Antibiofouling polymer interfaces: poly(ethylene glycol) and other promising candidates

Journal:	*Polymer Chemistry*
Manuscript ID:	PY-REV-10-2014-001356.R1
Article Type:	Review Article
Date Submitted by the Author:	22-Oct-2014
Complete List of Authors:	Lowe, Sean; The University of Melbourne, Chemical and Biomolecular Engineering O'Brien-Simpson, Neil; The University of Melbourne, Melbourne Dental School, Oral Health CRC Connal, Luke; The University of Melbourne, Chemical and Biomolecular Engineering

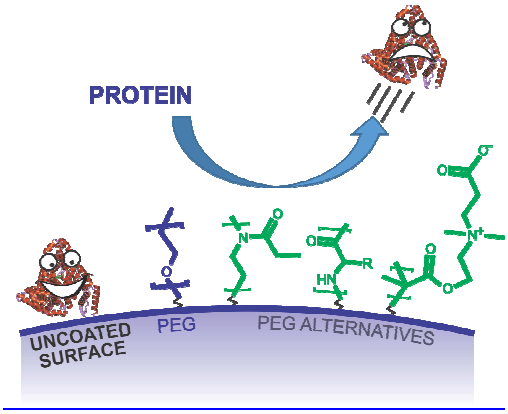
Antibiofouling polymer interfaces: poly(ethylene glycol) and other promising candidates

~~Antibiofouling polymer interfaces: design criteria for biomedicine and nano-therapeutics~~

Sean Lowe,[1] Neil M. O'Brien-Simpson,[2] Luke A Connal[1*]

1. Department of Chemical and Biomolecular Engineering, The University of Melbourne, Victoria, Australia 3010.
2. Melbourne Dental School, Oral Health CRC, The University of Melbourne, Victoria, Australia 3010.

*luke.connal@unimelb.edu.au



TOC one-line summary:

This review highlights antibiofouling polymer interfaces with emphasis on the latest developments using poly(ethylene glycol) and the design new polymeric structures.

Abstract

Nonspecific protein adsorption and/or microbial adsorption on biomedical materials adversely affects the efficacy of a range of biomedical systems, from implants and biosensors to nanoparticles. To address this problem, antibiofouling polymers can be coated on

biomedical devices or built into nanoparticles to confer protein and/or microbial repellent properties. The current review provides an overview of the range of synthetic polymers currently used to this end and explores their biomedical potential. The most widely-used antifouling polymer, poly(ethylene glycol) (PEG) is reviewed alongside several promising alternatives, including zwitterionic polymers, poly(hydroxyfunctional acrylates), poly(2-oxazoline)s, poly(vinylpyrrolidone), poly(glycerol), peptides and peptoids. For each material, notable applications for both nanomedicine and macroscopic surface coatings are highlighted.

Introduction

Biofouling from nonspecific ~~protein~~biomolecule or microbial adsorption is a ubiquitous and persistent challenge for any interface that is exposed to biological fluids or tissue. On *in vivo* devices, such as implants, catheters, biosensors or tissue engineered scaffolds, protein adsorption can lead to adverse immunological responses and may promote bacterial colonization and infection.[1, 2] Furthermore, on *in vitro* devices which come into contact with protein-rich biological material, such as microarrays or filtration membranes, nonspecific protein adsorption can either partially or entirely compromise functionality.[2] ~~Protein adsorption~~Biomolecule adsorption is also a major problem for nanoparticles, such as micelles, liposomes, and nanocapsules, used for drug delivery and bioimaging. ~~These systems often require long circulation times to sufficiently accumulate within target tissue or tumors.~~ Particles without antifouling moieties~~, however,~~ are usually quickly coated in biomolecules such as lipids, proteins, and sugars when they enter the bloodstream, creating a biomolecular corona.[3] The major constituent of the corona is a small subset of blood proteins,[3] and this nonspecific adsorption of blood proteins is termed opsonisation.[4] Opso~~blood proteins in a process called opsonization, forming a protein corona that reduces functionality.~~[3]

~~Opso~~nization can adversely affect the chemistry of conjugated drugs, causing aggregation or charge neutralization, and, moreover, typically leads to a rapid removal of nanoparticles from

the blood by phagocytic cells of the immune system.[5-7] Given ~~the given~~ that nonspecific protein adsorption is a key problem facing biomedical surfaces, this review ~~will~~ examines the ~~keydominant~~major polymer-based strategies to reduce it.

A common strategy to reduce protein fouling is to attach hydrophilic or zwitterionic synthetic polymers to surfaces. These polymers' electrical neutrality can help reduce electrostatic interactions with charged protein domains.[8] Their hydrophilic nature helps reduce nonpolar interactions between proteins and hydrophobic surfaces.[9] Moreover, these polymers may prevent protein adsorption by steric hindrance,[10-12] resulting in increased circulation times. Antifouling polymers have found applications as coatings for both macroscopic surfaces (such as implants and biosensors) and in nanoparticulate systems, endowing them with "stealth properties" (i.e., the ability to evade the body's normal clearance mechanisms).

The field of antifouling polymers is a fast growing area with a number of strategies and new polymer structures designed for this purpose. Poly(ethylene glycol) (PEG) has been the dominant polymer used for antifouling surfaces. However, PEG has limitations which have led to the development of a range of alternative polymeric materials, several of which are shown in **Figure 1**~~**Figure 1**~~. While several comprehensive reviews examine specific classes of antifouling polymers such as poly(ethylene glycol) PEG,[13-19] zwitterionic materials[19, 20] and poly(2-oxazolines),[21-25] the full range of antifouling polymers is rarely considered in one place. When it is, discussion typically focuses exclusively on nanoparticulate drug delivery systems[26-29] or macroscopic surface coatings[9, 12, 30, 31] rather than the whole range of nonfouling, biomedical applications.

This review will therefore provide a high level overview of the antifouling polymeric suite and its applications for biomaterials. It discusses both PEG and the most common synthetic PEG alternatives, with examples given for each material on both macroscopic surfaces and in

nanoparticulate systems. Emphasis is placed on the most recent advances and key foundational research. Focus will be placed on general antifouling polymer studies, rather than studies which focus on specialty applications like membranes and bioassays. Further, discussion will be limited to solution phase synthesis, rather than solid phase synthesis approaches such as chemical vapour deposition.[32] Nevertheless, the review will examine a broad range of some of the most salient and clinically applied antifouling polymers.

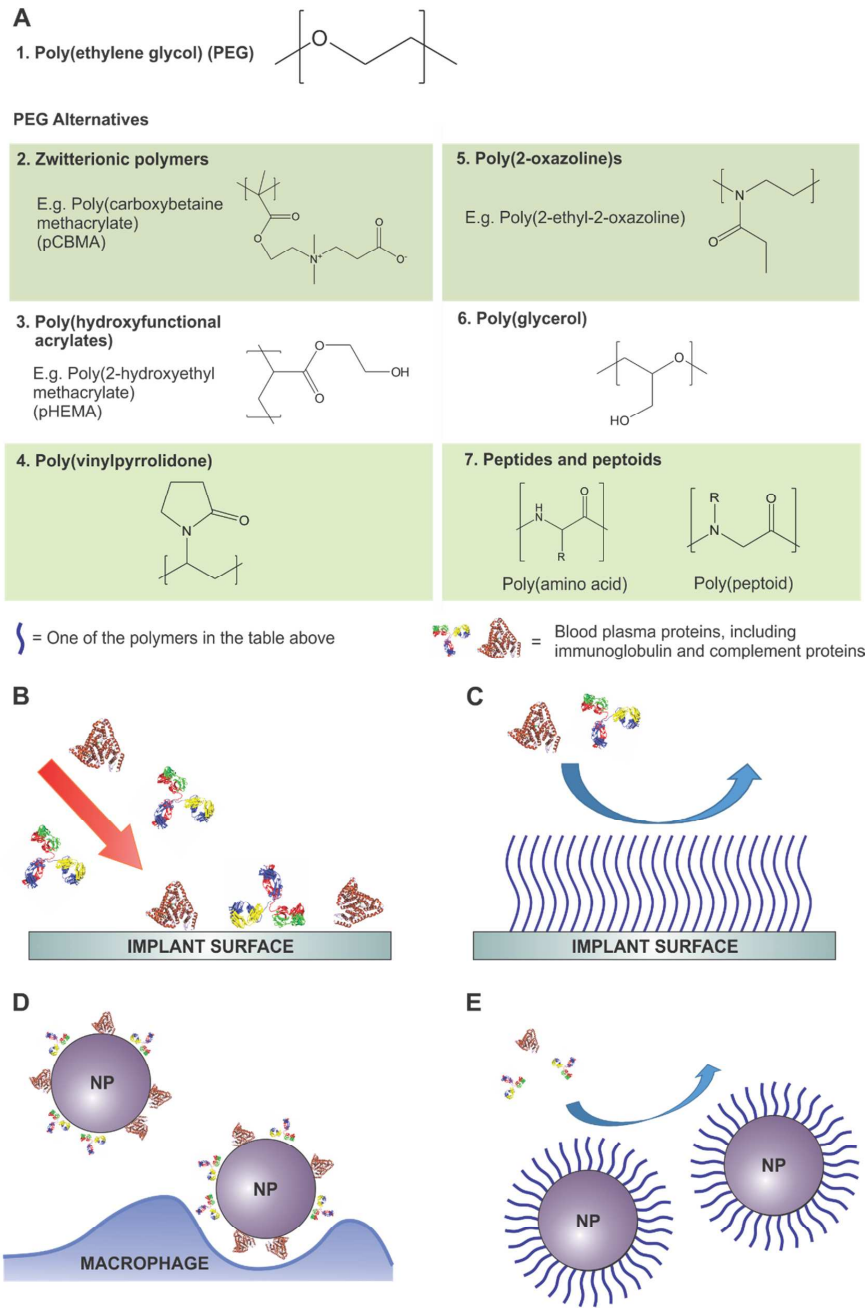


Figure 1. (A) Chemical structure of PEG and PEG alternatives designed as antifouling coatings. (B) A macroscopic surface without an antifouling polymer coating is quickly coated in proteins when it comes into contact with blood. (C) Coating the surface with PEG or PEG alternatives reduces protein adsorption to the surface. (D) As with macroscopic surfaces, nanoparticles (NPs) without antifouling polymers suffer from nonspecific protein adsorption in the body. Protein adsorption leads to phagocytosis by macrophages and removal of NPs from the bloodstream. (E) Attaching antifouling polymers to NP surfaces allows the particles to resist protein adsorption and phagocytosis and increases their blood circulation half-life.

1. Poly(ethylene glycol)

PEG has been the most widely used polymer for antifouling applications, such that it has often been termed the "gold standard" of antifouling polymers.[22, 28] In terms of surface applications, Prime and Whitesides first reported the potential for PEG-derivatives, showing that oligo(ethylene glycol) (OEG) self-assembled monolayers (SAMs) were effective protein-repellent coatings.[33, 34] However, OEG SAMs are prone to surface defects and have limited application to metal surfaces.[35] Given the limitations of these SAMs, alternative means of attaching PEG to surfaces have been explored.

