while mixed populations of MNP currently provide greater control over individual release kinetics for multiple factors, compartmental MNP for biomimetic multifactor delivery will likely be an area of increasing interest in the future.

2.4. Orchestrating Cell Trafficking with MNP that Establish Chemokine Gradients

Specific recruitment of immune and progenitor cells to various sites in the body is precisely orchestrated by various chemokines (chemotactic cytokines), which are secreted by cells at specific sites and diffuse outward to produce concentration gradients. Such gradients provide spatial organization of chemokine molecules, which can cause cells expressing corresponding chemokine receptors to migrate towards the source of the gradient. A hallmark example of this behavior is in the development of immunity, where recruitment of different populations of circulating cells to specific regions in secondary lymphoid organs allows these cells to be primed with antigen and stimulated to proliferate (or cause proliferation) in an extremely specific fashion.[9] Alternatively, in pathological conditions, malignant cells may actually secrete chemokines that recruit suppressive cells to facilitate tumor evasion of immune recognition.[58, 59] Drawing inspiration from these natural mechanisms for cell recruitment, biomimetic sustained release of chemokines from MNP presents a way to artificially orchestrate trafficking of immune cells for various therapeutic purposes. In particular, potential advances in vaccine efficacy could be realized by recruiting specific cells (e.g. APCs and/or T cells) to an immunization site with chemokine-releasing MNP. To that end, Zhao et al. and Wang et al. have both demonstrated the ability to direct *in vitro* migration of dendritic cells (DCs; professional APCs), monocytes (DC precursors), and T cells toward biodegradable PLGA and alginate microparticles releasing various chemokines, including pathogen-derived peptides (fN'LFN'YK), CCL19, CCL20, CXCL10, and CXCL12.[60, 61] As in nature, establishment of sufficient chemokine gradients is crucial to the efficacy of these microparticle delivery systems. Sustained release from microparticle point sources creates biomimetic concentration gradients; whereas, a bolus of chemokines will dissipate rapidly, failing to provide the spatial context needed for cell migration. Specifically, cells can respond to two percent concentration changes over the length of the cell (~10μm) at sufficient concentrations.[62] Thus, release kinetics of chemokines from microparticles must be designed accordingly.

In addition to vaccine applications, recruitment of effector immune cells may also be an effective way to generate anti-tumor immune responses. Notably, in an *in vivo* murine model of lung cancer, sustained intratumoral delivery of CCL21 from vault nanocapsules enhanced infiltration of effector T cells and DCs, which directly inhibited tumor growth and induced systemic antitumor immune responses.[63] The vault nanocapsules used in this study are recombinant ribonucleoprotein particles (~40×70nm), produced by a baculovirus expression system and engineered to encapsulate CCL21 within the vault cavity.[64] Importantly, sustained release of CCL21 eliminates the need for repeated intratumoral injections, a major advantage for clinical applications. In addition to recruiting immune cells, chemokine-releasing microparticles have been used in limited instances to recruit endogenous progenitor cells to local sites to promote wound healing and tissue regeneration.[65] For example, stromal-derived factor-1 (SDF-1 or CXCL12) released from

PLGA and alginate microparticles has been shown to stimulate migration of mesenchymal stem cells[66] and bone marrow-derived circulating progenitor cells expressing the SDF-1 receptor (CXCR4).[67] Recruitment of pro-angiogenic circulating progenitor cells with SDF-1 successfully restored perfusion in a murine hind-limb ischemia model,[67] and the ability to recruit progenitor cells to ischemic tissue also may be used to treat myocardial infarctions.[68]

For each of the aforementioned therapeutic applications, two key factors should be considered for the design and testing of future biomimetic MNP chemokine delivery systems to effectively orchestrate cell trafficking. First, an understanding of chemokine receptor heterogeneity is essential to selection of the appropriate chemokine to deliver for a given application. With different subpopulations of cells expressing diverse combinations of chemokine receptors,[69, 70] and multiple cells types potentially expressing comparable or distinct levels of the same receptor, both the efficacy *and* selectivity of different chemokines should be tested. Second, *in vitro* chemotaxis assays (e.g. transwell migration and video-microscopy cell tracking on substrates) likely will not mimic the chemokine concentration gradients and barriers to migration that would be experienced by circulating cells *in vivo*. Thus, the importance of *in vivo* migration studies (e.g. tracking cell recruitment to MNP with live animal imaging systems) cannot be overstated. Ultimately, our increasing knowledge of differential chemokine receptor expression and recognition of chemokines that interact with these receptors will likely advance biomimetic MNP to better direct more specific migration of desired subpopulations of cells for therapeutic applications.

3. Presentation of Surface-Bound Ligands

In addition to intercellular signaling mediated by soluble factors, which may be released in a paracrine or endocrine fashion, much cell-to-cell communication is mediated by contact between cells, or more precisely by presentation of surface-bound ligands to surface-bound receptors on adjacent cells. Examples of contact-dependent cell signaling in nature include immune synapses between lymphocytes and antigen presenting cells (APCs), adhesion between leukocytes and endothelial cells, differentiation-regulating interactions between stem cells and supporting cells in the stem cell niche, and interactions between osteoblasts and osteoclasts to control bone homeostasis. Surface-bound ligands provide additional context beyond that of soluble factors, which may be essential for appropriate cellular responses. First, surface-bound ligands confer high localization of stimuli. In other words, only cells that come in direct contact with a cell (or synthetic construct) presenting surface-bound ligands will receive stimulation. Second, presentation of ligands on the surface of a cell (or synthetic construct) confines the ligands to three degrees of freedom (e.g. x- and y-translation and z-rotation for a fluid cell membrane), or one (e.g. z-rotation for a fixed, static surface). Such restrictions in translation and rotation of ligands can actually stabilize and prolong ligand-receptor interactions, effectively decreasing dissociation constants by multiple orders of magnitude.[71] Thus, affinity and overall avidity (i.e. the combined synergistic strength of multiple ligand-receptor interactions) depend on the surface-bound presentation and density of ligands. Multivalent presentation of a single ligand to a constrained area on a responding cell may also be required for sufficient association of receptors. Furthermore, multiple different ligands can also be presented to cells in static or dynamic anisotropic patterns (Figure 3), providing additional context in the form of appropriate organization of distinct cytoplasmic receptor domains for signal transduction. Finally, shear forces on receptors, resulting from surface-bound ligands can actually mediate some signaling events.[72]

3.1. Fixed Random Distributions of Ligands (Isotropy)

To date, the vast majority of biomimetic MNP delivery systems that present surface-bound signaling molecules to cells are believed to do so in an isotropic fashion (Figure 3a). That is, various combinations of ligands are immobilized on the surfaces of MNP with random spatial distributions, with chief controllable parameters being ligand density and ratios of different ligands. Such MNP delivery systems present signaling biomolecules with contextual information, including directionality and positional stability (i.e. the relative position of ligands with respect to other ligands is fixed). More specifically, ligand-coated MNPs can present one or more ligands in a consistent parallel orientation, orthogonal to the surface of the particles. The mechanical stability or rigidity is dependent on the conjugation method, and avidity is dependent on the ligand density and affinity of individual ligand-receptor interactions. Furthermore, for systems that present multiple distinct surface-bound biomolecules, a cell's ability to integrate information from multiple signals proves to be essential to the overall response of the cell. With extensive advances in our understanding of contact-mediated cell-to-cell communication, recombinant protein and antibody production, and bioconjugation techniques, biomimetic MNP delivery systems that present surface-bound ligands (or antibodies for a ligand's receptor) have blossomed in the past decade. Herein, we will discuss many of these diverse synthetic constructs.

3.1.1. Leukocyte Mimics for Targeting Inflamed Endothelium—Inspired by the mechanisms of neutrophil arrest on inflamed endothelium, Hammer et al. recently developed leuko-polymersomes that mimic both the rolling and firm adhesion interactions of neutrophils with vascular endothelial cells lining blood vessels.[73] In nature, rolling adhesions (i.e. transient catch-slip interactions) are mediated by selectins, which are expressed exclusively on inflamed endothelium. Subsequent firm adhesion and arrest of rolling leukocytes is mediated by β2-integrins (e.g. ICAM-1), which are expressed on all endothelial cells, though upregulated with inflammation. Thus, synergy between selectin and integrin receptors is essential for arrest of leukocytes selectively on inflamed endothelium.[74] With biomimetic leuko-polymersomes, adhesivity on substrates coated with P-selectin and ICAM-1 can be fine-tuned by adjusting the ratio of selectin and integrin receptor mimics (sialyl Lewis X and anti-ICAM-1) presented on the surface of the polymersomes. Importantly, targeting ICAM-1 alone would result in unselective adhesion to all endothelium, while exclusively targeting P-selectin would not be sufficient for firm adhesion; however, the combination of both ligands improves avidity *and* selectivity of adhesion. Ultimately, leuko-polymersomes with optimized ratios of ligands were found to bind exclusively to inflamed HUVECs, but not uninflamed endothelial cells under conditions of hydrodynamic flow and physiological shear rates.[75] While similar results were previously demonstrated with polystyrene microspheres,[76] the use of polymersome (vesicles with membranes comprised of self-assembled amphiphilic block co-polymers) or biodegradable PLGA microparticle platforms[77] enables local delivery of encapsulated drugs or imaging contrast agents at sites of inflammation. Thus, leuko-polymersomes and PLGA leukocyte mimics have exciting potential applications for monitoring or treating inflammation, cancer, and cardiovascular disease.

3.1.2. Nanoparticle Pathogen Mimics as Vaccine Adjuvants—With the recent shift in vaccine formulations from whole microorganisms to antigenic protein subunits, which are less immunogenic, the need for novel, potent adjuvants to amplify immune responses has been recognized. The relatively poor immunogenicity of subunit vaccines likely results from a lack of context—namely, the absence of universal "danger signals," or pathogen-associated molecular patterns (PAMPs), that would be simultaneously presented by an intact pathogen and recognized by pattern recognition receptors (PRRs) on antigen presenting cells (APCs). Thus, presentation of relevant PAMPs on the surface of pathogen-mimicking

nanoparticle vaccine delivery vehicles can provide the necessary “pathogen-like” context for subunit antigen vaccines, thereby enhancing the immune response. As reviewed extensively by Demento et al. in 2011, a wide variety of PAMPs, especially those naturally found on the surfaces of bacteria, have been incorporated onto the surfaces of liposomes and biodegradable polymeric nanoparticles for vaccine delivery.[78] Some of the more commonly utilized PAMPs include bacterial flagella proteins (flagellin) and natural components of bacterial membranes, such as lipopolysaccharides (e.g. LPS and monophosphoryl lipid A (MPLA)), lipoproteins (e.g. Pam_3CAG), and peptidoglycans (e.g. muramyl dipeptide (MDP)).[79–84] An exhaustive list of PRR ligands incorporated on the surface of nanoparticle delivery vehicles can be found in reference [78]. The ligands presented by nanoparticle delivery vehicles interact with various PRRs, including Toll-like receptors (TLRs) and Nod-like receptors (NLRs), to induce signaling pathways that initiate and enhance activation and maturation of APCs, which is essential for generating potent, antigen-specific cellular and humoral responses.[85–87] Critical to the design of biomimetic nanoparticle-based adjuvants is the density of surface-immobilized ligands for PRRs. For example, antigen-loaded nanoparticles coated with antibodies specific for DEC-205 (a PRR), induced differential cytokine responses dependent on the density of ligands presented. Importantly, high densities of anti-DEC-205 resulted in significant production of the anti-inflammatory cytokine IL-10 by APCs, likely due to cross-linking of the DEC-205 PRRs. Given that anti-inflammatory responses would likely be detrimental to vaccine efficacy, titrating the density of different PRR ligands presented on the surface of nanoparticle antigen-delivery vehicles is essential.[88]

