Bio-inspired, bioengineered and biomimetic drug delivery carriers

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Abstract | Synthetic carriers such as polymer and lipid particles often struggle to meet clinical expectations. Natural particulates — that range from pathogens to mammalian cells — are therefore worth examining in more depth, as they are highly optimized for their specific functions *in vivo* and possess features that are often desired in drug delivery carriers. With a better understanding of these biological systems, in conjunction with the availability of advanced biotechnology tools that are useful for re-engineering the various natural systems, researchers have started to exploit natural particulates for multiple applications in the delivery of proteins, small interfering RNA and other therapeutic agents. Here, we review the natural drug delivery carriers that have provided the basis and inspiration for new drug delivery systems.

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Intrinsic issues that are associated with free drugs — particularly with small interfering RNA (siRNA) and other nucleic acids — include poor solubility, poor stability, unwanted toxicity and/or an inability to cross cell membranes. These issues have propelled the development of new drug delivery systems. The *in vivo* pharmacokinetics and therapeutic activity of drugs generally needs to be improved, as drug costs are rising and drug pipelines are constricting[1]. Fuelled by many advances in nanotechnology and biotechnology, the past decades have witnessed rapid growth in the research and development of drug delivery devices in the form of polymeric nano- and/or microparticles, liposomes and micelles, among others[2–4]. The success of these devices relies largely on the selection of appropriate design parameters to address the physicochemical limitations of free drugs (that is, solubility and stability) and to overcome biological hurdles in reaching the target (that is, the first-pass effect, immune clearance, cell entry and off-target deposition). The precise hurdle for drug delivery depends on the nature of the drug (whether it is a peptide, antibody or siRNA) as well as its route of administration — for example, oral, injection, transdermal or inhalation — each of which possesses its own benefits and limitations.

Although synthetic drug carriers are being actively developed for many applications, it remains important to critically examine natural particulates, which range from pathogens to mammalian cells, as they possess their own delivery mechanisms. The central dogma of drug delivery is to steer therapeutic cargos to target tissues and/or cells to achieve maximal therapeutic efficacy with minimal toxic effects. Natural particulates have evolved to accomplish this task; however, with the exception of viral vector systems, their delivery mechanisms have not been well recognized.

Pathogens such as viruses and bacteria have developed unique strategies to evade the host immune system and enter a target cell[5–8]. Conversely, red blood cells (RBCs) have a notable capability to circulate and deliver oxygen for a prolonged period of time, owing to their unique shape, mechanical properties and the presence of a self marker on their surface. Substantial effort has been undertaken towards understanding the key features of natural drug carriers, such as natural tropisms, self markers, cell entry mechanisms, antigenic components and physicochemical properties. This is motivated, in part, by efforts to mimic or modify these carriers for the delivery of various therapeutic payloads, which include DNA, vaccines, peptides and/or proteins, and small molecules. This Review focuses on recent advances in the design of such drug carriers, provides an overview of their current development status and highlights the various applications and limitations of each approach (TABLE 1).

Pathogens

Pathogens such as bacteria and viruses induce diseases by evading immune responses and inducing favourable interactions with target cells — a mechanism that bears a striking resemblance to the action of many drug delivery carriers. Accordingly, numerous efforts have been made to use pathogens for therapeutic delivery functions (FIG. 1).

Table 1 | **Current development status of bioengineered, bio-inspired and biomimetic drug delivery carriers**

Strategies	Key attributes/capabilities	Applications	Current status	Challenges and/or limitations	Refs
Bacteria					
Recombinant bacteria	• Full set of RNA polymerases that enable expression of various substances, including antigens, therapeutic proteins and siRNAs • Tumour tropism • No pathogenicity of GRAS bacteria	• Vaccine delivery • *In vivo* factory for therapeutic proteins at disease sites • RNA interference-based cancer therapy	Clinical trials (Phase I)	• Safety concerns associated with attenuated bacteria (reversion to virulence)	14,15, 31,32
Microbots	• Carry nanoparticles on the surface of bacteria • Neither bacterial disruption nor genetic manipulation is required • Take advantage of the invasive property of bacteria	• Gene or protein delivery	Preclinical	• Safety concerns associated with attenuated bacteria (reversion to virulence) • Applicability in actual disease models • Feasibility with biocompatible nanoparticles	34
Bacterial ghosts	• No cytoplasmic contents • Intact surface properties • Large drug-loading capacity • Natural tropism to various tissues, including tumours • Considerable safety and low production cost	• Drug or DNA delivery • Vaccine delivery	Preclinical	• Potential immunogenicity owing to lipopolysaccharide • Limited *in vivo* data	36,42
Viruses					
Viral vectors	• Replace viral genetic materials with desirable ones • Take advantage of transduction and self-replication ability of viruses • Allow long-term expression of target genes • Carry nanoparticles	• Gene therapy and/or imaging	Clinical trials (Phase I–III)	• Safety concerns (reversion to virulence) • Limited targeting ability (off-target effects) • Limited loading capacity	47,51
Virus-like particles	• Self-assembled particles that are composed of viral capsids • Easy to scale up at a low cost • Preserve antigenicity • Drug-loading capabilities • Natural tropism and targeting ability with further modification	• Vaccine delivery • Drug and DNA delivery	FDA-approved vaccines (Gardasil (Merck) and Cervarix (GlaxoSmithKline))	• Potential immunogenicity when used for non-vaccine delivery	55,62
Virosomes	• Reconstituted empty influenza virus envelope • Easy to produce with low toxicity • Adjuvant activity	• Vaccine delivery • Drug delivery	FDA-approved vaccines (Epaxal (Crucell), Invivac (Solvay influenza) and Inflexal V (Crucell)	• Potential immunogenicity when used for non-vaccine delivery	74,76
Eukaryotic cells					
RBCs	• Prolonged circulation (~120 days) • Large volume for drug encapsulation • Ability to carry nanoparticles and thrombolytics	• Drug delivery • Targeting the RES	Preclinical	• Difficult to maintain integrity • Limited targeting ability	102,117
Macrophages	• Natural homing tendency to disease sites • Ability to move through the BBB • Ability to phagocytose nanoparticles	• 'Trojan horse' delivery carriers	Preclinical	• Difficult to collect • Difficult to maintain integrity	125,126
Lymphocytes	• Ability to carry various sizes of particles • No damage to intrinsic functionality of the cells	• 'Cellular backpack' • Adoptive T cell therapy of cancer	Preclinical	• Difficult to collect • Difficult to maintain integrity	129,130
Stem cells	• Gene delivery by genetic engineering • Natural homing tendency to solid tumours • Ability to internalize nanoparticles	• Cancer therapy	Preclinical	• Difficult to collect • Difficult to maintain integrity	141,142
Pathogen-mimetic vaccines					
Pattern recognition mechanisms	• Ability to stimulate immune cells using danger signals from pathogens via pattern recognition mechanisms • Co-packaging of danger signals as adjuvants and antigens for improved immunization	• Vaccine delivery	Preclinical	• Limited to vaccine delivery	90

Table 1 (cont.) | **Current development status of bioengineered, bio-inspired and biomimetic drug delivery carriers**

Strategies	Key attributes/capabilities	Applications	Current status	Challenges and/or limitations	Refs
Virus mimetics					
pH-sensitive nanogels	• Capsid-like structure • pH-sensitive reversible swelling is followed by drug release and endosomal escape • Ability to migrate to neighbouring cells	• Targeting tumours	*In vitro*	• Vulnerable to immune recognition • *In vivo* validation needed	100
Filomicelles	• Flexible and filament-shaped micelles • Prolonged circulation time in blood (over one week)	• Targeting tumours	Preclinical	• Thorough investigation into PK/PD needed	101
Cell mimetics					
Synthetic RBCs	• Ability to mimic shape and mechanical property of RBCs • Drug-loading ability • Oxygen-carrying ability	• Drug delivery • Component of artificial blood	Preclinical	• Vulnerable to immune recognition • Detailed *in vivo* validation needed	143,145
Self marker CD47	• Membrane protein that is derived from RBCs • Contributes to self-recognition of RBCs by RES, thus enabling prolonged circulation time	• Evasion of RES	*In vitro*	• Limited resource	149
Compartmentalization					
Vesosomes	• Liposomes within a liposome: distinct inner compartments separated from the external membrane • Sustained release profile	• Drug delivery	Preclinical	• Vulnerable to immune recognition • *In vivo* validation needed	153
Nanocells	• Polymer nanoparticles within lipid vesicles • Dual drug release system: rapid release of one drug from the lipid layer and sustained release of the other drug from polymer nanoparticles	• Cancer therapy	Preclinical	• Vulnerable to immune recognition	154

BBB, blood–brain barrier; FDA, US Food and Drug Administration; GRAS, generally regarded as safe; PK/PD, pharmacokinetics/pharmacodynamics; RBC, red blood cells; RES, reticuloendothelial system; siRNA, small interfering RNA.