The effectiveness of PEG, and antifouling polymers in general, depends upon the surface grafting technique ~~and , achievable grafting densities,~~ and the polymer architecture .[36, 37] ~~For this reason~~Thus, a variety of grafting approaches have been developed to improve the effectiveness of PEG and other antifouling coatings. For example, f~~F~~irstly, ~~For example, linear~~ PEG ~~can behas been~~can be covalently attached to surfaces~~, for example,~~ using "grafting to" approaches to form linear polymer brushes~~[38-40]~~.[38-40] Secondly, PEG methacrylates ~~can also behave also been~~can be polymerized from ~~initiator pre-treated~~ surfaces using a "grafting from" ~~techniques~~approach (~~[34, 35] For this reason, a variety of grafting approaches have been developed to improve the effectiveness of PEG and other antifouling coatings. These include polymerization techniques such as s~~e.g. Surface-I~~i~~nitiated Atom Transfer Radical Polymerization (SI-ATRP)).~~,~~[31, 41-44] Using this ~~approach~~technique, ~~effective~~ antifouling brushes ~~are~~can be formed consisting of linear polymers with PEG side chains.

~~but also novel~~ Novel and ~~biomemetic~~Several novel ~~approaches~~ grafting strategies developed specifically for antifouling applications have also been developed. For example, a biologically inspired approach using components of muscle adhesive protein (i.e., L-3,4-

Formatted: Normal, Space After: 0 pt, Line spacing: single, No bullets or numbering

dihydroxyphenylalanine, L-DOPA) has been effectively used to attach PEG to surfaces via catechol groups (see **Figure 2~~Figure 2~~ (B)**).[45-51] In a separate approach, the amino acid lysine has been used as a polymer brush backbone with PEG.[47, 52-54 42, 47-49] PEG side chains are covalently linked through the primary amine on the lysine residues to form poly(lysine)-*graft*-PEG (PLL-*g*-PEG) polymers (see **Figure 2~~Figure 2~~ (A)**). Some lysine residues on the backbone are left in their native, cationic state, and these residues can adsorb to an anionic metal surface such as titanium oxide. ~~The~~ Further, the lysine side chain can ~~also~~ be functionalized with the integrin ligand, Arg-Asp-Gly (RGD), to endow the surface with the ability to specifically bind to host cells.[53]

The poly(lysine) strategy can be combined with a catechol strategy on the same polymer to create a multifunctional polymer anchor. Saxer et al.[47] developed such a polymer (**Figure 2~~Figure 2~~**), showing that the catechol groups led to increased coating resistance to high ionic salt solutions compared to PLL-*g*-PEG alone.[47]

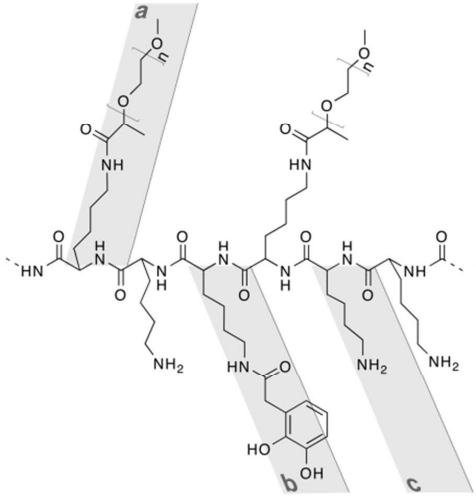


Figure 2. Poly(L-lysine)-*graft*-(3,4-dihydroxyphenylacetic acid, poly(ethylene glycol)). When bound to a surface, the polymer's PEG groups (a) conferred resistance to plasma fibrinogen adsorption, while the catechol (b) and lysine-containing (c) monomers anchored the polymer to the surface. ~~Reproduced from ref~~ Reprinted with permission from S. Saxer, C. Portmann, S.

Formatted: Font: Cambria, 11 pt, Font color: Accent 6

Formatted: Font: Cambria, 11 pt, Font color: Auto

Tosatti, K. Gademann, S. Zürcher and M. Textor, *Macromolecules*, 2010, **43**, 1050-1060, Copyright 2010 publisher. ~~[44].~~

Formatted: Font: Cambria, 11 pt, Font color: Auto

Formatted: Normal, Indent: Left: 0", Right: 0"

Despite significant research into PEG's applications on surfaces, its mechanism of action are still not fully understood.[9-11, 55] PEG's antifouling properties likely stem from steric repulsion associated with a loss of entropy when proteins attempt to adsorb to surfaces.[10, 11] Furthermore, it is likely PEG's hydrophilic nature leads to the formation of a hydration layer which inhibits protein encroachment.[9] Several variables which determine PEG's effectiveness include the grafting density (with higher densities being associated with greater protein repellence), chain length, and type of PEG branching architecture.[37, 56]

In addition to being useful for coating macroscopic surfaces, PEG has been effectively used in a variety of nanoparticulate systems.[13-19] Attaching PEG to nanoparticles, known as PEGylation, gained popularity when liposomes were first PEGylated in the early 1990s.[57-59] PEGylated liposomes encapsulating the anti-cancer drug doxorubicin became the first commercially available polymeric particulate drug delivery system (Doxil) in 1995.[60] In addition to liposomes, PEG has been used across the complete range of nanoscopic biomaterials, such as: drug conjugates,[61, 62] inorganic nanoparticles,[63] polymeric nanoparticles,[64-71] micelles, [72-74] star and hyperbranched polymers,[65, 75-79] and knedel-like polymers.[80]

PEGylation of nanoparticles confers a variety of benefits, including improved water solubility as well as reduced opsonization and associated improvements in blood circulation time.[5] Presumably due to this reduced opsonization and increased stability, PEG can increase the plasma half-life of conjugated drugs from several minutes to several hours.[57]

However, PEG has several notable weaknesses in biomedical contexts.[15, 28, 81] PEG can suffer oxidative damage in biological media and fluids, which limits its use for long term applications.[82, 83] Moreover, because PEG is not biodegradable, there are concerns about bioaccumulation in the lysosomes of healthy cells.[28, 84] Additionally, although PEG is typically ~~regarded as~~considered to be ~~immunologically inert a stealth polymer~~non-immunogenic, it has nevertheless ~~being~~ been shown to ~~be immunogenic~~provoke an immune response in some circumstances. For instance, when PEG has been used with drug delivery particles, antibodies to the PEG polymer have been shown to be generated.[85] Anti-PEG antibodies may result in faster clearance *in vivo,* reducing the effectiveness of PEGylated drugs. Moreover, it could lead to carrier-induced epitope specific suppression,[86] an effect where pre-immunization with a carrier can lead to a reduced immune response to antigens later presented on that same carrier. This phenomena would reduce PEG's applicability as an antigen carrier.

In some cases, PEGylated liposomes or other nanoparticles have greatly reduced blood circulation time after the first dose in animal models. This response appears to be IgM-mediated[87, 88] and is termed the accelerated blood clearance (ABC) phenomenon (see **Figure 3**~~**Figure 3**~~ **(A)**).[89-91] Interestingly, the effect is not observed for PEGylated liposomes encapsulating anti-cancer drugs; this is potentially because these drugs are cytotoxic to antibody secreting cells.[90, 91]

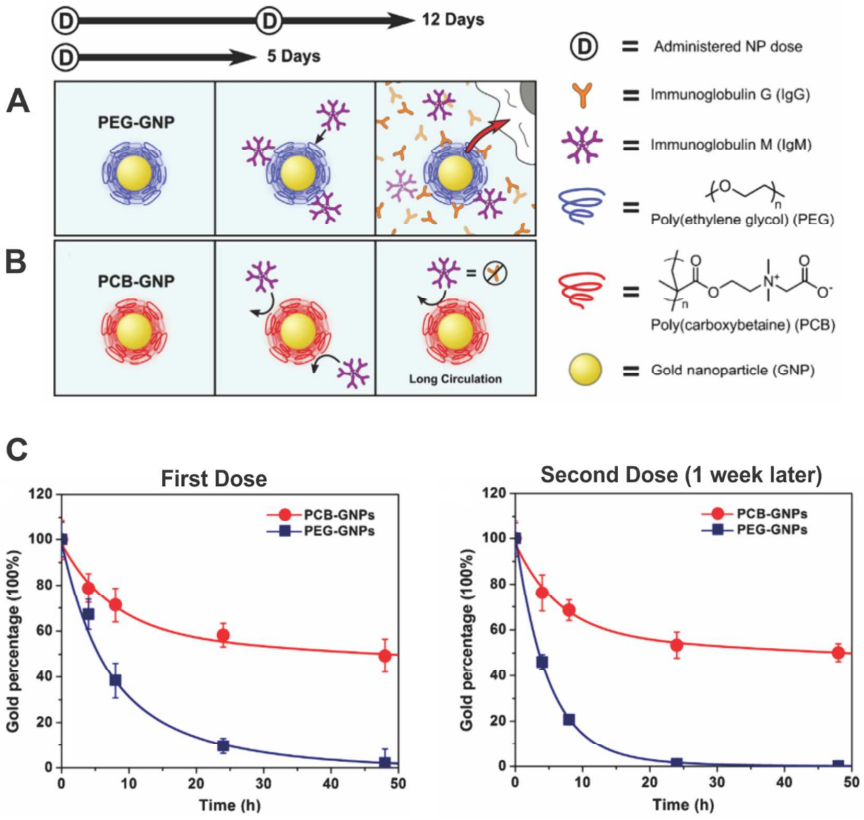


Figure 3. (A) Gold nanoparticles coated in PEG (PEG-GNP) stimulate the production of anti-PEG IgM over five days after the first injection in rats. Production of IgM leads to the production of IgG. (B) When GNPs are coated with ultralow fouling poly(carboxybetaine) (PCB), they resist the adsorption of IgM and thus the production of IgG. (C) The ABC phenomenon was tested by injecting rats with PCB-GNPs or PEG-GNPs at two time points (data represent average values across 6 rats). The PEG-GNP's half-life markedly decreased from 8.7 h in the first dose to 5.2 h in the second dose, while the half-life for PCB-GNPs remained relatively constant between the first and second dose (55.8 h and 55.6 h, respectively). ~~Adapted from ref. [87].~~Reprinted with permission from W. Yang, S. Liu, T. Bai, A. J. Keefe, L. Zhang, J.-R. Ella-Menye, Y. Li and S. Jiang, *Nano Today*, 2014, **9**, 10-16. Copyright 2014 ~~publisher~~Elsevier.

Formatted: Caption

2. Zwitterionic polymers

Zwitterionic polymers have recently emerged as promising alternatives to PEG.[8, 20, 92] Zwitterionic polymers are electrically neutral materials which contain both a positively charged species and a negatively charged species on the same monomer or, in the case of polyampholitic materials,[8, 93-95] on different monomers. The charged species appear

associated with an even stronger hydration effect than that created by PEG, which may in turn enhance the zwitterionic material's antifouling properties.[20, 96]

Several studies have indicated that zwitterionic surface coatings can have highly effective protein repellence. Surfaces coated in zwitterionic poly(2-methacryloyloxyethyl phosphorylcholine) brushes have shown protein resistance which surpasses comparable PEG coatings.[97] Similarly, poly(sulfobetaine methacrylate) (pSBMA)[98] and poly(carboxybetaine methacrylate) (pCBMA)[99, 100] have shown ultralow fouling properties. Use of an acrylamide[101, 102] or a norbornene[103] polymer backbone with zwitterionic betaine side chains can lead to undetectable levels of protein adsorption from undiluted human blood serum or plasma (see Figure 4~~**Figure 4**~~ for tests using carboxybetaine).

Zwitterionic surface coatings have recently been shown to have promise in clinically relevant implant models.[104, 105] For example, Liu et al.[105] reported that grafting pSBMA on to titanium alloy orthopaedic/dental implants led to increased osteointegration by promoting mineralization of the implant surface.

