1. Introduction

1.1 Antibody structure and function relevant to therapeutics
Antibodies or immunoglobulins constitute a large fraction of soluble glycosylated, highly diversified and specialised immune system-modulating proteins, found circulating in serum (~ 25 mg/ml) and bound to cell surfaces, and are the major agent of humoral immune protection in mammals. Major classes of normal antibodies produced in humans include (from most abundant to least, respectively) immunoglobulins IgG, -A, -D, -E and -M and their subclasses. Differences in the antibody heavy chain C-terminal sequences in their respective Fc regions distinguish each class of antibodies: IgG contains γ chains, IgM ε chains, IgA α chains, IgD δ chains and IgE ε chains. Light chains, comprising λ and κ peptide subunits that, in endless possible amino acid combinations within three hypervariable regions in the variable domain (Fv) antigen binding sites, allow combinatorial tailoring of antibody–antigen specificity through their pairing with three antigen binding loops on the heavy chain, forming unique complementarity-determining regions (CDRs) from the six combined hypervariable loops in the Fv heterodimer. Four subclasses of IgG are found in humans (IgG14), differing in segmental flexibility and capacity to trigger immune effector functions (1,2).
Antibody binding specificity to antigenic epitopes distinguishes the antibody function from all classes of drugs and receptor-ligand interactions. Target specificity and affinity are two critical properties that facilitate innate antibody immune responses, as well as permit therapeutic exploitation. Endogenous epitope equilibrium binding constants of micromolar for polyclonal antibodies, versus subnanomolar, for monoclonal variants, are commonly observed in the presence of numerous interferants (serum proteins, small molecule solutes). Antibody–antigen binding results in physical changes (membrane patching, capping, agglutination) and biochemical signals (receptor-mediated cell-based effector responses) responsible for both natural and therapeutic actions [2].

### 1.2 Therapeutic mechanisms

Antibody therapeutics are designed to select desired target antigenic epitopes, binding specifically to these antigens in order to produce direct and desired therapeutic actions [4,5,7-10]. Antibodies against numerous microbial, cellular and soluble targets produce and exploit the various effector functions for therapeutic purposes. Antimicrobial action can be elicited by antiviral binding and neutralisation against transfection, or antibacterial effects through either neutralising (lethal) binding or opsonisation, promoting Fc receptor-mediated phagocytosis and clearance [3]. Cancer therapy is often produced by antibody-promoted destruction of tumour cells by either direct cell targeting (e.g., anti-major histocompatibility complex binding and natural killer cell activation, 131I-Lym-1 [OncolytumTM; Peregrine]) or direct antigen receptor cell signalling death independent of effector mechanisms (apolizumab [RemitogenTM; Protein Design Labs]) or indirect 'starvation' by destruction of vasculature feeding tumours (e.g., anti-integrin, anti-VEGF (vascular endothelial growth factor) antibodies (bevacizumab [AvastinTM; Genentech]), receptor-induced cell apoptosis [11] or antibody-mediating toxic delivery (calicheamicin-conjugated anti-CD33, gemtuzumab ozogamicin [Mylotarg®; Celltech/Wyeth]). The most recent therapies use radiolabelled tumour-specific antibodies (e.g., ibritumomab tiuxetan [Zevalin®; IDEC] and tositumomab [Bexxar®; Corixa/GlaxoSmithKline]) to irradiate tumour sites upon binding.

Although therapeutic forms of other antibody types are possible (e.g., IgA is increasingly popular [4,12-14] and IgM has been reported [15]), IgG idiotype are currently the most attractive antibody therapeutic agents, probably due to several different factors unique to IgG shown in Box 1.