Bioengineered strategies based on bacteria

Recombinant bacteria. Advances in genetic engineering technologies have allowed the insertion of plasmid vectors that encode proteins (such as antigens, antibodies, cytokines and enzymes) into live bacteria. As bacteria possess the full complement of RNA polymerases, they can deliver or produce these proteins at the target site (FIG. 1Aa). Bacteria that are used for this purpose include non-pathogenic or GRAS ('generally regarded as safe') bacteria such as *Lactococcus lactis,* which is the most widely engineered bacterium for protein production, and commensal bacteria such as *Streptococcus gordonii,* which has an ability to colonize mucosal surfaces in the oral, nasal and vaginal cavity[9,10]. Such bacteria provide a potential vehicle for the production and delivery of biologically active proteins such as cytokines and enzymes[11].

L. lactis has been used to target the delivery of the anti-inflammatory cytokine interleukin-10 (IL-10) to intestinal mucosa for the treatment of inflammatory bowel disease[12,13], and this approach has been investigated in a Phase I clinical trial[14]. *L. lactis* (AG013) has also been modified to secrete trefoil factor 1 for the treatment of oral mucositis, and is currently in Phase Ib clinical trials[15]. An IL-1 receptor antagonist has also been produced by engineered *S. gordonii* and *Bacillus subtilis* strains to ameliorate symptoms of inflammatory bowel disease[16,17]. Bacteria have also been used as microbicides against HIV; they have been engineered to secrete HIV-1 fusion inhibitors or the prototypic virucidal compound cyanovirin-N, and these bacteria have been shown to be successful at inhibiting HIV infection *in vitro*[18,19] and *in vivo*[20].

Bacteria have also been engineered to deliver vaccines by expressing and secreting high levels of various heterologous antigens, including tetanus toxin fragment C[21], pneumococcal surface protein A[22] and the E7 antigen of the human papilloma virus (HPV-16)[23]. *Lactobacillus acidophilus* has been engineered to secrete the protective antigen of *Bacillus anthracis*. This antigen was fused to a dendritic cell-targeting peptide that specifically binds to mucosal dendritic cells and promotes endocytosis, thus eliciting safe and effective immune responses[24]. Although GRAS bacteria are available, live attenuated recombinant bacteria, such as *Salmonella enterica* subsp. *enterica* serovar Typhimurium, have also shown prominent progress in vaccine delivery[25]. In general, natural infection by live pathogenic microorganisms elicits strong mucosal and systemic immune responses. However, the immunogenicity of attenuated bacteria is not always favourable, especially if the bacteria are used for applications other than vaccine delivery in which immune responses represent a barrier that must be overcome.

Tumour-targeting bacteria. Despite considerable advances in tumour-targeting technologies, the lack of selectivity towards tumour cells is still the primary limitation of current cancer therapies. It has been discovered that some strains of bacteria — for example, *Clostridium beijerinckii, Bifidobacterium bifidum* and *S.* Typhimurium — have a natural tumour-targeting ability and they specifically colonize tumour cells[26]. Gram-positive anaerobes such as Clostridia and Bifidobacteria can colonize only within the necrotic and/or hypoxic areas of tumours, whereas Gram-negative facultative anaerobes

Free drugs
Drugs that are not modified or processed to improve their physicochemical properties and pharmacokinetics profile.

Natural tropisms
The natural movement of a biological organism preferentially towards specific cell types in response to environmental stimuli.

Attenuated bacteria
Viable bacteria with a reduced degree of pathogenicity.

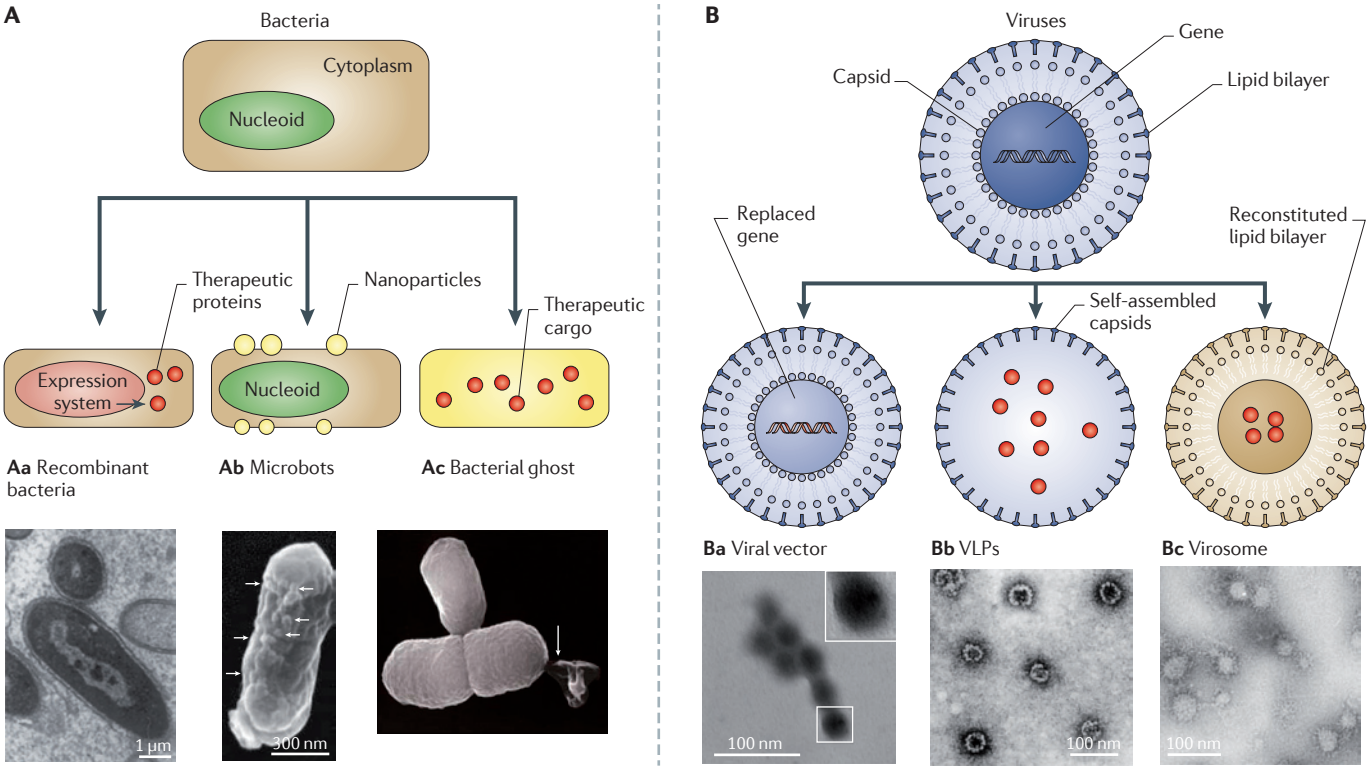

Figure 1 | **Bioengineered pathogens for drug delivery. A** | Various engineering strategies for bacteria. **Aa** | Recombinant bacteria are bacteria that are genetically modified by expression systems that encode antigens and biologically active proteins. The image in the bottom panel shows recombinant *Salmonella* Typhimurium internalized in macrophages[164]. **Ab** | Microbots are bacteria that carry nanoparticles on their surface. The image in the bottom panel shows *Listeria monocytogenes* bacteria carrying polystyrene nanoparticles[34]. The arrows indicate the nanoparticles. **Ac** | In bacterial ghosts, plasma components including genetic materials are removed. The image in the bottom panel shows protein E-lysed *Mannheimia haemolytica* bacterial ghosts[165]. The arrow indicates the efflux of bacterial cytoplasm at the time of the onset of lysis through the protein E-specific lysis tunnel. **B** | Various engineering strategies for viruses. **Ba** | In viral vectors, the viral gene is replaced with genes of interest. The image in the bottom panel shows adenoviral vectors that are coated with gold nanoparticles for tumour targeting[49]. **Bb** | Capsids that are derived from viruses are self-assembled to form virus-like particles (VLPs). The bottom panel shows VLPs that are loaded with peptides[166]. **Bc** | Virosomes are composed of viral membranes that are reconstituted with viral lipids and proteins. The bottom panel shows virosomes that are encapsulated with small interfering RNA[77]. Image **Aa** is reproduced, with permission, from REF. 164 © (2001) Elsevier Ltd. Image **Ab** is reproduced, with permission, from REF. 34 © (2007) Macmillan Publishers Ltd. All rights reserved. Image **Ac** is reproduced, with permission, from REF. 165 © (2004) The American Association of Immunologists, Inc. Image **Ba** is reproduced, with permission, from REF. 49 © (2006) American Chemical Society. Image **Bb** is reproduced, with permission, from REF. 166 © (2001) The Federation of American Societies for Experimental Biology. Image **Bc** is reproduced, with permission, from REF. 77 © (2006) Macmillan Publishers Ltd. All rights reserved.

such as *Salmonella* spp. can colonize and grow within both aerobic and anaerobic areas of tumours. These tumour-targeting bacteria have been genetically modified to secrete therapeutically active substances such as cytosine deaminase[27], tumour necrosis factor[28], herpes simplex virus thymidine kinase[29] and colicin E3 (REF. 30).