Field Code Changed
Formatted: Default Paragraph Font
Formatted: Font: Bold

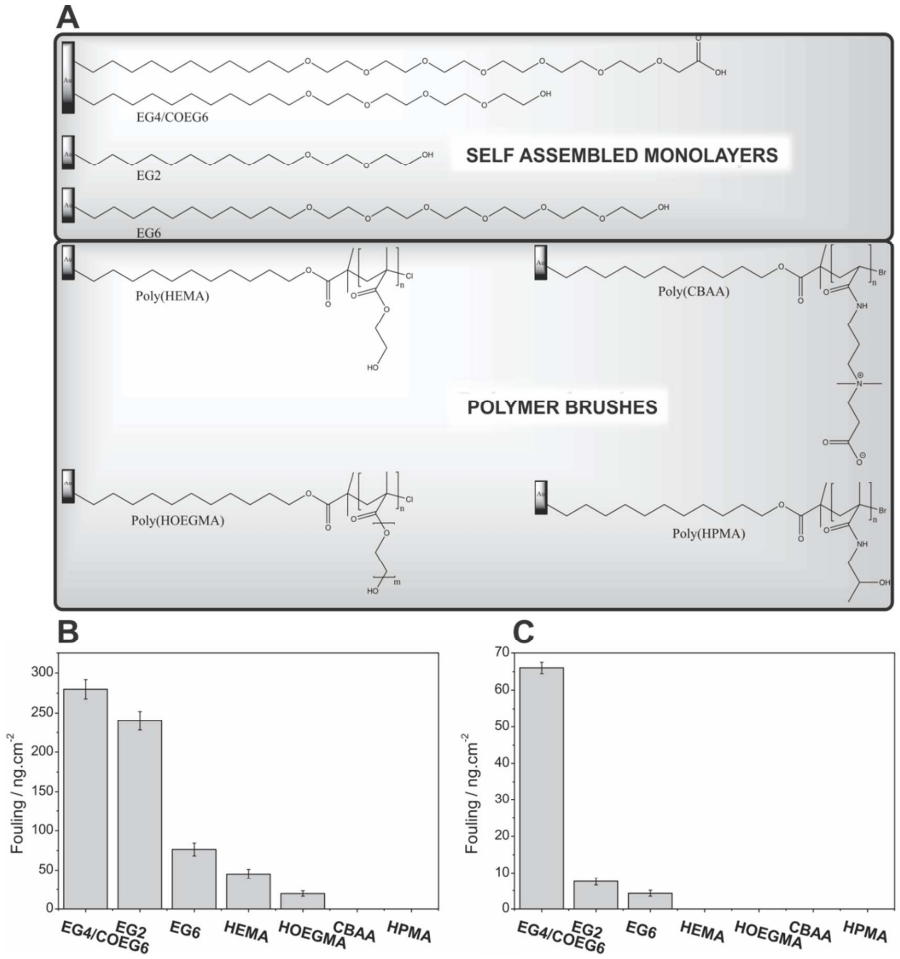


Figure 4. (A) Protein adsorption on different gold surfaces coated with various antifouling materials, including three self assembled monolayers (SAMs) based on ω-oligo(ethylene glycol) alkanethiols (EG4/COEG6, EG2, EG6), and polymer brushes produced using surface-initiated atom-transfer radical polymerization ~~(ATRP)~~ of oligo(ethylene glycol) methacrylate (HOEGMA), 2-hydroxyethyl methacrylate (HEMA), carboxybetaine acrylamide (CBAA) and *N*-(2-hydroxypropyl) methacrylamide (HPMA). The surfaces were exposed to undiluted blood plasma (B) or cerebrospinal fluid (C) for 15 minutes and the nonspecific protein adsorption was measured using surface plasmon resonance (SPR). Protein adsorption below the SPR detection limit (0.03 ng · cm^{-2}) was reported as zero. Reprinted with permission from C. Rodriguez-Emmenegger, M. Houska, A. B. Alles and E. Brynda, *Macromol. Biosci.*, 2012, **12**, 1413-1422. Copyright 2012 ~~publisher~~Wiley-VCH.~~Adapted from ref. [98].~~

In addition to surface-grafted polymer architectures, several new hydrogels with zwitterionic polymers appear to be particularly promising.[104, 106, 107] ~~[95, 98, 99]~~ Zhang et al.,[104] for example, developed a pCBMA hydrogel with long-term resistance to the foreign body reaction~~, the body's immunological response to implanted foreign objects.~~. In the foreign body reaction,

nonspecific protein adsorption is thought to signal the presence of ~~the~~an implant to the immune system ~~and set off a cascade of events~~ , ~~setting off a cascade of events leading to thewhich leads to the~~triggering the formation of a dense collagen layer around the implant to sequester ~~it~~the implant from surrounding tissue. This collagen layer can prevent effective mass transport and electrical communication between an implant and the body. ~~Remarkably, the pCBMA hydrogels implanted in mice resisted the foreign body reaction for three months.~~ Zhang et al. ~~The investigators~~ showed that pCBMA hydrogels resisted the foreign body reaction for three months when implanted in mice~~these zwitterionic polymers could reduce the formation of dense collagenous material at the implant site. This was presumably because the surface strongly resisted nonspecific protein adsorption~~. ~~This effect was attributed , an effect attributed to the surface's strong protein repellent propertiesrepellence~~The authors ~~hypothesized~~suggested that by resisting ~~preventing~~ protein adsorption, ~~allowed~~ the pCBMA hydrogels were able to~~to~~ prevent the chain of events leading to the formation of the collagen layer. ~~, as protein adsorption is thought to be the first step in the immune response which lead to the formation of the dense collagen layer~~ ~~From an applications viewpoint, this would~~By ~~preventing the foreign body reaction and the formation of a collagen layer, these pCBMA materials could~~ ~~potentially~~ improve mass transport and electrical communication between an implant and the body.~~

Zwitterionic polymers' chemical structure can allow for several unique functionalities. Carboxybetaines, for example, contain multiple functional groups amenable to conjugation. Further, due to the presence of oppositely charged species on each monomer, zwitterionic materials can be used as switchable surfaces, capable of shifting between cationic, zwitterionic, and anionic states based on the environmental pH.[108]

Zwitterionic polymers have also been successful at conferring protein resistant properties to nanoparticles.[20, 109] To this end, zwitterionic polymers have been used in a variety of nanoparticulate systems, including surface coatings for gold nanoparticles[110-112] and nanorods,[113] silica nanoparticles,[114] iron oxide nanoparticles,[115, 116] block copolymer micelles,[117] knedel-like polymers,[118] dendrimers,[119] polymeric immunobeads,[120, 121] and quantum dots[122, 123~~111, 112~~] and in protein conjugates.[124] In these applications, zwitterionic particles can strongly resist protein fouling.[110, 113-118] A direct comparison between PEG-coated and pCBMA-coated knedel-like nanoparticles found the two had similar size stability characteristics and biodistribution profiles in mice.[118]

Jiang and co-workers in fact found that in rats, pCBMA-coated gold nanoparticles (pCB-GNPs) had a longer half-life in the blood stream ~~in rats~~ than PEG-coated gold nanoparticles (PEG-GNPs)~~particles~~.[112]

A biodistribution study showed that ~~after~~ 5 days after the first injection, 30% of the original dose of pCBMA particles ~~were~~remained ~~found~~ in the blood stream, while almost all of the ~~PEGylated particles~~PEG-GNPs had ~~accumulated in the~~ been removed to the liver and other organs.

Moreover, unlike the ~~PEGylated particles~~PEG-GNPs, the pCBMA particles were able to avoid the ABC phenomenon.

As shown in ~~(~~**~~Figure 3Figure 3Figure 3Figure 3Figure 3Figure 3~~Figure 3**, for PEG-GNPs the rate of clearance ~~PEG particles~~ was greater after the second dose of ~~PEG GNPs~~ than the first dose. The clearance rate of pCBMA, on the other hand, did not appear to ~~accelerate~~change ~~between the first and second dosesafter the first dose~~ between doses. ~~).~~

Consistent with ~~this~~the blood residence time data, ~~blood samples taken after injection showed that the pCBMA~~PEG nanoparticles were~~ associated with lower~~ associated with elevated blood levels of IgM and IgG, two antibodies thought to underlie the ABC phenomenon~~,~~.

~~These two antibodies associated with opsonization and removal of nanoparticles from the bloodstreamwhich trigger the immune removal of foreign particles, and presumably cause the accelerated clearance of the PEGylated particles.~~.

~~The~~ pCB-GNP-injected rats~~BMA conditions~~ had similar levels of IgM and IgG as control rats ~~rats who were not injected with~~ without nanoparticles.

~~Moreover, unlike the PEGylated particles, the pCBMA particles were able to avoid the ABC phenomenon (**Figure 3**).~~ Thus~~Moreover, unlike the PEGylated particles, the pCBMA particles were able to avoid the ABC phenomenon (**Figure 3**).~~ Given such data, zwitterionic species appear to be a promising class of antifouling polymers which may be readily modified at their reactive functional groups for particular biomedical applications.

3. Poly(hydroxyfunctional acrylates)

Poly(hydroxyfunctional acrylates) include polymers such as poly(2-hydroxyethyl methacrylate) (pHEMA), poly(hydroxypropyl methacrylate) (pHPMA), and poly(N-hydroxyethylacrylamide) (pHEAA). Poly(hydroxyfunctional acrylates) are similar to other PEG alternatives in that they are electrically neutral and hydrophilic.

These polymers have a long history as biomaterials. pHEMA hydrogels, for example, have been conventionally used in a range of biomedical applications, including implants, tissue engineering scaffolds, and contact lenses, for over 25 years.[125, 126~~114, 115~~]

Recent work has explored both polymer brushes[101, 102, 127-131] and hydrogels[101, 132, 133] containing hydroxyfunctional acrylates for surface antifouling applications. *In vitro* protein absorbency tests with pHEMA demonstrate that it has protein repellence comparable to PEG.[127] Moreover, use of the acrylamide backbone (i.e., pHEAA) can produce surfaces with undetectable protein adsorption from undiluted human blood serum.[101] Similarly, pHPMA brushes can produce ultralow fouling properties similar to zwitterionic poly(carboxybetaine acrylamide)[102] (see **Figure 4**~~**Figure 4**~~).

While polymers like pHEMA[129, 134, 135] and pHPMA[136-138] have been incorporated into nanoparticles to confer antifouling properties, pHPMA drug conjugates have been the most prominent application of hydroxyfunctional acrylates for nanomedicine.[139, 140] pHPMA has been conjugated to cancer drugs (e.g., doxorubicin) in order to reduce drug toxicity or increase circulation time. In addition, by increasing a drug's effective molecular weight, pHPMA conjugation can be used to take advantage of the enhanced permeability and retention (ERP) effect associated with tumors.[139, 141-145] pHPMA is notable in that several commercial clinical trials involving pHPMA drug conjugates have commenced.[28]

4. Poly(vinylpyrrolidone)

Like the poly(hydroxyfunctional acrylates), poly(vinylpyrrolidone) (PVP) has a long history as a biocompatible polymer, being used as a plasma substitute in the 1930s and later as a food additive. PVP is a highly hydrated species,[146, 147] which likely accounts for its protein-repellence.[148]

Although macroscopic surfaces coated in PVP have received relatively less research attention, PVP does hold promise for surface applications.[149-151] Notably, Serrano et al.[151] recently tested PVP against human serum and found protein adsorption similar to PEG and

poly(2-ethyl-2-oxazoline), supporting PVP's utility for biomedical and similar antifouling applications.