3.1.3. Opsonin Coatings and Endocytic Receptor Ligands for Enhanced Uptake—Modulation, activation, or destruction of cells by intracellular delivery of siRNA, pharmaceutical agents, antigens, etc. often requires efficient uptake of MNP delivery vehicles.[89–91] To that end, biomimetic MNPs coated with opsonins, and/or natural ligands recognized by endocytic receptors, can enhance phagocytic or receptor-mediated endocytic uptake of those delivery vehicles. In nature, opsonization occurs when a pathogen is marked for phagocytosis by being coated with opsonins. These serum proteins, which include antibodies, complement proteins, and mannose-binding lectin, are then recognized by specific receptors on phagocytes (e.g. neutrophils and macrophages), which mediate phagocytosis.[86, 92] Considerable efforts in the field of drug delivery have been directed at extending circulation time of nanoparticles with “stealth” coatings that prevent adsorption of natural opsonins.[93–95] However, intentionally coating MNPs with biomimetic opsonins may be advantageous for delivery to phagocytes. For example, a recently discovered opsonin found in mineralized tissue fluids (osteopontin), which promotes phagocytosis of bone microparticulate during bone healing, also significantly increased phagocytosis of coated microspheres.[96] Similarly, nanoparticles coated with pulmonary surfactant-associated protein A showed significantly increased phagocytic uptake by alveolar macrophages.[97] Such opsonin-coated MNP delivery systems could potentially be used to enhance delivery to bone or lung-resident macrophages.

Like opsonin-coatings, MNP surface-presentation of ligands involved in receptor-mediated endocytosis can be used for selective intracellular delivery to certain cell populations. In particular, folate receptor-mediated endocytosis, which naturally facilitates uptake of the essential vitamin folate, has been used to selectively deliver nanoparticles to epithelial-derived tumor cells, which overexpress folate receptor 4 (FR4).[98–100] By conjugation of high-affinity FR4 ligands (e.g. low molecular weight folate and folic acid, or anti-FR4) to nanoparticle surfaces, internalization of the anticancer agent delivery vehicles by tumor cells can be enhanced selectively by ten to twenty fold. Preferential uptake by FR4-expressing tumor cells and tumor tissue site-specific accumulation of FR4 ligand-labeled nanoparticles has been demonstrated both *in vitro* and *in vivo*.[100–102] Finally, coating nanoparticles with

FR4 ligands could potentially be used for selective intracellular delivery to regulatory T cells (Treg), which also overexpress FR4 relative to other T cell populations.[103]

3.1.4. Red Blood Cell Inspired Biomimetic "Stealth" Coatings—The previous mention of "stealth" coatings bears some further discussion in the context of biomimetic MNP delivery systems. Traditional "stealth" coatings generally rely on polyethylene glycol (PEG), or other hydrophilic polymers, to prevent protein adsorption or particle aggregation, and have extensively been reviewed by several groups.[93–95] As such surface modifications are not truly biomimetic, they will not be addressed at depth in this review; however, two recently developed biomimetic "stealth" coatings that draw inspiration from nature's long-circulating delivery vehicles (i.e. erythrocytes) merit mentioning. In 2000, Oldenborg et al. first demonstrated the role of CD47 as a marker of "self" on the membrane of red blood cells. Specifically, as the ligand for an inhibitory receptor (SIRPα) expressed by macrophages, CD47 prevents phagocytosis of red blood cells.[104] Roughly ten years later, Tsai et al. coated polystyrene microparticles with CD47 and/or IgG (a potent opsonin) and demonstrated that at physiologically relevant densities of CD47 (~250 molecules/μm^2), phagocytosis by macrophages could be prevented, even if the microparticles were opsonized.[105] Thus, surface presentation of CD47 can effectively set much higher thresholds for macrophage-mediated phagocytosis of opsonized MNPs, and potentially facilitate increased circulation time of MNP delivery vehicles.

The aforementioned approach to extending MNP circulation by mimicking surface protein presentation by red blood cells represents a bottom-up approach to design. Similarly, most other biomimetic MNP delivery systems utilize bottom-up approaches that, while effective, are inherently limited in biomimicry by the complex protein composition of natural cell membranes. Recognizing this limitation, Hu et al. recently developed a novel top-down biomimetic approach to nanoparticle functionalization that involves translocating natural erythrocyte membranes (including both membrane lipids and associated membrane proteins) to the surface of PLGA nanoparticles.[106] A summary of the surface-functionalization process is presented in Figure 4. Remarkably, the erythrocyte membrane camouflage more than doubled the *in vivo* circulation half-life of nanoparticles relative to the gold standard "stealth" nanoparticles (PEG 2000-coated), and significant particle retention was observed in the blood even after 72 hours.[106] For future clinical applications, this biomimetic delivery platform could represent a form of personalized medicine, with nanoparticles coated with a patients' own red blood cell membranes.

3.1.5. Acellular Artificial Antigen Presenting Cells (aAPC's)—Of the isotropic surface-labeled biomimetic MNP systems, artificial antigen presenting cells (aAPCs) have been the most widely explored. The use of synthetic, acellular aAPCs to activate, expand, and differentiate naïve T lymphocytes has broad applications for vaccination,[107] cancer immunotherapy,[108] and immunosuppression.[109] Like their natural counterparts (DCs), biomimetic aAPC constructs may be either injected to interact with T cells *in situ*,[110, 111] or used to expand T cells *ex vivo* for adoptive transfer.[112, 113] Stimulation of both naïve $CD4^+$ and $CD8^+$ T cells by natural DCs and biomimetic aAPCs is achieved through simultaneous presentation of T cell receptor-binding recognition ligands (signal 1) and various costimulatory ligands (signal 2). Additionally, physiologically relevant adhesion ligands may be incorporated on the surface of aAPCs to stabilize interactions with T cells and enhance signaling.[114, 115]

The performance of various acellular aAPC constructs and their promise for clinical applications has been recently reviewed by Steenblock et al. in 2009,[116] and by Turtle et al. in 2010.[117] The vast majority of these aAPC constructs present combinations of surface-bound anti-CD3 and anti-CD28, which bind to T cell receptors (CD3) and costimulatory

receptors (CD28), respectively. One key for the design of efficient aAPCs is the demonstrated optimal T cell stimulation with intermediate densities of recognition ligands. More specifically, T cell activation and proliferation tends to exhibit symmetric, bell-shaped anti-CD3 concentration dependence curves—a result of T cell receptor aggregation.[116] Furthermore, consistent with expression on natural APCs, higher ratios of costimulatory anti-CD28 to anti-CD3 are required for optimal T cell stimulation by aAPCs.[118]

Of the microparticle-based aAPCs recently reviewed, substrates for ligand immobilization include nondegradable polystyrene latex and paramagnetic beads, as well as biodegradable PLGA microparticles.[116, 117] Magnetic bead aAPCs, such as the commercially available Dynabeads® T-Activator (anti-CD3, anti-CD28) and tosyl- or epoxy-activated beads (which can be covalently labeled with any antibodies), allow easy separation from expanded T cells *in vitro*.[119] Alternatively, biodegradable PLGA microparticles may be superior for *in vivo* use, where transient presence of the aAPCs is desired. Recently, Steenblock and Fahmy developed a comprehensive PLGA-based aAPC construct, which combined presentation of surface-bound anti-CD3 and anti-CD28 with release of soluble IL-2 from the particle interior.[120] The novelty of this particular biomimetic system is its ability to locally directly deliver to T cells both surface-bound ligands (signals 1 and 2) and soluble cytokines (signal 3), which otherwise must be added to culture media for optimal *ex-vivo* expansion of T cells with aAPC constructs. This aAPC delivery system, with a combination of surface-bound signals and paracrine delivery of soluble cytokines, dramatically enhanced T cell proliferation[120] and addressed one of the chief limitations of surface-labeled degradable microparticles—namely, that surface erosion can compromise long-term ligand presentation.[116] To that end, avidin-palmitic acid conjugates were incorporated into the PLGA microparticles during emulsion fabrication, with palmitic acid interacting with the PLGA core and avidin partitioning to the surface. The result was a dense and durable coating of avidin to which biotinylated ligands could be immobilized, and consequently, stable presentation of biotinylated ligands on the particle surfaces was recorded for more than 20 days in solution.[120]

Although surface-bound anti-CD3 can serve as a ligand for all T cell receptors (producing polyclonal T cells), aAPC systems that incorporate peptide-loaded major histocompatibility complexes (MHCs) instead of anti-CD3 can also be employed for activation of antigen-specific T cells.[121–124] These aAPC constructs, which typically use either HLA-Ig fusion proteins[121] or MHC tetramers,[122] have been used to activate and expand tumor or viral antigen-specific T cells, while leaving other T cells unaffected. The primary limitation of such antigen-specific aAPC constructs involves MHC heterogeneity between individuals. Since each person expresses different combinations of MHC isoforms, and an individual's T cells only recognize antigens presented by self-MHC isoforms, antigen-specific aAPCs (in their current state) would not represent a universal, off-the-shelf therapeutic.[9] Rather, different MHC isoforms would have to be used for different individuals.

A vast majority of the reported aAPC constructs, and those discussed thus far, present anti-CD28 as the costimulatory ligand; however, natural APCs can present multiple distinct costimulatory and adhesion ligands, which may generate different responses from T cells. Herein, we will focus on aAPC systems that have integrated additional costimulatory and adhesion ligands in order to enhance activation and proliferation of T cells, or encourage their differentiation toward a specific phenotype. The results of studies with such aAPC systems are summarized in Table 1. Overall, four key observations relevant to the design of future biomimetic aAPC delivery systems may be drawn from these studies: First, altering the nature of ligation of a specific receptor on T cells may qualitatively alter T cell responses. For example, stimulation of naïve $CD4^+$ T cells with aAPC beads presenting anti-CD3 and anti-CD28, CD80, or CD86 (all ligands of CD28) results in similar robust

proliferation and Th1 cytokine secretion (IFN-γ). However, Th2 cytokines (IL-4) are only secreted upon stimulation with CD80 or CD86. As anti-CD28 has a greater affinity for CD28 than natural costimulatory ligands (CD80 and CD86), these results are consistent with a "strength of signal" hypothesis, where the strength and/or duration of costimulation contributes to differentiation toward Th1 or Th2 phenotypes.[118] Second, integration of signaling through multiple different surface-bound costimulatory ligands may enhance, or alter, T cell responses, and the relative amount of different costimulatory ligands is important. For example, additional surface presentation of costimulatory anti-CD137 or anti-CD278 can enhance expansion of $CD8^+$ T cells, or promote differentiation and preferential expansion of Th17 cells.[125, 126] Furthermore, proliferation of $CD8^+$ T cells is dependent on the ratio of anti-CD137 to anti-CD28, with 3:1 ratios yielding the best results.[127] Third, addition of adhesion ligands may stabilize aAPC-T cell interactions and enhance signaling, thereby improving T cell responses. For example, additional presentation of anti-CD2, antibody against an adhesion molecule, further enhances T cell proliferation and cytokine secretion by stabilizing mRNA transcripts and lowering activation thresholds.[114] Finally, incorporation of suppressive ligands onto aAPCs can induce regulatory T cell (Treg) differentiation or suppress T cell proliferation altogether. For example, surface-bound PD-L1 enhances Treg induction by soluble TGF-β and improves maintenance of Treg function,[109] while engagement of PD-L2 on T cells suppresses proliferation.[128]