Bacteria have also been actively used as vectors for RNA interference (RNAi)-based cancer therapy. Recombinant *Salmonella* strains carrying siRNAs for multidrug-resistance genes[31] and signal transducer and activator of transcription 3-specific siRNAs[32] have demonstrated their ability to deliver plasmid siRNA to tumours and suppress *in vivo* tumour growth. In another study, the bacterial plasmid vector transkingdom RNAi plasmid was constructed to encode invasin for cellular internalization, listeriolysin O for endosomal escape and short hairpin RNA against the cancer gene, catenin β1 (REF. 33). The vector-bearing *Escherichia coli* exhibited significant gene silencing in *in vitro* and *in vivo* models of human colon cancer.

Nanoparticle-carrying bacteria. Although genetic engineering of bacteria has been actively investigated for vaccination and drug delivery, a novel approach to use unmodified bacteria for drug delivery has also been introduced. Akin *et al.*[34] designed bacteria-based nanoparticle delivery systems, which they termed 'microbots', using an attenuated form of the intracellular bacteria *Listeria monocytogenes* (FIG. 1Ab). Nanoparticles that were loaded with plasmid DNAs were conjugated — via biotin–streptavidin interactions — to the surface of the bacteria without any genetic manipulation, and the microbots successfully entered tumour cells and released nanoparticles, resulting in subsequent transcription and translation of the target proteins.

Non-living bacteria. Bacterial ghosts are non-living, non-denatured empty cell envelopes that are derived from Gram-negative bacteria by protein E-mediated lysis[35] (FIG. 1Ac). Despite the absence of cytoplasmic

contents (such as the genetic material), the intrinsic surface components such as fimbriae, flagella and polysaccharides enable the encapsulation or tethering of a range of cargos[36], and provide an intrinsic ability to target various cells such as dendritic cells[37], macrophages[38] and tumour cells[39]. In addition to their antigen-carrying ability, bacterial ghosts retain intrinsic adjuvant properties that are derived from membrane components such as lipopolysaccharides (LPSs) and peptidoglycan, which makes them excellent vaccine systems[40,41]. However, the presence of the immune-stimulating component LPS is not desirable in applications other than vaccine delivery, owing to unwanted immunogenicity. There are detailed reviews on the use of bacterial ghosts as drug and vaccine delivery carriers (see REFS 36,42).

Gram-positive enhancer matrix (GEM) particles that are loaded with a cell-wall binding antigen (the protein anchor) are another example of non-living bacteria that have been used as vaccine delivery carriers[43,44]. GEM particles are made from *L. lactis* bacteria; they are deprived of surface proteins and their intracellular content is largely degraded. As GEM particles are not genetically modified, they lack recombinant DNA. Therefore, compared to recombinant bacterial systems, GEM particles can minimize the risks that are associated with the dissemination of recombinant DNA.

Applications and limitations. In summary, bacteria are ubiquitous microorganisms that can be engineered to deliver vaccines and therapeutic cargos. As bacteria are generally considered to be pathogenic, there are some initial safety concerns associated with the use of bacterial systems. It is also worth noting that there are non-pathogenic (that is, GRAS) bacteria such as lactic acid bacteria. Although applications of GRAS bacteria are limited to local administration — owing to a lack of natural tropism — they have been used for the production of therapeutic proteins such as IL-10, and have entered Phase I clinical trials.

Attenuated pathogenic bacteria have more diverse applications than GRAS bacteria; for example, they can be used as tumour-targeting carriers and microbot delivery systems (discussed above). The potential to selectively colonize hypoxic areas of tumours that cannot be treated by chemotherapeutic drugs is an exciting therapeutic opportunity, and this could be combined with microbots. Specifically, attaching anticancer drug-loaded nanoparticles to the surface of genetically modified tumour-targeting bacteria represents a novel system that could be used as a dual form of tumour therapy. Imaging agents such as iron oxide could also be attached to the surface, and used for theranostic (that is, diagnostic therapy) applications.

Regarding safety concerns, attenuated bacteria have been proven to have no substantial pathogenicity. For example, repeated administration of attenuated strains of *Clostridium sporogenes* bacteria did not induce a severe host immune response, and systemically administered bacteria were completely removed by treatment with antibiotics[45]. Similarly, the pathogenicity or toxicity of an attenuated strain of *S.* Typhimurium is reduced or eliminated, and this system has demonstrated a good safety profile in Phase I studies in patients with cancer[46]. However, despite having a lack of pathogenicity, the potential immunogenicity of attenuated bacteria should not be ignored; rather, it should be thoroughly investigated before they are considered for clinical applications.

Bioengineered strategies based on viruses

Viral gene vectors. As viruses have naturally evolved into vehicles that efficiently transfer their genes into the host for self-replication, strategies that involve the engineering of viruses into vector systems to deliver specific genes of interest have shown considerable potential (FIG. 1Ba). Adenoviruses, adeno-associated viruses and retroviruses and/or lentiviruses represent some of the most commonly used viral vector systems. Each strain has its own advantages and limitations in terms of transfection efficiency and side effects (that is, induction of immune responses and oncogene activation).

One of the major concerns of viral vector systems is the unwanted side effects that are caused by off-target reactions owing to natural tropism. However, the natural tropism can be redirected by replacing the proteins that are responsible for virus–cell interactions with those from other viruses, or by replacing these proteins with chimeric proteins — a strategy that is referred to as pseudotyping[47]. Anchoring targeting ligands to viral vectors is another strategy that does not alter the integrity of vector structures and offers great flexibility, owing to a range of ligands and corresponding coupling reactions. For example, dendritic cell-targeting ligands such as CD40L have been used to enhance the accumulation of adenoviral vectors in target cells by approximately 10,000-fold compared to non-targeted vectors[48].

Viral vectors can be further engineered for combined photothermal therapy. For example, Everts *et al.*[49,50] attached hyperthermia-inducing gold nanoparticles to adenoviral vectors via covalent conjugation and, following this, they engineered these nanoparticles to re-target a tumour-associated carcinoembryonic antigen without altering the infectivity of the viral vectors. Imaging probes such as iron oxide nanoparticles[51] or quantum dots[52] have also been tagged on viral vectors. This system could potentially be used for gene therapy with simultaneous monitoring.

Virus-like particles. Virus-like particles (VLPs) are self-assembled particles of capsid or envelope proteins that are derived from viruses (FIG. 1Bb). Although VLPs have a homogenous size and morphology[53] they are not infectious, owing to the absence of genetic materials. VLPs offer certain advantages over conventional viral vectors in that they can be easily produced and scaled up at a low cost. Unlike viral vectors, VLPs are resistant to denaturation and harsh purification processes. More importantly, VLPs can carry cargos, which allows them to act as drug carriers.

Empty VLPs were initially developed for vaccination purposes, as an alternative to attenuated live viruses, because their antigenicity is comparable to the parent virus. For example, VLPs that were assembled from the

Photothermal therapy
A novel therapeutic use of electromagnetic radiation (for example, infrared) that is proposed to treat various medical conditions, including cancer, by producing heat to kill target cells.

Capsid
The protein shell of a virus that encloses and protects the genetic material inside the virus.