PVP has been extensively examined in the context of nanoparticulate systems. A major application for PVP is in micellar systems,[152-157] although PVP has also been used in liposomes,[158-160] nanostructured capsules,[161-164] and drug conjugates.[165, 166]

PVP appears to have favourable qualities for drug delivery, including resistance to opsonization and phagocytosis. For example, PVP-conjugated tumor necrosis factor-α (TNF-α) showed a longer blood circulation time and twofold increase in antitumor effect compared to PEGylated TNF-α.[167] However, while most studies in mouse models of cancer find a positive effect of PVP conjugation or coating,[165, 168-171] some report that PVP underperforms compared to PEG controls in terms of circulation time or immunogenicity. This has been observed ~~for~~with conjugation of PVP to the enzyme uricase~~.~~[165] and ~~for~~with coating of poly(D,L-lactide) (PLA)-based nanoparticles with PVP ~~(Gaucher et al., 2009).~~[171] Although the reason for PVP underperformance ~~is unclearhas not been established~~is unclear, it may be a function of ~~the capacity of the polymer to sterically hinder protein adsorption in the~~ the steric hindrance provided by a polymer in any ~~particulargiven~~particular system~~m under consideration~~. For example, as a ~~brush-like~~ coating for ~~with the~~ PLA nanoparticles, the relatively bulky side chain of PVP ~~maywould~~may confer ~~rigidity which prevents it from freely rotating in space and sterically hindering protein adsorption~~conformational rigidity.[171] ~~(Gaucher et al., 2009)~~ This may prevent PVP from forming a dense, conformational cloud which provides steric hindrance to protein adsorption. ~~and prevent the polymer from .~~ PEG, on the other hand, lacking this side chain, ~~would be~~is more flexible and~~.~~ ~~Being able to freely rotate in space, PEG may be better~~ therefore able to provide this steric hindrance~~avoid thisable to formforming a dense conformational cloud providing steric~~

~~hindrance necessary to sterically hinder protein adsorption~~. That said, ~~(Gaucher et al., 2009).~~ ~~That saidHoweverThatThat said, thet~~The ability of PVP to form such a confirmation cloud is a function of its molecular weight. Therefore, op~~, and op~~timisation of this parameter may yield more effective PVP coatings~~gs.~~

Formatted: Space After: 12 pt, Line spacing: Double

~~[151, 158] Thus, PEG as with most antifouling polymers, further characterization of the immunological qualities of PVP is justified.~~

5. Poly(2-oxazoline)s

Poly(2-oxazoline)s (POxs) such as poly(2-methyl-2-oxazoline) and poly(2-ethyl-2-oxazoline) have recently become prominent PEG alternatives.[21-25] POxs have generated considerable interest in part because they are able to maintain their antifouling character for longer periods than PEG, suffering less oxidative damage in biological and oxidative media.[172]

POxs have successfully been used as surface coatings, and POxs have similar protein repellence as PEG when the grafting density is optimised.[22] As with PEG, a variety of surface attachment techniques are available,[21] including "grafting-to,"[173, 174] "grafting-from,"[175] and the use of poly(lysine)-*g*-POx brushes.[176]

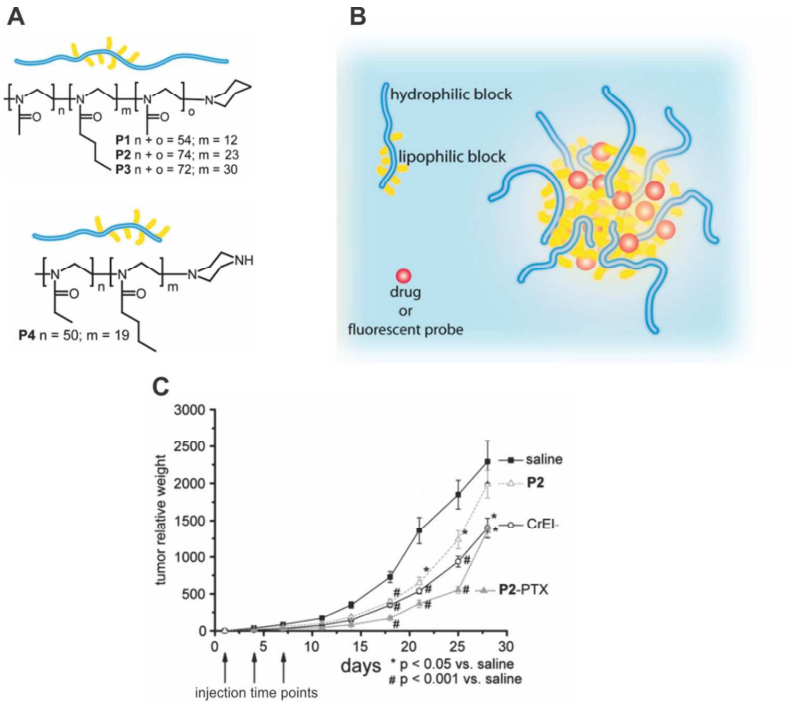


Figure 5. The synthesis and *in vivo* testing of micellar drug carriers constructed from poly(2-oxazoline)s (POxs). Four diblock or ABA-type triblock polymers were first produced entirely from POxs (A). To tune the hydrophilic/hydrophobic nature of the POx monomers, the length of the 2-alkyl side chains was varied. A methyl or ethyl side chain was used to produce a hydrophilic monomer and a butyl side chain was used to produce a hydrophobic monomer. When combined in an aqueous solution with hydrophobic drugs, these polymers self-assembled into micelles (B). Using this strategy, one of the polymers (polymer P2) was used to encapsulate the anticancer drug Paclixitel (PTX) (P2-PTX micelles). These micelles were tested against empty P2 micelles, a saline control, and a commercially available PTX carrier, Cremophor EL/ethanol (CrEL) in a mouse model of lung cancer (C). At all time points, the P2-PTX formulation performs similarly or better than the CrEL control. Reprinted with permission from R. Luxenhofer, A. Schulz, C. Roques, S. Li, T. K. Bronich, E. V. Batrakova, R. Jordan and A. V. Kabanov, *Biomaterials*, 2010, **31**, 4972-4979. Copyright 2010 Elsevier. ~~Adapted from ref. [170].~~

Formatted: Font: Cambria, 11 pt, Italic

Formatted: Font: Cambria, 11 pt, Bold

POxs have been used in place of PEG in several nanoparticulate systems. Notably, POxs appear promising as the hydrophilic component of block copolymer micelles designed to deliver hydrophobic drugs (see **Figure 5**~~**Figure 5**~~ for an example).[177-183] POxs have also been coated on the surfaces of liposomes[184, 185] and nanocapsules[186] to successfully impart stealth properties.

POxs appear to have similar *in vivo* characteristics as PEG in terms of circulation time and uptake by the reticuloendothelial system.[184, 187] Several recent studies have found that POxs have limited cytotoxicity or immunogenicity *in vitro*.[183, 188-193] For instance, Pulkkinen et al.[193] have shown *in vivo* that poly-ε-caprolactone cross-linked with 2, 2-bis(2-oxazoline) is biocompatible, non-toxic, and is slowly enzymatically degraded over a 12 week period when implanted in rat heart, lung, liver, kidney, spleen and brain tissue. These studies help to allay previous uncertainties about POxs' biocompatibility[28] and encourage further exploration of POxs for nanomedicine and controlled drug release applications.

6. Poly(glycerol)

Poly(glycerol) (PG) is a common, biocompatible polyol widely used in the food and pharmaceutical industries. Its polyether structure is similar to PEG but also contains hydroxyl groups amenable to functionalization. PG has received attention as an antifouling surface coating over the last 10 years. While surfaces with linear PG have been synthesized,[194-196] the hyperbranched form appears to be more resistant to protein adsorption[196] and is more extensively studied. PG has been affixed to surfaces both as SAMs[194-199] and as polymer grafts.[200-205] While PG coatings have strong resistance to model proteins such as fibrinogen and albumin,[201, 202, 204, 205] they have not yet been extensively tested in complex biological media such as human blood plasma.

PG has been used to impart stealth properties to a number of nanoparticulate systems, including inorganic nanoparticles,[206, 207] liposomes,[208-210] nanogels,[211] and micelles.[212] *In vitro*[213, 214] and *in vivo*, mice studies[215, 216] show that PG is biocompatible and ~~immunologically inert~~evades the immune system. For instance, Kainthan and Brooks report a plasma half-life for hyperbranched glycerol of approximately 57 hours with no notable toxicity.[216] However a significant build-up and retention of hyperbranched glycerol in organs of the reticuloendothelial system (i.e., the liver and spleen) was observed, an effect which increased with molecular weight. This was presumably due to a lack of biodegradable groups in the molecule, a limitation shared with PEG.

7. Peptides and peptoids

Synthetic peptides and peptoids may serve as effective antifouling polymers and may overcome the biodegradability issues of PEG and other PEG alternatives. Indeed, peptide-based SAMs,[217, 218] peptoid-based SAMs[219] and peptoid-based polymer brushes[36, 220-225] have recently been found to be highly effective as antifouling macroscopic surface coatings. Peptoids, or poly(*N*-substituted glycine) (see **Figure 1**~~**Figure 1**~~), are synthetically produced mimics of natural peptides with the side chain attached to the nitrogen atom, rather than the α-carbon. The shift of the side chain appears to endow peptoids with increased resistance to proteolytic degradation.[226, 227]

Statz and co-workers developed peptoid-coated surfaces that were able to resist protein adsorption from repeated washings with fresh serum for up to five months (**Figure 5**).[220] The antifouling character arises from the peptoid's methoxyethyl side chain, whose structure resembles PEG. The impressive long-term resilience of the surface may be attributed to the anchoring group, a peptide designed to mimic mussel adhesive protein.

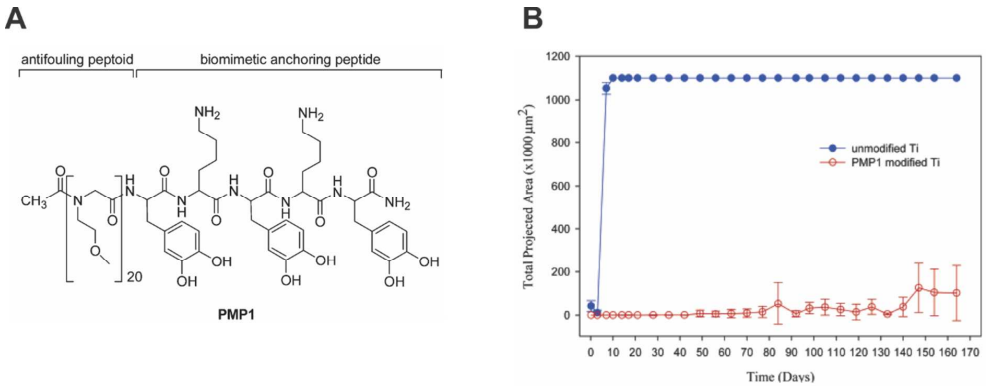


Figure 6. (A) An antifouling peptoid with catechol-containing peptide anchoring group attached to titanium surfaces (B) resists fibroblast attachment for up to five months. Both peptide-modified and control surfaces were incubated continuously with fresh fibroblasts in a solution of 10% fetal bovine serum, and the area of the surface covered by fibroblasts was measured. Because fibroblast attachment is mediated by protein adsorption, limited fibroblast attachment to the experimental surface suggests resistance to protein fouling. Reprinted with permission from A. R. Statz, R. J. Meagher, A. E. Barron and P. B. Messersmith, *J. Am. Chem. Soc.*, 2005, **127**, 7972-7973. Copyright 2005 American Chemical Society.~~Reproduced from ref. [212].~~