Though most synthetic aAPCs present ligands to T cells for the purposes of activation, expansion, and differentiation purposes, unique "killer" aAPCs (KaAPCs) have recently been developed for antigen-specific immunosuppression. In the mid-1990s, researchers identified high expression of Fas ligand (FasL; an apoptosis-inducing ligand) on the surface of malignant cells as potent mechanism by which tumors could evade T cell-mediated immune responses.[130] Drawing inspiration from these findings, Schütz et al. affixed both apoptosis-inducing ligands (anti-Fas IgM) and recognition ligands (HLA-A2-Ig) to the surface of microparticles. When cocultured with polyclonal populations of T cells, these KaAPCs depleted CD8 T cells in an antigen-specific fashion by inducing Fas/FasL-dependent apoptosis in less than one hour.[131] As observed with previously discussed biomimetic MNP systems, ligand density and ratios of different ligands could dramatically affect the response of T cells. Specifically, relative presentation of too much recognition ligand (HLA-A2-Ig) promoted antigen-specific T cell activation and expansion, while relative presentation of an overabundance of anti-Fas led to nonspecific killing of all T cells. Titration of ligands revealed an optimal ratio, with moderate amounts of both ligands, to induce apoptosis in antigen-specific T cells.[131] With the ability to deplete different antigen-specific T cells, such KaAPC delivery systems offer great potential for the treatment of autoimmune diseases and transplant rejection. In fact, a recent study used KaAPCs to deplete alloantigen-specific T cells and reduce graft rejection in a murine skin transplant model. Intravenous injections of KaAPCs with H-2Kb (an MHC allotype from C57BL/6 mice) significantly prolonged graft survival in BALB/c mice (H-2Kd) that had received skin from C57BL/6 mice. This improved graft survival was likely a result of a 60 percent decrease in H-2Kb alloreactive T cells in the recipient BALB/c mice. Finally, this novel approach to immunosuppression did not inhibit overall immune responsiveness.[132]

The use of synthetic aAPCs to simultaneously present multiple stimuli to T cells offers several advantages over cellular-based systems. Synthetic constructs afford engineers flexibility and precise, reproducible control over ligand presentation (e.g. ligand density and relative amounts of different ligands). Consequently, synthetic aAPCs can potentially generate more consistent T cell responses than natural DCs, which may vary considerably between individuals.[133] Additionally, such off-the-shelf biomimetic delivery systems may eliminate much of the time and cost associated with isolation and expansion of specific subsets of autologous DCs, and the concerns with genetically engineered cell-based

aAPCs.[116] Still, synthetic aAPCs tend to be less efficient at activating, expanding, and differentiating T cells than natural DCs or cell-based aAPCs.[134, 135] This is most likely a result of incomplete biomimicry. Specifically, synthetic aAPCs with random (isotropic) distributions of ligands fail to capture the spatial organization (anisotropy) of ligands presented by natural DCs. Furthermore, static ligand presentation by synthetic aAPCs fails to capture the dynamic reorganization of ligands at the immune synapse between DCs and T cells. Thus, next-generation synthetic aAPCs will likely incorporate surface-presentation of additional costimulatory and adhesion ligands with physiologically relevant spatial distributions potentially on dynamic surfaces.

3.2. Static Patterns of Ligands (Anisotropy)

While isotropic (spatially uniform) presentation of ligands on the surfaces of MNP has been used successfully for various delivery systems, in some cases, a more biomimetic approach with anisotropic, or patterned, presentation of ligands may enhance cellular responses. In particular, the well-documented anisotropy of immune synapses between APCs and T cells (i.e. a bull's eye pattern with concentric regions of recognition, costimulatory, and adhesion ligand-receptor pairs, as depicted in Figure 3c) suggests that presentation of relevant ligands in a biomimetic pattern may enhance the efficacy of existing synthetic aAPCs by providing additional contextual information.[136–138]

Though several methods for tailored multicomponent protein patterning on 2D planar surfaces exist (e.g. dip-pen nanolithography, micro- and nanocontact printing, electron beam lithography, and photolithography), none are particularly suitable for patterning of ligands on the 3D curved surfaces of MNP.[139, 140] However, recent breakthroughs have led to three novel techniques to produce surface anisotropy on MNP.[51, 141, 142] As one example, Roh et al. developed a novel electrohydrodynamic jetting technique to create Janus particles with hemispherical surface anisotropy. In this case, simultaneous parallel streams of two different polymer solutions, under the influence of an electric field, can yield biphasic nanoparticles that can present distinct ligands on opposite halves.[51] As depicted in Figure 5a, surface modification of biphasic nanoparticles with two different ligands can be achieved by selective bioconjugation. That is, two different conjugation chemistries can be used to attach two different ligands to distinct functional groups present on the surfaces of the two phases.

Using a vastly different approach to generate anisotropic microparticles, Zhang et al. effectively demonstrated the possibility of depositing gold nanodots on the surface of microspheres via a form of colloidal lithography. With polystyrene microspheres arranged in colloidal crystals, and the top two layers (etched by O_2-plasma) serving as a mask for gold vapor deposition, two to five gold nanodots can be deposited. Furthermore, the number and size of gold nanodots (20–400 nm) can be controlled by varying the crystal structure (e.g. hexagonal close-packed (hcp) vs. face-centered cubic (fcc) arrays) and incidence angle of the gold vapor beam.[141] While these gold nanodots could be used direct the organization of microspheres and mediate colloidal self-assembly, they may also provide a means for anisotropic surface-presentation of ligands. For example, ligand-alkanethiol conjugates could be covalently bound to the gold nanodots, and a second ligand could potentially be attached to the background microsphere surface via a second bioconjugation scheme, as depicted in Figure 5b.

Finally, Kamalasanan et al. recently reported a novel technique to systematically produce patches on the surface microspheres via interfacial condensation of a liquid mask, with the mask pattern determined by contact points between adjacent microspheres. Notably, when liquid polydimethylsiloxane (PDMS) is applied to the desired array microspheres (e.g. colloidal crystal, monolayer, linear array, etc.), dewetting occurs at the bulk particle surface,

with selective solidification of PDMS at contact points between microspheres. At this point, one ligand can be conjugated to the exposed microsphere surface, followed by removal of the PDMS masks and conjugation of a second ligand to the newly exposed patches.[142] This approach (schematically depicted in Figure 5c) offers considerable flexibility in terms of bioconjugation strategies, as well as in the number of patches and different ligands used. For example, to mimic the patterning of a natural immune synapse, patches containing a mixture of recognition and costimulatory ligands (e.g. anti-CD3 and anti-CD28) could be surrounded by a background field of adhesion ligands (e.g. ICAM-1). Future anisotropic MNP formulations with more complex, physiologically relevant ligand patterns (e.g. bull's eye arrays for synthetic immune synapses) could even further improve T cell activation and proliferative responses to synthetic aAPCs. At this point, however, with patterned presentation of ligands on MNP in its infancy, it still remains to be seen whether anisotropic MNP delivery systems will prove to be superior to existing isotropic constructs in performing various tasks both in vitro and in vivo.

3.3. Dynamic Distribution of Ligands (Responsive Anisotropy)

Though anisotropic ligand patterning on MNP may represent a major step toward more complete biomimicry, such synthetic constructs may even better replicate cell-mediated stimulation by capturing the dynamic nature of ligand presentation on naturally fluid cell membranes. Importantly, the lateral diffusion of ligands and associated receptors on interacting cell membranes has important roles in contact-mediated signaling between cells. For example, the formation of immune synapses between APCs and lymphocytes is a dynamic process that involves spatial reorganization of recognition, costimulatory, and adhesion ligand-receptor complexes after initial contact. The transition from a disperse isotropic spatial distribution of ligands and receptors to a highly structured supramolecular activation complex (SMAC; Figure 3c) allows lymphocytes to initially sample multiple distinct MHC-peptide complexes presented on the surface of a single APC, and then form sustained interactions, resulting in lymphocyte activation and proliferation, when a specific antigen is recognized.[136] Furthermore, the ability of relatively low total quantities of ligands on a given cell to cluster at the interface with another cell, thereby forming a localized high density patch of ligands, permits more efficient signaling. For example, if T cell activation requires a minimum ligand density of 60 MHC-peptide/μm^2,[136] an APC with a fluid membrane theoretically would need only 60 MHC-peptide complexes on its membrane surface. In dramatic contrast, one cell-sized microsphere (d=10μm) with isotropic surface presentation would theoretically require nearly 19,000 recognition ligands to elicit T cell activation (~300X less efficient).

Though immune synapses are of the most widely cited example of "responsive anisotropy" in nature (where the spatial organization of ligands on one cell surface changes over time in response to interactions with another cell), responsive anisotropy in contact-mediated communication between other cells is becoming increasingly documented.[143, 144] Thus, biomimetic MNP delivery vehicles that mimic the responsive anisotropy seen in nature would provide the most natural temporospatial context for surface presentation of ligands, which may enhance their ability to delivery information to and elicit desired responses from cells. Two key classes of MNP with fluid lipid bilayer surfaces—liposomes and supported lipid bilayers (SLBs), or protocells, provide platforms for what could potentially be the most biomimetic presentation of surface-bound ligands. First described by Bangham and Horne in 1964,[145] liposomes (vesicles formed by self-assembly of spherical lipid bilayer membranes in aqueous environments) have been widely used for drug delivery.[146, 147] For biomimetic surface presentation of ligands on liposomes, different ligands can be readily conjugated to diverse head groups on the phospholipid derivatives.[147] Alternatively, liposomes may be formed by hypotonic treatment of cells followed by extrusion of the natural cell membranes,

in which case the lipid particle would already contain the corresponding natural surface-bound ligands.[148] Furthermore, by using different phospholipid derivatives (with different transition temperatures), and adjusting the amount of cholesterol, overall fluidity of liposome membranes can be controlled.[149] Importantly, this provides temporal control of lateral diffusion rates for ligand presentation to cells.

While liposome-based aAPCs were first reported in 1978,[150] the biomimetic interactions between such aAPCs and T cells, involving responsive anisotropy, have become evident through more recent studies. In particular, Prakken et al. demonstrated dynamic clustering of ligand-receptor (MHC-TCR) pairs at biomimetic immune synapses formed between liposome aAPCs and T cells. In essence, local densities of recognition ligands (MHC-peptide complexes) required for T cell activation were attained by lateral diffusion of ligands toward the initial interaction site.[151] More recently, it was discovered that biomimetic pre-clustering of T cell ligands in membrane microdomains on the surface of liposomal aAPCs can dramatically enhance T cell activation and expansion.[115, 152] Specifically, co-localization of recognition (anti-CD3), costimulatory (anti-CD28), and adhesion (anti-LFA-1) ligands on neutravidin lipid rafts (i.e. microdomains) results in significantly greater expansion of CD8+ T cells.[115] Such biomimetic constructs with microdomains actually represent an instance where reorganization of initially anisotropic ligand presentation occurs in response to interactions with a cell. Despite the benefits of biomimetic dynamically anisotropic ligand presentation by liposomes, mechanical stability is a major limitation of liposomes.[153] Though liposome stability can be improved for delivery of soluble factors, most of these approaches also result in reduced membrane fluidity[154] and hence could compromise their potential for dynamically anisotropic ligand presentation.