L1 major capsid protein from HPV subtypes 16 and 18 — the subtypes that cause most human cervical cancers — were produced in recombinant yeast expression systems. The HPV-like particles resembled authentic HPV virions and induced protective immune responses against HPV[54,55]. These safe, well-tolerated and highly immunogenic VLP HPV vaccines were approved by the US Food and Drug Administration for marketing under the trade names Gardasil (Merck) and Cervarix (GlaxoSmithKline). Many other VLP vaccine candidates are also under development.

VLPs can be used as delivery carriers for various cargos including antigens, adjuvants, nucleic acids, and peptides or proteins. Exogenous DNA or oligonucleotides have been packaged into VLPs by osmotic shock[56,57], and peptides and proteins have also been encapsulated into VLPs during *ex vivo* self-assembly of capsid and/or envelope proteins[58]. Attachment of various drugs to VLPs by chemical conjugation has also been used for imaging contrast agents[59], fluorescent dyes[60] and positron emission tomography agents[61]. For example, an antitumour agent, paclitaxel (Taxol; Bristol-Myers Squibb), was conjugated to VLPs that were derived from the bacteriophage MS2; paclitaxel did not compromise capsid functionality[62].

VLPs can provide a range of natural tropisms owing to the diversity of available parental viruses[63]. Some examples of this natural targeting include tropisms of hepatitis B VLPs to the liver[64] and polyoma VLPs to the spleen[65]. In addition to the natural tropisms, VLPs have been engineered to obtain selectivity via chemical conjugation of various ligands such as peptides and antibodies[66–68].

Virosomes. A virosome is a reconstituted virion-like phospholipid bilayer spherical vesicle that contains integrated surface glycoproteins that are derived from viruses with a diameter of 20–150 nm[69,70] (FIG. 1Bc). Virosomes are devoid of both capsid proteins and genetic material. Virosomes are generally produced by detergent solubilization of the influenza virus and subsequent reconstitution with two influenza envelope glycoproteins, haemagglutinin (HA) and neuraminidase (NA). Unlike conventional liposomes, the unique features of these two envelope proteins — HA and NA — provide virosomes with excellent adjuvant properties and the ability to carry various drugs, including antigens and nucleic acids[71]. These glycoproteins are responsible not only for the structural stability and homogeneity of virosomes but also for targeting, receptor-mediated endocytosis and endosomal escape after endocytosis[72,73].

For a long time, virosomes have been studied for the purpose of vaccination, and they have now been successfully adopted as a vaccine delivery system against the hepatitis A virus (Epaxal; Crucell) and influenza viruses (Invivac (Solvay-Influenza) and Inflexal V (Crucell))[74]. They have also gained attention as potential drug and gene delivery carriers, owing to their ease of production and modification as well as their low toxicity. Their *in vivo* applications, however, are limited by the potential risk of immunogenicity. To overcome this obstacle and to reduce off-target effects, virosomes have been modified with polyethylene glycol (PEG) and targeting ligands[75]. In another study, the antitumour agent doxorubicin was effectively delivered to breast cancer cells by virosomes that were modified with a PEG-conjugated, ERBB2 (also known as HER2)-specific antibody[76]. Virosomes have also been used as carriers for siRNAs[77].

Unlike bacteria, all human viruses are pathogens so there are important safety concerns associated with the use of engineered viruses. Therefore, although viral vectors have been extensively investigated as a promising gene delivery vehicle, their clinical applications are often limited to life-threatening diseases owing to potential risks such as immune recognition and mutagenesis. Efforts have been made to improve the safety and efficacy of viral vectors — for example, by using re-targeting technologies such as pseudotyping and surface modifications with targeting ligands, as discussed above. Viral vectors have been well characterized as nanoparticles with a uniform shape, size and modifiable surface properties, and therefore have promising applications — for example, as versatile delivery carriers of tumour therapeutics and imaging agents.

Advantages and limitations. VLPs and virosomes have made substantial progress in vaccine delivery because they are composed of viral components that retain the antigenicity of the parent virus. The major advantages of both systems over viral vectors include ease of fabrication and the ability to scale up at a low cost. In addition, VLPs and virosomes are able to load various exogenous cargos — such as siRNA, nucleic acids, peptides and/or proteins, and antitumour drugs — while sparing the beneficial traits of the parent viruses, such as natural tropisms and modifiable surface properties. Most studies of viral drug carriers, however, have been performed *in vitro*, and their *in vivo* efficacy has not been well established. Although the use of VLPs and virosomes has been approved for application in humans and their practical uses as drug carriers are very promising, the immunogenicity derived from viral components still exists and further modifications are required to adapt VLPs and virosomes for drug delivery applications.

Pathogen-based biomimetic strategies

Pathogen-mimicking vaccines. The poor immunogenicity of soluble antigens has led to the development of antigen-carrying synthetic particles that mimic the structure and/or composition of microbes in a reductionist fashion[78]. The particulate nature of pathogens has an important role in their recognition by immune cells: professional antigen-presenting cells (APCs) that internalize the particle-associated proteins process these antigens for presentation to $CD8^+$ T cells (a process known as cross-presentation) up to 1,000-fold more efficiently than if the same extracellular proteins are internalized in a soluble form[79,80]. In addition, antigen presentation to $CD4^+$ T cells is amplified, which triggers improved T cell assistance for both $CD8^+$ T cell and antibody responses[81,82]. Consequently, polymer particles such as poly(lactic-co-glycolic acid) (PLGA) micro- and

nanoparticles have been developed to enable the continuous release of antigens within APCs, which might sustain T cell priming *in vivo*[83].

The size of pathogens also regulates, in part, their dissemination *in vivo*; viral particles that are small enough to diffuse freely through the extracellular matrix are drained rapidly from the peripheral tissue sites to lymph nodes, where primary immune responses are generated[84]. By mimicking this size-dependent transport process, synthetic polymer particles that carry antigens and are <100 nm in size have been shown to efficiently reach lymph nodes following their injection into the skin, thereby yielding more efficient immune responses[85,86].

The internalization of antigens in a particulate form represents only one of the many factors that dictate the generation of potent immune responses by natural pathogens. Conserved molecular motifs in the structure of microbes (for example, unmethylated cytosine–guanine units (CpG DNA), double-stranded RNA, LPS, flagellin, and so on) are danger signals that are recognized by immune cells, and they engage receptors such as Toll-like receptors (TLRs). Incorporation of defined TLR agonists or other molecular danger signals into antigen-carrying particles mimics the coexistence of both antigens and stimulatory signals that are present in pathogens, and promotes both antibody and cellular immune responses.

Co-delivery of danger signals and antigens to a common phagosomal compartment by antigen-carrying particles has been suggested as a key mechanism by which APCs distinguish harmless environmental antigens from genuine pathogenic threats[89]. Pathogen-derived danger signals have been incorporated into synthetic particulate vaccines via encapsulation or surface immobilization[90–93]. Antigen-carrying particles can also be designed to trigger components of the host response, such as complement[86]. In addition to triggering the activation of pattern recognition receptors, pathogens may be sensed by intracellular sensors of cellular damage and/or stress. It has also been discovered that the disruption of phagosomes and/or endosomes by pathogens induces a complex intracellular signalling network that is known as the inflammasome[94]. Synthetic particles, which include the classic adjuvant alum[95,96] and degradable PLGA particles[97], have the potential to activate this pathway, thus providing a previously unappreciated mechanism for the design of effective vaccine carriers.

Preclinical studies have clearly demonstrated the benefits of pathogen-mimicking vaccines over classical vaccines; these benefits include elevated antibody titres and cytotoxic T cell responses[78,98]. However, this field is in very early stages and only a few studies in non-human primates and Phase I clinical trials have been performed so far; further studies will ultimately determine the value of these approaches for therapeutic or prophylactic vaccination.

Current designs adopt some of the most basic principles of pathogens: antigen presentation from a surface and simultaneous presentation of danger signals. The development of readily translatable synthetic and/or manufacturing schemes and the demonstration of increased efficacy in patients (as opposed to equivalent performance to existing adjuvants such as alum) will be required for these engineered materials to become widely used. However, additional aspects of pathogen-mimicking vaccines remain to be explored, particularly as additional features of host–pathogen interactions are elucidated. Future studies that are focused on developing an in-depth understanding of this landscape will prove to be crucial for the advancement of this field.

