In the past three decades since the launch of recombinant human insulin, biopharmaceutical drugs — including peptides, recombinant therapeutic proteins, enzymes, monoclonal antibodies and antibody–drug conjugates — have transformed the pharmaceutical industry. From 1989 to 2012 the number of marketed biotechnology products grew from 13 to 210, while worldwide product sales increased to US$163 billion. Biotech products accounted for 71% of the worldwide revenue generated by the ten top-selling pharmaceuticals in 2012, up from 7% in 2001 (REF. 1). This transformation has similarly affected development pipelines. Over 900 biopharmaceutical products are currently in development, targeting diseases across a wide range of therapeutic areas and representing nearly 20% of the total number of drugs in the pipeline. About 40% of these products are being developed by large pharmaceutical companies. As a class, biopharmaceutical drugs offer the advantages of high specificity and potency compared to small molecules. These features arise from their macromolecular nature, which provides the structural complexity that is often required for specificity. However, this structural complexity also makes them some of the most challenging molecules to formulate and deliver. Indeed, the formulation and delivery issues of biopharmaceuticals were recognized as major potential liabilities from the earliest days of biotechnology, which partly made them less appealing drug candidates. Loss of activity in response to environmental triggers such as moisture or temperature, which can occur during storage or in the body, puts a substantial burden on formulation technologies. The high molecular mass of biopharmaceutical drugs also creates delivery challenges, namely a substantial reduction in permeability across biological barriers such as skin, mucosal membranes and cell membranes, which means that injection is currently the primary mode of administration. Delivery of biopharmaceutical drugs to specific sites — for example, intracellular targets — is also challenging owing to their poor membrane permeation.

In spite of these challenges, novel parenteral formulations and delivery strategies have enabled the launch onto the market of numerous successful products, including products based on luteinizing hormone-releasing hormone (LHRH; also known as GnRH) analogues, such as leuprolide depot (Lupron Depot; AbbVie). This suggests that the advantages of biopharmaceuticals far exceed their limitations. This Review highlights the recent progress and unmet needs in the formulation and delivery of biopharmaceutical drugs. The advances reviewed here suggest that biopharmaceutical drugs will have an even greater impact in the future if the remaining unmet needs are addressed.

Abstract | The formulation and delivery of biopharmaceutical drugs, such as monoclonal antibodies and recombinant proteins, poses substantial challenges owing to their large size and susceptibility to degradation. In this Review we highlight recent advances in formulation and delivery strategies — such as the use of microsphere-based controlled-release technologies, protein modification methods that make use of polyethylene glycol and other polymers, and genetic manipulation of biopharmaceutical drugs — and discuss their advantages and limitations. We also highlight current and emerging delivery routes that provide an alternative to injection, including transdermal, oral and pulmonary delivery routes. In addition, the potential of targeted and intracellular protein delivery is discussed.

In the past three decades since the launch of recombinant human insulin, biopharmaceutical drugs — including peptides, recombinant therapeutic proteins, enzymes, monoclonal antibodies and antibody–drug conjugates — have transformed the pharmaceutical industry. From 1989 to 2012 the number of marketed biotechnology products grew from 13 to 210, while worldwide product sales increased to US$163 billion. Biotech products accounted for 71% of the worldwide revenue generated by the ten top-selling pharmaceuticals in 2012, up from 7% in 2001 (REF. 1). This transformation has similarly affected development pipelines. Over 900 biopharmaceutical products are currently in development, targeting diseases across a wide range of therapeutic areas and representing nearly 20% of the total number of drugs in the pipeline. About 40% of these products are being developed by large pharmaceutical companies.

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Advanced formulations and chemistry

Intravenous, intramuscular and subcutaneous injections are currently the most commonly used ways of delivering biopharmaceuticals. Subcutaneous injections are the most convenient as they require minimal skills and are the least invasive, whereas intramuscular injections
Microparticles. Microparticles are generally used for the long-term delivery (1 week or longer) of proteins, peptides and some small molecules, and are generally administered by intramuscular or subcutaneous injection (TABLE 1). They control both the level of the drug and its lifetime in the body1, and can affect drug pharmacokinetics by enabling sustained release of the drug. Poly(lactic-co-glycolic acid) (PLGA) polymers are the most common material used for the encapsulation of peptides or proteins, as they are mechanically strong, hydrophobic, biocompatible and degrade into toxicologically acceptable products that are eliminated from the body2. Other potentially useful materials include polyanhydrides3 and cyclodextrins4. PLGA polymers have been widely used in marketed products — for example, for delivering risperidone (Risperdal; Johnson & Johnson) to treat schizophrenia5 and naltrexone for treating alcoholism and narcotic addiction6. They have also been used to deliver peptides such as LHRH analogues for treating advanced prostate cancer and endometriosis7, glucagon-like peptides (GLPs) for treating type 2 diabetes8 and proteins such as human growth hormone for treating pituitary dwarfism9 (Supplementary information S1 (table)).

Encapsulation, biocompatibility and protein release from microspheres are closely related to their structural properties. For example, the release rates of proteins depend on the degradation of the polymer and/or diffusion of the protein from the microsphere, which in turn depends on the molecular mass of the polymer and the protein, the lactic/glycolic molar ratio, particle size and the loading of proteins within microspheres, as well as microsphere size and porosity (FIG. 1). The shape of a particle also influences its behaviour, in particular its interactions with macrophages10. Because the diffusion transport of proteins through PLGA is limited, the rate of polymer degradation has an important role in the mechanism and rate of release of the drug from microspheres. In addition, diffusion of the protein through the biological fluid surrounding the microsphere is dependent on the size of the protein, and this will affect the rate of absorption after release11.

The most common method of microencapsulating peptides is through the use of solvent evaporation, but many other approaches such as atomization methods are also used12. The encapsulation of proteins is substantially more challenging, and additional properties of proteins must be considered. Proteins can lose their structure and a covalent dimer formed in a microsphere. The addition of magnesium hydroxide and a covalent dimer formed in a microsphere formulation of darbepoetin alfa (Aranesp; Amgen)15. In addition, the local degradation of polymers at the injection site can lower the pH inside the microsphere, which can further add to the potential for protein inactivation16. The addition of magnesium hydroxide and other antacids within the microsphere has been shown to negate the adverse effects of low pH17.

Although clear advances have been made in the use of microparticles for delivering biopharmaceutical drugs, challenges remain. Specifically, further advances in controlling the burst release can increase the duration of drug release. In addition, methods that provide active control of the rate of drug release, including on-demand termination of release, could open up new opportunities. A reduction in the size of the microsphere (but still within the micron scale) could lead to a subsequent reduction in the required needle size for administration while maintaining the rate of sustained release.
Advantages

- Controlled release can be achieved
- Delivery is possible using subcutaneous injections

Depot injections

- The same parent drugs can be formulated in several dosage forms (such as weekly, monthly, quarterly or semi-annual formulations)
- The delivery technology can be applied to a large number of compounds
- Lower burst release (in implants)
- Averts the requirement for reconstitution and/or suspension (in implants)

Nanoparticles

- Targeted delivery: small size allows enhanced permeation into tumours and retention in tumours
- Has high adjuvancy for vaccine applications

Jet injections

- Allows a needle-free approach
- Has a long history of use
- Works with injectable formulations
- Broad applicability without the need to modify the design of injectors
- Rapid systemic uptake

Pumps

- Precise control over rates of delivery
- Long duration of delivery

Transdermal delivery

- Painless and sustained delivery
- Allows for active control and discontinuation of delivery
- High patient compliance

Pulmonary delivery

- High bioavailability
- Rapid systemic uptake
- Ease of use

Oral delivery

- Ease of use
- High patient compliance

Other mucosal routes (vaginal, nasal and buccal)