Target specificity, protein stability and binding site affinity are important innate antibody traits exhibited in therapeutics. However, higher affinity is not necessarily reflected in improved efficacy. In vivo antibody affinities are typically $K_d \sim 10^{-9}$ M [18]. Recent work with high-affinity anti-anthrax recombinant single-chain antibody fragments correlates monoclonal antibody (mAb) affinity with protection against anthrax infection in mice [19]. However, high-affinity binding does not always correlate with therapeutic efficacy, particularly in solid tumours. On binding, therapeutic antibodies must extensively
penetrate the target tumour tissue to effect comprehensive target neutralisation, immune system elimination or destruction [20]. Although conventional wisdom claims that high-affinity binding does not allow adequate antibody tissue penetration and tumour site saturation [20], this has recently become controversial following analysis of more extensive antibody antitumour data using theoretical models [21]. Affinity is important to specificity and tumour retention, but possibly not as simply as previously thought; low-affinity antibodies penetrate tumours, but bind poorly and produce low-grade effector functions, whereas high-affinity antibodies do penetrate tumours, while retaining high specificity and high effector efficiencies. Newer engineered diabodies [4-6] exhibit extended tumour site retention, with higher tumour: blood ratios than single-chain analogues with lower affinities [22]. In addition, therapeutic antibodies in antimicrobial applications must be selected for neutralising titres versus simply antibody binding or opsonisation (blocking) titres [23]. These titres are distinctly different between viral and bacterial pathogen targets and therapeutic predictions from in vitro binding or neutralisation titres are not straightforward [24,25]. High antiviral efficacy against respiratory syncytial virus (RSV) using palivizumab (Synagis\textsuperscript{TM}; MedImmune) [26] can be compared with failed therapy trials using the high-affinity anticytomegalovirus human antibody, sevirumab (Proovir\textsuperscript{TM}; Protein Design Labs) [27], attributed to the inability of sevirumab to manifest therapeutic antiviral neutralisation in vivo, as also demonstrated for anti-HIV mAbs [28]. Finally, circulation half-lives for antibodies can be crudely tailored: Fc domain-containing antibodies exhibit the innate FcRn receptor retention mechanism that maintains circulation far longer than engineered antibody fragments lacking an Fc region and, hence, this route to retention [29]. In addition, murine and other xenogeneically sourced antibodies exhibit shorter circulating half-lives in humans than humanised forms. Some therapies require long antibody circulating half-lives, versus others (e.g., radio- or toxin-labelled antibodies) where rapid clearance might be therapeutically desired. Covalent conjugation of the water-soluble polymer modifier polyethylene glycol (PEG, process known as pegylation) is known to increasing the circulating half-lives of whole antibodies and their fragments [30]. Glycosylation patterns are also recognised to influence protein circulating half-life and target efficacy including Fc receptor interactions [4]. In addition, polysialic acid conjugation has been reported to improve the circulating half-life for protein therapeutics, although as yet, this has not been achieved for antibodies or their fragments [31]. Antibody–albumin conjugation or affinity binding in serum using chemical or genetic (fusion protein) methods also prolongs protein circulating half-life in serum [32,33]. New bispecific diabodies have recently been used in approach to bind endogenous serum IgG, invoke intrinsic FcRn receptor rescue pathways and extend the circulating serum half-life [34]. An interesting result showing mAb therapeutic antitumour potency enhancement in mice lacking the FcγRIIB inhibitory receptor indicates that blocking inhibitory effectors, or selectively targeting Fc-activating receptors, could increase therapeutic indices in certain tumours [95]. Hence, antibody bioavailability (circulating kinetics and clearance dynamics) is possible to control, using antibody engineering and bioconjugate chemistry to alter antigen specificity, interactions with effector components, effector functions and dosing regimens.

1.3 Serum-pooled antibodies in immunotherapy

Immunotherapy, the use of exogenously introduced antibodies to prevent or mitigate disease, dates back to the nineteenth century [35-37]. This clinical practice typically uses intravenous doses of human-derived (or infrequently in antiserum, xenogeneically sourced) antibodies, usually pooled from several to thousands of individuals, and containing significant amounts of non-specific antibodies and other proteins, to improve the immune response of the host at a time of deficiency. Intravenous immunoglobulin (IVIg) is an FDA-approved therapy comprising human polyclonal antibodies pooled from thousands of human serum donors and administered to thousands of humans annually, most of whom suffer from immune deficiencies resulting from disease (e.g., AIDS) or adjunct to other therapy (chemotherapy, transplant immunosuppression) [38-42]. Advantages of the IVIg treatment include wide population diversity of antibody families against many possible targets and compatibility with clinically standard drugs in tandem use (e.g., antibiotics, diuretics, sedatives, analgesics) [7,43,44]. Disadvantages include a large nonspecific antibody component with possible side effects and no rational efficacy against disease. This dilutes effector function from antibody fractions specific to desired targets and effectors. Despite a strong, scientifically sound rationale for use [38,42,43], cost, inconsistent efficacy and questions surrounding reliability, toxicity and safety as a pooled human blood-derived protein product [46-49] plague the clinical acceptance of IVIg as an antibody therapy.

1.4 Bioengineered and artificially-selected monoclonal humanised antibodies

Ehrlich’s early conception of the therapeutic ‘magic bullet’ [50], and subsequent heavy biomedical exploitation of antibodies isolated to specific therapeutic sites for this targeting role over 100 years have dominated perceptions in therapeutic utility of antibodies in antibody-conjugated drugs targeted directly to disease sites. In this capacity, the antibody is simply a reliable delivery vehicle and a carrier, an important function still exploited in mAb therapeutic applications using radio-isotope (\(^{90}\)Y, \(^{131}\)I) tags [51] or conjugated toxins (e.g., Mylotarg, ricin A chain fragments and diphtheria) [7,52] to selectively destroy cell targets. However, use of non-native, bioengineered or artificially-selected, purified mAbs as new drugs, not necessarily involved in either drug delivery or traditional immunotherapy is an emerging opportunity in protein-based therapeutics [48,53,54]. In this new role, the antibody frequently exerts intrinsic pharmacology at a specific target site without a drug payload [45,8,9]. Targeting cell surface receptors critical to cell signalling,
Box 2. Therapeutic applications of antibodies in clinical trials.

- Transplant rejection
- Rheumatoid arthritis
- Crohn's disease
- Non-Hodgkin's lymphoma
- Breast cancer
- Leukaemia
- Respiratory syncytial virus
- Herpes simplex virus
- HIV and its secondary complications
- Osteoporosis
- Asthma
- Bacterial infections
- Arthritis
- Colon cancer
- Stroke
- Psoriasis

targets (e.g., TNF-α in sepsis or rheumatoid arthritis) [66], by genetically combining variable domains with human antibody base molecules. Early 'chimeric' mAbs comprising larger murine fractions (~33%) from complete mouse variable antigen-binding domains produced human antichimeric antibody reactions [69]. Newer chimeric antibodies grafting only murine CDR regions to human clones (e.g., 'humanised' trastuzumab [Herceptin™; Genentech, Inc.]) eliminate this adverse reaction [8]. Other novel 'de-immunising' approaches for designing and constructing mAbs have been recently reviewed [9].

