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REVIEW

Recent Biomedical Advances with Polyampholyte Polymers

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ABSTRACT: Polyampholyte polymer systems are composed of varying mixtures of charged monomer subunits. These polymeric systems have gained increasing attention because it is possible to design the final material properties through careful selection of the charged monomer subunits and controlling the polymer architecture. Characteristics that have been manipulated include the hydration, mechanical properties, pH responsive swelling, temperature responsive swelling, resistance to nonspecific protein adsorption, and protein conjugation capability. This had led researchers to propose the use of polyampholyte polymers as biosensor platforms, fouling release membranes, drug delivery vehicles, and tissue engineering scaffolds. This review is focused on advances that have been made over the last 5 years to develop polyampholyte polymers for these biomedical applications. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40069.

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INTRODUCTION

Polyampholyte polymers are polymeric systems composed of mixed charge monomer subunits. These copolymer systems have been receiving increasing attention in the polymeric research community because it is possible to incorporate specific polymer features simply through the selection of the specific charged monomer subunits. For example, it is possible to tailor both the mechanical properties and the polymer response to external stimuli by understanding these features for polymers composed of the individual monomers. This control can be designed by developing random copolymer distributions with specific ratios of the individual monomers or through the development of block copolymers with controlled molecular weight distributions.

The unique properties of polyampholyte polymers make them useful for a variety of applications. They have recently been used as adsorptive materials for the removal of bisphenol A, indigo carmine, aromatic compounds, lead, cadmium, and other heavy metals from various sources.[1–5] They have also been applied as a stabilizer for catalysts composed of copper II and oxometalates.[6–9] However, this review is focused on recent advances and breakthroughs (2008 and later) of biomedical applications for polyampholyte materials. First, we will discuss in more detail the general characteristics of polyampholytes that make them attractive for biomedical applications. Then we will highlight recent advances in the areas of biosensor and surface coatings, membrane based bioseparations, drug delivery, and tissue engineering.

GENERAL POLYAMPHOLYTE CHARACTERISTICS

The properties of polyampholyte polymers have been linked to the characteristics of proteins as far back as 1950.[10] Previous reviews have extensively detailed the solution properties of polyampholyte polymers, so only a brief summary is provided here.[11–13] One of the first distinctions that must be made is the subclass of polyampholyte because this will impact their overall properties. There are four subclasses of polyampholytes based on the strength of their anionic and cationic functional groups.[12] The first subclass contains polyampholytes composed of both weak anionic and cationic functional groups. The second subclass contains a weak anionic group and a strong cationic group. The third subclass consists of polyampholytes with a strong anionic group and a weak cationic group. The final subclass includes those polyampholytes composed of both strong anionic and cationic functional groups. The distinction between a weak and strong functional group is important to make because the weak functional groups have a greater response to changes in pH. This is one feature of polyampholyte polymers that allows them to mimic biological entities. Table I summarizes the most common monomer subunits used to develop polyampholyte polymers and the relative strength of the charged functional group.

The second distinction that must be made regarding polyampholyte polymers is the polymeric structure. Polyampholytes can be prepared as random copolymers where there is a

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distribution of the oppositely charged monomers throughout the polymer, as block copolymers where the charged monomers are polymerized in separate regions of the polymer, or as non-linear star polymer networks. Pafiti et al. recently demonstrated clear differences between the solution properties of linear polyampholytes prepared as either random or block copolymers.[14] Specifically they prepared polyampholytes composed of DMAEM (D) and MAA (A) with an ethylene glycol dimethacrylate cross-linker (E), as block copolymers with the sequences of E_3-grad-A_{25}-grad-D_{50}-grad-A_{25}-grad-E_3 and E_3-grad-D_{25}-grad-A_{50}-grad-D_{25}-grad-E_3, and statistical copolymers with the sequences of E_3-grad-(A_{50}-*co*-D_{50})-grad-E_3 and D_{50}-*co*-A_{50}-*co*-E_6. Although these polyampholytes were composed of identical macroscopic compositions, they exhibited different isoelectric points (IEP) and swelling behaviors as a function of pH. These differences, along with representative polyampholyte swelling behaviors in general, can be seen in Figure 1. In this figure, it can easily be seen that all the polyampholyte polymers have a significantly lower degree of swelling when they are at their IEP, as compared to both higher and lower pH values. This phenomena is directly attributed to the electrostatic interactions that occur between the charged subunits. At the IEP, there is charge balance throughout the polyampholyte, and electrostatic interactions between the positively and negatively charged functional groups lead to a collapse of the polymer structure. As the pH gets well beyond the IEP, the polyampholyte becomes charged and electrostatic repulsions within the structure lead to the expansion of the polymer network. A related relationship has also been demonstrated for the viscosities of polyampholytes in solution, with the viscosities reaching a minimum at the IEP.[12,15,16] However, the magnitudes of the changes in both size and viscosity are clearly dependent on the composition and architecture of the polymer as demonstrated