By choosing amino acid sequences with hydrophilic traits similar to PEG (e.g., L-asparagine and L-glutamine)[228-231] or by creating polyampholitic sequences,[232] peptides may be used for antifouling purposes in nanoparticulate systems. Indeed, poly(amino acids) have been used to confer stealth properties in several drug delivery systems, including liposomes,[228-231] inorganic nanoparticles,[232, 233] and micelles.[234]

Peptides have a distinct appeal as building blocks for nanoparticles. Because of their biodegradability, peptide-based nanoparticles can potentially avoid the bioaccumulation problems associated with PEG. In addition, peptides can be designed such that they are degraded by proteases at target sites, a trait that can be used for drug targeting.[234] Furthermore, peptides may be able to avoid the ABC phenomenon. Liposomes coated with poly(hydroxyethyl-L-asparagine) were shown to have a reduced ABC effect, with a longer circulation time associated with the second dose, compared to PEG-coated liposomes.[230]

By combining poly(amino acids) and PEG, advantages of both can be obtained in a single molecule.[235] For example, ring-opening polymerization of amino acid N-carboxyanhydrides

has been effectively used to produce peptide star polymers with a PEG corona and a core containing cationic[236, 237] or anionic[238] poly(amino acids). These star peptides have high biocompatibility and can be functionalized to target cancer cells[236] and deliver and release drug cargo in a pH-dependent manner.[238]

Peptides and proteins are commonly used as signalling molecules within the body, a feature which can be utilized to create stealth particles. Rodriguez et al.[239] designed peptides based on human CD47 protein, a molecule which signals to the immune system that its carrier is part of the body and should not be removed by phagocytes. Conjugating these "self-peptides" to nanobeads led to improved stealth-like properties, with approximately four times as many self-peptide presenting nanobeads remaining in circulation than PEGylated controls at 40 minutes post-injection into mice.

Peptoid-based nanoparticles may also have a nonfouling character. The non-ionic, hydrophilic poly(sarcosine) has been used in block copolymers with poly(γ-methyl L-glutamate)[240] and poly(L-lactic acid)[241] to make opsonization-resistant vesicles for bioimaging.[240, 241] However, despite the apparent promise of peptoids for biomedical applications, limited research has specifically examined their biocompatibility[225] and future work will be needed in this area.

Conclusion

Several polymeric materials, including PEG and a range of PEG alternatives, have been developed to combat protein-based fouling. Interestingly, antifouling properties observed on relatively flat, macroscopic surfaces are maintained at the nanoscale and on surfaces with a variety of geometries, including spherical interfaces.

Although the outlook for antifouling polymers is positive, much of the work is at an early-stage of application. For instance, many of the macroscopic surface coatings reviewed (e.g.,

polyglycerols) have been tested primarily *in vitro* with a limited number of model proteins, rather than whole blood fluids. Additional research will be required to test these surfaces against complex biological media and *in vivo*.

Considerable work has examined protein repellent nanoparticles in clinical settings; noteworthy examples include PEG and HPMA, which have in fact entered the commercial pharmaceutical product development pipeline. PEG has been the most successful polymer in this regard, and its application has served as a model for nanoparticulate drug delivery systems. Given its success, PEG has become the standard antifouling polymer. A challenge for nanomedicine is to bring many of the promising PEG alternatives, such as zwitterionic materials and POxs, to the clinic.

There is still significant scope to develop more effective materials based on novel structures. We believe numerous research opportunities exist to develop new zwitterionic monomers or monomers based on peptoids or unnatural amino acids. In addition to the development of new monomers, scope also exists for developing improved grafting/surface anchoring strategies. Continued application of existing anchoring strategies (e.g., using biomimetic catechol groups) as well as the development of new approaches will likely continue to be a basis for new antifouling surfaces. Indeed, combining these surface attachment strategies with new or existing monomers will pave the way for the next generation of antifouling polymer architectures.

Acknowledgements

This work was supported by the Victorian endowment for science knowledge and innovation (LAC).

References

1. D. Shi, *Biomaterials and Tissue Engineering*, Springer, Berlin, 2004.
2. C. J. Wilson, R. E. Clegg, D. I. Leavesley and M. J. Pearcy, *Tissue Eng.*, 2005, **11**, 1-18.
3. M. P. Monopoli, C. Aberg, A. Salvati and K. A. Dawson, *Nat Nano*, 2012, **7**, 779-786.
4. Y. Yan, K. T. Gause, M. M. J. Kamphuis, C.-S. Ang, N. M. O'Brien-Simpson, J. C. Lenzo, E. C. Reynolds, E. C. Nice and F. Caruso, *ACS Nano*, 2013, **7**, 10960-10970.
5. D. E. Owens and N. A. Peppas, *Int. J. Pharm.*, 2006, **307**, 93-102.
6. P. P. Karmali and D. Simberg, *Expert Opin. Drug Deliv.*, 2011, **8**, 343-357.
7. M. A. Dobrovolskaia and S. E. McNeil, *Nat. Nanotechnol.*, 2007, **2**, 469-478.
8. Y.-J. Shih, Y. Chang, D. Quemener, H.-S. Yang, J.-F. Jhong, F.-M. Ho, A. Higuchi and Y. Chang, *Langmuir*, 2014, **30**, 6489-6496.
9. S. Chen, L. Li, C. Zhao and J. Zheng, *Polymer*, 2010, **51**, 5283-5293.
10. S. I. Jeon, J. H. Lee, J. D. Andrade and P. G. Degennes, *J. Colloid Interface Sci.*, 1991, **142**, 149-158.
11. S. I. Jeon and J. D. Andrade, *J. Colloid Interface Sci.*, 1991, **142**, 159-166.
12. I. Banerjee, R. C. Pangule and R. S. Kane, *Adv. Mater.*, 2011, **23**, 690-718.
13. H. Otsuka, Y. Nagasaki and K. Kataoka, *Adv. Drug Delivery Rev.*, 2003, **55**, 403-419.
14. F. M. Veronese and G. Pasut, *Drug Discov. Today*, 2005, **10**, 1451-1458.
15. D. Vllasaliu, R. Fowler and S. Stolnik, *Expert Opin. Drug Deliv.*, 2014, **11**, 139-154.
16. R. Gref, A. Domb, P. Quellec, T. Blunk, R. Müller, J. Verbavatz and R. Langer, *Adv. Drug Delivery Rev.*, 1995, **16**, 215-233.
17. H. Otsuka, Y. Nagasaki and K. Kataoka, *Adv. Drug Delivery Rev.*, 2012, **64**, 246-255.
18. S. Jevševar, M. Kunstelj and V. G. Porekar, *Biotechnol. J.*, 2010, **5**, 113-128.
19. M. Binazadeh, M. Kabiri and L. D. Unsworth, in *Proteins at Interfaces III: State of the Art*, eds. T. Horbett, J. L. Brash and W. Norde, 2012, vol. 1120, pp. 621-643.

20. Z. Q. Cao and S. Y. Jiang, *Nano Today*, 2012, **7**, 404-413.

21. L. Tauhardt, K. Kempe, M. Gottschaldt and U. S. Schubert, *Chem. Soc. Rev.*, 2013, **42**, 7998-8011.

22. R. Konradi, C. Acikgoz and M. Textor, *Macromol. Rapid Commun.*, 2012, **33**, 1663-1676.

23. R. Luxenhofer, Y. Han, A. Schulz, J. Tong, Z. He, A. V. Kabanov and R. Jordan, *Macromol. Rapid Commun.*, 2012, **33**, 1613-1631.

24. R. Hoogenboom, *Angew. Chem., Int. Ed.*, 2009, **48**, 7978-7994.

25. O. Sedlacek, B. D. Monnery, S. K. Filippov, R. Hoogenboom and M. Hruby, *Macromol. Rapid Commun.*, 2012, **33**, 1648-1662.

26. Z. Amoozgar and Y. Yeo, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*, 2012, **4**, 219-233.

27. D. Mishra, J. R. Hubenak and A. B. Mathur, *J. Biomed. Mater. Res. Part A*, 2013, **101**, 3646-3660.

28. K. Knop, R. Hoogenboom, D. Fischer and U. S. Schubert, *Angew. Chem., Int. Ed.*, 2010, **49**, 6288-6308.

29. N. Larson and H. Ghandehari, *Chem. Mater.*, 2012, **24**, 840-853.

30. C. Blaszykowski, S. Sheikh and M. Thompson, *Chem. Soc. Rev.*, 2012, **41**, 5599-5612.

31. A. Hucknall, S. Rangarajan and A. Chilkoti, *Adv. Mater.*, 2009, **21**, 2441-2446.

32. R. Yang, A. Asatekin and K. K. Gleason, *Soft Matter*, 2012, **8**, 31-43.

33. K. L. Prime and G. M. Whitesides, *Science*, 1991, **252**, 1164-1167.

34. K. L. Prime and G. M. Whitesides, *J. Am. Chem. Soc.*, 1993, **115**, 10714-10721.

35. Y. Stetsyshyn, K. Fornal, J. Raczkowska, J. Zemla, A. Kostruba, H. Ohar, M. Ohar, V. Donchak, K. Harhay, K. Awsiuk, J. Rysz, A. Bernasik and A. Budkowski, *J. Colloid Interface Sci.*, 2013, **411**, 247-256.

36. K. H. A. Lau, C. Ren, S. H. Park, I. Szleifer and P. B. Messersmith, *Langmuir*, 2012, **28**, 2288-2298.

37. J. Satulovsky, M. A. Carignano and I. Szleifer, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 9037-9041.

38. R.-V. Ostaci, D. Damiron, S. Al Akhrass, Y. Grohens and E. Drockenmuller, *Polymer Chemistry*, 2011, **2**, 348-354.

39. B. S. Flavel, M. Jasieniak, L. Velleman, S. Ciampi, E. Luais, J. R. Peterson, H. J. Griesser, J. G. Shapter and J. J. Gooding, *Langmuir*, 2013, **29**, 8355-8362.

40. S. Jo and K. Park, *Biomaterials*, 2000, **21**, 605-616.

41. S. Tugulu and H.-A. Klok, *Biomacromolecules*, 2008, **9**, 906-912.

42. N. Nath, J. Hyun, H. Ma and A. Chilkoti, *Surf. Sci.*, 2004, **570**, 98-110.

43. V. Stadler, R. Kirmse, M. Beyer, F. Breitling, T. Ludwig and F. R. Bischoff, *Langmuir*, 2008, **24**, 8151-8157.

44. H. Ma, J. Hyun, P. Stiller and A. Chilkoti, *Adv. Mater.*, 2004, **16**, 338-341.

45. J. L. Dalsin, B.-H. Hu, B. P. Lee and P. B. Messersmith, *J. Am. Chem. Soc.*, 2003, **125**, 4253-4258.

46. H. Lee, S. M. Dellatore, W. M. Miller and P. B. Messersmith, *Science*, 2007, **318**, 426-430.

47. S. Saxer, C. Portmann, S. Tosatti, K. Gademann, S. Zürcher and M. Textor, *Macromolecules*, 2010, **43**, 1050-1060.

48. B. Malisova, S. Tosatti, M. Textor, K. Gademann and S. Zürcher, *Langmuir*, 2010, **26**, 4018-4026.

49. S. Heo, Y.-S. Jeon, S. I. Kim, S. H. Kim and J.-H. Kim, *Macromol. Res.*, 2013, **22**, 203-209.

50. J. L. Dalsin, L. J. Lin, S. Tosatti, J. Voros, M. Textor and P. B. Messersmith, *Langmuir*, 2005, **21**, 640-646.

51. X. Ding, C. Yang, T. P. Lim, L. Y. Hsu, A. C. Engler, J. L. Hedrick and Y.-Y. Yang, *Biomaterials*, 2012, **33**, 6593-6603.