1.5 Therapeutic antibody production economics

At the present time, mAb therapies represent ~16% of the value of the total global biotechnology industry with approximately one of every five drugs in clinical trials being antibodies. Nonetheless, production issues remain extremely important to mAb therapeutic potential and clinical utility. With the possibility of >100 therapeutic antibodies approved by 2010, production capacity will be severely strained, unless antibody dosing requirements (pegylation [50], local delivery), therapeutic antibody alternatives (fragments, mimics) or production methods (transgenics) are innovated. Production of mAbs for all purposes currently exceeds 500 kg/year. Several methods are now available: cell culture, bacterial and fungal culture, transgenic animals and transgenic plants. Since therapeutic whole antibody efficacy, linked to bioavailability, clearance factors and particularly for mAbs in tumour therapy, is frequently linked to proper antibody glycosylation [4,5], mammalian cell culture systems (e.g., Chinese hamster ovary [CHO] cells) are used primarily because they reliably glycosylate recombinant antibodies. Interestingly, antibody engineered fragments (Fab or [Fab']₂ fragments [5]), or antibody diads [6], are expressed in their fully functional form from Escherichia coli without apparent glycosylation requirements. This may be due to the elimination of Fc domain-associated glycosylation that may elicit specific abnormal effector (receptor-mediated and complement) functions.

Most recombinant antibodies are expressed in the range 10–2000 μg/l in various bioreactor cultures, making production issues a challenge. This comes at a cost (Genentech's CHO cell fermentors, 2 g/l yield, cost US$333/g of antibody), limitations in scalability (to 50,000 l) limit production to 500 kg/year of antibody and there is a risk of contamination. Successful genetic manipulation techniques are not only essential for humanisation and target affinity selection, but also to the ease and reliability of mAb biopharmaceutical production. Genencor's Asperillus fungal expression system has shown much higher yields possible (40 g/l but not with mammalian protein expression), less expensive fermentation, robust at high volumes (>50,000 l) and no animal virus contamination problems, but is anticipated to exhibit post-translational glycosylation problems. Transgenic approaches offer, so far unproven, opportunities for substantial cost reduction (estimated US$100/g, eventually to US$1/g according to optimistic speculation) [70]. Proving product equivalence to mammalian cell
Table 1. FDA-approved marketed therapeutic monoclonal antibodies (2004).

<table>
<thead>
<tr>
<th>Product</th>
<th>Company</th>
<th>Year approved</th>
<th>Type of molecule</th>
<th>Cell line</th>
<th>Production method</th>
<th>Indications</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT*3 (muromonab-CD3)</td>
<td>Ortho Biotech</td>
<td>1986</td>
<td>Murine</td>
<td>Not applicable</td>
<td>Ascites</td>
<td>Organ transplant</td>
</tr>
<tr>
<td>Reopro* (abciximab)</td>
<td>Centocor</td>
<td>1994</td>
<td>Chimeric</td>
<td>rSP2/0</td>
<td>Perfusion</td>
<td>Prevention of complications from coronary intervention and angioplasty</td>
</tr>
<tr>
<td>Panorex* (17-1A)</td>
<td>Centocor</td>
<td>1995 (Germany only)</td>
<td>Murine</td>
<td></td>
<td></td>
<td>Colorectal cancer</td>
</tr>
<tr>
<td>Rituxan* (rituximab)</td>
<td>IDEC</td>
<td>1997</td>
<td>Chimeric</td>
<td>rCHO</td>
<td>Suspension</td>
<td>Non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>Zenapax* (daclizumab)</td>
<td>Protein Design Labs</td>
<td>1997</td>
<td>Humanised</td>
<td>rNS/0</td>
<td>Suspension</td>
<td>Kidney transplant rejection</td>
</tr>
<tr>
<td>Herceptin* (trastuzumab)</td>
<td>Genentech</td>
<td>1998</td>
<td>Humanised</td>
<td>rCHO</td>
<td>Suspension</td>
<td>Metastatic breast cancer</td>
</tr>
<tr>
<td>Synagis™ (palivizumab)</td>
<td>MedImmune</td>
<td>1998</td>
<td>Humanised</td>
<td>rNS/0</td>
<td>Suspension, fed batch</td>
<td>Prevention of respiratory syncytial viral disease</td>
</tr>
<tr>
<td>Simulect* (basiliximab)</td>
<td>Novartis</td>
<td>1998</td>
<td>Chimeric</td>
<td>Recombinant myeloma</td>
<td>Suspension</td>
<td>Kidney transplant rejection</td>
</tr>
<tr>
<td>Remicade* (infliximab)</td>
<td>Centocor</td>
<td>1998, 1999</td>
<td>Chimeric</td>
<td>rSP2/0</td>
<td>Perfusion</td>
<td>Colorectal cancer, rheumatoid arthritis</td>
</tr>
<tr>
<td>Mylotarg™ (gemtuzumab ozogamicin)</td>
<td>Celltech/Wyeth</td>
<td>2000</td>
<td>Humanised</td>
<td>rNS/0</td>
<td>Suspension</td>
<td>Acute myeloid leukaemia</td>
</tr>
<tr>
<td>Campath* (alemtuzumab)</td>
<td>Millenium/LEX</td>
<td>2001</td>
<td>Humanised</td>
<td>rCHO</td>
<td>Suspension</td>
<td>B cell leukaemia</td>
</tr>
<tr>
<td>Xolair* (omalizumab)</td>
<td>Genentech</td>
<td>2003</td>
<td>Humanised</td>
<td>rCHO</td>
<td>Suspension</td>
<td>Asthma</td>
</tr>
<tr>
<td>Humira™ (adalimumab)</td>
<td>Abbott/ Cambridge Antibody Tech.</td>
<td>2003</td>
<td>Humanised</td>
<td>rCHO</td>
<td>Suspension</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>Zevalin™ (ibritumomab tiuxetan)</td>
<td>IDEC</td>
<td>2002</td>
<td>Murine</td>
<td></td>
<td>Suspension</td>
<td>Non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>Bexxar™ (tosotumomab)</td>
<td>Corixa/GSK</td>
<td>2003</td>
<td>Murine</td>
<td></td>
<td>Suspension</td>
<td>Non-Hodgkin's lymphoma</td>
</tr>
<tr>
<td>Avastin™ (bevacizumab)</td>
<td>Genentech</td>
<td>2004</td>
<td>Humanised</td>
<td>rCHO</td>
<td>Suspension</td>
<td>Metastatic colorectal cancer</td>
</tr>
</tbody>
</table>