Virus-mimicking particles, including effects of shape. An increased understanding of the mechanisms of viral infection has motivated researchers to adapt viral structures and functions towards the design of synthetic drug carriers. Self-assembled liposomes that mimic viral structures have been developed as tumour-targeted gene delivery carriers. Liposomes that are fabricated using lipids, transferrin and DNA have been shown to yield highly compact nanostructures with a uniform size distribution (50–90 nm); these nanostructures are characterized by a multicentre lamellar core structure and a transferrin-coated membrane, thus mimicking the architecture of envelope viruses such as influenza virus or herpesvirus[99]. These virus-mimicking transferrin nanostructures enhanced the efficacy of the delivery of the gene encoding the cellular tumour antigen p53 into human prostate cancer tumours *in vivo*[99].

A pH-sensitive nanogel system that resembles the structural and functional traits of a virus has also been developed[100]. This virus-mimetic nanogel consists of a hydrophobic core and two layers of hydrophilic shells with tumour-targeting ligands (FIG. 2a). The anticancer drug, doxorubicin, was loaded into the hydrophobic core. To mimic the capsid-like structure, PEG was linked to the core polymer (this functioned as the inner shell) and bovine serum albumin was bound to the other end of PEG (this functioned as the outer shell). These virus-mimicking particles were pH-sensitive such that a pH reduction from a physiological (pH 7.4) to endosomal (pH 6.4) level induced reversible swelling of the nanogel and caused an increase in size from 55 nm to 340 nm; this facilitated endosomal escape and the release of doxorubicin into the cytosol. Once the cells were killed by doxorubicin and disintegrated, the nanogels moved to the neighbouring cells and repeated the same cycle, thus replicating the infection cycle of viruses.

As several viruses are filamentous, 'filomicelles' — which are wormlike filamentous micelles — have been synthesized to take advantage of the morphological features of viruses[101] (FIG. 2b). The long and flexible filomicelles that are composed of self-assembling amphiphilic block copolymers effectively evade the reticuloendothelial system (RES), which results in remarkably long blood circulation times (approximately 1 week). Interestingly, the optimal length of filomicelles is similar to the ~8 μm diameter of circulating RBCs; longer filomicelles fragmented rapidly to this size, whereas shorter filomicelles were cleared more quickly. When they were loaded with the widely prescribed anticancer drug paclitaxel, filomicelles also shrank tumours more effectively than either

Reticuloendothelial system (RES). A component of the immune system, which consists of phagocytic cells that are capable of engulfing abnormal cells) and foreign substances. Also called the mononuclear phagocyte system.

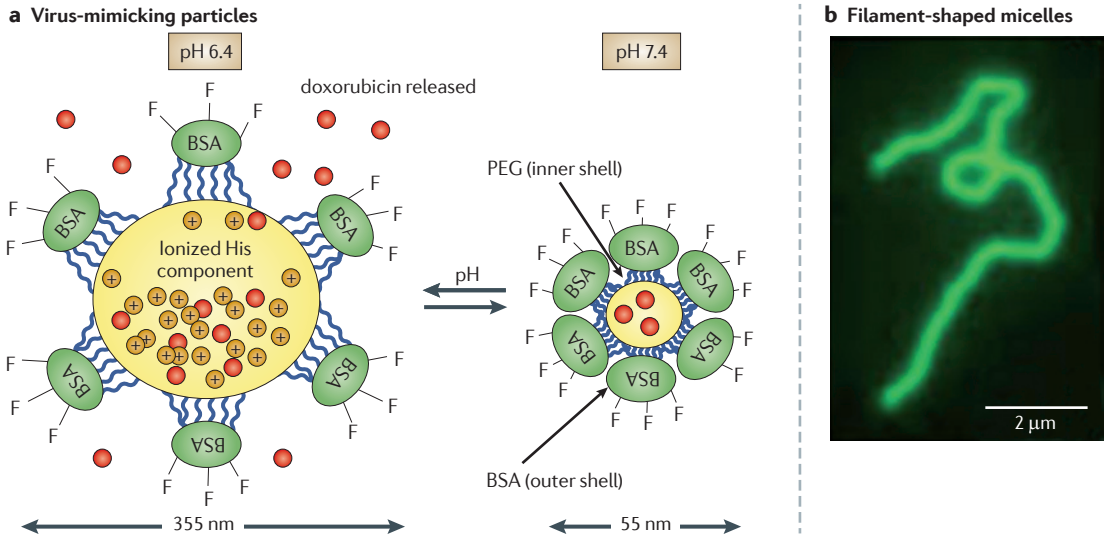

Figure 2 | **Virus-mimicking synthetic drug carriers. a** | Virus-mimicking nanogels[100]: the nanogel system consists of a hydrophobic core and two layers of hydrophilic shells. It resembles the viral capsid structure and acts like a virus as it swells and shrinks repeatedly in response to a change in intracellular pH, to release drugs and kill the cell. **b** | Filomicelles[101]: filament-shaped self-assembled micelles have an exceptionally long circulation time in the blood as they can evade the reticuloendothelial system, and they have been shown to reduce tumour size when they are loaded with paclitaxel. BSA, bovine serum albumin; F, folate; His, histidine; PEG, polyethylene glycol. Image a is reproduced, with permission, from REF. 100 © (2008) John Wiley & Sons, Inc. Image b is reproduced, with permission, from REF. 101 © (2007) Macmillan Publishers Ltd. All rights reserved.

the free drug or spherical nanocarriers. The *in vivo* effectiveness of such carriers with nonspherical shapes is likely to be studied in more depth in the coming years.

Strategies that are based on mimicking the structure of viruses have demonstrated clear advantages over simple particulate carriers. The effect of shape and flexibility on the circulation and targeting ability of filomicelles is very promising. The ability of pH-sensitive nanogels to escape from endosomes and continuously infect adjacent cells is also promising. Overall, these strategies support the advanced engineering — beyond simple surface modification — of viruses into synthetic drug delivery carriers, and they broaden the applications of these virus-mimicking particles in targeted drug delivery. However, these strategies are in early stages and their utility in the clinic is not yet proven.

Bioengineered strategies based on cells

Various types of cells — including RBCs, macrophages, dendritic cells and stem cells — have either been used as drug delivery carriers or they have prompted the design of new drug delivery carriers (FIG. 3).

RBCs. RBCs are the most abundant cells in the human body and they have been widely studied as drug carriers for over 30 years[102]. They have a unique biconcave discoidal shape with an average diameter of 7–8 µm in humans (mouse RBCs have a smaller diameter) and they are highly flexible, which enables them to squeeze through small capillaries (that are ~3 µm in diameter) while maintaining a constant surface area. RBCs have many beneficial features that make them efficient carriers[103]; these include biocompatibility, prolonged circulation (~120 days) and eventual clearance from the blood by the RES. In addition, their large volume (90 μm^3) provides sufficient space for the encapsulation of various types of cargos, including peptides and/or proteins (FIG. 3Aa).

RBCs can be used either for the continuous release of drugs into the circulatory system or for targeted drug delivery to specific organs. Owing to their prolonged circulation time and slow rate of drug release[104], RBCs have been used as drug delivery carriers for various intravenous long-acting drugs, such as antiretroviral drugs[105], antiparasitic drugs[106], antineoplasmic drugs[107], steroids[108] and cardiovascular drugs[109]. Targeting the RES is an important application of carrier RBCs. Membrane damage[110] and opsonization[111] invariably accelerates their recognition by the RES, which may make RBCs suitable for the treatment of macrophage-related hepatic diseases. RBCs have also been used to target organs other than the spleen and liver by photosensitization[112] and incorporation of magnetic particles[113] or antibodies[114].

Owing to the extended circulation times of RBC-bound pathogens, RBCs have been investigated as drug delivery carriers for polymeric nanoparticles to avoid the rapid clearance of these polymeric particles by the RES. It has been demonstrated that polymeric particles that are attached to rat RBCs by non-covalent adhesion remained in the circulation for over 10 hours; when they were not attached to RBCs, the particles were eliminated within a few minutes[115] (FIG. 3Ab). Further improvement of circulation times was also obtained by modifying the surface of particles with PEG[116]. The combination of RBC attachment and additional targeting modifications

on both polymeric particles and RBCs may lead to new opportunities in drug delivery, such as sequential targeting.