- Non-invasive
- Ease of use

Table 1 | Comparison of different delivery technologies for biopharmaceutical drugs*

| Method              | Advantages                                      | Limitations                                      |
|---------------------|------------------------------------------------|-------------------------------------------------|
| Microparticles      | • Controlled release can be achieved            | • Burst release can occur, which causes the      |
|                     | • Delivery is possible using subcutaneous       | potential for local toxicity and wastage        |
|                     | injections                                       | • Burst release can be associated with adverse   |
|                     |                                                 | events related to peak serum exposure           |
| Depot injections    | • The same parent drugs can be formulated in    | • Larger gauge needle can be required            |
|                     | several dosage forms (such as weekly, monthly, | (for injections), or incisions need to be made   |
|                     | quarterly or semi-annual formulations)         | into the skin (for implants)                    |
|                     | • The delivery technology can be applied to a   |                                                 |
|                     | large number of compounds                      |                                                 |
|                     | • Lower burst release (in implants)             |                                                 |
|                     | • Averts the requirement for reconstitution     |                                                 |
|                     | and/or suspension (in implants)                 |                                                 |
| Nanoparticles       | • Targeted delivery: small size allows enhanced | • Non-specific uptake in reticuloendothelial      |
|                     | permeation into tumours and retention in        | system (RES) organs                             |
|                     | tumours                                         | • Immunotoxicity can occur                       |
|                     | • Has high adjuvancy for vaccine applications   |                                                 |
| Jet injections      | • Allows a needle-free approach                 | • Cause occasional pain and bruising            |
|                     | • Has a long history of use                     | • Inconsistent delivery of drug                  |
|                     | • Works with injectable formulations            |                                                 |
|                     | • Broad applicability without the need to modify|                                                 |
|                     | the design of injectors                         |                                                 |
| Pumps               | • Precise control over rates of delivery        | • Implanted devices are invasive                 |
|                     | • Long duration of delivery                     | • Infection can occur with patch pumps          |
| Transdermal delivery| • Painless and sustained delivery               | • Low bioavailability (potentially addressable   |
|                     | • Allows for active control and discontinuation | with advanced technologies)                    |
|                     | of delivery                                      | • Some devices are bulky                         |
|                     | • High patient compliance                       | • Potential for local toxicity and immunogenicity|
| Pulmonary delivery  | • High bioavailability                         |                                                 |
|                     | • Rapid systemic uptake                         |                                                 |
|                     | • Ease of use                                   |                                                 |
| Oral delivery       | • Ease of use                                   | • Low bioavailability (potentially addressable   |
|                     | • High patient compliance                       | with advanced technologies)                     |
|                     |                                                 | • Enzymatic degradation in the stomach or liver |
|                     |                                                 | • Variable absorption                            |
|                     |                                                 | • Interference in absorption rates from food    |
| Other mucosal       | • Non-invasive                                  | • Low bioavailability (potentially addressable   |
| routes (vaginal,    | • Ease of use                                   | with advanced technologies)                     |
| nasal and buccal)   |                                                 | • Enzymatic degradation                         |
|                     |                                                 | • Variable absorption                            |

*The summary presented in this table offers a generalized description of several delivery methodologies. Exceptions to generalized descriptions exist in certain cases and ongoing research is actively addressing the limitations discussed throughout the main text.

Additional unmet needs include the requirement for a robust manufacturing process that can be achieved at a reasonable cost, the need for stabilization and sterilization strategies to be broadly applicable to many biopharmaceutical drugs, and a need for preclinical tools and/or models for addressing the risk of immunogenicity. In addition, it is important to consider issues such as ease of injection — that is, addressing the reconstitution of the lyophilized product and the clogging of needles with drugs. Many of these issues are not unique to protein- and peptide-based drugs, however, and are in fact common to all microsphere-based drugs.

Nanoparticles. Nanoparticles — which are composed of materials such as polymers, lipids and dendrimers — have been actively studied as carriers, especially for targeted delivery of small-molecule drugs. Indeed, several nanoparticles are currently on the market, including doxorubicin liposomes (Doxil; Janssen) for the treatment of ovarian cancer and AIDS-associated Kaposi’s sarcoma, and paclitaxel-containing albumin nanoparticles (Abraxane; Célène) for second-line treatment of patients with breast cancer.

There are fewer examples of the use of nanoparticles for delivering proteins and peptides, and these agents are in the early stages of development. There are several examples of nanoparticle-mediated delivery of small-molecule chemotherapeutic agents at clinical and preclinical stages (reviewed in REF. 28). An example of nanoparticle-mediated protein delivery that shows promise for clinical application is the delivery of cytokines for tumour immunotherapy. Specifically, immunosuppressive factors associated with the tumour microenvironment were targeted using a combination...
Injectable implants of drugs are an alternative to particulate formulations for sustained drug release, and have been used for both small and large molecules. Surgically implantable depots — that is, of a small-molecule transforming growth factor-β (TGFβ) inhibitor and the immunostimulant interleukin-2 (IL-2). Core-shell nanoparticles were developed to encapsulate both the water-soluble protein (IL-2) and the hydrophobic small-molecule cargo (the TGFβ inhibitor) into a single nanoparticle that was designed to enable simultaneous release of both drugs. This method led to sustained local delivery, thereby reducing the toxicity of IL-2, and the combination therapy delayed tumour growth and increased survival in a mouse model of melanoma.

In another example, which aimed to increase the viability and efficacy of a cell-based therapy, sustained pseudoucocrine stimulation was provided to donor cells in a mouse model of adaptive T cell cancer therapy by conjugating adjuvant nanoparticles to the T cell surface. The approach mitigates the dose-limiting toxicities that are associated with conventional approaches to adjuvant administration. Multilamellar lipid nanoparticles were directly conjugated to free thiol groups on the surface of various cell types, including CD8+ T cells. Nanoparticle-tethered T cells trafficked to antigen-expressing tumours as effectively as their untethered counterparts. When the particles were bound to melanoma-specific T cells and injected into mice with established lung and bone marrow metastases, complete clearance of the tumour was achieved.

In general, systemically delivered nanoparticles face several physiological barriers before reaching their target. Technological advances for overcoming these hurdles are important to enable future success in this field; these include pegylation for prolonging the circulation of nanoparticles and enhancing tissue penetration, optimization of particle size and the use of substances to enhance deep tumour penetration of nanoparticles.

Although a limited number of studies can be found on the use of nanoparticles for the delivery of therapeutic proteins, nanoparticles have been actively used for delivering vaccines or as vaccine adjuvants. The primary advantage of nanoparticle vaccines is effective antigen presentation to dendritic cells, which yields a stronger immune response than soluble antigens.

Depot injections. Injectable implants of drugs are an alternative to particulate formulations for sustained drug release, and have been used for both small and large molecules. Surgically implantable depots — that is,
formulations that are too large to be injected by needles — have also been developed for small molecules (reviewed in REF. 37), such as the chemotherapeutic drug bis-chloro-ethylnitrosourea (BCNU). Among biopharmaceutical drugs, an injectable formulation of the LHRH agonist goserelin acetate contained in a PLGA rod (Zoladex; AstraZeneca) is the most commercially successful example (Supplementary information S1 (table)). Other polymers such as hyaluronic acid are also being explored for their use in depot injections48.

Injectable monoliths can circumvent some of the manufacturing complexities and challenges associated with particulate systems, such as the suspension and reconstitution of particulate drug formulations. In addition, the lower specific surface area of monoliths can often result in a reduced burst release of drugs. The use of additional ingredients such as PEG 400 in the formulation has been shown to reduce the initial burst49. Blends of block copolymers and PLGA are also effective in offering prolonged release of protein drug. Injectable in situ-forming depot systems, including those based on injectable solutions of PLGA in sucrose acetate isobutyrate40,41 or N-methyl pyrrolidone42,43, offer similar potential benefits44.

Reverse thermal gelling systems45 are based on polymers that form solutions at room temperature but become gels at body temperature following injection. The in situ-forming system PB1023, which is based on a fusion of the drug cargo GLP1 with elastin-like polypeptide (ELP), recently completed a Phase II clinical trial for the treatment of diabetes46 (ClinicalTrials.gov identifier: NCT01658501). ELPs undergo a phase transition at body temperature to form a depot that leads to sustained release. A single injection of the GLP1–ELP fusion protein reduced glucose levels for 5 days, which is 120 times longer than with GLP1 alone.