GSK: GlaxoSmithKline; rCHO: Recombinant Chinese hamster ovary

In addition to therapeutic applications, proteomic analysis of both eukaryotes and prokaryotes is a substantial driver of antibody discovery, mAb production technologies and attractive economics. So far, ~5000 antibodies have been produced to target known soluble proteins – mammalian and otherwise. Estimates for the number of possible proteins in the human proteome are in the range 100,000 – 250,000, with 10,000 secreted in plasma. Hence, only a small fraction of the anticipated antibody need for proteomics is currently satisfied. The market for antibodies was ~US$5 billion in 2003, an increase of 27% from 2001, and expected to rise further to a total of US$12.1 billion by 2010 [201]. New biotechnology and biopharmaceutical methods for rapid evolution of, and selection from, combinatorial antibody libraries, as well as scalable methods to produce target
Box 3. Controlled-release advantages and disadvantages for therapeutic antibodies.

**Advantages:**
- Direct local delivery of antibodies to diseased sites
- Spatial control over antibody distribution, producing lower dosing requirements for local effects
- Reduced systemic antibody exposure (select cases) and potential toxicity
- Longer duration between dosing intervals
- Additivity or synergy with co-administered clinically accepted antibiotics and small molecule drugs at local sites of delivery and reduced doses over systemically administered antibody
- Improved control over release kinetics (pulsatile, off-on, slow basal, longer-term)
- Diverse local site selection with potential to reduce costs of drug administration and dosing over intravenous infusion
- No antibiotic resistance mechanisms known for antimicrobial antibodies

**Disadvantages:**
- Low antibody bioavailability from certain devices from poor loading capabilities
- Poor antibody stability within the release device
- Reduced systemic antibody exposure (some cases where antibody systemisation is required)
- Increased costs associated with formulation development, production and royalty expenses, increasing device cost
- Low efficacy for controlled release in certain pathologies (large bolus doses required)

antibodies reliably are presently the focus of substantial research and development.

1.6 Antibody clinical efficacy

Genetically engineered or selected humanised mAbs are showing promise in applications to several distinct disease classes. Table 1 lists therapeutic antibodies now approved as drugs, including other details about their source, commercial developer and clinical indications.

OKT3 (Ortho Biotech), a murine-derived mAb for the treatment of acute transplant rejection, was first introduced clinically in 1986. However, its acute immunogenicity problems typified patient response to murine-sourced antibodies, replacing anticipated therapeutic value with adverse reactions [4,8,9]. Several subsequent clinical failures for other murine-derived monoclonal drug trials diminished enthusiasm until nearly a decade later when chimeric and humanisation methods substantially improved immunogenicity problems, improving patient response to murine-derived antibody drugs. All commercial antibodies listed in Table 1 have substantial reporting histories in both primary immunology and clinical therapeutic literature bases, as well as websites of companies that developed and sell these therapeutic antibodies. Full description of these drugs has already been done and is beyond the scope of this review [4,5,9,17,69].

2. Controlled-release strategies for therapeutic antibodies

The raw material costs for therapeutic antibodies, generally exceeding US$100/g, coupled with multiple intravenous dosing requirements of up to 1 g/70 kg patient, intravenous infusion kits and requisite assistance, together make antibody therapy comparatively costly. Health insurance reimbursement policies subsidise this standard of care for intravenous infusion, limiting incentives to change delivery modalities. Nonetheless, numerous reports for controlled-release or local delivery of therapeutic antibodies describe alternative delivery methods with therapeutic efficacy and potential benefits. Controlled drug delivery of proteins in general is a sophisticated formulation strategy with specific requirements to suit biomacromolecular drugs that exhibit limited stability and shelf-life. The controlled-release of antibodies seeks to deliver therapeutic levels of antibodies at the intended site of action using delivery matrices. Antibody controlled-release devices are designed to be implantable, topical (skin, buccal, nasal, vaginal, rectal) or orally administered, generally comprising compatible polymeric materials loaded with bioactive antibodies. Anticipated enhancements to antibody therapy exploiting polymer controlled-release strategies were reviewed more than a decade ago [76]. In addition to the typical benefits of patient compliance, dose control and kinetics, general advantages of controlled-release formulations for antibodies are shown in Box 3.