RBCs have also been used as delivery carriers of thrombolytic agents. Tissue-type plasminogen activators (tPAs) or recombinant soluble urokinase plasminogen activator receptors have been conjugated to the surface of RBCs *ex vivo*. RBC-coupled tPA circulated in the blood for a tenfold longer period of time than free tPA[117], and pre-injected RBC–tPA complexes in mice showed remarkable alleviation of brain ischaemia and stroke to an extent that could not be attained by injecting free tPA at a dose tenfold higher than that of the RBC–tPA complex[118]. In another study, tPA was directly coupled to circulating RBCs in the blood using a monoclonal antibody against complement receptor type 1, which is expressed on RBCs, and tPA showed similar *in vivo* therapeutic effects compared to those that were exhibited by *ex vivo* RBC–tPA complexes[119].

Although RBCs circulate in the bloodstream for a prolonged period of time and have the capacity to load several drug compounds in addition to carrying particles, RBCs by themselves are not ideal drug carriers owing to their restricted space of activity (that is, within blood vessels). However, this restriction could be an advantage for specific purposes of drug delivery. As shown above, RBC–tPA complexes are ideal carriers for thrombolytic agents that only exert their pharmacological activity in the blood. Likewise, the biomedical applications of RBCs are promising for other blood or endothelium-related diseases.

Macrophages. For a long time it has been proposed that macrophages, which are an essential component of the immune system, can be exploited as carriers for delivering therapeutic cargos, as they have a natural tendency to home in to disease sites in response to signalling molecules such as cytokines and/or chemokines that are secreted from diseased tissues and/or neighbouring blood vessels[120]. In recent years, this concept has developed in conjunction with advances in nanotechnology. As macrophages are able to phagocytose nanoparticles, therapeutic nanoparticles can be loaded *ex vivo* into macrophages. Nanoparticle-bearing macrophages are then re-injected into the body as 'Trojan horse' delivery carriers.

Macrophages have been studied for the delivery of antiretroviral drugs to the targeted disease sites where active HIV-1 replication occurs. Dou *et al.*[121] demonstrated that bone marrow-derived macrophages that carry solid lipid nanoparticles of indinavir (Crixivan; Merck) can accumulate in HIV-infected sites and release this drug via dissolution of the nanoparticles, which is followed by diffusion of the drug out of macrophages. Drug release was observed for over 2 weeks without

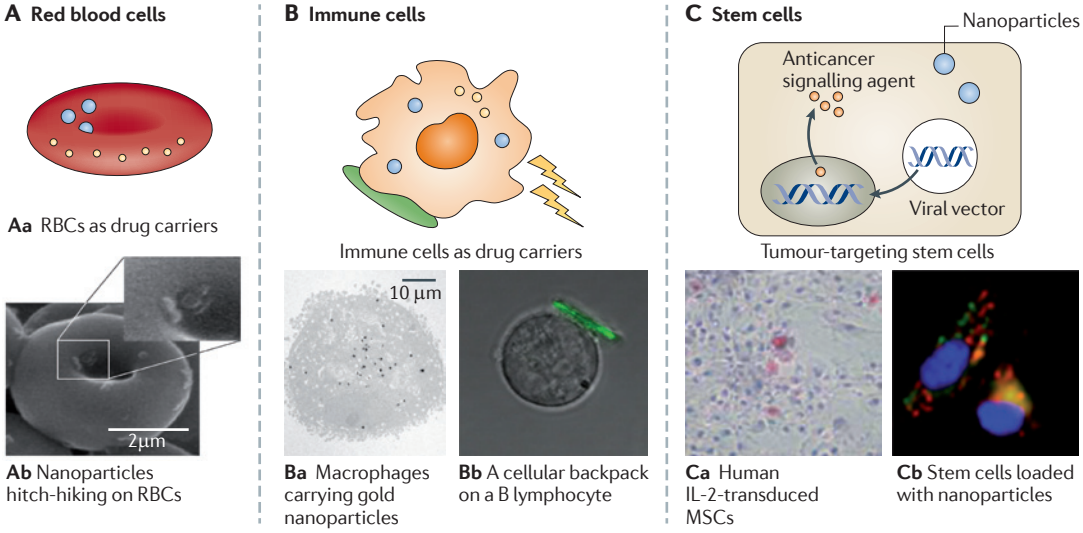

Figure 3 | **Bioengineered eukaryotic cells for drug delivery. A** | Red blood cells (RBCs). **Aa** | RBCs have been used as drug carriers, by encapsulation, immobilization or hitch-hiking. **Ab** | Nanoparticles that hitch-hike on RBCs show prolonged circulation[115]. Nanoparticles that were otherwise eliminated within a few minutes circulated for over 10 hours when they were non-covalently attached to RBCs. **B** | Immune cells. Immune cells have been used as carriers for various cargos. **Ba** | Gold nanoparticles were phagocytosed by macrophages. The gold nanoparticle-bearing macrophages were recruited by solid tumours and used for photothermal therapy[125]. **Bb** | The image shows a cellular backpack on a B lymphocyte[128]. Lymphocyte attachment allows targeted accumulation of particles at tumour sites. **C** | Stem cells. Stem cells have been genetically modified or used as carriers for nanoparticles. **Ca** | The image shows human interleukin-2 (IL-2)-transduced mesenchymal stem cells (MSCs)[139]. **Cb** | The image shows stem cells that have been loaded with nanoparticles (shown in green)[142]. The nanoparticles were internalized by stem cells without affecting cellular functions. Image **Ab** is reproduced, with permission, from REF. 115 © (2004) Elsevier Ltd. Image **Ba** is reproduced, with permission, from REF. 125 © (2007) ACS Publications. Image **Bb** is reproduced, with permission, from REF. 128 © (2008) ACS Publications. Image **Ca** is reproduced, with permission, from REF. 139 © (2004) Macmillan Publishers Ltd. All rights reserved. Image **Cb** is reproduced, with permission, from REF. 142 © (2010) Elsevier Ltd.

any significant toxicity, which increased the therapeutic index[121]. In a related study, migration of macrophages to the HIV-infected blood–brain barrier was observed in mouse models[122]. Based on these findings, macrophages carrying indinavir nanoparticles were used to target the HIV-infected brain[123]. These macrophages successfully passed through the blood–brain barrier and accumulated in infected areas in the brain, where they released antiretroviral drugs for 14 days.

Another application of the 'Trojan horse' macrophage is in targeting hypoxic areas of solid tumours. This characteristic, isolated, non-vascularized hypoxic area of a solid tumour practically restricts the access of chemotherapeutic agents or antitumour drug delivery carriers based on enhanced permeability and retention effects. One of the unique responses of the body to such hypoxic areas is the recruitment of macrophages. Tumour-infiltrated macrophages rapidly differentiate into tumour-associated macrophages (TAMs). Endometrial, breast, prostate and ovarian tumours recruit a high number of TAMs to their hypoxic areas[124]. One example that is based on photothermal ablation therapy has used TAMs as delivery carriers of gold nanoshells[125] (FIG. 3Ba). In another study, TAMs that were associated with cyclodextrin nanoparticles showed an enhanced ability to migrate through the blood–brain barrier to hypoxic areas of brain tumours[126]. Therefore, macrophages constitute promising drug delivery systems for inflammatory diseases such as rheumatoid arthritis[127].

Most studies that have used macrophages as drug carriers have focused on the recruitment of macrophages to targeted disease sites. However, their off-target recruitment has not been well investigated, which is a crucial issue for the clinical applications of carrier macrophages in terms of their side effects. The ideal carrier macrophages will need to either selectively accumulate at diseased sites or selectively exert their therapeutic activities at target sites, and remain inert elsewhere. If it is possible to address these issues, macrophage-based systems are likely to have applications in the treatment of various disorders in which macrophages are known to accumulate at the disease site, including cancer and atherosclerosis.

Lymphocytes. Lymphocytes have also been engineered as therapeutic drug carriers. Cell engineering has also been carried out on lymphocytes by conjugating synthetic drug carriers to the surfaces of T cells or B cells, and thereby using lymphocytes as chaperones for exogenous drug cargos. In the first example of this approach, B cells and T cells were grafted with polyelectrolyte multilayer patches — disc-shaped thin polymer films that are ~300 nm in thickness and several micrometres in diameter — that had a cell-adhesive face to enable cell attachment, termed a 'cellular backpack'[128,129] (FIG. 3Bb). Cell migration was not inhibited by the attachment of these patches.