Table I. Common Polyampholyte Monomer Subunits

Chemical name	Acronym	Monomer formula	Strength of functional group
Acrylamide	AM	$CH_2{=}CHCONH_2$	Weak cation
N-[3-(Dimethylamino) propyl] acrylamide	DMAPAA	$CH_2{=}CHCONH(CH_2)_3N(CH_3)_2$	Weak cation
2-(Dimethylamino)ethyl methacrylate	DMAEM	$CH_2{=}C(CH_3)COOCH_2CH_2N(CH_3)_2$	Weak cation
2-(Diethylamino)ethyl methacrylate	DEAEM	$CH_2{=}C(CH_3)CO_2CH_2CH_2N(C_2H_5)_2$	Weak cation
[2-(Methacryloyloxy) ethyl] trimethylammonium chloride	TM	$CH_2{=}C(CH_3)CO_2CH_2CH_2N(CH_3)_3Cl$	Strong cation
2-(Acryloyloxy ethyl) trimethyl ammonium chloride	TMA	$CH_2{=}CHCO_2CH_2CH_2N(CH_3)_3Cl$	Strong cation
[3-(Methacryloylamino) propyl] trimethylammonium chloride	MAPTAC	$CH_2{=}C(CH_3)CONH(CH_2)_3N(CH_3)_3Cl$	Strong cation
2-Carboxyethyl acrylate	CAA	$CH_2{=}CHCO_2(CH_2)_2CO_2H$	Weak anion
Methacrylic acid	MAA	$CH_2{=}C(CH_3)COOH$	Weak anion
Acrylic acid	AA	$CH_2{=}CHCOOH$	Weak anion
Itaconic acid	IA	$HO_2CCH_2C(=CH_2)CO_2H$	Weak anion
3-Sulfopropyl methacrylate potassium salt	SA	$H_2C{=}C(CH_3)CO_2(CH_2)_3SO_3K$	Strong anion
2-Sulfoethyl methacrylate	SE	$H_2C{=}C(CH_3)CO_2(CH_2)_2SO_3H$	Strong anion

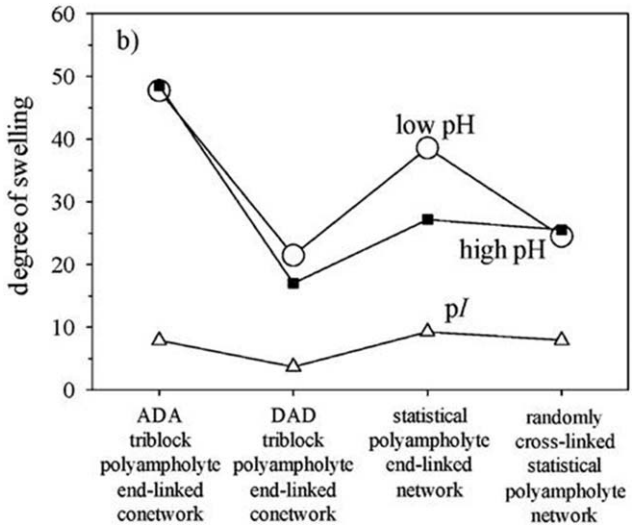

Figure 1. Degrees of swelling of polyampholyte copolymers as a function of their polymer architecture. The figure is reprinted from Ref. 14 with permission. Copyright 2011 American Chemical Society.