Oral antibody dosing shows almost zero bioavailability (except in neonates where abundant gut FeRn receptors facilitate absorption), consistent with generally poor absorption of protein-based drugs from the gastrointestinal tract. Intravenous infusions of antibodies are cleared from the plasma with first-order kinetics (with a half-life of a few days). Bolus antibody injections into subcutaneous or intramuscular compartments exhibit plasma half-lives and clearance kinetics similar to intravenous infusions [77,78]. Figure 1 shows one published pharmacokinetic profile for IgG antibodies administered to humans using several common strategies. The limitations of the most popular systemic dosing approaches for antibodies are evident: generally low bioavailability and poor control over pharmacokinetics. Bioavailability and therapeutic indices for many therapeutic antibodies are critically dependent on the circulating half-life. Plasma half-lives and bioavailability for antibodies can be altered using controlled-release methods, based on alterations of release devices geometries, architectures, size, materials, antibody loading and, importantly, sites for device placement and local release [78]. Rather than depend on pharmacodynamics intrinsic to each selected therapeutic antibody plasma systemic clearance mechanism, controlled-release methods access further kinetic variables where device dose-loading, rates of antibody delivery and, hence, local clearance are rate-limited or governed by many other possible factors. Unfortunately, accurate model predictions for antibody plasma concentrations over time as a function of many of these variables in slow, controlled-release systems are very difficult.
Figure 1. Anti-Rh antibody (IgG) levels measured in human plasma following various routes of systemic administration. Bioavailabilities and clearance profiles are distinct for each method: uptake rates for intramuscular injection (0.43 ± 0.11/day) are higher than for subcutaneous injection (0.22 ± 0.025/day), whereas peak plasma levels after 3 days are higher for intramuscular (~30%) and intravenous (~40%) injections. All these IgG administration methods converge on a common plasma kinetic profile (<~25% of total dose remaining) almost 2 weeks following the chosen route of IgG administration, indicating systemic clearance mechanism-dependent pharmacokinetics beyond this point. Hence, antibody bioavailability following systemic administration is apparently independent of administration route beyond this time point when antibody dose is primarily systemically circulating. Controlled-release antibody technologies could significantly change total therapeutic antibody dosing requirements through site administration, device loading and release kinetics that influence systemic availability, host clearance mechanisms and, hence, total bioavailability profiles. Although pharmacokinetic analysis has been applied to analogous systemic administration cases to predict antibody plasma profiles, similar, accurate model predictions for IgG time-dependent concentrations in local and systemic sites when delivered from controlled-release devices still remain challenging. Adapted from [77] with permission from Elsevier.

The extended-release of bioactive proteins from synthetic polymer vehicles was first reported nearly three decades ago [79,80]. A pioneering study first reported the release of antibodies from polymer non-degradable vehicles comprising non-degradable, hydrophobic poly(ethylene-co-vinyl acetate) (PEVAc) for nearly 24 months [81]. A follow-on basic study of release mechanisms [82] confirmed the ability to release immunoglobulins from dense, polymer matrices over extended time periods. The expected first-order release profiles extending from days to months, depending on matrix variables, were generally observed. Polymer hydrogels polymerised ‘around’ solutions of IgG, then assayed for IgG release in vitro, show only a 7% release from a 4 mol% photo-crosslinked polydimethylacrylamide disks swollen to 700% in media after 80 h [83], suggesting that polymer matrix pore size (mesh or network size) must be carefully assessed to control rates of antibody release [78]. Two more recent studies have provided further details on this same polymer formulation morphological influences and effects on IgG release kinetics [84,85]. These studies showed, using ethylene-co-vinyl acetate (EVA) polymer matrices of slab and microsphere geometries, that controlled-release antibody dosing could provide similar antibody circulating concentrations as current systemic antibody infusions >50 times greater on a weight/weight basis [84]. Whereas therapeutic indices for mAbs are either not well-established or widely correlated with various disease states and exact dosing requirements for local delivery are unknown, this work assesses a value for controlling circulating antibody much more effectively and efficiently. A follow-on study showed that antibody release from EVA as a combination diffusion-dissolution controlled mechanism, regardless of device matrix geometry [85].

Other polymeric delivery forms have also demonstrated controlled-release of bioactive antibodies [86], including those using supercritical fluid processing [87]. Formulations of antibodies in many polymer vehicles often require organic solvents deleterious to protein structure and function. Precedent indicates that solid, dry antibody particles suspended in organic solvent can survive polymer device formulation, releasing to exhibit high levels of bioactivity [86,88]. Therapeutic performance requires that polymer processing methods do not inactivate antibody dose; protein release should correlate with preservation of large fractional bioactivity. Other developments should improve antibody formulating capabilities, including the fundamental understanding of, and functional improvements in, antibody structural stability [61,87].