Another example of a protein depot injection consists of a conjugate of poly-L-glutamic acid and α-tocopherol, which forms non-covalent complexes with proteins and peptides through electrostatic and hydrophobic interactions. This results in the formation of an in vivo drug depot after injection, with sustained drug release over 1–14 days47. Proof of concept of this proprietary system was shown for a formulation of IL-2 in patients with renal cell carcinoma48, and a formulation of interferon-alpha-2b is currently in clinical trials for the treatment of hepatitis C virus (HCV) infection (Supplementary information S1 (table); ClinicalTrials.gov identifier: NCT01010646). Another example of an injectable depot technology consists of a solution of naturally occurring polar lipids that self-assemble into reversed liquid crystal phases when injected into the body (known as FluidCrystal injection depots)49. This system has been
used for sustained delivery of proteins and peptides, such as leuprolide acetate (CAM2032) and octreotide (CAM2029; orphan designation EU/3/09/645), which are under clinical evaluation for prostate cancer and acromegaly, respectively (Supplementary information S1 (table); see the Camurus website for further information).

The emergence of biosimilars and the availability of analytical technologies with increased sensitivity for the detection of degradation products have resulted in increasingly high standards for the analytical purity of conventional protein formulations, and recognition from the scientific community that these higher standards also need to be applied to sustained-release protein formulations.

Although the stresses imposed on protein drugs through the fabrication, storage and administration of sustained-release formulations have been long appreciated, a better understanding of the molecular basis of protein degradation and aggregation pathways could potentially lead to new stabilization approaches or the design of new delivery systems that are more suited to labile macromolecules. Recent examples include a dendritic polyglycerol nanogel system that was used to encapsulate asparaginase under surfactant-free conditions using hydrophilic components. A pH-sensitive benzacetol crosslinking agent enabled the triggered release of payload under acidic conditions with almost 100% recovery of enzymatic activity compared to a control. Asparaginase that was recovered at the end of matrix degradation after 7 days had the same activity as a control, and its secondary structure was unchanged.

In another example, an alginate depot formulation of a fully humanized immunoglobulin G1 (IgG1) monoclonal antibody was developed to exploit the electrostatic interactions that occur between the protein and the polyanionic gel matrix to enable local sustained delivery. The system was designed to provide a stable pH environment and to avoid protein aggregation at a high payload concentration. In vitro analysis showed 90–100% recovery of intact IgG1, with monomer content >94%. Preliminary evaluation of IgG1 release over 28 days in rats showed bioavailability was comparable to that observed with a conventional solution formulation.

Most depot formulation technologies, as well as the particle technologies discussed above, rely on non-covalent association between the drug and the matrix. Thus, their implementation does not require the creation of a new molecular entity. This could potentially simplify their development owing to the applicability of analytical methods that were developed for conventional formulations of the same drug, as well as preclinical pharmacology and safety data generated with those formulations.

**Types of protein modifications.** Chemical modification with hydrophilic polymers can increase the hydrodynamic radius such that glomerular filtration is reduced or eliminated, thus extending the circulating half-life to a commercially attractive range. There are many examples of pegylated drugs on the market, and alternatives to polyethylene glycol (PEG) — such as silicic acid, as well as the naturally occurring polysaccharides hyaluronic acid and hydroxyl ethyl starch — are currently under preclinical and clinical investigation (Supplementary information S1 (table)).

Approaches that exploit the neonatal Fc receptor recycling (FcRn recycling) pathway have also been developed to increase the half-life of macromolecules (FIG. 4). This approach, which involves the creation of genetic fusions between the macromolecule and the Fc region of IgG, has resulted in many approved products, including the blockbuster etanercept (Enbrel; Amgen) and several candidates that are currently in advanced stages of clinical development (Supplementary information S1 (table)).

Efforts have also been made to extend circulation times by exploiting the favourable circulating half-life of albumin, thus enabling weekly dosing. For example, an albumin fusion of GLP1(7–36) (albiglutide; ClinicalTrials.gov identifier: NCT01098539) was approved by the US Food and Drug Administration (FDA) in April 2014 for the improvement of glycaemic control in adults with type 2 diabetes, and an albumin fusion of factor IXα is in Phase III clinical evaluation for the prevention of bleeding episodes in children and adults with severe haemophilia B (ClinicalTrials.gov identifier: NCT02053792). In addition, an albumin fusion of granulocyte colony-stimulating factor (G-CSF), neugranin, is under development for the treatment of chemotherapy-induced neutropenia (ClinicalTrials.gov identifier: NCT00837265). A fusion of albumin with two different single-chain variable fragments (scFv fragments), which bind to HER2 and HER3, has been created to form a single-polypeptide-chain bispecific antibody that is in Phase II clinical trials for the treatment of certain types of oesophageal cancer (MM-111; ClinicalTrials.gov identifier: NCT01774851). A related approach, which uses chemical conjugation of albumin with a synthetic peptide, has been applied to exendin-4; the conjugate (known as GJC-1134-PC) is under clinical evaluation for the treatment of type 2 diabetes (Supplementary information S1 (table); ClinicalTrials.gov identifier: NCT01514149).

**Advantages and disadvantages of protein modification.** Although pegylation can decrease protein immunogenicity and increase solubility, the principal benefit of this modification is the ability to reduce the frequency of dosing owing to the longer circulating half-life of the drug. The approach provides sustained — but not controlled — exposure; clearance from the body is still exponential, and so a longer duration of exposure can only be attained by increasing the dose. For compounds with large therapeutic indices (which is typical for many biopharmaceutical drugs), the cost of goods
Conjugation approaches include protein modification with polymers such as polyethylene glycol (PEG) and hyaluronic acid. The advantages of the conjugation approach include: the availability of a variety of established chemistries; ease of evaluation at a discovery stage using well-known approaches such as N-hydroxy succinimide or maleimide chemistries; reduction of protein immunogenicity; and a proven history with multiple products. Their limitations include the creation of a new molecular entity, polydispersity and potential immunogenicity of polymers. Fusions offer the advantage of being developed and formulated as conventional protein therapeutics, avoiding additional downstream processing such as encapsulation and associated costs. In addition, there is a proven history of several products based on this approach. Their limitations include the creation of a new molecular entity and the associated safety issues and testing, the possibility of generating an immune response to the modified protein and potential formulation challenges owing to the increased molecular complexity.

Reactions of the immune system to non-native conjugates or fusion structures pose a potential safety issue. In the case of fusions with native human proteins, cross-reactivity with endogenous homologues could pose a long-term safety threat in addition to potentially altering the clearance of subsequent doses. However, for many Fc- or albumin-fusion products this risk has not proven to be detrimental (Supplementary information S1 (table)).
REVIEWS

New developments in protein modification. Early approaches to conjugation (reviewed in REF. 55) illustrated the potential to affect properties such as potency and pharmacokinetics56. More recent advances in long-circulating biopharmaceutical polymer conjugates have increased the precision with which conjugates are defined at a molecular level, either through polymer chemistry or through site-specific conjugation. At its most advanced stage, conjugate research applies the tools of medicinal chemistry by incorporating insights from structure–function studies into the design of the molecule. A study64 of the site-specific pegylated WW domain of the human protein peptidyl-prolyl cis-trans isomerase NIMA-interacting 1 (PIN1)65 showed that conformational stability depends on the molecular mass of PEG; longer oligomers increased the folding rate and reduced the rate of protein unfolding.

Pegylated proteins are generally considered to have reduced immunogenicity compared to their nonmodiﬁed equivalents, and one of the earliest pegylated products, pegademase bovine, exploited the ability of PEG to mitigate the immunogenicity of a bovine enzyme for use as a biopharmaceutical. Nevertheless, the formation of antibodies against PEG itself has recently gained attention; PEG end-group chemistry (that is, the substituent at the end of the PEG molecule) is reported to have a substantial role in determining immunogenicity. The use of hydroxyl-PEG, as opposed to the widely used methoxy-PEG, may offer a safety advantage under some circumstances66,67. These types of insights could lead to the future development of biopharmaceutical conjugates with superior properties. Reversible pegylation has been explored as a way of mitigating the reduction in potency induced by conjugation and achieving greater control of drug concentration over time68. The creation of PEG prodrugs that are minimally or partially active could potentially make it possible to administer higher doses — and thus achieve a longer duration of action — of compounds that have toxicities related to Cmax in their fully active form.