By combining the advantages of a fluid lipid bilayer for ligand presentation and a solid particle platform for mechanical stability and controllable factor release,[155] supported lipid bilayers (SLBs) offer great potential for biomimetic MNP delivery. Such systems include both anchored and unanchored lipid bilayers on hydrogel and silica MNP. In the case of anchored lipid bilayers, fatty acids covalently bound to particle surfaces drive spontaneous bilayer shell assembly in the presence of liposomes, with the inner lipid layer comprised of anchored fatty acid tails instead of intact phospholipids.[156] Importantly, such constructs retain membrane fluidity comparable to that of unanchored lipid bilayers, which are formed by liposome fusion on silica MNP.[155] As with liposomes, multiple ligands may be covalently attached to the SLB surface by conjugation to phospholipid head groups. In the past year, two key reports of biomimetic SLB that present ligands to cells and viruses with dynamic temporospatial context highlight the potential of these systems.[157, 158] For instance, Smith et al. studied how low densities of ligands on fluid cell surfaces can aid in avoiding nonspecific interactions with many other cells in the body, yet still interact strongly with certain, specific cell populations. Notably, with fluid SLBs, multivalent binding occurs regardless of overall ligand density, dramatically increasing specific affinity for, and receptor-mediated endocytosis by cancer cells relative to non-fluid SLBs and liposomes. Dynamic interactions between SLB protocells and Hep3B cancer cells are depicted in Figure 6. Interestingly, support of lipid bilayers on nanoporous silica particles actually enhances membrane fluidity relative to liposomes and SLBs on non-porous silica particles, and the more responsive membranes can even provide more efficient interactions with cells.[157]

In a separate study, Porotto et al. used similar protocells with biomimetic surface presentation of specific viral entry receptors (EFNB2) to inactivate enveloped viruses that would normally infect human cells via membrane fusion. These protocells effectively acted as decoys, with interactions between G-protein on the viral envelope and EFNB2 receptors on the protocells triggering premature fusion and rendering the viruses unable to

subsequently infect cells. Notably, viral inactivation was dependent on protocell membrane fluidity, as less fluid membranes and static presentation of receptors on sepharose microparticles led to less efficient viral inactivation.[158] This could be a result of the increased time required for receptors to accumulate near and interact with the viruses (i.e. inappropriate temporal context). Ultimately, the dynamic nature of ligand presentation on fluid MNP membranes has the potential to provide the most biomimetic temporospatial context for interactions with cells (or viruses).

4. Biomimetic Sizes, Shapes, and Mechanical Properties

Given the wide variety of sizes, shapes, and mechanical properties of human cells and microbes, perhaps it is not surprising that these features should be important considerations for the design of MNP that intend to mimic these natural “particles”. Indeed, in recent years, scientists and engineers have begun to investigate how pertinent information can be included by manipulating the physical properties of the MNP themselves, and several of these considerations are reviewed by Mitragotri and Lahann [159]. As will be reviewed here, biomimetic sizes, shapes, and mechanical properties can affect the distribution of MNP delivery vehicles in the body, or even dictate cellular responses. Thus, these physical properties may be considered forms of information delivered by the MNP.

4.1. Size Effects

With adjustments of MNP size to mimic the natural dimensions of typical viruses (~20–300nm), bacteria (~0.5–3μm), or human cells (~6–20μm),[160] various effects on contextual delivery have been observed. These include effects on the distribution of MNP throughout the body, the ability of MNP to be phagocytosed, and the ability of MNP to interact with cells via surface presentation of ligands. For biomimetic MNP design, general dimensions are largely dictated by the desired biodistribution. For example, large microparticles (especially >10μm), whose size mimics that of most human cells, will typically persist at sites of injection or application. As such particles are too large to pass through most biological barriers in the body or travel through microvasculature, they would be appropriate for local delivery, but not for systemic or targeted delivery applications.[161] As the size of particles decreases, however, they become increasingly able to penetrate physiological barriers, circulate through microvasculature, and reach distant tissues in the body. For example, small microparticles (~1–5μm) that mimic the size of (deformed) red blood cells or bacteria may be injected into the blood stream with less risk of embolism and can accumulate in diverse tissues.[161] Even smaller virus-size nanoparticles can pass through mucus barriers (<200nm),[162] endothelial barriers (<100nm),[163] and even the blood-brain barrier (<50nm),[164] facilitating delivery to sites that were previously unreachable.

In addition to affecting the ability of MNP to pass through biological barriers, size also has a definitive effect on uptake of particles by phagocytic cells. While most cell types may be able to ingest sub-micron size particles (i.e. those the size of viruses or small intracellular bacteria) by pinocytosis, only phagocytic cells can take up microparticles (especially 0.5–10μm). Multiple studies with different phagocytic cells have demonstrated that maximal phagocytosis occurs for particles that are 2–3μm in diameter, regardless of whether they are opsonized.[165, 166] In particular, recent investigation by Champion et al. suggests that the intermediate-sized (2–3μm) particles are consistent with the dimension of membrane ruffles on macrophages, thereby affording maximal contact between the microparticles and macrophage membrane. Elimination of the natural membrane ruffles by osmotic swelling eliminated the size dependence of phagocytosis, supporting this hypothesis.[166] It is especially interesting, albeit not surprising, that this particular microparticle size is consistent with that of most bacteria which the immune system would have evolved to recognize and phagocytize. Finally, the ability of MNP to interact with cells via surface-

bound ligands may depend on the size of the particle substrate. Essentially, interactions between MNP and cells may require contact and ligation of receptors over a sufficient contiguous surface area. This is most evident with synthetic aAPCs, where particle sizes of at least 4–5μm were necessary to provide optimum stimulation of T cells, with responses decreasing dramatically with smaller aAPC sizes.[167] Overall, these findings further reinforce the importance of MNP size for providing context of signals presented to cells.

4.2. Shape Effects

While the effects of particle size on phagocytosis have been investigated since the late 1980's, the effects of particle shape are only beginning to come to light in the past decade. The recent explosion in the study of shape-effects has partly resulted from the development of novel techniques to produce non-spherical polymeric particles, as reviewed by Champion et al.[168] When motivated by biomimesis, one may question whether or not the diversity in bacterial shape, including spheroidal or ellipsoidal cocci, cylindrical or rod-shaped bacilli, and various spirals may play a critical role in bacterial function. Observations of how differently shaped bacteria travel throughout the body, are ingested by phagocytes, and/or infect other cells do indeed suggest that shape may also provide important context for MNP delivery. A comprehensive discussion of the value and evolutionary rationale of different bacterial shapes can be found in reference [169].

In terms of biomimetic MNP, several recent studies have demonstrated that the shape of MNP (especially curvature and aspect ratios) can have a dramatic effect on attachment and internalization of MNP for phagocytosis and endocytosis.[170–173] Champion and Mitragotri first observed that microparticles with identical volumes, but various shapes (e.g. spheres, oblate ellipsoids, prolate ellipsoids, and elliptical discs), are all capable of initiating phagocytosis when presented in at least one orientation to macrophages; however, the local particle curvature from the perspective of the macrophage dictates whether the particle will be internalized. In particular, internalization occurs when the initial point of contact with the phagocyte has high curvature.[170] Following up on those results, they further demonstrated that phagocytosis could be largely inhibited by particles with extremely high aspect ratios (>20), as only the two ends of such particles have high curvature surfaces required for internalization. Any attachment of phagocytes to the predominant low curvature surface would prevent internalization.[172] Consistent with such observations, Sharma et al. most recently showed that particle shape affects attachment and internalization independently, with relative particle attachment favoring prolate ellipsoids > oblate ellipsoids > spheres, but internalization favoring oblate ellipsoids ≫ spheres > prolate ellipsoids. Notably, though prolate ellipsoids attached most efficiently, their large aspect ratios necessitate more actin remodeling in the phagocyte, and thus internalization is reduced considerably.[173] Finally, recent work by Gratton et al. reveals that biomimetic particle shapes can play a significant role in internalization by non-phagocytic cells. Remarkably, HeLa cells can actually internalize biomimetic rod-shaped particles as large as 3μm by endocytosis, and rod-shaped MNP with an aspect ratio of 3 are internalized more rapidly than those of similar volume and lower aspect ratios.[171] These findings resonate with the adeptness of rod-shaped bacteria for infecting non-phagocytic cells.[174] Furthermore, such results suggest that traditional upper-limits of nanoparticle size for endocytic uptake (150nm)[175] may need to be reconsidered for novel biomimetic particle shapes.

In addition to directly affecting phagocytosis, the shape of MNPs can also affect their circulation time in the body via hydrodynamic phenomena. The following examples of biomimetic microparticle shapes illustrate this effect. First, Muro et al. demonstrated that elliptical discs (0.1×1×3μm), injected intravenously in mice, have longer half-lives in circulation than microspheres (1–10μm) with the identical materials and coatings.[176] One potential explanation for this result is that the elliptical discs may align with flow in the

bloodstream, thereby minimizing their collisions with blood vessel walls.[176] The use of such disc-shaped microparticles to extend circulation time in the blood stream would be consistent with nature's use of disc-shaped red blood cells for extended circulation. In a separate study, Geng et al. also demonstrated that particle shape could dramatically affect circulation half-life.[177] Specifically, filament-shaped particles (filomicelles), with lengths of up to 8μm, remain in circulation for up to a week following intravenous injection. This is roughly ten times longer than the spherical counterparts, and three times longer than PEG-coated "stealth" microspheres.[177] As with the aforementioned disc-shaped particles, these particles, which mimic the shape of filamentous viruses (e.g. Ebola and H5N1 influenza), align with blood flow. Furthermore, longer filomicelles (≥ 3μm) are less readily phagocytosed than shorter filomicelles and microspheres because hydrodynamic shear forces wrench the long filament-shaped particles away from any phagocytes they contact.[177]

Based on observations that many bacteria can change their shape (e.g. from rod to coccus, or rod to filament)[169] and the knowledge that certain biomimetic particle shapes can either prolong circulation in the bloodstream or enhance phagocytosis, it stands to reason that shape-changing MNP could be extremely advantageous for delivery systems. To that end, Yoo and Mitragotri recently engineered polymeric particles that change shape in response to various stimuli (e.g. temperature, pH, or chemicals). Dynamic transformations of these PLGA constructs relies on a delicate balance of polymer viscosity and interfacial tension, with ellipsoid discs relaxing to energetically favorable spherical particles as the polymer viscosity is reduced by a stimulus.[178] Importantly, such a change in shape modulates interactions of the particles with phagocytes, effectively altering the context of delivery. Elliptical disc-shaped particles, which are poorly phagocytosed by macrophages, could potentially circulate through the body until they reach a particular target site, where a stimulus (e.g. temperature elevated by ultrasound, or the acidic tumor environment) would initiate a shape change to spheres that would be more readily internalized by phagocytes.[178] Given the expanding knowledge base of non-spherical MNP for delivery, future work in this field will likely focus on production of non-spherical MNP with diverse surface-bound ligands and encapsulated factors, control of shape-changing particles and novel shape transformations, and reversible shape-changes and alternative stimuli. Each of these areas of exploration will further expand the toolbox available to drug delivery specialists, and permit additional control over the context of information presented by biomimetic MNP delivery vehicles.