Figure 2 shows an important precedent set of data comparing release of whole IgG and purified IgG-derived fragments (F_{ab}, F_{ab'}, [Fab']_2) over time from hydrophobic
Figure 2. Antibody (human polyclonal and mouse monoclonal anti-HCG IgG) and human antibody fragment (Fab, Fc and [Fab']2) levels released from solvent-cast EVAc polymer matrices into well-stirred PBS at 37°C. (A) Polymer devices are diameter 4 mm, thickness 1 mm, mass 7–10 mg, comprising 65 wt% EVAc and 35 wt% unsieved solid antibody solids (7 wt% protein, 4–20% salts, 72–88% 70 kDa Ficoll), 200 µg total protein. (B) Polymer disks are diameter 8.5 mm, 1.6 mm thickness, mass 80–100 mg, comprising 60 wt% EVAc and 40 wt% sieved solid antibody solids (<178 µm diameter, 0.7 wt% protein, 0.3–1.6% salts, 98–99% 400 kDa Ficoll), 250 µg total protein. Inset: Comparison of ELISA quantified binding versus release of mouse monoclonal anti-HCG IgG from polymer rings (diameter 4 mm, thickness 1.5 mm, mass 17 mg) of 50% EVAc and 50% sieved solid particles (35 µg total protein) containing 0.4 wt% anti-HCG, 1.1% salts and remaining 400 kDa Ficoll, indicating quantitative retention of binding activity postrelease from polymer. (C) Rates of antibody release (human IgG and IgG fragments) into well-stirred PBS as indicated for conditions in Figure 2A: (square) human IgG, (circle) human antibody fragment (Fab'), (cross) Fab and (triangle) Fc. Rates are calculated from cumulative release shown in Figure 2B. The high inclusion percents and high molecular weights of the Ficoll polysaccharide filler as porogens in the dense non-swelling EVAc polymer seems to control the rate of protein release through pore generation and connectivity in the EVAc matrix. Despite significant differences in IgG molecular weights, rates of release show only small differences. Adapted from [95] with permission.

ELISA. Enzyme-linked immunosorbent assay. HCG. Human chorionic gonadotropin. PBS. Phosphate buffered saline.

polymer devices. IgG release is slower than smaller derived fragments. Fragments can also have higher tissue permeability. However, antibody fragments have their disadvantages (i.e., lack of recognised Fc regions or glycosylation patterns as described above that influence their half-life and therapeutic potential) [45]. Figure 3A shows the release of polyclonal human IgG from a hydrogel matrix comprising mostly water. Diffusion controlled-release is observed but with much more rapid release rates and reduced duration (<12 h). This hydrogel release translates directly to rapid systemic bioavailability when antibodies release interpartitionally, as shown in Figure 3B. Hence, local dosing and release also provides a route to antibody systemisation when desired. In addition, polymer devices should permit tailoring of antibody total dose, release rates and appropriate geometries or morphologies to provide versatile local or direct antibody delivery strategies for application to various tissue or disease sites. The avoidance of systemic dosing limitations and clearance mechanisms is a primary objective in formulating such devices.

Of the > 200 different reports or patents describing controlled or direct antibody delivery that have appeared over the past decade, several studies stand out as examples of approaches, methods, therapeutic effects and current challenges that demonstrate the trends important for this field in terms of antibody therapy. These examples have been selected from a substantial literature base and are described in further detail below.

2.1 Topical antibody delivery

The issues and advantages of topical delivery of therapeutic antibodies to mucosal surfaces in several significant disease scenarios have been reviewed [89]. Several examples of topical immunotherapy against dermatitis [90] and keratitis [91] using immunoglobulins date back nearly a decade. Carrier materials for controlling the application were not a particular focus of these studies. Topical delivery in a murine mucosal model exploited PEVA monolith devices containing sieved solid ~100 µm antibody particles dispersed in the polymer matrix providing interconnected chaotic paths for release [92]. The direct delivery of antibodies using this matrix to mucosa has proven protective, effectively preventing herpes simplex viral transmission in a vaginal infection model with topically applied full antibodies, F(ab)² and Fab fragments [93]. Long-term dose-dependent protection was observed, approximately equal for all three proteins delivered [94]. PEVA disks containing either radiolabelled mAbs or
antilactate dehydrogenase-C4 antibodies were also released both in vitro [95] and topically to murine vaginal mucosa. High antibody levels were measured on the mucosal surface as well as in systemic mouse circulation. Biologically-active antibodies measured in serum constituted 1% of those measured topically from vaginal mucosa [96]. A recent study reports the use of topically applied human polyclonal IgG antibodies to block adhesion of *P. aeruginosa* to cultures of corneal cells in the context of contact lens-based infection [97]. Secretory IgA has also been shown to inhibit pathogen adhesion to corneal epithelium to protect against keratitis [98]. Antibody fragments have been shown to penetrate corneal tissue [99], yet fluxes are low, equilibrium delivery amounts undetermined and the potential to exploit this delivery route for therapeutic purpose remains unclear.
2.2 Implantable antibody controlled-release devices