in Figure 1. Electrostatic interactions have also been demonstrated to play a role in the swelling behaviors of star conetworks, with a minimum again being found at the IEP.[17] Furthermore, electrostatic interactions between the arms of star copolymers have been demonstrated to be responsible for the pH dependent self-assembly of micelles and other structures when star copolymers are in solution.[17–22]

The degree of swelling and viscosity of polyampholytes are also impacted by the concentration and type of salt ions present. In what is known as the antipolyelectrolyte effect, the size and viscosity of a polyampholyte increase with greater concentrations of salt at or near the IEP.[12,15,16] As the concentration of salt increases, the ions shield the electrostatic interactions between the charged regions of the polyampholyte. The size and viscosity continue to increase until the salt concentration reaches a threshold value, where all of the electrostatic interactions between the polyampholyte charged regions are shielded, leading to a plateau value. As before, the magnitudes of these responses depend on both the composition and architecture of the polymer as well as the ionic composition of the salt.[16] These general swelling behaviors are summarized in Figure 2.

The potential to influence the properties of polyampholytes has led numerous investigators to pursue controlled polymerization methods to tune the final material characteristics. Although a detailed review of the polymerization techniques is beyond the scope of this review, recently investigators have pursued the formation of polyampholytes through the copolymerization of monomer subunits[23–28] or through modifications of a polymer backbone to add in charged functional groups.[16,29–31] In addition, there has been increasing interest in the modification of bio-based polymeric substrates to give them polyampholyte characteristics. Examples of this include the modification of chitosan through either its pendant —OH or —NH_2 groups,[32–34] cellulose,[35] dextran,[36] and konjac glucomannan (a component of the tuber *Amorphophallus konjac*).[37] Finally, there have been recent efforts in the synthesis of star and miktoarm copolymer structures with carefully controlled compositions of the star arms. These polymer systems have been shown to have unique self-assembly and interfacial behaviors as a function of composition,[17,18,20] architecture,[17,20] temperature,[18] and pH.[17–22] The remainder of this review is focused on recent advances demonstrating biomedical applications of polyampholyte polymers.

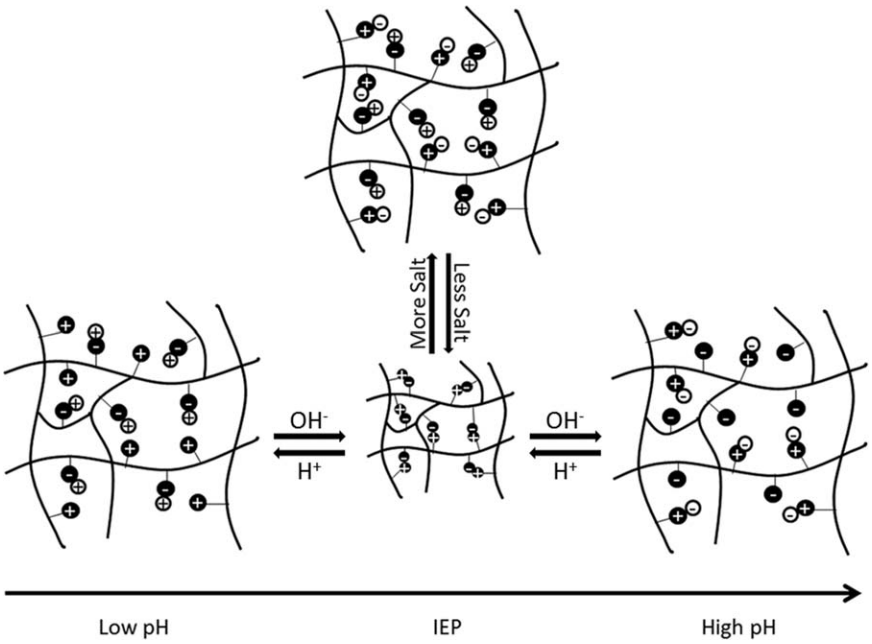

Figure 2. Schematic showing the impact that changes in pH and salt concentration have on the electrostatic interactions in polyampholyte polymer systems.