4.3. Mechanical Properties

Mechanical properties of MNP may also play an important role in providing additional contextual information to responding cells. While the effects of mechanical properties on stem cell differentiation and functional maturation of various tissues (e.g. muscle, bone, nerve, etc.) have been widely studied, these studies primarily involve culture of cells on scaffolds with different stiffness.[179, 180] There have been, however, only a handful of reports of the influence of microparticle stiffness on interactions with cells and transport through the body. In particular, Beningo and Wang demonstrated that mechanical stiffness of microparticles alone can radically affect phagocytosis by macrophages. Macrophages respond differently to rigid or soft polyacrylamide microspheres, with disparities in adhesion, cytoskeletal reorganization, and even intracellular phosphorylation. Consequently, *in vitro* phagocytosis of rigid particles can be six-fold greater in extent than that of soft particles.[181] In another study, Robbins et al. noted that rigid leuko-polymersomes (discussed previously) adhered less avidly to substrates with inflammatory ligands (mimicking inflamed endothelium) than flaccid vesicles, despite similar rolling interactions.[182] Such effects of particle mechanical properties on interactions with cells are

consistent with trends seen in nature, where leukocytes with relatively deformable membranes are able to adhere to inflamed endothelium.

In a separate area of drug delivery, recent efforts by two independent groups have led to development of microparticles that mimic the mechanical properties of red blood cells (RBCs), with deformability permitting flow through capillaries that are narrower than their dimensions. In nature, the development of discoidal RBCs from spherical reticulocytes follows a dramatic decrease in the elastic modulus from ~3 MPa to ~15 kPa.[183] Drawing inspiration from this process, Doshi et al. collapsed hollow microspheres to form biconcave discoid templates, onto which relevant proteins (e.g. hemoglobin and albumin) could be deposited and cross-linked in a layer-by-layer fashion (Figure 7a).[184] Subsequent dissolution of the PLGA template left flexible protein RBC-shaped shells with an elastic modulus reasonably comparable to that of natural RBCs (92.8±42 kPa vs. ~15 kPa), allowing the 7±2μm discs to deform and flow through 5μm diameter glass capillaries.[184]

With a vastly different approach, Merkel et al. used an established, proprietary nano-molding technique (Particle Replication in Non-Wetting Templates; PRINT®), as depicted in Figure 7b, to fabricate RBC-shaped hydrogel microparticles, with elasticity (8, 17, 40, or 64 kPa) controlled by the amount of cross-linker.[185] Like the RBC mimics developed by Doshi et al., the softer RBC mimics were able to deform and flow through channels roughly 2μm smaller than their un-deformed diameter. Furthermore, the hydrogel RBC mimics showed minimal interactions with endothelial cells, and an eight-fold decrease in modulus corresponded to a thirty-fold increase in circulation half-life, when the particles were injected intravenously in mice.[185] Ultimately, both of these biomimetic, flexible RBCs have potential for extending circulation time of microparticles by preventing entrapment in microvasculature (and potentially by reducing phagocytosis). It would be interesting to see if the RBC surface coatings discussed in the previous sections could be combined with these mimetic particles (i.e. combine surface-presentation of ligands with the biomimetic context of shape and stiffness) to extend circulation times even further. Regardless, the studies with synthetic RBC mimics and phagocytosis of soft vs. rigid particles illustrate the importance of considering mechanical properties for the future design of MNP delivery vehicles.

5. Biomimetic Delivery as Encoding Information for Biological “Communication”

The remarkable capacity for cells to detect multiple biological signals and their associated temporospatial context, integrate the information contained therein, and then respond appropriately, appears to be a critical part of development, tissue regeneration, immunity, and even normal tissue homeostasis (as just a few examples). Without this sophisticated ability to present, receive, and process information, the trillions of cells in the body, acting with complete autonomy, could never form a cohesive organism—a productive “community” of cells. With breakdowns in appropriate cell-to-cell communication responsible for many diseases and disorders, the goal of many biomimetic MNP delivery systems is to restore (or supplement) missing (or insufficient) “information” in the form of signals with appropriate temporospatial context. As with the numerous examples presented above, the ability of these systems to effectively “communicate” the necessary information (in the form of temporal or spatial cues) to the various cells in the body will be paramount to their therapeutic efficacy.

Indeed, the biomimetic MNP delivery systems discussed in this review present different modes of information to cells in the form of soluble factors, surface-bound ligands, and physical properties, with the goal of achieving a specific response from the cells with which these systems interact. Importantly, incomplete communication (i.e. the absence of one

signal) or minor changes in one mode of delivery (i.e. a change in the presentation of one signal) can result in dramatically different cellular responses. For example, particles that present surface-bound recognition and costimulatory ligands (signals 1 and 2; Figure 8a) to T cells in the absence of soluble TGF-β (signal 3) would generate effector T cells and elicit an inflammatory response; whereas, the same presentation of activating ligands in the presence of TGF-β would generate immunosuppressive Treg.[46] In other words, the information provided to the cell through one mode of delivery (e.g. surface-bound ligand presentation) may be ambiguous, as the cellular response to this information may require additional information.

Analogously, the exchange of various types of information between two people can rise above a level of uncertainty when semantics (the study of words and their meaning), grammar (the composition of words), and non-verbal cues are integrated to yield pragmatics (the study of context with respect to meaning). For example, a rather complex impression can be derived from a combination of the following variables: eye contact, a handshake, and an exchange of words (Figure 8b). For instance, a combination of a weak handshake and the absence of eye contact would communicate one message, despite the verbal exchange. Conversely, a firm and persistent handshake with unrelenting eye contact could serve to communicate quite the opposite message. In other words, not only the presence of each individual variable, but also the integration of various spatial combinations in context to one another determines the response to the perceived message. Further, the temporal organization of spoken words can significantly affect the meaning of an exchange. For example, “See, I can’t help!” and “Help, I can’t see!”, would evoke vastly different responses from an individual. Likewise, changing the order of growth factor delivery may have dramatically different impacts on cells involved with tissue regeneration. Finally, spatial context can also be important to communicating information. For instance, one can certainly imagine using audible cues to locate another individual in a dark room. Yet, this information is completely lost if the sound emanated from speakers placed evenly around the room (i.e. the sound is not enough information to determine location). Similarly, chemokine delivery for cell recruitment depends on the establishment of concentration gradients (i.e. the soluble factor itself is not enough information to evoke a desired migratory response). In all of the ways described above, the central role of temporospatial context and the integration of multiple modes of exchange in interpersonal communication becomes an apt analogy for the interaction between biomimetic delivery vehicles and cells, or tissues in the body.

When we consider the analogy between cell-to-cell (or biomimetic MNP-to-cell) communication and interpersonal communication, we may even begin to draw inspiration for biomimetic delivery from the established infrastructure people use to communicate with one another across great distances (e.g. telecommunications networks, postal service, internet, and even public address systems). Similarly, the body contains “communications infrastructure” of its own, such as the complex vascular networks and coagulation cascade. For instance, in an approach to amplify delivery to tumor cells, Maltzahn et al. recently developed a biomimetic delivery system that harnesses the coagulation cascade to broadcast the location of tumor-targeted nanoparticles to circulating, clot-targeted liposome delivery vehicles carrying anti-tumor therapeutic agents.[186] This delivery system mimics platelets, which amplify their own accumulation at damaged blood vessels through the coagulation cascade.[187] Thus, biomimetic MNP can not only communicate directly with cells in the body, but also can take advantage of existing communication infrastructures to indirectly accomplish various tasks from a distance. Ultimately, further advancement of the field of biomimetic MNP delivery systems will be advanced by understanding the body’s own complex communication pathways (in both temporal and spatial organization of biological cues) so that synthetic formulations can appropriately “encode” the information that will

ultimately be “decoded” by target cells who have learned to speak this language over millennia of evolution.

6. Regulatory Implications of Biomimetic Delivery with MNP

Recognizing the importance of incorporating various modes of communication into biomimetic delivery systems with appropriate temporospatial context also sheds light on potential limitations of many of today’s pharmaceuticals. Though traditional drugs are not likely to become obsolete anytime in the foreseeable future, novel and increasingly biomimetic drugs will likely begin to appear in our arsenal to treat a range of diseases, disorders, and injuries. Unfortunately, the existing regulatory systems may not be geared toward fostering clinical translation of this type of complex therapeutic. With pre-clinical regulatory processes requiring homogeneity in formulations, it will be considerably more expensive to ensure strict reproducibility with intricately “programmed” delivery formulations, and perhaps even prohibitively so. With traditional drugs, reproducibility involves relatively few parameters, which are generally straightforward to measure, such as drug mass per tablet, purity, and bioactivity. With novel biomimetic delivery systems, however, numerous other factors must be rigorously tested and controlled. Reproducible quantities of multiple proteins, uniform distributions of multiple ligands on all particles (and even over an individual particle surface), orientation of ligands on a particle surface, release kinetics for multiple factors, membrane fluidity, and bioactivity of factors over the entire time period of release are only a few examples of factors that may need to be considered. Furthermore, whereas slight deviations in the quantity of active ingredient in a pill may have minimal effect on patients (e.g. 202mg ibuprofen vs. 200mg), the consequences of seemingly minor deviations in a biomimetic delivery vehicle could be dramatic. Thus tighter tolerances may be imposed, despite being considerably more difficult to attain, or even measure. Furthermore, in terms of therapeutic development, it becomes critical to test biomimetic MNP at early stages from a “systems” approach with rigorous in vivo studies, as in vitro assays have little value (i.e. the natural context and complexity of the body becomes paramount to development). Endogenous factors could have serious, unexpected roles on the cell and tissue responses to biomimetic delivery, and thus the implications of patient-to-patient variability may be greater with biomimetic delivery than traditional medicines. Ultimately, as biomimetic delivery systems become increasingly more sophisticated and “cell-like”, we may actually require a shift in the existing regulatory paradigm of treating them as “new drugs” toward treating them as “cellular therapies.”

7. Conclusions

The nascent field of biomimetic delivery using micro and nanoparticles (MNP) has advanced with great leaps and bounds in the past five to ten years, with advances in programming controlled release formulations, surface patterning on particles, and methods to generate non-spherical particles. Drawing inspiration from the ways that cells communicate in the body, several different modes of “delivery” (i.e. temporal or spatial presentation of biological signals) have been investigated in a number of therapeutic contexts. Presentation of surface-bound ligands on MNP may be used to target cells or tissues in the body, deliver cues for proliferation or differentiation, direct immune responses, and promote or prevent particle uptake by cells (each in similar ways as their native, live cell counterparts). While the majority of approaches to date involve randomly distributed ligands, static and dynamic anisotropic ligand presentation has been made possible by recent advances in protein patterning on particle surfaces and supported lipid bilayers. In addition, physical properties, including particle size, shape, and mechanical stiffness, can also deliver contextual information, especially to promote or prevent particle uptake by cells. Importantly, the context provided by multimodal, or multifactor delivery represents a key

element of most biomimetic MNP systems, a concept emphasized by the analogy to human interpersonal communication. In the future, we anticipate that systems that (1) combine multiple modes of delivery, (2) incorporate additional biomolecules, and (3) do so with improvements in natural context will begin to replace current medicine with those that are much more potent and targeted to accomplish specific tasks. Ultimately, and ironically, the resulting medical treatments will ever increasingly imitate the very life that they are designed to save.

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Biographies

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Dr. Steven Little received his Ph.D. in Chemical Engineering at the Massachusetts Institute of Technology in 2005 in the laboratories of Robert Langer. He currently holds the position of Assistant Professor and Bicentennial Alumni Faculty Fellow at the University of Pittsburgh in the Departments of Chemical Engineering, Bioengineering, Immunology, and the McGowan Institute for Regenerative Medicine. His laboratory focuses on advanced drug-delivery strategies, including biomimetic systems, as applied to both immunotherapeutics and tissue regeneration.