Controlled-release devices in this category generally exploit polymers as carriers in various geometries (e.g., slabs, films, gels, cylinders, microspheres) where the matrix morphology (porous or dense), geometry (altered drug diffusion paths and transport kinetics), chemistry (swellable, erodable or hydrophobic) and drug–polymer interactions can all be tailored to change device release properties to tissue sites. Subcutaneously injected biodegradable polymer (poly(lactic acid) microspheres injected into rats presented serum-measurable antibodies for 30 days [100]. Polymer non-degradable vehicles comprising PEVAc have been formulated with human antibodies, antibody fragments and mouse mAbs directed against human chorionic gonadotropin. In addition, this same study reports human antibody release from surface-eroding poly(anhydride) devices [95]. Poelestra et al. [101] demonstrated rapid release of polyclonal human antibodies from carboxymethylcellulose gel applied topically to polymer abdominal implants containing lethal amounts of introduced \textit{P. aeruginosa} in a murine surgical implant infection model. Substantial antibody-based benefits were observed against implant infections from several bacterial strains [101]. Analogous antibody therapeutic benefits were reported for a closed lethal murine peritonitis model where direct injection of polyclonal human antibodies into the peritoneal cavity produced substantial protection against systemised infection from several Gram-negative bacterial strains [102,103]. In these studies, applied human antibodies were rapidly detected in murine serum, detectable ≥ 1 week. Antibody clearance to systemic circulation correlated with reduced sepsis marker, IL-6 and protection from sepsis and death in these lethal infection models. Importantly, human polyclonal antibodies delivered locally provided synergistic benefits when co-administered in these models with ceftriaxone antibiotic systemic infusions [103]. Tricalcium phosphate microcapsules containing anti-HER-2/NEU antibodies against breast carcinoma placed intraperitoneally in rats rapidly cleared these antibodies to systemic circulation detectable in saliva by enzyme-linked immunosorbent assay (ELISA) after several days [104]. Antibody aqueous lavages were also directly applied to a lapine orthopaedic implant acute surgical infection model reported for \textit{Staphylococcus aureus} colonisation of spinal hardware [105]. Human polyclonal antibodies applied directly to spine Gram-positive \textit{S. aureus} infection sites failed to show therapeutic benefit compared with the antibiotic, vancomycin, when given intravenously [106]. Despite substantial antistaphylococcus antibody titres detected using ELISA \textit{in vitro}, the study indicates that Gram-positive pathogens require more sophisticated antibody-neutralising mechanisms to be effective therapeutically \textit{in vivo} [25,47,106,107]. Several antibody commercial development efforts are focusing on this particular antimicrobial targeting challenge (i.e., Inhibitex, Biosynexus, Nabi Biopharmaceuticals).

Fundamental studies of postnatal neogenesis in rats used controlled-release of antibodies against the transiently expressed axonal surface glycoprotein, TAG-1, present only in premigratory granule cells in the postnatal cerebellum, and HNK-1, a carbohydrate epitope found on cerebellar cell membrane during postnatal development. FVAc square matrices with dispersed solid IgG and IgM particles up to 1.2% w/w antibody. At this loading, 50% of the loading releases in 15 days, whereas at lower loadings, a maximum of 22% release is observed. IgMs are released more slowly than IgG, with both exhibiting only slight (< 20%) loss of bioactivity [108].

2.3 Pulmonary and respiratory antibody delivery

Aerosolised dry powder, liquid nebulised or directly instilled antibody delivery routes have been explored typically against airway or pulmonary disease targets or respiratory toxins [109-113]. Various prophylactic biodefence-related antibody antibodies have been reported against toxins Shiga [114], tetanus [115] and anthrax [19], amenable to direct lung administration of specific antibodies.

Lipid-derived hollow-porous microparticulate delivery of antibodies to the respiratory tracts of BALB/C mice via non-aqueous liquid instillation, demonstrated first-order release kinetics with 80% antibody release in 6 h [116]. Dry powder inhalation is a possible alternative delivery method and would change these release kinetics and antibody bioavailability. These studies indicate consistent correlations between inhaled dose and systemic antibody bioavailability, demonstrating therapeutic utility for both local (airway) and systemic antibody delivery. Delivery vehicle activation of abundant macrophage populations in the lung is always a concern; solid particle of sizes below a few microns are readily phagocytosed by airway phagocytic cells. Phagocytic activation by particle carriers can lead to adjuvant immune modulatory effects via antigen-presenting cells or respiratory burst-induced cell damage to pulmonary tissues [116].

Aerosolised antibodies have been applied topically to RSV infections in the lung [117] although controversy persists about efficacy [118,119]. Topical intranasal delivery of IgA has been shown to protect against RSV in a murine model [120].

2.4 Other routes of antibody controlled-release

Pooled human polyclonal antibodies injected subcutaneously directly into a murine lethal. Gram-negative, full-thickness burn wound infection model demonstrate efficacy in preventing systemisation of several different common burn wound pathogens, substantially reducing mortality [121]. Further intriguing results are reported for the same treatment against analogous lethal murine full-thickness burn wound infections against a clinically-isolated, moderately antibiotic-resistant \textit{P. aeruginosa} pathogens [122]. Synergistic therapeutic benefit was reported using both locally injected antibodies at these wound-sites with clinically indicated ceftriaxone intravenous infusion against lethal burn wound infections in both of these studies.

A recent report of the delivery of bioactive anticocaine antibodies from biodegradable polymer (poly(lactide-co-glycolic acid) [PLGA]) microspheres injected subcutaneously into mice indicated a 15% protein loading capability with a
substantial (80%) burst release within 24 h in vitro with slow residual release out after 1 week. In vitro, anticancer antibodies could be detected beginning at 1 h in serum, and peaking at 1 week. However, in vivo release detected in serum was a small fraction of the in vitro release, indicating detection assay problems from serum or poor bioavailability from the PLGA microspheres [123].