Stephan *et al.*[130] also demonstrated that T cells and B cells can be modified by surface conjugation of drug-loaded liposomes or polymer particles, using endogenous free thiols at the surface of intact cells as a chemical handhold for particle attachment. Cytotoxic T cells could be conjugated with up to ~100 particles, each one being 200–300 nm in diameter, without interfering with the cell's intrinsic ability to proliferate, kill target cells or home in to tumours *in vivo*. Notably, cell attachment dramatically altered the biodistribution of nanoparticles *in vivo*. Because this approach can be used with numerous drug carriers (including liposomes, polymer particles, and so on), it appears to be promising for applications in adoptive T cell therapy for cancer; this approach is currently in clinical trials in which autologous antitumour T cells are infused into patients with cancer[131,132].

Key challenges in the use of lymphocytes for drug delivery include difficulties in their harvesting and preservation of their integrity. The development of strategies for direct, *in vivo* placement of cellular backpacks on the cell surface may help to address these issues. This could be accomplished by the incorporation of cell-targeting peptides or antibodies in these cellular backpacks. Such advanced cellular backpacks could have applications in the treatment of immune disorders.

Stem cells. Stem cells have been extensively studied as a gene delivery system, especially for cancer therapy. As the process of forming tumour stroma is similar to wound healing, signalling molecules that are secreted from malignant cells mediate the recruitment and proliferation of stem cells, mostly mesenchymal stem cells (MSCs), for tissue construction[133]. Genetic engineering as well as the tumour tropism of stem cells makes it possible for stem cells to express therapeutic gene products that encode antitumour proteins — such as interferons (IFNs) and interleukins — and target tumours (FIG. 3Ca).

Transduced MSCs that express IFNβ have been widely studied for targeted delivery to various tumours. Despite the highly potent antiproliferative and anti-apoptotic activities of this cytokine, *in vivo* applications of IFNβ have been limited owing to its systemic toxicity. However, genetically modified MSCs that produce IFNβ have been successfully integrated into target tumour cells — including breast tumour carcinoma[134], human glioma[135] and prostate cancer lung metastasis[136] — after their intravascular or local administration, which results in extended survival with reduced toxicity in animal models. In addition, other genes that correspond to therapeutic signalling agents — such as IFNα[137], IL-2 (REFS 138,139), IL-12 (REF. 140) and cytosine deaminase[141] — have been inserted into stem cells for targeted cancer therapies.

Another possible use of stem cells is as cellular carriers for nanoparticles. Roger *et al.*[142] have demonstrated that nontransformed, non-immortalized adult human MSCs are able to internalize polymeric and lipid nanoparticles without affecting the viability, differentiation or ability of MSCs to migrate to brain tumours (FIG. 3Cb). Nanoparticle-carrying stem cells are therefore likely to have many biomedical applications, although a more thorough understanding of the mechanisms underlying MSC migration is necessary.

Tumour-associated macrophages
(TAMs). Macrophages that are derived from peripheral blood monocytes and recruited into the tumour stroma. Following their activation, TAMs release various growth factors, cytokines and inflammatory mediators for tumour progression.

Cell-based bio-inspired and -mimetic strategies

Particles that mimic cell morphology and functions. RBC-mimicking biocompatible polymeric particles have been studied[143–145] (FIG. 4Aa,c). Some of these particles were synthesized using layer-by-layer building onto a PLGA template to resemble natural RBCs in size, shape, mechanical flexibility and oxygen-carrying ability[143] (FIG. 4Aa). These polymeric particles were also capable of encapsulating various compounds, including drugs and imaging agents. Haghgooie *et al.*[144] synthesized PEG particles that mimic various aspects of the size, shape and flexibility of RBCs; these PEG particles were capable of passing through thin capillary channels (FIG. 4Ab). In another study, Merkel *et al.*[145] designed RBC-like hydrogel microparticles with tunable elasticity, and they found that circulation time in the blood was greatly increased as the modulus of these particles was tuned to that of RBCs (~26 kPa) or lower (FIG. 4Ac).

In addition to the size, shape and mechanical flexibility of RBCs, a 'marker of self' recognition system has been considered to be a key factor that contributes to the exceptionally long circulation time of RBCs (~120 days). A membrane protein, CD47, that is present on all cells was shown to signal inhibition of the phagocytic activity of macrophages[146]: CD47-knockout RBCs were cleared very rapidly from the bloodstream in normal mice. Given the desire to prolong the circulation of nanoparticles in the blood for applications in nanomedicine[147,148], this 'marker of self' is now being attached to polymer particles. For example, Tsai *et al.*[149] attached a recombinant version of the immunoglobulin-like domain of CD47 to polystyrene particles to control *in vitro* phagocytosis (FIG. 4B). Particle uptake studies showed that CD47 inhibited phagocytosis by human macrophages and monocytes in a dose-dependent manner. Incorporation of such a 'marker of self' into RBC-mimicking systems and many other particles is expected to generally improve immunocompatability *in vivo*.

Platelet-mimicking nanoparticles have also been developed for promoting haemostasis[150]. Synthetic platelets consisting of poly-L-lysine conjugated to PLGA (PLGA–PLL) block copolymers were conjugated to PEG and functionalized by RGD (Arg-Gly-Asp) peptides, which have a specific binding affinity for activated platelets (FIG. 4C). *In vitro* and *in vivo* studies have demonstrated that synthetic platelets are able to adhere to activated platelets at the bleeding site and successfully halt bleeding.

Particles that mimic compartmental cellular architecture. The complex chemistry and function of living cells is facilitated by the organization of the cellular machinery within spatially defined compartments. Initial studies of hierarchical drug carrier structures that mimic, at a simple level, the compartmental organization of eukaryotic cells have been motivated by the goal of designing advanced drug delivery systems that are capable of sequestering diverse compounds within a single particulate carrier[151].

Kisak *et al.*[152] have developed multiple internal bilayer-enclosed compartments, termed 'vesosomes', by exploiting a reversible vesicle-to-bilayer sheet transition (FIG. 4Da). As vesosomes contain multiple non-nested internal compartments, each of which may have distinct membrane compositions that encapsulate drugs, drug cargos must first diffuse through the internal vesicle wall into the cytosol of the vesosome and, following this, they must permeate through the external bilayer, which results in a sustained release profile (over ~10 hours)[153].