Alternatives to PEG that could offer improvements in chemical definition, biocompatibility or manufacturability have been explored. Among the most advanced are polymers that are based on poly(2-oxazoline). These are synthesized by a technique known as living polymerization, which allows for good control of the molecular definition; hydrophobicity can be tuned according to the composition of the side chains. Amphiphilic block copolymers of poly(2-oxazoline) have been shown to increase the uptake of conjugated macromolecules into cells69.

Alternative approaches to protein modification based on genetic engineering. Lessons learned from the success of pegylation and Fc-fusion-based products have helped guide the application of genetic engineering tools to improve the delivery of biopharmaceuticals. The most commercially successful example, darbepoetin alfa, is a variant of erythropoietin that is genetically engineered to include two additional sialic acid chains70. Introducing non-structured polyamino acid tails on the amino and/or carboxy termini of several therapeutic proteins achieved a PEG-like effect on the hydrodynamic radius and on

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Fusions of recombinant factor VIII’s62 and factor IX’s63, which are currently in advanced development for the treatment of haemophilia (ClinicalTrials.gov identiﬁers: NCT02093897 and NCT02053792, respectively; Supplementary information S1 (table)), are intended for chronic lifelong use, and as such their approval could be an important new milestone in establishing the safety of fusion approaches. The immunogenicity of fusion proteins, however, is an ongoing concern. Many of the approved Fc-fusion drugs (Supplementary information S1 (table)), such as etanercept, which is approved for the treatment of rheumatoid arthritis and psoriasis, are themselves immunosuppressive therapies, whereas others, such as aflibercept (Zaltrap; Regeneron), which is approved for the treatment of metastatic colorectal cancer, have been used for indications where patients’ immune systems are not functioning normally. As a result, effects related to immunogenicity are possibly obscured. The bar for the safety of clotting factors is likely to be higher. Importantly, the Phase III study for a factor IX–Fc fusion protein reported no evidence of immunogenicity63, and the FDA approved this product in March 2014.

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Liquid jet injections subcutaneous space. A wearable infusion pump mechanism to regulate surgical intervention), a control thus avoiding the need for patch pumps have also been developed to achieve more precise control over delivery and have been used to deliver human pharmaceutical drugs, especially insulin pumps. Implantable, patch and microfluidic pumps. Newer, microtechnology-based implantable pumps are lightweight and can be worn discreetly on the skin address the limitations of implantable pumps as they have limited longevity owing to the limited duration of the sterility of the skin. The ultimate goal of an insulin pump is to develop a closed-loop device that offers automated control of blood glucose levels. Robust algorithms that utilize the output of an implanted glucose sensor to determine requisite doses have been developed and are in clinical trials (ClinicalTrials.gov identifiers: NCT01271023, NCT01472406).

Needle-free injectors. Liquid jet injections offer several advantages, including the ability to use currently approved injectable formulations — which will greatly facilitate clinical adoption — as well as the ability to work without electronics and the provision of small portable devices. Liquid jet injectors have been used for mass immunization programmes against infectious diseases, as well as for the delivery of insulin and growth hormones. However, the acceptance of liquid jet injectors has been low owing to occasional pain and bleeding at the site of injection. Therefore, efforts are underway to improve the jet injection process, such as the use of pulsed microjets, variable velocity injectors and feedback-controlled injectors. Although needle-free liquid injectors are the only currently available, broadly applicable, needle-free approach for protein delivery, they have not had a strong impact on the field. Future research must focus on addressing the variability in the amount of drug that is delivered via injection, which originates in part from the variation in skin properties among patients, as well as within different regions of the body. In addition, the devices must be designed so they are inexpensive and sufficiently simple to use.

Alternative routes of drug delivery

Owing to the limitations of injections, alternative delivery routes — including pulmonary, nasooral, transdermal, vaginal and ocular delivery of biopharmaceutical drugs — have been explored to increase patient compliance (Fig. 5). Each route offers its own unique advantages and limitations (Table 1). However, the delivery of biopharmaceutical drugs through these alternative routes is limited by the associated biological barriers, the biophysical properties of which are reviewed in Refs 99,100. Nevertheless, device- or formulation-based approaches have been developed to overcome these barriers.

Pulmonary delivery. To date, most research on the delivery of aerosol formulations has focused on the delivery of small molecules to the lung itself, and has addressed issues such as aggregation of drug particles and decreasing the clearance of drugs by macrophages. Nevertheless, the delivery of protein drugs though the pulmonary route has been achieved. Inhaled insulin received FDA approval in 2006; however, it was discontinued in 2007 owing in part to high costs as well as poor patient uptake of the bulky device. Recent studies have shown that a more compact device can effectively deliver insulin by inhalation in patients with type 2 diabetes, and the product has recently received regulatory approval (see the 27 Jun 2014 press release on the MannKind Corporation website). Although advances have been made in the field of

$K_d$
The dissociation constant; a type of equilibrium constant that characterizes the propensity of a complex to separate reversibly into its constituents.

Implantable pumps

Small devices that can be placed within the body and used to deliver a drug. The pumps carry a drug reservoir (which, in some cases, can be refilled through a port, thus avoiding the need for surgical intervention), a control mechanism to regulate delivery, and the delivery catheter.

Insulin patch pumps

A wearable infusion pump that is attached to the skin and delivers insulin into the subcutaneous space.

Liquid jet injections

A type of injection that enables the delivery of drugs into the skin and subdermal tissues — without using needles — by accelerating a stream of drug solution to high velocities.
pulmonary delivery, unmet needs remain; these include potential safety issues, the limited delivery efficiency associated with inhaled proteins and the ease of use of the product, as well as potentially the relatively short duration of action, which is the same physiological factors that enable the drug to have a quick onset of action also make sustained delivery challenging.

**Transdermal administration.** Transdermal patches offer a painless alternative to injections; however, their applications are generally limited to low-molecular-mass hydrophobic drugs. An in-depth review of transdermal drug delivery can be found in [Ref. 103]. Several methods have been developed to temporarily disrupt the skin structure to deliver macromolecular drugs, including the use of peptides such as TD-1 [Ref. 104], SPACE (skin-permeating and cell-entering) peptides[105] and cell-penetrating peptides such as polyarginine[106]. TD-1 and SPACE peptides were discovered specifically to penetrate skin using in vivo phage display and effectively delivered large molecules such as insulin[107] and hyaluronic acid[108] into mice. Several chemical-based formulation strategies[109] have also been developed to enhance transdermal drug delivery; however, their clinical applications are generally limited to small molecules.

Devices that are based on the use of ultrasound, electric fields or microneedles have been particularly effective in increasing the permeability of the skin[109]. Ultrasound and electric-field-based methods such as iontophoresis and electroporation have been used to deliver biopharmaceutical drugs — including insulin[110,111] and parathyroid hormone[112] — in animal models. Neither method is currently used in the clinic for large molecules, but ultrasound and iontophoresis have been approved by the FDA for the delivery of small molecules in a clinical setting[113]. Microneedles can penetrate into skin as far as the epidermis and thereby enhance drug penetration without causing pain. Microneedles have successfully delivered insulin, vaccines and parathyroid hormone in clinical studies[98], and are proving to be particularly appealing for the delivery of vaccines as they have been shown to induce immune responses that are comparable to or better than intramuscular injections in mouse models[114,115]. Microneedles are also being developed for the systemic delivery of parathyroid hormone and are in clinical studies of parathyroid hormone-related protein (ClinicalTrials.gov identifier: NCT01368796). Microneedles have been approved by the FDA for the delivery of small molecules, including insulin[116]. Microneedles can be used for the delivery of vaccines; however, they have also been used for the delivery of parathyroid hormone and are in clinical studies of parathyroid hormone-related protein (ClinicalTrials.gov identifier: NCT01368796).

Collectively, the technologies described above have enabled progress in the transdermal delivery of proteins over the past two decades. There are several future challenges in this field; the complexity of the devices, including the size and required expertise, can make their adoption challenging, and so the device-based methods must be simple to use. At the same time, the
bioavailability of the drug must be sufficiently high to make the technology economically feasible. Finally, the variability of drug delivery must be sufficiently reduced to allow the administration of drugs that have a narrow therapeutic window.