52. L. G. Harris, S. Tosatti, M. Wieland, M. Textor and R. G. Richards, *Biomaterials*, 2004, **25**, 4135-4148.

53. R. R. Maddikeri, S. Tosatti, M. Schuler, S. Chessari, M. Textor, R. G. Richards and L. G. Harris, *J. Biomed. Mater. Res. Part A*, 2008, **84A**, 425-435.

54. G. L. Kenausis, J. Vörös, D. L. Elbert, N. Huang, R. Hofer, L. Ruiz-Taylor, M. Textor, J. A. Hubbell and N. D. Spencer, *The Journal of Physical Chemistry B*, 2000, **104**, 3298-3309.

55. M. Binazadeh, H. Zeng and L. D. Unsworth, in *Biomaterials Surface Science*, Wiley-VCH Verlag GmbH & Co. KGaA, 2013, pp. 45-61.

56. G. Gunkel, M. Weinhart, T. Becherer, R. Haag and W. T. S. Huck, *Biomacromolecules*, 2011, **12**, 4169-4172.

57. A. L. Klibanov, K. Maruyama, V. P. Torchilin and L. Huang, *FEBS Lett.*, 1990, **268**, 235-237.

58. G. Blume and G. Cevc, *Biochim. Biophys. Acta*, 1990, **1029**, 91-97.

59. D. Papahadjopoulos, T. Allen, A. Gabizon, E. Mayhew, K. Matthay, S. Huang, K. Lee, M. Woodle, D. Lasic and C. Redemann, *Proc. Natl. Acad. Sci. U. S. A.*, 1991, **88**, 11460-11464.

60. Y. Barenholz, *J. Controlled Release*, 2012, **160**, 117-134.

61. Y. Ding, P. Zhang, X.-Y. Tang, C. Zhang, S. Ding, H. Ye, Q.-L. Ding, W.-B. Shen and Q.-N. Ping, *Polymer*, 2012, **53**, 1694-1702.

62. L. Zhang, B. D. Klein, C. S. Metcalf, M. D. Smith, D. R. McDougle, H.-K. Lee, H. S. White and G. Bulaj, *Mol. Pharm.*, 2013, **10**, 574-585.

63. T. Gillich, C. Acikgoz, L. Isa, A. D. Schluter, N. D. Spencer and M. Textor, *ACS Nano*, 2013, **7**, 316-329.

64. L. A. Connal, C. R. Kinnane, A. N. Zelikin and F. Caruso, *Chem. Mater.*, 2009, **21**, 576-578.

65. E. D. Pressly, R. A. Pierce, L. A. Connal, C. J. Hawker and Y. Liu, *Bioconjug. Chem.*, 2012, **24**, 196-204.

66. J. Cheng, B. A. Teply, I. Sherifi, J. Sung, G. Luther, F. X. Gu, E. Levy-Nissenbaum, A. F. Radovic-Moreno, R. Langer and O. C. Farokhzad, *Biomaterials*, 2007, **28**, 869-876.

67. K. Liang, J. J. Richardson, H. Ejima, G. K. Such, J. Cui and F. Caruso, *Adv. Mater.*, 2013, **26**, 2398–2402.

68. V. C. F. Mosqueira, P. Legrand, A. Gulik, O. Bourdon, R. Gref, D. Labarre and G. Barratt, *Biomaterials*, 2001, **22**, 2967-2979.

69. E. Ahmed, S. W. Morton, P. T. Hammond and T. M. Swager, *Adv. Mater.*, 2013, **25**, 4504-4510.

70. M. K. M. Leung, G. K. Such, A. P. R. Johnston, D. P. Biswas, Z. Zhu, Y. Yan, J.-F. Lutz and F. Caruso, *Small*, 2011, **7**, 1075-1085.

71. M. M. J. Kamphuis, A. P. R. Johnston, G. K. Such, H. H. Dam, R. A. Evans, A. M. Scott, E. C. Nice, J. K. Heath and F. Caruso, *J. Am. Chem. Soc.*, 2010, **132**, 15881-15883.

72. D. Roy, G. Y. Berguig, B. Ghosn, D. D. Lane, S. Braswell, P. S. Stayton and A. J. Convertine, *Polymer Chemistry*, 2014, **5**, 1791-1799.

73. S.-W. Lee, M.-H. Yun, S. W. Jeong, C.-H. In, J.-Y. Kim, M.-H. Seo, C.-M. Pai and S.-O. Kim, *J. Controlled Release*, 2011, **155**, 262-271.

74. C. Liu, F. Liu, L. Feng, M. Li, J. Zhang and N. Zhang, *Biomaterials*, 2013, **34**, 2547-2564.

75. K. Wang, H. Peng, K. J. Thurecht, S. Puttick and A. K. Whittaker, *Polymer Chemistry*, 2014, **5**, 1760-1771.

76. A. Ardana, A. K. Whittaker and K. J. Thurecht, *Macromolecules*, 2014, **47**, 5211-5219.

77. D. J. Coles, B. E. Rolfe, N. R. B. Boase, R. N. Veedu and K. J. Thurecht, *Chem. Commun.*, 2013, **49**, 3836-3838.

78. K. J. Thurecht, I. Blakey, H. Peng, O. Squires, S. Hsu, C. Alexander and A. K. Whittaker, *J. Am. Chem. Soc.*, 2010, **132**, 5336-5337.

79. B. E. Rolfe, I. Blakey, O. Squires, H. Peng, N. R. B. Boase, C. Alexander, P. G. Parsons, G. M. Boyle, A. K. Whittaker and K. J. Thurecht, *J. Am. Chem. Soc.*, 2014, **136**, 2413-2419.

80. A. Ibricevic, S. P. Guntsen, K. Zhang, R. Shrestha, Y. Liu, J. Y. Sun, M. J. Welch, K. L. Wooley and S. L. Brody, *Nanomedicine*, 2013, **9**, 912-922.

81. M. Barz, R. Luxenhofer, R. Zentel and M. J. Vicent, *Polymer Chemistry*, 2011, **2**, 1900-1918.

82. E. Ostuni, R. G. Chapman, R. E. Holmlin, S. Takayama and G. M. Whitesides, *Langmuir*, 2001, **17**, 5605-5620.

83. L. Li, S. Chen and S. Jiang, *J. Biomater. Sci. Polym. Ed.*, 2007, **18**, 1415-1427.

84. R. Duncan and R. Gaspar, *Mol. Pharm.*, 2011, **8**, 2101-2141.

85. H. Schellekens, W. E. Hennink and V. Brinks, *Pharm. Res.*, 2013, **30**, 1729-1734.

86. L. A. Herzenberg and T. Tokuhisa, *J. Exp. Med.*, 1982, **155**, 1730-1740.

87. X. Wang, T. Ishida and H. Kiwada, *J. Controlled Release*, 2007, **119**, 236-244.

88. T. Ishida, M. Ichihara, X. Wang and H. Kiwada, *J. Controlled Release*, 2006, **115**, 243-250.

89. T. Ishida, M. Ichihara, X. Wang, K. Yamamoto, J. Kimura, E. Majima and H. Kiwada, *J. Controlled Release*, 2006, **112**, 15-25.

90. A. S. Abu Lila, H. Kiwada and T. Ishida, *J. Controlled Release*, 2013, **172**, 38-47.

91. P. Laverman, O. C. Boerman, W. J. G. Oyen, F. H. M. Corstens and G. Storm, *Crit. Rev. Ther. Drug Carrier Syst.*, 2001, **18**, 551-566.

92. S. Jiang and Z. Cao, *Adv. Mater.*, 2010, **22**, 920-932.

93. M. E. Schroeder, K. M. Zurick, D. E. McGrath and M. T. Bernards, *Biomacromolecules*, 2013, **14**, 3112-3122.

94. Q. Yang, L. Wang, W. Lin, G. Ma, J. Yuan and S. Chen, *J. Mater. Chem. B*, 2014, **2**, 577-584.

95. Y. Chang, S.-H. Shu, Y.-J. Shih, C.-W. Chu, R.-C. Ruaan and W.-Y. Chen, *Langmuir*, 2009, **26**, 3522-3530.

96. C. Leng, K. A. Gibney, Y. W. Liu, G. N. Tew and Z. Chen, *ACS Macro Letters*, 2013, **2**, 1011-1015.

97. K. Yoshimoto, T. Hirase, J. Madsen, S. Armes and Y. Nagasaki, *Macromol. Rapid Commun.*, 2009, **30**, 2136-2140.

98. R. S. Smith, Z. Zhang, M. Bouchard, J. Li, H. S. Lapp, G. R. Brotske, D. L. Lucchino, D. Weaver, L. A. Roth and A. Coury, *Sci. Transl. Med.*, 2012, **4**, 132-153.

99. G. Cheng, G. Li, H. Xue, S. Chen, J. D. Bryers and S. Jiang, *Biomaterials*, 2009, **30**, 5234-5240.

100. W. Yang, H. Xue, W. Li, J. Zhang and S. Jiang, *Langmuir*, 2009, **25**, 11911-11916.

101. C. Zhao, J. Zhao, X. Li, J. Wu, S. Chen, Q. Chen, Q. Wang, X. Gong, L. Li and J. Zheng, *Biomaterials*, 2013, **34**, 4714-4724.

102. C. Rodriguez-Emmenegger, M. Houska, A. B. Alles and E. Brynda, *Macromol. Biosci.*, 2012, **12**, 1413-1422.

103. S. Colak and G. N. Tew, *Langmuir*, 2012, **28**, 666-675.

104. L. Zhang, Z. Cao, T. Bai, L. Carr, J.-R. Ella-Menye, C. Irvin, B. D. Ratner and S. Jiang, *Nat. Biotechnol.*, 2013, **31**, 553-556.

105. P. Liu, E. Domingue, D. C. Ayers and J. Song, *ACS Appl. Mater. Interfaces*, 2014, **6**, 7141-7152.

106. B. Cao, L. Li, Q. Tang and G. Cheng, *Biomaterials*, 2013, **34**, 7592-7600.

107. T. Shimizu, T. Goda, N. Minoura, M. Takai and K. Ishihara, *Biomaterials*, 2010, **31**, 3274-3280.

108. H. S. Sundaram, J.-R. Ella-Menye, N. D. Brault, Q. Shao and S. Jiang, *Chem. Sci.*, 2014, **5**, 200-205.

109. K. P. Garcia, K. Zarschler, L. Barbaro, J. A. Barreto, W. O'Malley, L. Spiccia, H. Stephan and B. Graham, *Small*, 2014, **10**, 2516-2529.

110. W. Yang, L. Zhang, S. Wang, A. D. White and S. Jiang, *Biomaterials*, 2009, **30**, 5617-5621.

111. D. F. Moyano, K. Saha, G. Prakash, B. Yan, H. Kong, M. Yazdani and V. M. Rotello, *ACS Nano*, 2014, **8**, 6748-6755.

112. W. Yang, S. Liu, T. Bai, A. J. Keefe, L. Zhang, J.-R. Ella-Menye, Y. Li and S. Jiang, *Nano Today*, 2014, **9**, 10-16.