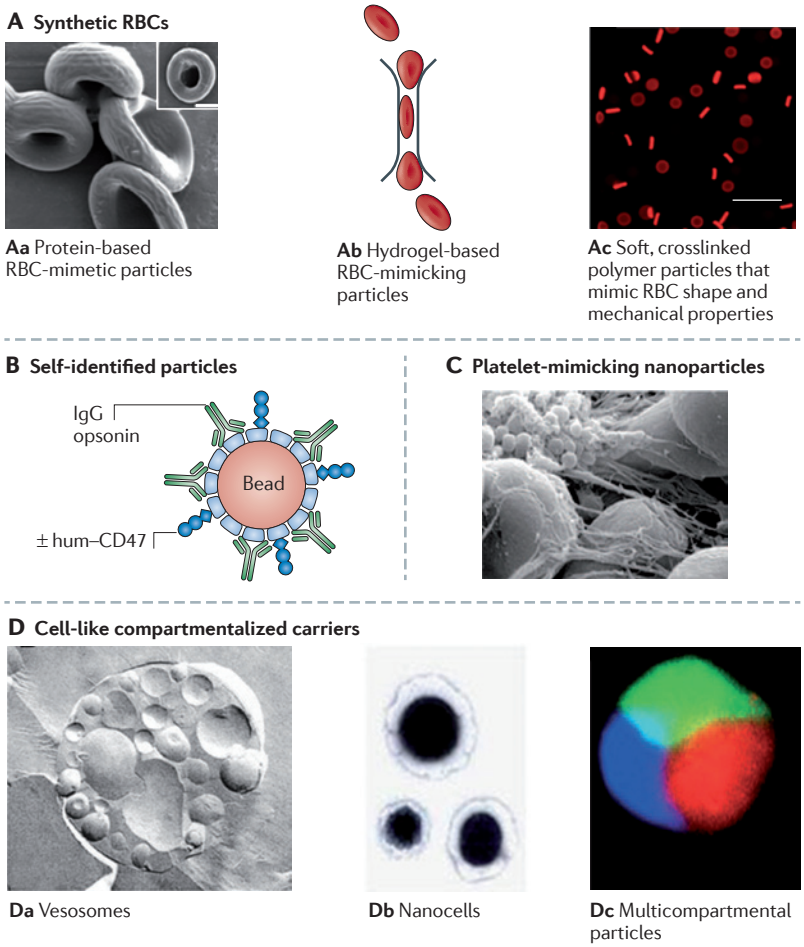

Figure 4 | **Cell-mimicking synthetic drug particles. A** | Synthetic red blood cells (RBCs). **Aa** | Protein-based RBC-mimetic particles[143]. These particles are prepared by layer-by-layer assembly of proteins on polymeric particles. **Ab** | Like their natural counterparts, RBC-mimetic particles flow through small capillaries[144]. **Ac** | The figure shows hydrogel-based RBC-shaped particles that exhibit an elasticity-dependent circulation half-life in the blood[145]. **B** | Self-identified particles. The figure shows a synthetic particle that is coated with a 'marker of self', CD47 (REF. 149). **C** | Platelet-mimicking nanoparticles. The image shows synthetic platelets accumulating within clots in an injured artery[150]. **D** | Cell-like compartmentalized carriers. **Da** | Vesosomes (liposomes within liposomes) have been used for chemotherapy[152]. **Db** | Nanocells (polymer particles within liposomes) have been used for drug delivery[154]. **Dc** | Multicompartmental particles have been fabricated by the electrohydrodynamic co-spinning method[161]. Image **Aa** is reproduced, with permission, from REF. 143 © (2009) The National Academy of Sciences. Image **Ab** is reproduced, with permission, from REF. 144 © (2010) John Wiley & Sons, Inc. Image **Ac** is reproduced, with permission, from REF. 145 © (2011) The National Academy of Sciences. Image **B** is reproduced, with permission, from REF. 149 © (2008) The Rockefeller University Press. Image **C** is reproduced, with permission, from REF. 150 © (2009) The American Association for the Advancement of Science. Image **Da** is reproduced, with permission, from REF. 152 © (2002) ACS Publications. Image **Db** is reproduced, with permission, from REF. 154 © (2005) Macmillan Publishers Ltd. All rights reserved. Image **Dc** is reproduced, with permission, from REF. 161 © (2009) John Wiley & Sons, Inc.

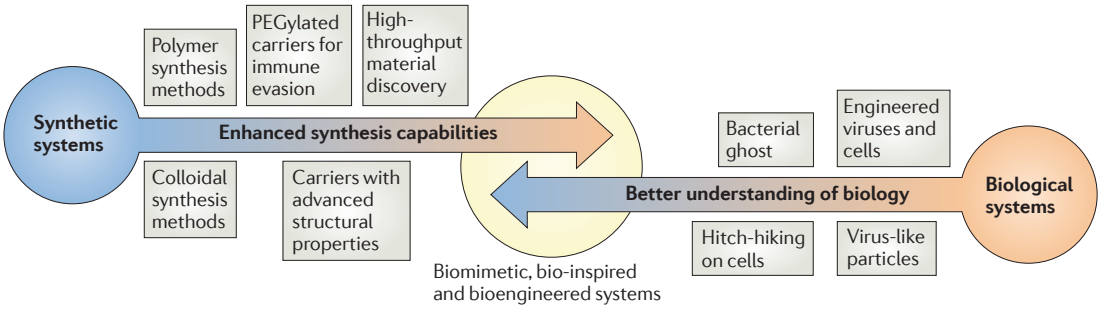

Figure 5 | **Bioengineered, bio-inspired and biomimetic systems.** The gap between synthetic and biological systems has traditionally been very large. However, recent advances in the synthesis of novel materials and understanding of biological systems have paved the way towards bridging this gap. Combining perspectives from the synthetic and biological fields will provide a new paradigm for the design of drug delivery systems. PEG, polyethylene glycol.

To co-deliver two antitumour drugs with distinct release kinetics, hybrid lipid and/or polymer 'nanocells' have been devised, which are based on the encapsulation of biodegradable polymer nanoparticles within lipid vesicles[154] (FIG. 4Db). The nanoparticles were formed from a doxorubicin–PLGA conjugate, whereas an anti-angiogenesis agent, combrestatin, was trapped within the surrounding lipid bilayer compartment. This design aimed for rapid release of the anti-angiogenesis agent from the outer compartment, as these intravenously injected particles accumulated in tumours and stimulated the collapse of blood vessels within the tumours, thus irreversibly trapping the nanocells within the tumour environment. This was followed by a steady release of the cytotoxic doxorubicin cargo from the nuclear compartment of the nanocell, which killed the remaining tumour cells. This two-pronged strategy greatly decelerated tumour growth compared to the single-drug treatment controls. This approach could therefore be extended to several chemotherapeutic drug combinations with known or potential treatment synergy.

Multicompartmental solid particles and micellar structures have also been devised. Multicompartmental micelles are formed by designing block copolymers with multiple distinct block chemistries that assemble to form stable structures in water[155,156]. These structures can be used to sequester multiple drug cargos of distinct physical properties within discrete nanoscale zones of individual micelles. On the micrometre scale, particles with well-defined 'core-shell' structures have been prepared using microfluidic reactors[157,158] as well as emulsion spray-drying strategies[159], which provide concentric compartments for drug loading.

More complex morphologies can be accessed using electrohydrodynamic spray-dying strategies[160,161], in which controlled phase separation in polymer solutions is used to fabricate complex multicompartmental particle structures (FIG. 4Dc). Approaches involving the fabrication of hydrogel particles with defined internal compartments of varying composition and chemistry have been demonstrated using techniques such as continuous- and stop-flow lithography[162,163]; these approaches permit the synthesis of monodispersed microparticles with well-defined internal structures. Although approaches involving engineered micelle and polymer particle structures are generally less advanced towards medical applications than lipid vesicle-based strategies (discussed above), they may offer novel properties that cannot be accessed by simple phospholipid-based materials.

As the importance of carrier properties (such as size, shape, mechanical flexibility, surface property and internal architecture) in particle–cell interactions for drug delivery has been revealed, engineering technologies have advanced and more complex particles have been developed. Researchers have therefore started to take advantage of the morphologies and functions of cells by mimicking their key properties. Accordingly, particles that are capable of partially mimicking known properties of cells have been developed. For example, the size, shape and mechanical properties of RBCs have been combined into biocompatible particles, but their internal structure and CD47 — a pivotal maker for surface recognition — have not yet been combined into a single particle. Key desirable properties of different cells or pathogens can be combined into a single synthetic particle so that tailored drug delivery carriers can be designed and optimized for specific purposes. As our knowledge of known key cellular properties expands, synthetic particle systems that mimic cells will have great potential for future drug delivery carrier systems.

Challenges and future prospects

The development of drug delivery carriers that are based on natural particulates is a rapidly emerging field, which takes advantage of the remarkable delivery mechanisms that are used by pathogens and mammalian cells, such as selective targeting and prolonged circulation by evasion of the immune system. The field of biologically inspired drug carriers is, however, still in its infancy and there are several challenges that need to be overcome.

First, processes that are required for the development of such carriers, such as genetic engineering or *ex vivo* treatments to produce or incorporate therapeutic substances, make it difficult to maintain the integrity of natural particulates, particularly that of eukaryotic cells. For example, the surface integrity that is important for the prolonged circulation of RBCs may be compromised during *ex vivo* engineering, which may result in a more

rapid clearance of the drug from the blood. Further optimization is therefore required to minimize structural alterations and boost delivery.

Second, delivery carriers that are based on pathogens such as bacteria and viruses are potentially immunogenic. A certain degree of immunogenicity can be ideal if pathogen-based carriers are intended for vaccine delivery, owing to their adjuvant ability. However, for applications other than vaccine delivery, the immunogenicity always elevates the safety concerns of pathogen-based carriers. The potential immunogenic components of pathogens therefore need to be removed or inactivated, and their *in vivo* safety should be thoroughly addressed. However, it is worth noting that there are several GRAS bacteria such as food-grade and commensal bacteria, which are free from safety issues and therefore have the potential to proceed to clinical applications until the potential safety issues of other pathogen-based systems can be resolved.

Some of the latest attempts to improve drug delivery have focused on mimicking key attributes of biological carriers, such as physical morphologies (for example, the shape, structure and cellular compartments), self markers (for example, CD47) and molecular danger signals (for example, TLRs), in synthetic systems. Developing a clearer understanding of the delivery mechanisms that are used by biological carriers and improving synthesis techniques that will allow the adoption of this understanding into synthetic systems are necessary to meet the complexity of the requirements (FIG. 5). However, combining the advantages of synthetic systems — such as controllability and mass production — with the extraordinary delivery functions of biological systems has great potential for the advancement of effective drug delivery technologies.

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Competing interests statement
The authors declare no competing financial interests.