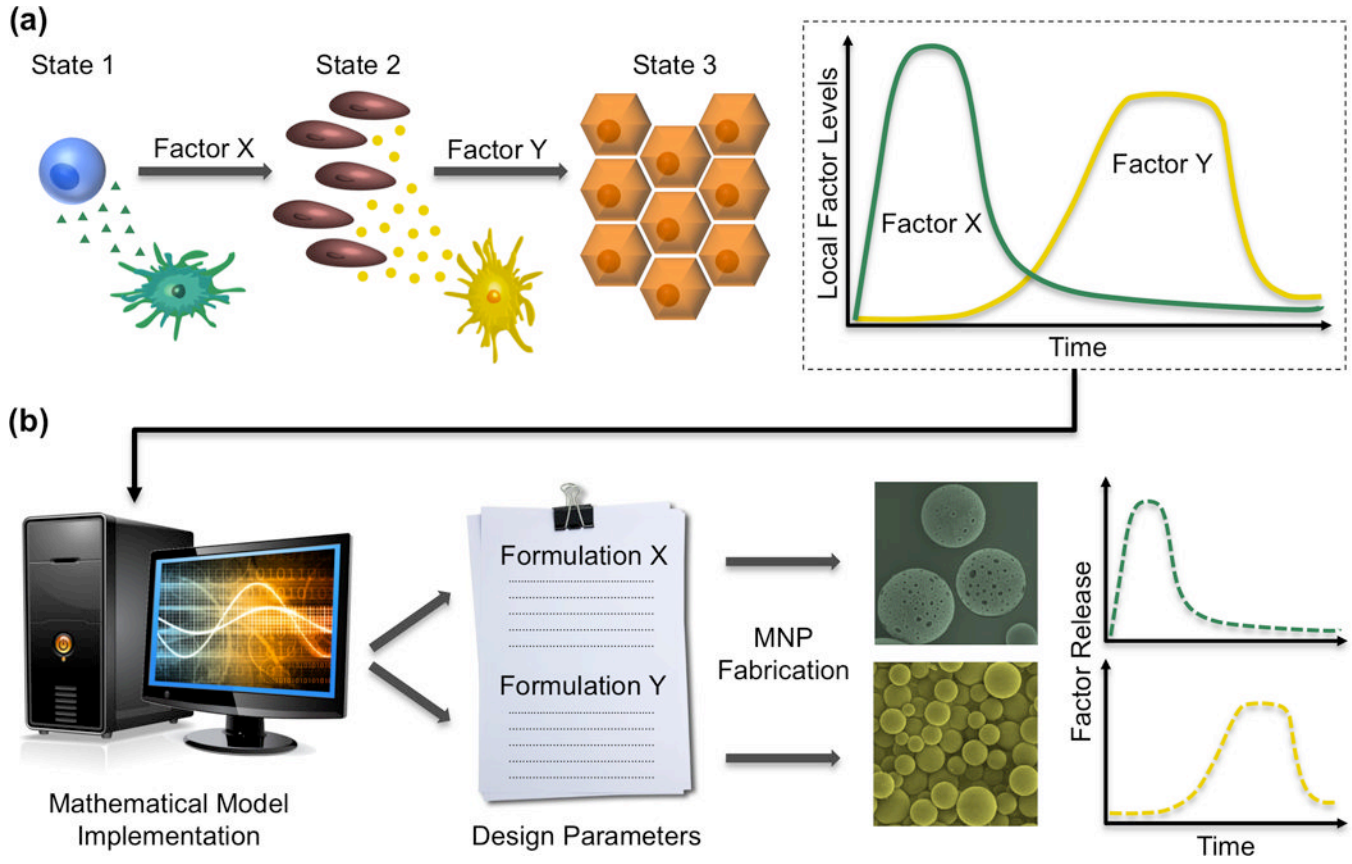

Figure 1.

Schematic illustrating effects of soluble paracrine signaling factors and rational design of biomimetic MNP. **(a)** Soluble factors locally released by cells may promote proliferation, differentiation, and/or reorganization of cells to form structured tissues. Different paracrine factors act as instructions for diverse transitions from one “state” to another, where a “state” refers to the particular quantity, phenotype, and organization of cells. Although sequential secretion of factors X and Y is depicted, simultaneous secretion of multiple factors, and more complex temporal patterns with two or more factors also occur in nature. Such factors may also be secreted by the same cell or by multiple different cells. **(b)** For rational design of biomimetic MNP, the temporal patterns of local concentrations of factors can be input into mathematical models, used to guide the design of controlled release formulations. Outputs of the model include design parameters, in this case for two different MNP formulations. These design parameters serve as recipes for fabrication of MNP with release profiles that mimic the natural temporal patterns of factor secretion.

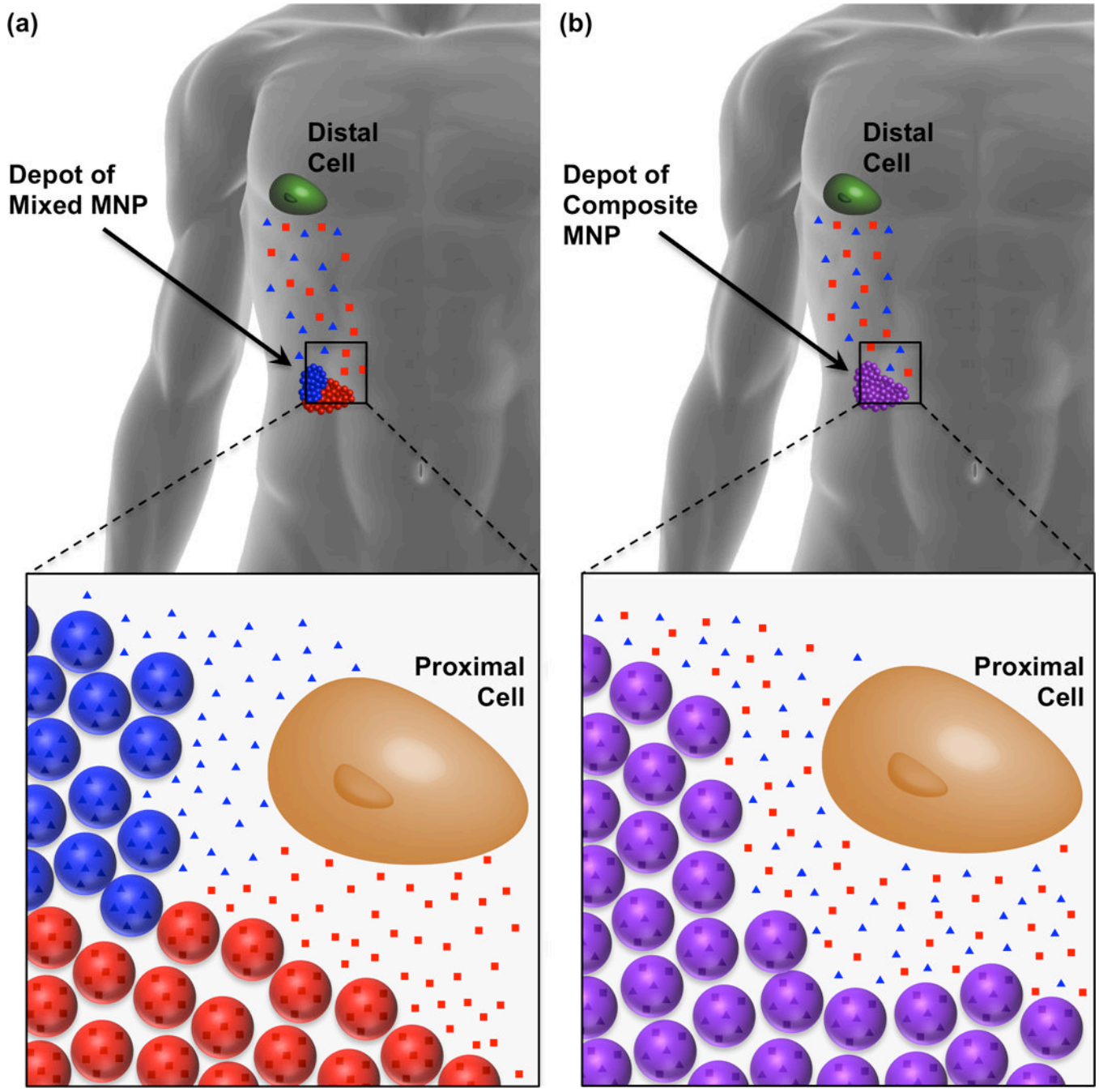

Figure 2.

Two approaches to multifactor delivery. **(a)** An injected depot containing a mixture of two MNP formulations (red and blue) that each release distinct individual factors (squares and triangles) mimics the secretion of factors by different cell populations in a local area. Cells not immediately adjacent to a depot (i.e. distal cell) with segregated particles perceive a mixture of both factors coming from the same direction (top). In contrast, cells approaching the depot site would “see” the two factors originating from a different location (bottom). **(b)** A depot containing composite, multi-compartmental MNP (purple), which release both factors, can mimic dual factor secretion by a single cell. As with the mixed MNP depot, distal cells perceive both factors coming from the same source (top). However, cells

immediately adjacent to composite particles now “see” both factors originating from the same location (bottom).

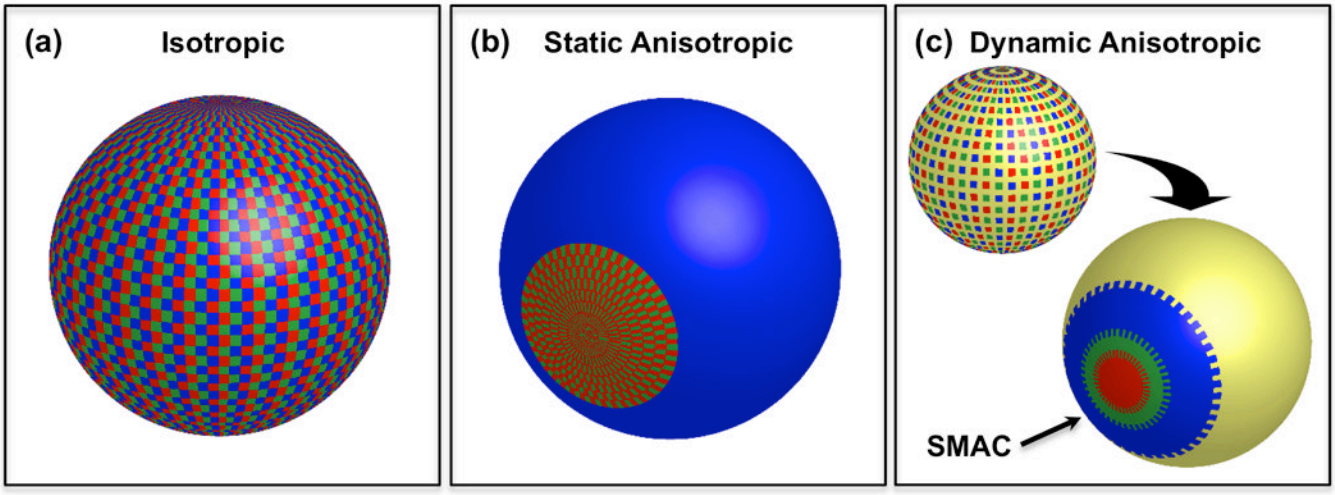

Figure 3.