113. X. Chen, J. Lawrence, S. Parelkar and T. Emrick, *Macromolecules*, 2013, **46**, 119-127.

114. G. Jia, Z. Cao, H. Xue, Y. Xu and S. Jiang, *Langmuir*, 2009, **25**, 3196-3199.

115. L. Zhang, H. Xue, C. Gao, L. Carr, J. Wang, B. Chu and S. Jiang, *Biomaterials*, 2010, **31**, 6582-6588.

116. X. Zhang, W. Lin, S. Chen, H. Xu and H. Gu, *Langmuir*, 2011, **27**, 13669-13674.

117. Z. Cao, Q. Yu, H. Xue, G. Cheng and S. Jiang, *Angew. Chem., Int. Ed.*, 2010, **49**, 3771-3776.

118. A. Li, H. P. Luehmann, G. Sun, S. Samarajeewa, J. Zou, S. Zhang, F. Zhang, M. J. Welch, Y. Liu and K. L. Wooley, *ACS Nano*, 2012, **6**, 8970-8982.

119. R. R. Ramireddy, A. V. Subrahmanyam and S. Thayumanavan, *Chem. Eur. J.*, 2013, **19**, 16374-16381.

120. G. Kim, C. E. Yoo, M. Kim, H. J. Kang, D. Park, M. Lee and N. Huh, *Bioconjugate Chem.*, 2012, **23**, 2114-2120.

121. G. Kim, Y. Yong, H. J. Kang, K. Park, S. I. Kim, M. Lee and N. Huh, *Biomaterials*, 2014, **35**, 294-303.

122. E. Giovanelli, E. Muro, G. Sitbon, M. Hanafi, T. Pons, B. Dubertret and N. Lequeux, *Langmuir*, 2012, **28**, 15177-15184.

123. E. Muro, T. Pons, N. Lequeux, A. Fragola, N. Sanson, Z. Lenkei and B. Dubertret, *J. Am. Chem. Soc.*, 2010, **132**, 4556-4557.

124. A. J. Keefe and S. Jiang, *Nat. Chem.*, 2012, **4**, 59-63.

125. A. S. Hoffman, *Adv. Drug Delivery Rev.*, 2002, **54**, 3-12.

126. D. Luensmann and L. Jones, *Cont. Lens Anterior Eye*, 2012, **35**, 53-64.

127. H. Vaisocherová, V. Ševců, P. Adam, B. Špačková, K. Hegnerová, A. de los Santos Pereira, C. Rodriguez-Emmenegger, T. Riedel, M. Houska, E. Brynda and J. Homola, *Biosens. Bioelectron.*, 2014, **51**, 150-157.

128. C. Zhao, L. Li, Q. Wang, Q. Yu and J. Zheng, *Langmuir*, 2011, **27**, 4906-4913.

129. C. Zhao and J. Zheng, *Biomacromolecules*, 2011, **12**, 4071-4079.

130. C. Rodriguez-Emmenegger, O. A. Avramenko, E. Brynda, J. Skvor and A. Bologna Alles, *Biosens. Bioelectron.*, 2011, **26**, 4545-4551.

131. C. Rodriguez-Emmenegger, E. Brynda, T. Riedel, M. Houska, V. Subr, A. B. Alles, E. Hasan, J. E. Gautrot and W. T. S. Huck, *Macromol. Rapid Commun.*, 2011, **32**, 952-957.

132. C. Zhao, X. Li, L. Li, G. Cheng, X. Gong and J. Zheng, *Langmuir*, 2013, **29**, 1517-1524.

133. C. Zhao, L.-Y. Li, M.-M. Guo and J. Zheng, *Chem. Pap.*, 2012, **66**, 323-339.

134. S. N. Guntari, E. H. H. Wong, T. K. Goh, R. Chandrawati, A. Blencowe, F. Caruso and G. G. Qiao, *Biomacromolecules*, 2013, **14**, 2477-2483.

135. R. P. Johnson, Y.-I. Jeong, E. Choi, C.-W. Chung, D. H. Kang, S.-O. Oh, H. Suh and I. Kim, *Adv. Funct. Mater.*, 2012, **22**, 1058-1068.

136. S. Keller, J. T. Wilson, G. I. Patilea, H. B. Kern, A. J. Convertine and P. S. Stayton, *J. Control. Release*, 2014, **191**, 24-33.

137. J. G. Schellinger, J. A. Pahang, R. N. Johnson, D. S. Chu, D. L. Sellers, D. O. Maris, A. J. Convertine, P. S. Stayton, P. J. Horner and S. H. Pun, *Biomaterials*, 2013, **34**, 2318-2326.

138. B. B. Lundy, A. Convertine, M. Miteva and P. S. Stayton, *Bioconjugate Chem.*, 2013, **24**, 398-407.

139. J. Kopecek and P. Kopeckova, *Adv. Drug Delivery Rev.*, 2010, **62**, 122-149.

140. R. Duncan and M. J. Vicent, *Adv. Drug Delivery Rev.*, 2010, **62**, 272-282.

141. R. Duncan, *Nat. Rev. Cancer*, 2006, **6**, 688-701.

142. E. Kopansky, Y. Shamay and A. David, *J. Drug Targeting*, 2011, **19**, 933-943.

143. B. Rihova, T. Etrych, M. Pechar, M. Jelinkova, M. Stastny, O. Hovorka, M. Kovar and K. Ulbrich, *J. Controlled Release*, 2001, **74**, 225-232.

144. M. Sirova, M. Kabesova, L. Kovar, T. Etrych, J. Strohalm, K. Ulbrich and B. Rihova, *Curr. Med. Chem.*, 2013, **20**, 4815-4826.

145. H. Kostkova, T. Etrych, B. Rihova and K. Ulbrich, *J. Bioact. Compat. Polym.*, 2011, **26**, 270-286.

146. Z. H. Ping, Q. T. Nguyen, S. M. Chen, J. Q. Zhou and Y. D. Ding, *Polymer*, 2001, **42**, 8461-8467.

147. D. D. Eley, M. J. Hey and B. L. Winteringham, *J. Solution Chem.*, 1976, **5**, 787-797.

148. Z. Q. Wu, W. F. Tong, W. W. Jiang, X. L. Liu, Y. W. Wang and H. Chen, *Colloids Surf., B*, 2012, **96**, 37-43.

149. X. L. Liu, Y. J. Xu, Z. Q. Wu and H. Chen, *Macromol. Biosci.*, 2013, **13**, 147-154.

150. L. Yuan, Q. Yu, D. Li and H. Chen, *Macromol. Biosci.*, 2011, **11**, 1031-1040.

151. Â. Serrano, O. Sterner, S. Mieszkin, S. Zürcher, S. Tosatti, M. E. Callow, J. A. Callow and N. D. Spencer, *Adv. Funct. Mater.*, 2013, **23**, 5706-5718.

152. Z. S. Zhu, C. Xie, Q. Liu, X. Zhen, X. C. Zheng, W. Wu, R. T. Li, Y. Ding, X. Q. Jiang and B. R. Liu, *Biomaterials*, 2011, **32**, 9525-9535.

153. A. Veeren, A. Bhaw-Luximon and D. Jhurry, *Eur. Polym. J.*, 2013, **49**, 3034-3045.

154. A. Leiva, F. H. Quina, E. Araneda, L. Gargallo and D. Radic, *J. Colloid Interface Sci.*, 2007, **310**, 136-143.

155. Z. Zhu, Y. Li, X. Li, R. Li, Z. Jia, B. Liu, W. Guo, W. Wu and X. Jiang, *J. Controlled Release*, 2010, **142**, 438-446.

156. N. Bailly, M. Thomas and B. Klumperman, *Biomacromolecules*, 2012, **13**, 4109-4117.

157. N. Bailly, G. Pound-Lana and B. Klumperman, *Aust. J. Chem.*, 2012, **65**, 1124-1131.

158. V. P. Torchilin, M. I. Shtilman, V. S. Trubetskoy, K. Whiteman and A. M. Milstein, *Biochim. Biophys. Acta, Biomembr.*, 1994, **1195**, 181-184.

159. V. P. Torchilin, V. S. Trubetskoy, K. R. Whiteman, P. Caliceti, P. Ferruti and F. M. Veronese, *J. Pharm. Sci.*, 1995, **84**, 1049-1053.

160. D. Le Garrec, J. Taillefer, J. E. Van Lier, V. Lenaerts and J. C. Leroux, *J. Drug Targeting*, 2002, **10**, 429-437.

161. S. L. Ng, G. K. Such, A. P. R. Johnston, G. Antequera-Garcia and F. Caruso, *Biomaterials*, 2011, **32**, 6277-6284.

162. A. N. Zelikin, A. L. Becker, A. P. R. Johnston, K. L. Wark, F. Turatti and F. Caruso, *ACS Nano*, 2007, **1**, 63-69.

163. A. P. R. Johnston, M. M. J. Kamphuis, G. K. Such, A. M. Scott, E. C. Nice, J. K. Heath and F. Caruso, *ACS Nano*, 2012, **6**, 6667-6674.

164. C. R. Kinnane, G. K. Such, G. Antequera-García, Y. Yan, S. J. Dodds, L. M. Liz-Marzan and F. Caruso, *Biomacromolecules*, 2009, **10**, 2839-2846.

165. P. Caliceti, O. Schiavon and F. M. Veronese, *Bioconjugate Chem.*, 2001, **12**, 515-522.

166. P. Caliceti, O. Schiavon, M. Morpurgo, F. M. Veronese, L. Sartore, E. Ranucci and P. Ferruti, *J. Bioact. Compat. Polym.*, 1995, **10**, 103-120.

167. H. Kamada, Y. Tsutsumi, Y. Yamamoto, T. Kihira, Y. Kaneda, Y. Mu, H. Kodaira, S.-i. Tsunoda, S. Nakagawa and T. Mayumi, *Cancer Res.*, 2000, **60**, 6416-6420.

168. Z. Zhu, Y. Li, X. Li, R. Li, Z. Jia, B. Liu, W. Guo, W. Wu and X. Jiang, *J. Controlled Release*, 2010, **142**, 438-446.

169. X. R. Liu, J. B. Sun, X. M. Chen, S. S. Wang, H. Scott, X. Zhang and Q. Zhang, *J. Pharm. Pharmacol.*, 2012, **64**, 775-782.

170. X. Y. Ding, C. J. Hong, Y. Liu, Z. L. Gu, K. L. Xing, A. J. Zhu, W. L. Chen, L. S. Shi, X. N. Zhang and Q. Zhang, *Int. J. Nanomedicine*, 2012, **7**, 1723-1735.

171. G. Gaucher, K. Asahina, J. H. Wang and J. Leroux, *Biomacromolecules*, 2009, **10**, 408-416.

172. B. Pidhatika, M. Rodenstein, Y. Chen, E. Rakhmatullina, A. Muehlebach, C. Acikgoez, M. Textor and R. Konradi, *Biointerphases*, 2012, **7**.

173. Y. Chujo, E. Ihara, S. Kure and T. Saegusa, *Macromolecules*, 1993, **26**, 5681-5686.

174. S. Yoshikawa, N. Tsubokawa, K. Fujiki and M. Sakamoto, *Composite Interfaces*, 1999, **6**, 395-407.

175. N. Zhang, T. Pompe, I. Amin, R. Luxenhofer, C. Werner and R. Jordan, *Macromol. Biosci.*, 2012, **12**, 926-936.

176. R. Konradi, B. Pidhatika, A. Muehlebach and M. Textort, *Langmuir*, 2008, **24**, 613-616.

177. S. C. Lee, C. Kim, I. C. Kwon, H. Chung and S. Y. Jeong, *J. Controlled Release*, 2003, **89**, 437-446.

178. R. Luxenhofer, A. Schulz, C. Roques, S. Li, T. K. Bronich, E. V. Batrakova, R. Jordan and A. V. Kabanov, *Biomaterials*, 2010, **31**, 4972-4979.