**Oral delivery.** Oral delivery offers increased patient acceptance, but the oral delivery of macromolecules is limited by enzymatic degradation in the gastrointestinal tract and limited permeation across the intestinal epithelium. In addition, the mucus layer on the epithelium provides a barrier for the diffusion of macromolecules. These limitations lead to poor drug absorption into systemic circulation and insignificant bioavailability of macromolecules.

Several absorption enhancers, including fatty acids, surfactants and bile salts, have been tested for their ability to improve the oral delivery of macromolecules in animal models. Sodium caprate enhances the delivery of macromolecules such as oligonucleotides and low-molecular-weight heparin in animal models and humans. Another absorption enhancer is tetradecylmaltoside, which also enhances the uptake of low-molecular-weight heparin. The screening of a large library of chemicals and chemical mixtures to identify compositions that enhance the uptake of peptides and proteins led to the identification of the zwitterionic surfactant dimethyl palmitoyl ammonio propanesulfonate (PPS), which enhanced the intestinal permeation of calcitonin in mice. Other methods of increasing absorption include pH-sensitive hydrogels to protect the drug from low pH in the stomach, chitosans and chitosan derivatives to induce mucoadhesion of the drug carrier and increase intestinal permeation, and acrylate-based polymers to increase mucoadhesion.

Device-based approaches are also being actively pursued for oral drug delivery. Mucoadhesive devices that mimic the structure of skin patches have been tested for the oral delivery of insulin and calcitonin in rodent models. These devices continue to be researched and developed, but have yet to be tested in humans. The current design of these mucoadhesive devices is based on layered structures that comprise a mucoadhesive layer that attaches and retains the device on the mucous membrane. This design creates a depot of high-concentration drug on the surface of the intestine, which enhances the concentration gradient across the intestine and increases oral drug bioavailability.

The use of nanoparticles, which are absorbed across the intestinal epithelium, has also been explored for the oral delivery of biopharmaceuticals. The uptake, which can be mediated by Peyer’s patches, M cells and enterocytes, depends on several parameters, including the size and surface chemistry of nanoparticles. Although unmodified particles are known to permeate the epithelium, targeted nanoparticles further enhance uptake. In this regard, several targeting ligands — including lectin, the M cell-targeted monoclonal antibody and the Fc region of antibodies — have been explored for the oral uptake of nanoparticles in mice. Further testing of such novel technologies in larger animal models is necessary to understand their clinical potential.

The ability of penetration enhancers, nanoparticles and mucoadhesive devices to deliver insulin, calcitonin and other biopharmaceutical drugs has been established in small animals. Oral delivery of proteins and peptides, however, has had limited clinical success. Clinical studies have shown oral delivery of salmon calcitonin using the permeation enhancer 5-CNAC but the clinical programme was terminated owing to lack of efficacy in Phase III clinical trials (see the 14 Dec 2011 press release on the Emisphere website). Permeation enhancers from the same family have also been shown to enhance the oral uptake of heparin in humans. Another enhancer, sodium caprate, has been shown to increase the delivery of oligonucleotides in humans and of low-molecular-weight heparin in rats. Advances have also been made using other medium-chain fatty acids as enhancers to enable the oral delivery of peptides, especially octreotide.

Another technology that is based on the use of protease inhibitors and an absorption enhancer has been tested in humans for the delivery of insulin. Orally delivered insulin reduced the frequency of high glucose readings (>200 mg per dl) when compared to control. The same technology has also been tested in humans for delivery of the GLP1 analogue exenatide. Orally delivered exenatide increased peak plasma insulin levels by 28% in healthy individuals. A technology based on the use of citric acid (which has proteolysis-inhibiting properties) has also been tested for the oral delivery of salmon calcitonin for the treatment of postmenopausal osteoporosis in women; oral calcitonin improved lumbar spine bone mineral density by about threefold compared to placebo.

In spite of substantial research over the past two decades, the goal of oral protein delivery remains difficult. Limited bioavailability and variability in absorption remain general challenges in the field. The appeal of oral protein delivery, however, has not diminished. Future research must focus on enhancing bioavailability to economically feasible levels, which will be different for each drug. In addition, issues related to the variability in absorption and interference from food must be addressed. Other delivery routes that are not discussed in this Review include the buccal, nasal, vaginal and ocular routes.

**Targeted and intracellular delivery.** Although the focus of biopharmaceutical delivery has largely been on systemic delivery, targeted and intracellular delivery are important goals. The local delivery of conventional formulations is the simplest option for targeting specific tissues — for example, the intravenetically administered vascular endothelial growth factor (VEGF) inhibitors bevacizumab, ranibizumab (Lucentis; Genentech/Novartis) and afibercept (Eylea; Regeneron) for the treatment of neovascular age-related macular degeneration. Controlled-release depot formulations provide more consistent local exposure, which is important for the delivery of growth factors for tissue engineering (box 2).
The active targeting of biopharmaceuticals to specific tissues following systemic administration using ligands of, or antibodies against, cell surface receptors is an attractive concept. Recent advances with antibody–drug conjugates and a small-molecule nanoparticle formulation of docetaxel enabled preferential delivery of cytotoxic agents to cancerous tissue; this could potentially increase the therapeutic index and enhance the efficacy of such cytotoxic drugs. The extension of this approach to the delivery of macromolecular cargo is, however, in the early stages of development.

Interest in the intracellular delivery of biopharmaceuticals in part originates from recent advances in RNA interference (RNAi), where small interfering RNA (siRNA) must overcome the cellular barrier to access the RNAi machinery; these advances have focused attention on the potential opportunity for developing protein and peptide drugs against intracellular targets. Several technologies have been developed to deliver siRNA to the skin, liver, brain and tumours, as well as other tissues. siRNA delivery technologies are not discussed here; see REF. 150 for an overview of the field.

**Targeted delivery to the brain.** The blood–brain barrier poses a unique challenge for the targeted delivery of biopharmaceuticals to the brain (FIG. 7). Nevertheless, there has been growing interest in this area, which has been fuelled in part by the therapeutic promise seen in the peripheral administration of antibodies for the treatment of neurodegenerative disease. Antibodies are attractive owing to their high specificity and reduced off-target effects, and peripherally administered monoclonal antibodies result in measurable, albeit low, central nervous system exposure that probably results from a nonspecific mechanism of uptake across the blood–brain barrier. For example, systemically circulating IgG levels in the CNS are 0.1% of their levels in plasma.

Neurodegenerative disease is an area of particular focus, in part owing to the difficulty of developing CNS-penetrant, specific, small-molecule therapeutics. An antibody inhibitor of β-secretase, the enzyme that processes amyloid precursor protein (APP), reduced peripheral and CNS amyloid-β concentrations in mice and in non-human primates following systemic administration. An antibody against α-synuclein, which accumulates abnormally in certain forms of dementia, alleviated behavioural deficits in an α-synuclein transgenic mouse model when dosed systemically, trafficking and histology studies showed that the antibody crossed into the CNS and resulted in reduced levels of α-synuclein aggregates in the cortex and hippocampus.

Antibodies targeted to reduced tau pathology and delayed the onset of motor function decline in transgenic mouse models when delivered peripherally and reduced cognitive impairment when delivered by intracerebroventricular infusion. Several antibodies against amyloid-β have been evaluated in the clinic for the treatment of Alzheimer’s disease, most notably bapineuzumab (ClinicalTrials.gov identifier: NCT01254773) and solanezumab (ClinicalTrials.gov identifier: NCT01900665). Although both recently failed to meet their primary end points in several Phase III trials — calling into question the amyloid-targeted approach to treating Alzheimer’s disease — passive immunotherapy for the treatment of neurodegenerative disease remains an active area of investigation. A humanized IgG4 antibody binding to semaphorin 4D attenuated disease severity in rodent models of multiple sclerosis, including chronic experimental autoimmune encephalomyelitis (EAE), including chronic experimental autoimmune encephalomyelitis (EAE). The antibody is being evaluated in a Phase I trial for safety and tolerability in patients with multiple sclerosis (ClinicalTrials.gov identifier: NCT01764737).