Three ligands (recognition (red), costimulatory (green), and adhesion (blue)) that could be presented on the surface of artificial antigen presenting cells (aAPCs) in various ways. **(a)** Isotropic surface presentation of randomly distributed ligands. All three ligands are presented uniformly over the particle surface. **(b)**Anisotropic presentation of ligands in a patch pattern on the surface of a particle. Recognition and costimulatory ligands are randomly distributed in the patch, with a surrounding field of adhesion ligands. **(c)** Dynamic anisotropic presentation of ligands on a fluid supported lipid bilayer (SLB; yellow). Before interactions with a cell (e.g. T cell), lower initial surface density of randomly distributed ligands may be placed on the SLB surface. Anisotropic reorganization of ligands occurs in response to interactions with a cell. The resulting bull's eye pattern would be characteristic of a natural supramolecular activation clusters (SMAC) formed at immune synapse between a T cell and APC.

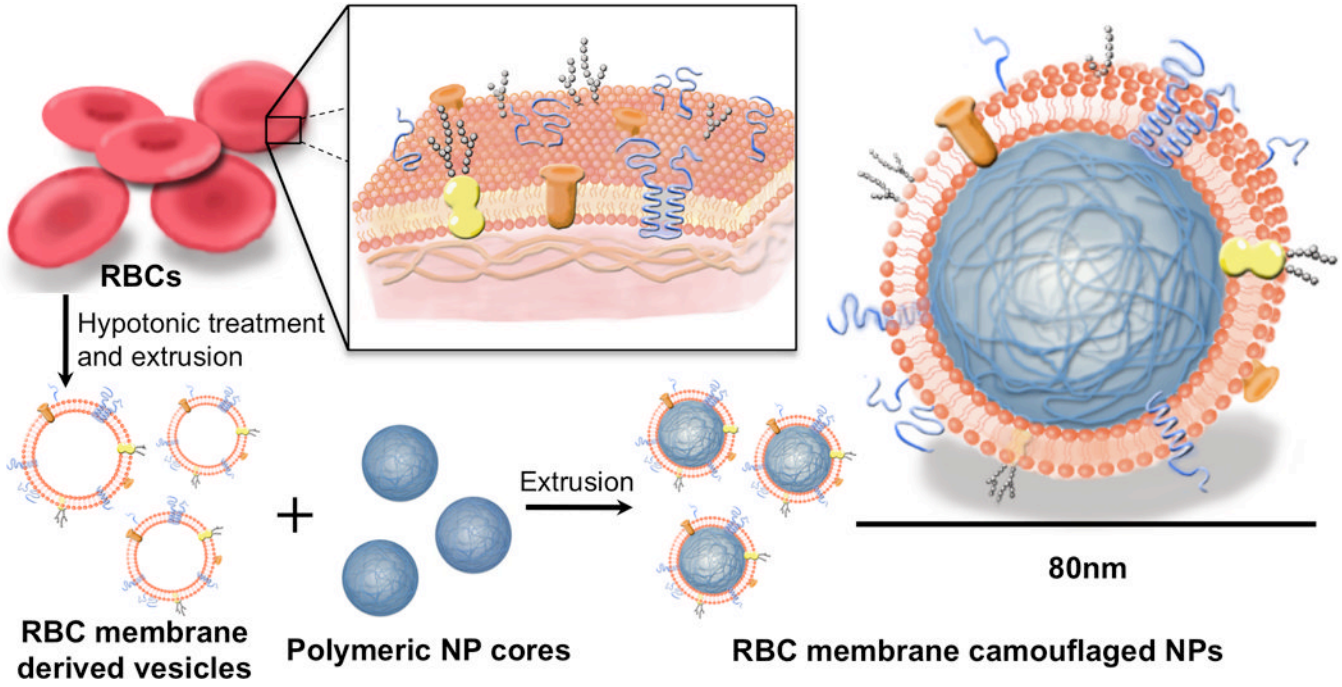

Figure 4.

A method to achieve biomimetic ligand composition on the surface of nanoparticles (NPs) by coating them with cellular membranes, including associated membrane proteins. Reproduced with permission from [106]. Copyright 2011, National Academy of Sciences, USA.

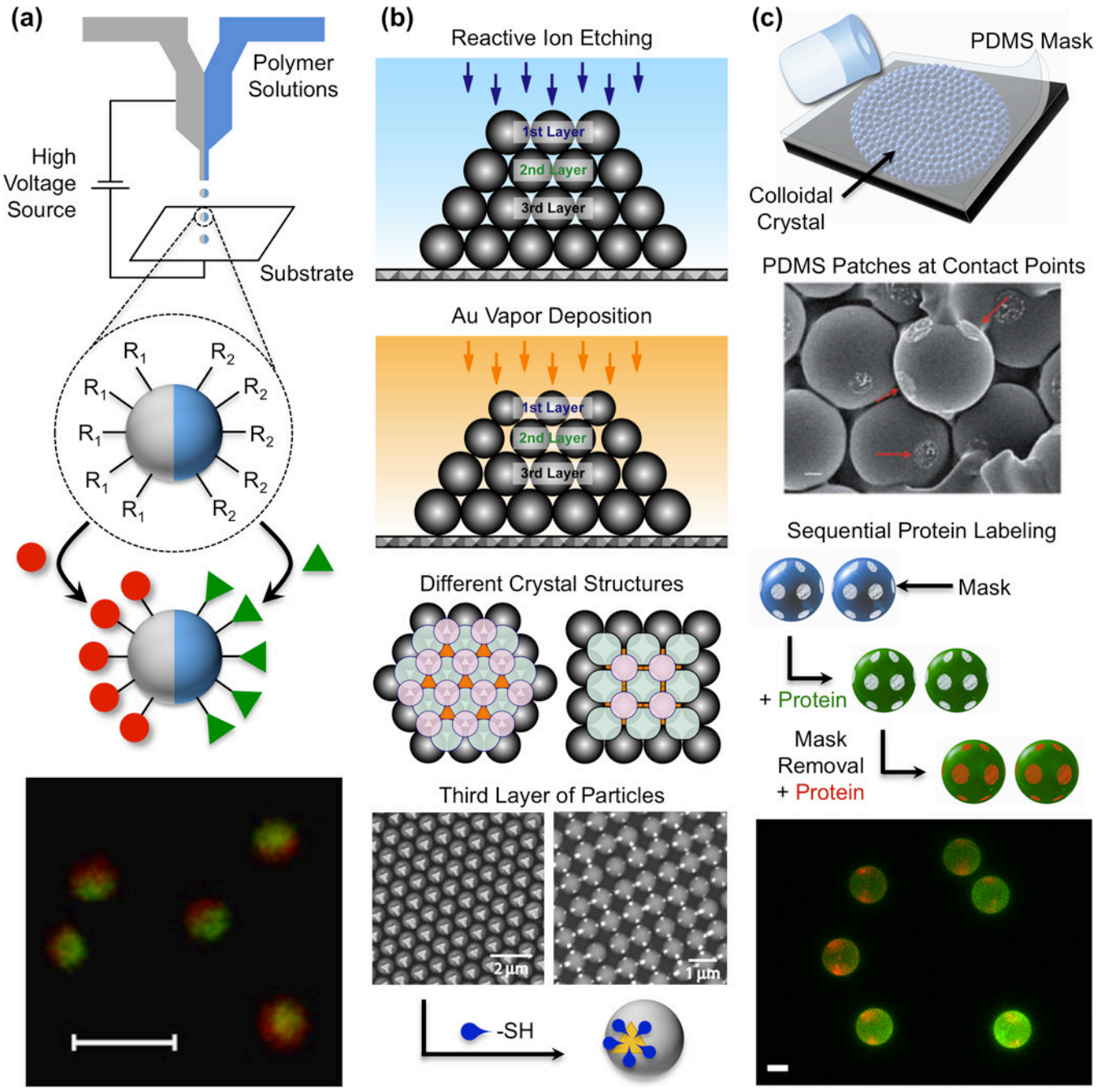

Figure 5.

Three approaches to protein ligand patterning on the surface of particles. **(a)** Electrodynamic jetting with parallel streams of two distinct polymer solutions (blue and grey) results in biphasic Janus particles. The presence of different functionalities (R1 and R2) on opposite hemispheres enables selective surface modification with two ligands (red circles and green triangles) via two bioconjugation schemes. A fluorescence micrograph shows Janus particles dually labeled with rhodamine (red) and BODIPY dye (green) (bar = 2µm). Adapted with permission from [51]. Copyright 2005, Nature Publishing Group. **(b)** Reactive ion etching, followed by gold vapor deposition on a colloidal crystal of microparticles, results in different patterns of gold patches on the third layer of particles, depending on the crystal

structure. SEM images depict two possible patterns of gold patches achieved with the corresponding crystal structures (bars = 2μm). Subsequent conjugation of ligands (blue teardrops) to the gold patches may be possible through thiol (-SH) conjugation chemistry. Adapted with permission from [141]. **(c)** Liquid polydimethylsiloxane (PDMS) applied to a colloidal crystal selectively solidifies at points of contact between microspheres. The resulting patchy masks allows for sequential protein labeling of the different regions (masked and unmasked) via various bioconjugation methods. Fluorescence micrograph shows patchy particles with regions presenting different fluorescently labeled proteins (bar = 10μm). Adapted with permission from [142].

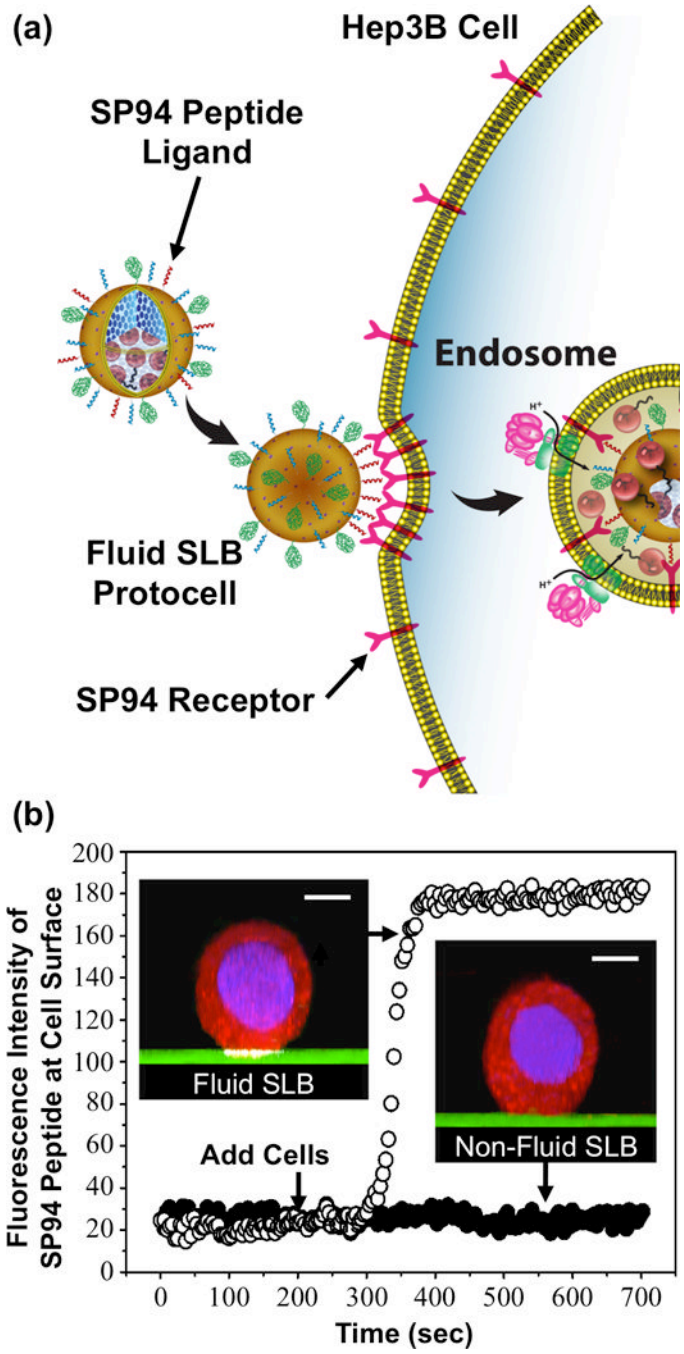

Figure 6.