The controlled-release of solid dispersed antibodies against leukocyte adhesion molecules from PEVAc disk devices has been studied to inhibit leukocyte–epithelium interactions [124]. An inert water-soluble filler polysaccharide (Ficoll) was used to enhance release. Release duration was limited to 1 week. However, antibody bioactivity was maintained and possibilities exist to increase release duration with device modifications and drug loading alternatives.

Local Pluronic™-based gel delivery of antitumor factor antibodies in vivo to vein graft surfaces was shown to hinder early CD18+ leukocyte infiltration into vein graft surgical sites, but failed to improve intimal hyperplasia outcomes, a common clinical failure mechanism for these grafts [125].

Given the dosing, combined with the size of most antibodies (150 kDa), and even their effective fragments (Fab, Fv domains, diabodies) [65,126] and single-chain Fv antibodies [60,127], transdermal and iontophoresis routes of delivery are unlikely to become viable unless antibody mimics of distinctly different physicochemical properties are developed. Hence, topical (inhalable or epithelia-applied), oral, surgically implant-based or Injectable device-associated routes (liquid in situ-gelling depot devices placed subcutaneously or intramuscularly) have remained a major focus of antibody delivery strategies so far.

3. Expert opinion and conclusion

Because of the observed pharmacodynamic and pharmacokinetic constraints of circulating antibodies, alternative delivery routes for antibodies must be developed to avoid repeated parenteral administrations and unwieldy dosing regimens. Innovations in antibody delivery that promote intrinsic antibody targeting, controlled-release of active antibody and control of sustained effective doses are necessary in order to increase therapeutic value and reduce costs. All of these therapeutic indices rely on further understanding of site-specific antibody release, correlated target-site concentrations, systemic concentrations and clearance or metabolism. Controlled-release systems should be able to deliver the bioactive antibody from days (rapid) to months (slow) and target sustained doses in serum (systemic), at a desired tissue site (local), to epithelial surfaces (mucosa) or from implanted medical devices (combination products). A particularly attractive use of antibody therapy resides in additive or synergistic action with existing clinically prescribed drugs using novel delivery vehicles and dosing kinetics. Significantly, pharmacokinetic models that accurately predict in vivo concentration profiles from device release at tissue sites or systemically must be developed to provide a rational approach for such device design. This will require substantial new research on specific antibody properties in devices, tissue sites and clearance mechanisms.

So far, many reported approaches substantiate the therapeutic potential for antibody-controlled-release strategies. Most promise is shown in cancer therapies, modulating inflammation and mitigating infection. Biotechnology innovations will contribute immensely to the potential and value of antibody-based therapies in the future. Recombinant antibody production costs will continue to fall and techniques for custom antibody selection for more effective human therapeutic action will continue to improve. Disease mechanisms and critical targets for antibody binding to produce therapies will also evolve in parallel. In addition, it is likely that antibody-like molecules (mimics or truncated versions are already reported) [4,5,126], exhibiting improved stability, especially in polymer delivery vehicles, lower molecular weight, improved specificity and reduced clearance or side effects, will be developed. Already, trends in reducing antibody size, improving specificity and affinity, enhancing stability and engineering potency are observable. This anticipates an eventual scenario when antibody therapy is more affordable, more reliable and widely versatile in addressing many types of disease. Although antibody intravenous infusion remains the delivery mode of choice, it is costly, inconvenient, unsophisticated and unsuited to optimal therapeutic dosing in several disease scenarios. With these anticipated improvements in antibody-based drug molecular designs, economics and efficacy, many other modalities for dosing and delivery might also become commonplace, bringing associated clinical benefits. Current controlled-release limitations, involving protein stability and antibody dosing requirements, should be overcome by development of smaller antibody fragments, non-protein synthetic antibody mimics that retain the innate specificity and host immune advantages or stabilised recombinant antibody designs specific to desired targets, but capable of satisfying device loading (dose), formulation (kinetics, bioavailability) and shelf-life (storage) requirements. Combinations of local antibody release with clinically standard chemotherapeutic antineurourm drugs [129], or antibiotics [103,121,122], delivered systemically (or locally) also offer new therapeutic opportunities.

An interesting new application with little reported study so far lies in the area of regenerative medicine, where antibodies could play key roles when released locally in tissue sites. The use of antibodies to trigger, stimulate or block local angiogenesis or neurogenesis, bind or release tissue growth factors and cytokines, control cell phenotypic properties, or work in tandem with implanted scaffold materials to help stimulate new tissues morphogenesis and function in situ appear promising. Controlled-release of new cell-signalling-specific antibodies to control cellular pathways
for gene transduction and apoptosis mechanisms could also be both fundamentally interesting and clinically fruitful. Both external cell membrane receptors and channels, as well as internal intracellular (cytoplasmic and nuclear) targets (54), are expanding rapidly and represent interesting new sites of antibody-modulated action requiring newer, sophisticated delivery methods. Versatile combinations of agonist and antagonist effects with mixtures of different therapeutic antibodies released specifically under dynamic conditions of disease or therapy are possible using new sophisticated scaffolds that respond to disease states or local physiological signals (129,130).

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