179. C. H. Wang, C. H. Wang and G. H. Hsiue, *J. Controlled Release*, 2005, **108**, 140-149.

180. C. H. Wang, W. T. Wang and G. H. Hsiue, *Biomaterials*, 2009, **30**, 3352-3358.

181. C. Kim, S. C. Lee, J. H. Shin, I. C. Kwon and S. Y. Jeong, *Macromolecules*, 2000, **33**, 7448-7452.

182. C. Kim, S. C. Lee, S. W. Kang, I. C. Kwon and S. Y. Jeong, *J. Polym. Sci. Part B Polym. Phys.*, 2000, **38**, 2400-2408.

183. R. Luxenhofer, G. Sahay, A. Schulz, D. Alakhova, T. K. Bronich, R. Jordan and A. V. Kabanov, *J. Controlled Release*, 2011, **153**, 73-82.

184. M. C. Woodle, C. M. Engbers and S. Zalipsky, *Bioconjugate Chem.*, 1994, **5**, 493-496.

185. S. Zalipsky, C. B. Hansen, J. M. Oaks and T. M. Allen, *J. Pharm. Sci.*, 1996, **85**, 133-137.

186. K. Kempe, S. L. Ng, K. F. Noi, M. Müllner, S. T. Gunawan and F. Caruso, *ACS Macro Letters*, 2013, **2**, 1069-1072.

187. N. Adams and U. S. Schubert, *Adv. Drug Delivery Rev.*, 2007, **59**, 1504-1520.

188. M. Bauer, C. Lautenschlaeger, K. Kempe, L. Tauhardt, U. S. Schubert and D. Fischer, *Macromol. Biosci.*, 2012, **12**, 986-998.

189. M. Bauer, S. Schroeder, L. Tauhardt, K. Kempe, U. S. Schubert and D. Fischer, *J. Polym. Sci., Part A: Polym. Chem.*, 2013, **51**, 1816-1821.

190. J. Kronek, Z. Kronekova, J. Luston, E. Paulovicova, L. Paulovicova and B. Mendrek, *J. Mater. Sci. Mater. Med.*, 2011, **22**, 1725-1734.

191. J. Kronek, E. Paulovicova, L. Paulovicova, Z. Kronekova and J. Luston, *J. Mater. Sci. Mater. Med.*, 2012, **23**, 1457-1464.

192. J. Kronek, E. Paulovicova, L. Paulovicova, Z. Kronekova and J. Luston, *Journal of Materials Science-Materials in Medicine*, 2012, **23**, 1457-1464.

193. M. Pulkkinen, M. Malin, J. Böhm, T. Tarvainen, T. Wirth, J. Seppälä and K. Järvinen, *Eur. J. Pharm. Sci.*, 2009, **36**, 310-319.

194. M. Weinhart, T. Becherer, N. Schnurbusch, K. Schwibbert, H.-J. Kunte and R. Haag, *Adv. Eng. Mater.*, 2011, **13**, B501-B510.

195. M. Weinhart, I. Grunwald, M. Wyszogrodzka, L. Gaetjen, A. Hartwig and R. Haag, *Chem. Asian J.*, 2010, **5**, 1992-2000.

196. P.-Y. J. Yeh, R. K. Kainthan, Y. Zou, M. Chiao and J. N. Kizhakkedathu, *Langmuir*, 2008, **24**, 4907-4916.

197. C. Siegers, M. Biesalski and R. Haag, *Chem. Eur. J.*, 2004, **10**, 2831-2838.

198. M. Wyszogrodzka and R. Haag, *Biomacromolecules*, 2009, **10**, 1043-1054.

199. J. I. Paez, V. Brunetti, M. C. Strumia, T. Becherer, T. Solomun, J. Miguel, C. F. Hermanns, M. Calderon and R. Haag, *J. Mater. Chem.*, 2012, **22**, 19488-19497.

200. M. Khan and W. T. S. Huck, *Macromolecules*, 2003, **36**, 5088-5093.

201. T. Weber, M. Bechthold, T. Winkler, J. Dauselt and A. Terfort, *Colloids Surf., B*, 2013, **111**, 360-366.

202. M. Lange, S. Braune, K. Luetzow, K. Richau, N. Scharnagl, M. Weinhart, A. T. Neffe, F. Jung, R. Haag and A. Lendlein, *Macromol. Rapid Commun.*, 2012, **33**, 1487-1492.

203. D. Ekinci, A. L. Sisson and A. Lendlein, *J. Mater. Chem.*, 2012, **22**, 21100-21109.

204. Q. Wei, T. Becherer, R.-C. Mutihac, P.-L. M. Noeske, F. Paulus, R. Haag and I. Grunwald, *Biomacromolecules*, 2014, **15**, 3061-3071.

205. Q. Wei, T. Becherer, P.-L. M. Noeske, I. Grunwald and R. Haag, *Adv. Mater.*, 2014, **26**, 2688-2693.

206. L. Wang, K. G. Neoh, E. T. Kang, B. Shuter and S.-C. Wang, *Adv. Funct. Mater.*, 2009, **19**, 2615-2622.

207. K. G. Neoh and E. T. Kang, *Polymer Chemistry*, 2011, **2**, 747-759.

208. K. Maruyama, S. Okuizumi, O. Ishida, H. Yamauchi, H. Kikuchi and M. Iwatsuru, *Int. J. Pharm.*, 1994, **111**, 103-107.

209. A. M. Hofmann, F. Wurm, E. Huehn, T. Nawroth, P. Langguth and H. Frey, *Biomacromolecules*, 2010, **11**, 568-574.

210. A. Ishihara, M. Yamauchi, T. Tsuchiya, Y. Mimura, Y. Tomoda, A. Katagiri, M. Kamiya, H. Nemoto, T. Suzawa and M. Yamasaki, *J. Biomater. Sci. Polym. Ed.*, 2012, **23**, 2055-2068.

211. D. Steinhilber, M. Witting, X. Zhang, M. Staegemann, F. Paulus, W. Friess, S. Kuechler and R. Haag, *J. Controlled Release*, 2013, **169**, 289-295.

212. S. Gupta, B. Schade, S. Kumar, C. Boettcher, S. K. Sharma and R. Haag, *Small*, 2013, **9**, 894-904.

213. R. K. Kainthan, S. R. Hester, E. Levin, D. V. Devine and D. E. Brooks, *Biomaterials*, 2007, **28**, 4581-4590.

214. R. K. Kainthan, J. Janzen, E. Levin, D. V. Devine and D. E. Brooks, *Biomacromolecules*, 2006, **7**, 703-709.

215. R. Chapanian, I. Constantinescu, N. Medvedev, M. D. Scott, D. E. Brooks and J. N. Kizhakkedathu, *Biomacromolecules*, 2013, **14**, 2052-2062.

216. R. K. Kainthan and D. E. Brooks, *Biomaterials*, 2007, **28**, 4779-4787.

217. A. K. Nowinski, F. Sun, A. D. White, A. J. Keefe and S. Jiang, *J. Am. Chem. Soc.*, 2012, **134**, 6000-6005.

218. R. Chelmowski, S. D. Koester, A. Kerstan, A. Prekelt, C. Grunwald, T. Winkler, N. Metzler-Nolte, A. Terfort and C. Woell, *J. Am. Chem. Soc.*, 2008, **130**, 14952-+.

219. M. Schneider, C. Fetsch, I. Amin, R. Jordan and R. Luxenhofer, *Langmuir*, 2013, **29**, 6983-6988.

220. A. R. Statz, R. J. Meagher, A. E. Barron and P. B. Messersmith, *J. Am. Chem. Soc.*, 2005, **127**, 7972-7973.

221. A. R. Statz, J. P. Park, N. P. Chongsiriwatana, A. E. Barron and P. B. Messersmith, *Biofouling*, 2008, **24**, 439-448.

222. A. R. Statz, A. E. Barron and P. B. Messersmith, *Soft Matter*, 2008, **4**, 131-139.

223. K. H. A. Lau, C. Ren, T. S. Sileika, S. H. Park, I. Szleifer and P. B. Messersmith, *Langmuir*, 2012, **28**, 16099-16107.

224. K. H. A. Lau, *Biomaterials Science*, 2014, 627-633.

225. R. Luxenhofer, C. Fetsch and A. Grossmann, *J. Polym. Sci., Part A: Polym. Chem.*, 2013, **51**, 2731-2752.

226. S. M. Miller, R. J. Simon, S. Ng, R. N. Zuckermann, J. M. Kerr and W. H. Moos, *Drug Dev. Res.*, 1995, **35**, 20-32.

227. R. J. Simon, R. S. Kania, R. N. Zuckermann, V. D. Huebner, D. A. Jewell, S. Banville, S. Ng, L. Wang, S. Rosenberg, C. K. Marlowe, D. C. Spellmeyer, R. Y. Tan, A. D. Frankel, D. V. Santi, F. E. Cohen and P. A. Bartlett, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, **89**, 9367-9371.

228. B. Romberg, J. M. Metselaar, L. Baranyi, C. J. Snel, R. Bunger, W. E. Hennink, J. Szebeni and G. Storm, *Int. J. Pharm.*, 2007, **331**, 186-189.

229. J. M. Metselaar, P. Bruin, L. W. T. de Boer, T. de Vringer, C. Snel, C. Oussoren, M. H. M. Wauben, D. J. A. Crommelin, G. Storm and W. E. Hennink, *Bioconjugate Chem.*, 2003, **14**, 1156-1164.

230. B. Romberg, C. Oussoren, C. J. Snel, M. G. Carstens, W. E. Hennink and G. Storm, *Biochim. Biophys. Acta, Biomembr.*, 2007, **1768**, 737-743.

231. B. Romberg, W. E. Hennink and G. Storm, *Pharm. Res.*, 2008, **25**, 55-71.

232. A. K. Nowinski, A. D. White, A. J. Keefe and S. Jiang, *Langmuir*, 2014, **30**, 1864-1870.

233. H.-M. Yang, C. W. Park, T. Ahn, B. Jung, B.-K. Seo, J.-H. Park and J.-D. Kim, *J. Colloid Interface Sci.*, 2013, **391**, 158-167.

234. C. Dittrich, C. J. Burckhardt and G. Danuser, *Biomaterials*, 2012, **33**, 2746-2753.

235. A. Sulistio, P. A. Gurr, A. Blencowe and G. G. Qiao, *Aust. J. Chem.*, 2012, **65**, 978-984.

236. A. Sulistio, J. Lowenthal, A. Blencowe, M. N. Bongiovanni, L. Ong, S. L. Gras, X. Zhang and G. G. Qiao, *Biomacromolecules*, 2011, **12**, 3469-3477.

237. A. Sulistio, A. Widjaya, A. Blencowe, X. Zhang and G. Qiao, *Chem. Commun.*, 2011, **47**, 1151-1153.

238. Y. Yan, L. Liao and D. Liu, *J. Controlled Release*, 2011, **152, Supplement 1**, e60-e61.

239. P. L. Rodriguez, T. Harada, D. A. Christian, D. A. Pantano, R. K. Tsai and D. E. Discher, *Science*, 2013, **339**, 971-975.

240. H. Tanisaka, S. Kizaka-Kondoh, A. Makino, S. Tanaka, M. Hiraoka and S. Kimura, *Bioconjugate Chem.*, 2007, **19**, 109-117.

241. A. Makino, S. Kizaka-Kondoh, R. Yamahara, I. Hara, T. Kanzaki, E. Ozeki, M. Hiraoka and S. Kimura, *Biomaterials*, 2009, **30**, 5156-5160.