The above developments have increased interest in approaches to enhance the efficiency of biopharmaceutical drug delivery to the CNS. Local administration, active transport and conjugate approaches are being evaluated. The CNS exposure of systemically administered idursulphaspe, a lysosomal enzyme that is approved for the treatment of Hunter’s syndrome (a lysosome storage disorder), is insufficient to treat the cognitive impairment associated with the disease. Intrathecal administration via intracerebroventricular and lumbar routes resulted in widespread distribution of the drug in brain parenchyma, including its deposition in neuronal and oligodendrocyte lysosomes in normal non-human primates. The clinical evaluation of intrathecal...
Box 2 | Biopharmaceutical drug delivery and its relationship with tissue engineering

Engineered tissues and artificial organs have an important role in maintaining and improving the quality of life of patients suffering from illnesses and injuries; for example, skin substitutes and cartilage replacements have already helped thousands of patients, and several other tissues such as the liver, cornea, and spinal cord are being studied in animals or in clinical trials. Engineered tissues may have a role in the assessment of drug toxicity in the form of organs on a chip. There are several forms of engineered tissues, including cell aggregates, three-dimensional polymer cell constructs, decellularized tissue constraints and entire functioning organs.

The delivery of proteins has a major role in tissue engineering by providing controlled and localized release of cytokines, growth factors and nucleic acids to improve cell survival or otherwise contribute to tissue growth. The spatiotemporal control of the release of growth factors and cytokines can be crucial for tissue growth. These factors also stimulate delivered cells to proliferate or perform specific functions, such as recruiting immune cells to the site of implantation of the engineered tissue. In addition, the delivery of growth factors that promote vascular neoogenesis is essential for tissue growth. Various approaches have been developed to encapsulate and release proteins, peptides and nucleic acids from matrices that are used for tissue engineering. These delivery approaches include matrix embedding, encapsulation of drug-loaded microparticles and the immobilization and covalent attachment of drugs to the scaffolds.

dosed idursulphase in conjunction with its systemic administration is underway for the treatment of paediatric patients with Hunter’s syndrome and the associated cognitive impairment (ClinicalTrials.gov identifiers: NCT00920647, NCT02055118).

Several groups have explored approaches to increase penetration within the brain or reduce the frequency of administration relative to what is achievable with local administration of conventional formulations. Following intracranial infusion in a non-human primate, ultrasound increased the distribution in the brain of liposomes and model compounds with a high molecular mass. Encouraging results were reported when depot formulations of epidermal growth factor were administered epicotically in a mouse model of stroke, and when brain-derived neurotrophic factor was incorporated in polymeric implants and injected into the rat hippocampus for evaluation in a model of depression.

Active transport utilizes the internalization of receptors with high expression levels in the brain, such as the transferrin and insulin receptors. The approach has long been considered to be an attractive option for increasing the uptake of drugs into the brain following systemic administration. The fusion of the lysosomal enzyme arylsulphatase A with an insulin receptor-targeted IgG increased uptake to 1–2% of the injected dose in a non-human primate model. In another study, a bispecific antibody was designed to increase CNS exposure via transcytosis by combining high-affinity binding to the therapeutic target (the enzyme β-secretase) with low-affinity binding against the transferrin receptor. When the binding affinity of the transferrin receptor was reduced, brain uptake was maximized and brain distribution improved. Although promising, the approach resulted in acute clinical signs (including profound lethargy and spastic movements in some animals) and a reduction in reticulocytes in mice. These effects were partially mitigated by removing the Fc effector function from the antibody.

In a related approach, a single transferrin receptor-binding Fab was fused to the Fc region of an amyloid-β-targeted monoclonal antibody, which increased target engagement in a mouse model of Alzheimer’s disease by over 55-fold. Last, receptor-mediated transcytosis via the low-density lipoprotein receptor-related protein 1 (LRP1) is being evaluated for the delivery of antibodies to treat cancer. Preliminary data suggested that ANG4043, a HER2-targeted monoclonal antibody conjugated to a peptide that binds LRP1, increased survival in mice with intracranial BT-474 breast tumour cells. ANG1005, a related molecule using LRP1 for the delivery of paclitaxel, is being evaluated in patients with breast cancer who have recurring brain metastases (ClinicalTrials.gov identifier: NCT02048059).

Conjugated forms of several biopharmaceutical drugs have improved brain penetration compared to their unmodified counterparts when administered intrathecally or systemically. For example, chemical modification of leptin with poly(ethylene oxide)-poly(propylene oxide) block copolymers improved the delivery of leptin across the blood–brain barrier when injected intravenously in a mouse model. This strategy is potentially attractive for the treatment of leptin resistance (which is attributed to impaired leptin transport) and the consequent obesity. The approach resulted in a modest (less than twofold) increase in uptake with less than 0.5% of the injected dose detected in the brain, but therapeutic levels were nevertheless achieved. Improved pharmacokinetics of the conjugates may have been responsible for the sustained pharmacological effect observed.

As a second example, intrathecal administration of a PEG conjugate of fibroblast growth factor 2 (FGF2) in a blend of hyaluronan and methylcellulose (HAMC) to rats resulted in enhanced penetration in spinal cord tissue. Finally, when amphiphilic block copolymers of poly(2-oxazoline) were conjugated to superoxide dismutase 1 (SOD1), there was increased uptake of the conjugate into MDCK cells and Caco-2 cells. The conjugate also accumulated in mouse parenchyma by a non-saturable mechanism following intravenous administration. The self-association of the SOD1 conjugates to form aggregates of 8–20 nm (depending on the composition of the side chain) may have affected their uptake into neurons; this effect was reported to occur through a lipid raft- or caveolae-dependent mechanism.
**Intracellular delivery.** Owing to their hydrophilicity and poor membrane permeability, biopharmaceuticals have generally been restricted to targets in the vascular compartment or on the surface of cells — often referred to as the ‘accessible target space’. Various approaches have been undertaken to expand this space to include intracellular targets that were traditionally considered to be druggable only by small-molecule therapeutics, or not druggable at all [FIG. 7]. A second area of potential opportunity for intracellular delivery involves human gene therapy. Current approaches to targeted genome editing require the intracellular delivery of nucleases or genetic material that encodes them (such as a plasmid DNA or mRNA).

One strategy for accessing intracellular targets focuses on altering the permeability of a biomolecule through the cell membrane. The ability of proteins and peptides to permeate the cell membrane can be enhanced through chemical conjugation or mutagenesis. Stapled peptides, which are a class of helical peptides that are modified by the introduction of aliphatic bridges to enhance cell penetration, are potentially promising as mitochondria-targeted agents for the treatment of cancer. A stapled BCL-2 homology 3 (BH3) peptide was shown to bind to intracellular BAX, a mult domain pro-apoptotic BCL-2 family protein, and to overcome apoptotic resistance in mouse models of haematological cancers. Stapled peptides that were designed for the therapeutic modulation of protein–protein interactions involving the intracellular transcription factor Notch had antiproliferative effects in a mouse model of T cell acute lymphoblastic leukaemia. Moreover, a potent stapled peptide that was a dual inhibitor of MDM2 and MDX restored p53-dependent cell cycle arrest and apoptosis in p53-positive mouse xenografts.

Zinc finger nucleases are fusions of the nonspecific cleavage domain of the FokI endonuclease and zinc finger proteins that are custom-designed for genome editing applications, and they were recently reported to undergo cellular uptake in culture in an unmodified form; this was attributed to their net positive charge. In a related approach, macromolecular cargo have been fused to proteins engineered with a high net positive charge (so-called ‘supercharged proteins’) and shown to penetrate mammalian cells; proof of concept was demonstrated by in vivo delivery to mouse retina of Cre recombinase that was fused to a supercharged green fluorescent protein.