Dynamic surface ligand-receptor interactions between SLB protocells and Hep3B cancer cells. **(a)** A fluid SLB binds to a cell with high avidity by recruitment of SP94 peptide ligands to the cell surface, and internalization by receptor-mediated endocytosis follows the dynamic binding event. **(b)** When presented on a fluid SLB (green), Alexa Fluor 647-labeled SP94 peptide ligands (white) are recruited to the surface of a Hep3B cell (red). Such dynamic reorganization of presented ligands is not seen with a non-fluid SLB. Adapted with permission from [157]. Copyright 2011, Nature Publishing Group.

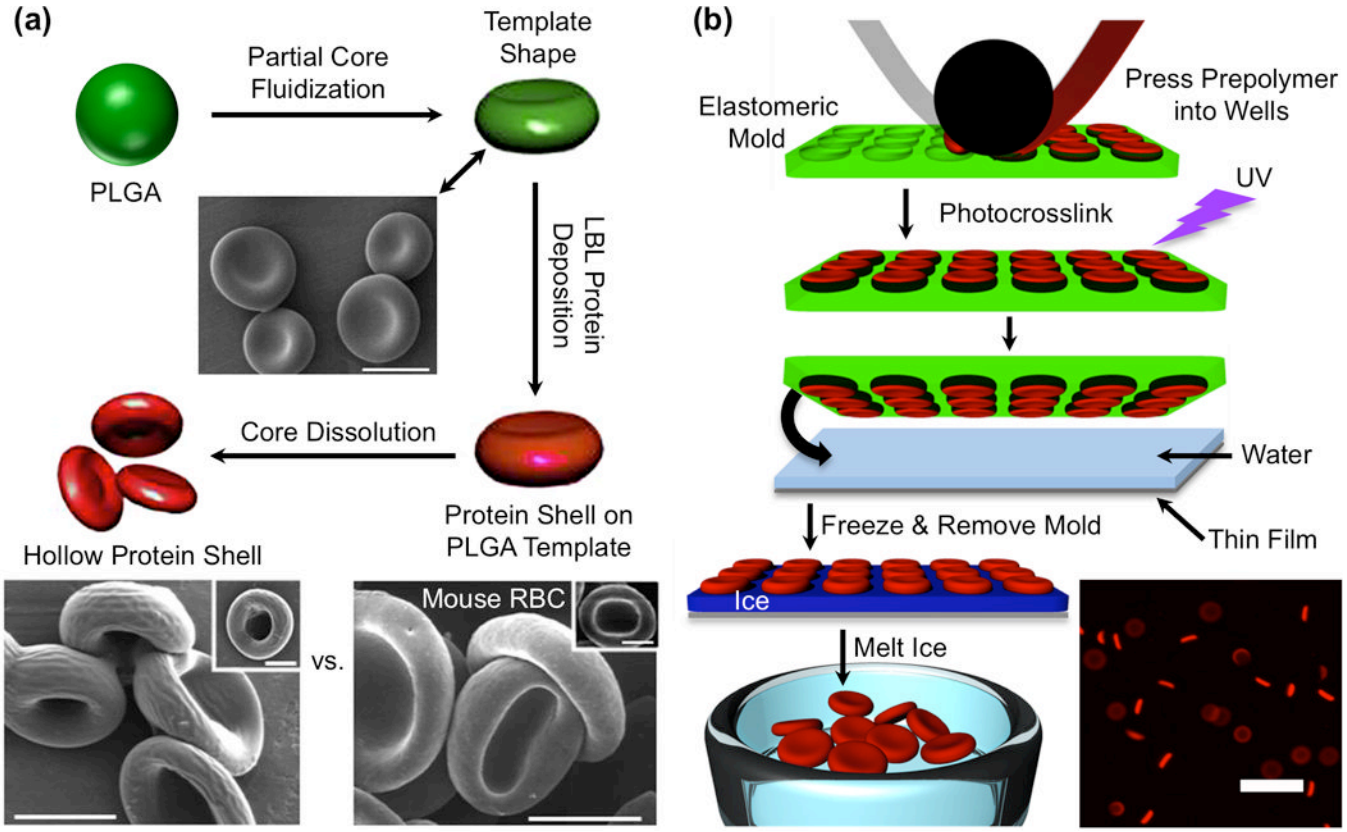

Figure 7.

Two methods to produce particles that mimic the shape and mechanical properties of red blood cells (RBC). **(a)** PLGA microspheres produced by electrodynamic jetting collapse into a discoidal shape due to partial fluidization of the PLGA core by 2-propanol. The resulting template is uniformly coated with cross-linked proteins in a layer-by-layer (LbL) process, and the PLGA core dissolved by organic solvents, leaving flexible protein shell particles. The number of protein layers dictates particle stiffness, allowing for physiologically relevant mechanical properties. SEM images show the semblance between RBC-mimics and mouse RBCs (bars = 5μm). Adapted with permission from [184]. Copyright 2009, National Academy of Sciences, USA. **(b)** Cross-linked hydrogel, RBC-shaped particles fabricated by the PRINT® (Particle Replication in Non-wetting Templates) process. Prepolymer is pressed into the RBC-shaped wells of a mold, and cross-linked by UV light, with the degree of cross-linking dictating particle stiffness. Freezing in water, peeling away the mold from particles trapped in ice, and allowing the ice to melt results in free particles. Fluorescent image shows RBC-shaped hydrogel particles (bar = 20μm). Adapted with permission from [185]. Copyright 2011, National Academy of Sciences, USA.

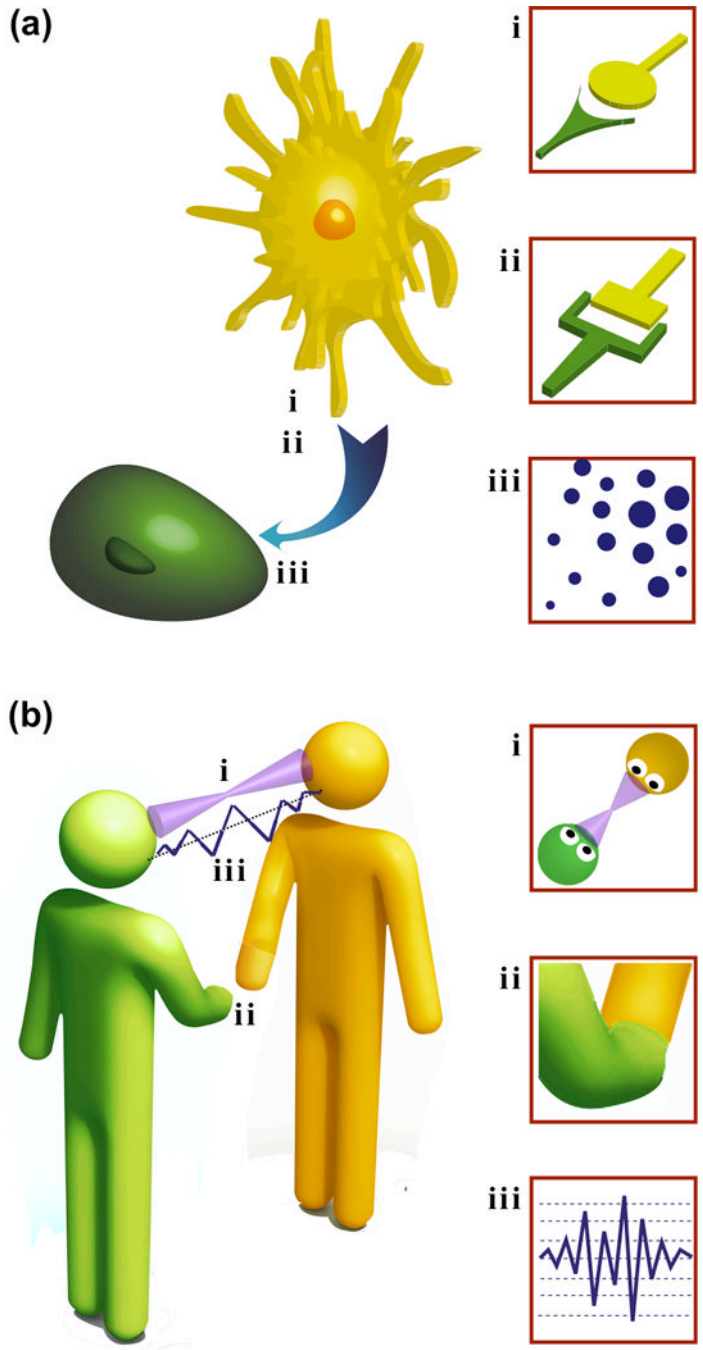

Figure 8.
Combinations of various types of information produce an integrated message. **(a)** Cell-to-cell communication between a dendritic cell (yellow) and a T cell (green) involves at least three signals: (i) MHC-peptide presentation to TCR, (ii) costimulatory ligand presentation to T cell, and (iii) secreted soluble cytokines. **(b)** Similarly, interpersonal communication between two individuals generally involves multiple forms of exchange, such as (i) eye contact, (ii) a handshake, and (iii) a verbal exchange.

Table 1

T cell responses to different costimulatory and adhesion ligands presented by synthetic aAPC.

Costimulatory Ligands (with anti-CD28)	Summary of T cell Responses	Reference(s)
CD80 (B7.1) CD86 (B7.2)	$CD4^+$ T cell costimulation with CD80 or CD86 leads to a Th2 response (IL-4), whereas costimulation with higher affinity anti-CD28 results in a Th1 response (IFN-γ).	[118]
Anti-CD137 (4-1BBL)	Costimulation with anti-CD137 (especially at a 3:1 CD137:CD28 ratio) enhances activation of $CD8^+$ T cells and preferentially expands memory T cells.	[125, 127, 129]
Anti-CD278 (ICOS)	Costimulation with ICOS (as compared to anti-CD28) promotes induction and expansion of human Th17 cells.	[126]
PD-L1	Surface-bound PD-L1 synergizes with soluble TGF-β to induce differentiation of naïve $CD4^+$ T cells to Treg.	[109]
Anti-PD-L2 (B7-DC)	PD-L2 engagement by aAPCs coated with anti-PD-L2 inhibits T cell division and proliferation.	[128]
Adhesion Ligands (with anti-CD28)		
Anti-CD2	Anti-CD2 enhances T cell proliferation and expression of IL-2 and IFN-γ, though anti-CD2 alone is less efficient than anti-CD28. Anti-CD2 may also stabilize aAPC-T cells interactions, lowering activation thresholds.	[114]
Anti-LFA-1	Anti-LFA-1 (with anti-CD3 and anti-CD28) on liposome surfaces increases efficiency of immune synapse formation and enhances CD8+ T cell expansion relative to anti-CD3/anti-CD28 beads.	[115]