Internalizing cell-surface receptors can offer access to intracellular drug targets or an avenue for increasing the efficiency of delivery, in a manner similar to the active transport approaches to CNS delivery discussed above. The conjugation of a zinc finger nuclease with transferrin via a reducible disulphide linkage resulted in rapid and efficient uptake in several cell lines, including primary mouse and human haematopoietic stem cell progenitors. The approach was reported to match the efficiency of cDNA approaches but with improved temporal control of nuclease activity.
When internalization occurs via a vesicular mechanism, the endosomal membrane presents an additional delivery barrier to accessing the cytosol. Various approaches have addressed this challenge. A polymeric system that incorporates a pH-responsive endosomolytic feature has been developed for the intracellular delivery of several biomolecules. Intracellular delivery of a BH3 peptide with the polymeric system increased apoptotic activity in a HeLa cervical cancer cell line, providing an alternative to the stapled peptide approach discussed above. The system was also evaluated for use as a prophylactic tumour vaccine by intracellularly delivering a model protein antigen to potentially access the cytosolic major histocompatibility class I (MHC-I) antigen presentation pathway and enhance the CD8⁺ cytotoxic lymphocyte response. This resulted in increased tumour-free survival in a mouse xenograft model when the model protein antigen ovalbumin was incorporated in the polymeric carrier.

**Concluding thoughts**

The impact of biopharmaceutical drugs over the past decade has been facilitated by technological breakthroughs associated with manufacturing, formulation and delivery. Although outstanding progress has been made in the discovery and delivery of biopharmaceutical drugs, challenges and unmet needs remain. Current biopharmaceutical drugs are primarily aimed at extra-cellular targets owing to the challenges associated with intracellular delivery, and so improving intracellular delivery could result in new classes of biopharmaceutical drugs, including drugs against new intracellular targets.

Another area for future research is the development of new delivery devices for biopharmaceutical drugs that could overcome the hurdles that cannot be addressed by new formulations. Devices that make use of non-parenteral routes are especially appealing owing to their non-invasive nature. Efforts should be focused on making the devices safer and simpler to use. Perhaps one of the most exciting future opportunities lies in the expansion of the therapeutic target space. This will be enabled by new delivery technologies that allow access to sites (intracellular targets and the local tissue environment, such as the nervous system) that are not routinely available to injected molecules and can thus pave the path to unexplored therapeutic opportunities. Future research should focus on the discovery and development of methods that enable intracellular delivery.
REVIEWS

39. Chalilabor, Z., Korb, M. & Bodmeier, R. Immunogenicity and release properties of poly(lactide-co-glycolide) implants prepared by hot-melt extrusion. Pharm. Res. 27, 571–579 (2010).

40. Okumu, F. W. et al. Sustained delivery of human growth hormone from a novel gel system SAB-ABE (factor VIII) 503–509 (2002).

41. Pechon, S., Shenoy, B., Yang, M. X., Basu, S. K. & Margolin, A. Injectable controlled release formulations incorporating protein crystals. J. Microencapsul. 14, 149–158 (2004).

42. Brodieck, K. J., Pushpala, S. & McHugh, A. J. Sustained release of human growth hormone from PLGA depot injections. Pharm. Res. 16, 1825–1829 (1999).

43. Ravivarapu, H. B., Moyer, K. L. & Dunn, R. L. Sustained suppression of pituitary-gonadal axis with LAR: randomized, open-label, Phase I, repeat-dose study in healthy volunteers. J. Med. Chem. 49, 1138–1150 (2006).

44. Lundstrom, K. Review on Medusa: a polymer-based antigenic formulation. Biomacromolecules 5, 157–166 (2004).

45. Chan, Y. P., Meyrueix, R., Kravtzoff, R., Nicolas, F. & Zentner, G. M. Biodegradable dendritic polyglycerol nanogels by hot-melt extrusion. Biomaterials 26, 1699–1711 (2005).

46. Misko, P. T., Basu, S. K., Brodieck, K. J., Pushpala, S. & McHugh, A. J. Injectable controlled release technology: a clinical overview. J. Control Release 123, 3–17 (2007).

47. Wang, J., Wu, K. L. & Dunn, R. L. Sustained delivery of human insulin. A placebo-controlled trial using sustained-release implants of insulin in T1DM patients. Diabetes Technol. Ther. 10, 1058–1070 (2008).

48. Chen, M., Gupta, V., Anselmo, A. C., Muraski, J. A. & Ashley, G. W. Predictable and tunable half-life of antibodies induced by methoxyPEG-proteins. Bioconjug. Chem. 24, 243–252 (2013).

49. Cleland, J. L., Shore, C. R. & Kipnes, M. S. A placebo-controlled single ascending dose study of Phase 1 safety, tolerability, pharmacokinetics and pharmacodynamics of VRS-859 in patients with T2DM (Poster). Diabetologia 54, S318 (2011).

50. Cleland, J. L. Long-acting human growth hormone fusion protein (VRS-317): enhanced in vivo potency and half-life. J. Pharm. Sci. 91, 2744–2754 (2002).

51. Yuen, K. C. et al. A long-acting human growth hormone with delayed clearance (VRS-315): results of a double-blind, placebo-controlled, single ascending dose study in normal adult men. J. Clin. Endocrinol. Metab. 98, 2595–2603 (2013).

52. Zalevsky, J. et al. Enhanced antibody half-life improves in vivo viral protection. Nature Biotechnol. 28, 157–159 (2010).

53. Suzuki, T. et al. Importance of neonatal FCR in regulating the serum half-life of therapeutic proteins containing the Fc domain of human IgG1: a comparative study of the affinity of monoclonal antibodies and Fc-fusion proteins to human neonatal Fc receptor. J. Immunol. 184, 1968–1976 (2010).

54. Yeung, Y. A. et al. Engineering human IgG1 affinity to human neonatal Fc receptor: impact of affinity improvement on the pharmacokinetics of recombinates. J. Immunol. 182, 7665–7671 (2009).

55. Andersen, J. T. et al. Structure-based mutagenesis reveals the albumin-binding site of the neonatal Fc receptor. Nature Commun. 3, 610 (2012).

56. Sleep, D., Cameron, J. & Evans, L. R. Albumin as a versatile platform for improving the half-life extension of proteins. J. Controlled Release 179, 526–534 (2013).

57. Kermode, M. Unsafe injections in low-income country health settings: need for injection safety promotion to prevent the spread of blood-borne viruses. Health Promot. Int. 19, 95–103 (2004).

58. Hirose, M., Beverly, E. A. & Weinger, K. Quality of life and technology use in children and families with diabetes. Curr. Diab. Rep. 12, 711–720 (2012).

59. Ricotti, L., Assaf, T., Dario, P. & Menciassi, A. Wearable and implantable pancreas substitutes. J. Artif. Organs 16, 9–22 (2013).

60. Papapgyri, P. et al. An observational 7-year study of continuous subcutaneous insulin infusions for the treatment of type 1 diabetes mellitus. Endocrinol. Nutr. 61, 141–146 (2013).

61. Schaperlyck, P. et al. Advances in pump technology: insulin patch pumps, combined pumps and glucose sensors, and implanted pumps. Diabetes Metab. 37, S85–S90 (2011).

62. Richards Grayson, A. C. et al. Multi-pulse drug delivery from a resorbable polymeric microchip device. Nature Mater. 2, 767–772 (2003).

63. Farrant, S. & White, S. In vivo characterisation of a wirelessly controlled drug delivery microchip. Sci. Transl. Med. 4, 132ra21 (2012).

64. Ziser, K. Clinical update on optimal prandial insulin dosing using a refined run-to-run control algorithm. J. Diabetes Sci. Technol. 3, 487–491 (2009).

65. Engwiler, E. E., Abbink, E. J., Tack, C. J. & de Galan, B. E. Improved pharmacokinetic and pharmacodynamic profile of rapid-acting insulin using needle-free jet injection technology. Diabetes Care 34, 1804–1808 (2011).

66. Baxter, J. & Mitragotri, S. Needle-free liquid jet injections: mechanisms and applications. Expert Rev. Med. Devices 3, 565–576 (2006).

67. Jackson, L. A. et al. Safety and immunogenicity of varying dosages of trivalent inactivated chicken flu vaccine administered by needle-free jet injectors. Vaccine 19, 4703–4709 (2001).

68. Arora, A. et al. Needle-free jet injection of macromolecules across the skin by nanoliter-volume pulsed microjets. Proc. Natl Acad. Sci. USA 104, 4297–4300 (2007).

69. Staackiw, J. C., LeH, T., H. A., Mitragotri, S., & Fletcher, D. A. Dynamic control of needle-free jet injection. J. Control Release 135, 104–112 (2009).

70. Taberner, A., Hogan, N. C. & Hunter, I. Needle-free jet injection using real-time controlled linear Lorentz-force actuators. Med. Eng. Phys. 34, 1228–1235 (2012).

71. Edwards, D. A. & et al. Large porous particles for pulmonary drug delivery. Science 276, 1868–1871 (1997).

72. Pesch, P. et al. Salmon calcitonin nasal spray treatment for postmenopausal women after hip fracture with total hip arthroplasty. J. Bone Miner. Res. 26, 243–252 (2005).

73. Whitehead, K., Shen, Z. & Mitragotri, S. Oral delivery of macromolecules using intestinal patches: applications for insulin delivery. J. Control Release 98, 37–45 (2004).

74. Mitragotri, S., Blankschtein, D. & Langer, R. Ultrasound mediated transdermal protein delivery. Science 269, 850–855 (1995).

75. Alba, N., Naik, A., Guy, R. H. & Kalia, Y. N. Effect of charge and molecular weight on transdermal peptide delivery by iontophoresis. Pharm. Res. 22, 2069–2078 (2005).

76. Lennernas, H. Intestinal permeability and its relevance for absorption and elimination. Xenobiotics 37, 1015–1051 (2007).

77. Wertz, P. W. Current understanding of skin biology pertinent to skin penetration: skin biochemistry. Skin Pharmacol. Physiol. 26, 217–226 (2013).

78. Rosenstock, J. et al. Safety and efficacy of inhaled human insulin (exubera) during discontinuation and readministration of therapy in adults with type 2 diabetes: a 5-year randomized controlled trial. Diabetes Technol. Ther. 11, 697–705 (2009).

79. Ziser, H. et al. Technosphere insulin effectively controls postprandial glycaemia in patients with type 2 diabetes mellitus. Diabetes Technol. Ther. 14, 997–1001 (2012).

80. Prasunzit, R. & Langer, R. Transdermal drug delivery. Nature Biotech. 26, 1261–1268 (2008).

81. Chen, Y. et al. Transdermal protein delivery by a coadministered peptide identified via phage display. Nature Biotech. 24, 455–460 (2006).

82. Hsu, T. & Mitragotri, S. Delivery of siRNA and other macromolecules to cells using a peptide enhancer. Proc. Natl Acad. Sci. USA 108, 15816–15821 (2011).

83. Rosenthal, J. et al. Conjugation of arginine oligomers to cyclopertin A facilitates topical delivery and inhibition of inflammation. Nature Med. 6, 1255–1257 (2000).

84. Chen, M., Gupta, V., Anselmo, A. C., Muraski, J. A. & Mitragotri, S. Topical delivery of hyaluronic acid into skin using SAP-PEC peptide carriers. J. Control Release 173, 67–74 (2014).

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Investigation on the synergistic effect of a combination of chemical enhancers and modulated iontophoresis

In combination with other techniques, such as nanotechnology, microneedle vaccination with chemical enhancers can achieve therapeutic levels of the antigen in the skin. This approach is particularly useful for vaccines that require a strong immune response. The use of chemical enhancers to improve the uptake of antigens is an active area of research, with several promising candidates for future clinical trials.

Mimetic delivery of HSN1 influenza virus-like particles to the skin induces long-lasting B- and T-cell responses in mice. Clin. Vaccine Immunol. 17, 1381–1389 (2010)

Poly(ethylene glycol) modification enhances intranasal delivery of low molecular weight heparin by reducing postglacial sodium caprate excitation in a porcine model. In: Ninth Annual Diabetes Technology Meeting, 5–7 November 2009, San Francisco. J. Diabetes Sci. Technol. 4, A71 (2010)

Yale, T. A., Thuringer, J., Thuringer, F., and Thuringer, G. Development of anti-HIV-1 antibodies in two transgenic models: reduction of F40 pathology and delay of disease progression. J. Biol. Chem. 286, 1456–1465 (2011)

Sajadi, M. N., Jabbari, H., and Jabbari, A. Inhibition of amyloid-β production by the use of engineered nucleases for genome modification. Nucleic Acids Res. 41, 3617–3627 (2013)

Khan, S. A., Khan, M. A., and Khan, A. Anti-tau antibodies that block tau aggregation and normalize disease-related biomarkers in vitro. J. Neurochem. 126, 201–211 (2013)

152. Yanamandra, K. et al. Anti-tau antibodies that block tau aggregation and normalize disease-related biomarkers in vitro. J. Neurochem. 126, 201–211 (2013)

153. Khan, S. A., Khan, M. A., and Khan, A. Anti-tau antibodies that block tau aggregation and normalize disease-related biomarkers in vitro. J. Neurochem. 126, 201–211 (2013)

154. Yanamandra, K. et al. Anti-tau antibodies that block tau aggregation and normalize disease-related biomarkers in vitro. J. Neurochem. 126, 201–211 (2013)
Biotechnol. Bioeng. 48, 4812–4829 (2010).

Different monoclonal antibodies.

J. Pharm. Sci. 94, 11234–11238 (1995).

Aggregation.

Carpenter, J. F. Hydrogen bonding between sugar and dextran on protein stability and conformation induced protein unfolding. Biophys. J. 106, 1763–1770 (2014).

Jiang, P. et al. Effective targeting of the tumor microenvironment for cancer therapy. Anticancer Res. 32, 1205–1212 (2012).

Domansky, K. et al. Perfused multiwell plate for 3D liver tissue engineering. Lab. Chip 10, 51–58 (2010).

Sasaki, S. et al. In vivo evaluation of a novel scaffold for artificial corneas prepared by using ultrahigh hydrostatic pressure to decellularize porcine corneas. Proc. Natl Acad. Sci. USA 99, 3024–3029 (2002).

Wagner, I. et al. A dynamic multi-organ-chip for long-term cultivation and substance testing proven by 3D human liver and skin tissue co-culture. Lab. Chip 13, 3538–3547 (2013).

Orlando, G. et al. Production and implantation of renal extracellular matrix scaffolds from porcine kidneys as a platform for renal bioengineering investigations. Ann. Surg. 256, 365–370 (2012).

Mountzouris, P. M. et al. Effect of temporally patterned TNF-a delivery on in vitro osteogenic differentiation of mesenchymal stem cells cultured on biodegradable polymer scaffolds. J. Biomater. Sci. Polym. Ed. 24, 1794–1815 (2013).

Huang, Y. C., Simmons, C., Kaigler, D., Rice, K. G. & Mooney, D. J. Bone regeneration in a rat cranial defect with delivery of PEI-condensed plasmid DNA encoding for bone morphogenetic protein-4 (BMP-4). Gene Ther. 12, 418–426 (2005).

Cao, L. & Mooney, D. J. Spatiotemporal control over growth factor signaling for therapeutic neovascularization. Adv. Drug Deliv. Res. 59, 1340–1350 (2007).

Silva, E. A. & Mooney, D. J. Spatiotemporal control of vascular endothelial growth factor delivery from injectable hydrogels enhances angiogenesis. J. Thromb. Haemost. 5, 590–598 (2007).

Brunger, J. M. et al. Scaffold-mediated lentiviral transduction for functional tissue engineering of cartilage. Proc. Natl Acad. Sci. USA 111, E798–806 (2014).

Ravichandran, R., Sundarrajan, S., Venugopal, J. R., Mukherjee, S. & Ramakrishna, S. Advances in polymeric systems for tissue engineering and biomedical applications. Macromol. Biosci. 12, 286–511 (2012).

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Competing interests statement

The authors declare competing interests: see Web version for details.

FURTHER INFORMATION

Emisphere — 14 Dec 2011 press release (“Salmon Calcitonin for Osteoarthritis and Osteoporosis”): http://www.emisphere.com/salmon_calcitonin.html

MannKind Corporation — 27 Jun 2014 press release (“MannKind Corporation Announces FDA Approval of AFREZZA®: A Novel, Rapid-Acting Inhaled Insulin for the Treatment of Diabetes”): http://www.news.mannkindcorp.com/phoenix.zhtml?c=147953&p=irol-newsArticle&ID=2041339

SUPPLEMENTARY INFORMATION

See online article: S1 (table)

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