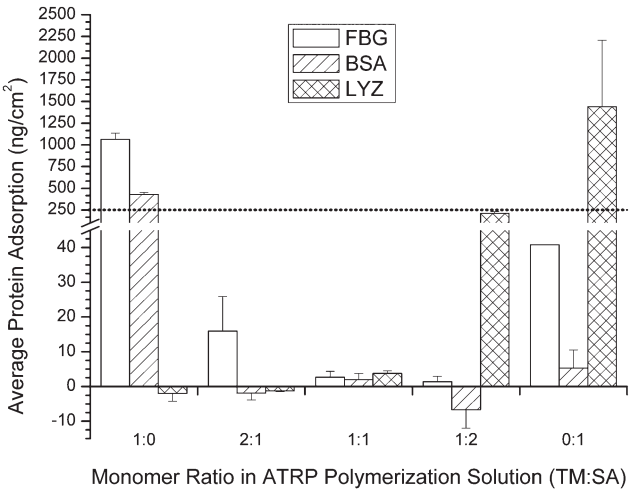

Figure 3. Nonspecific protein adsorption of fibrinogen (FBG), lysozyme (LYZ), and bovine serum albumin (BSA) on polyampholyte polymer brushes composed of varying ratios of TM and SA. The figure is reprinted from Ref. 42 with permission. Copyright 2008 American Chemical Society.

SURFACE GRAFTED POLYAMPHOLYTE POLYMERS

Polyampholyte polymer brush coatings can be formed through either the covalent attachment of an end-functionalized polymer chain to an activated substrate (grafting to) or through surface-mediated polymerization reactions (grafting from). In both instances, polyampholyte polymer chains maintain some of the characteristics of their solution based analogs. For example, several investigators have probed the pH responsive swelling behaviors of polyampholyte polymer brush coatings as a function of their composition and architecture.[38–41] These polymer brush coatings have consistently been shown to collapse upon themselves when exposed to a solution at their IEP.[38,39,41] This collapse is due to an increase in the electrostatic interactions between the charged monomer subunits in both individual polymer brush chains and between neighboring polymer brush chains. Yu and Han found that the collapsed polymer brushes have significantly more surface roughness and attributed this surface feature to the polymer conformation.[41] This collapsed state retains water, as confirmed by neutron reflectivity, and this attribute is important for biomedical applications of polyampholyte polymer brushes.[39]

It has recently been demonstrated that polyampholyte polymer brushes are resistant to nonspecific protein adsorption, opening the door for their use in many biomedical applications.[42] However, as demonstrated in Figure 3, this nonfouling property is only found in statistical copolymers with an equimolar distribution of the charged monomer subunits. Furthermore, it was suggested that electrostatic interactions dictate the initial protein adsorption behavior based on the observed trends between the protein adsorption and the polyampholyte polymer composition. A similar resistance to nonspecific protein adsorption has also been observed for mixed polyelectrolyte brushes with overall charge neutrality.[43] Chang et al. further probed the nonfouling properties of statistical polyampholyte polymers as a function of temperature, pH, and salt concentration.[44] More importantly, they demonstrated through surface plasmon resonance (SPR) biosensor investigations that the nonspecific protein adsorption from 20% platelet poor plasma (ppp) was only 7.65 ng/cm^2. Furthermore, even following exposure to 20% ppp, the polyampholyte polymer brush coatings had no visible blood cell or platelet adhesion.

These studies have all suggested that polyampholyte polymer brushes have excellent potential as a coating to prevent interactions between bio-molecules and the underlying substrate. One drawback, however, is that most of the polymer brush investigations have been completed with polymer brushes formed on either silicon[38–41] or gold[42,44] based on the compatibility of these substrates for the immobilization of polymerization initiators. To address this limitation, Li proposed the use of 3,4-dihydroxyphenyl-L-alanine (DOPA) molecules as a more universal adhesive for the attachment of polyampholyte polymer chains to a variety of surfaces.[45] In this work two separate DOPA containing polymerization initiators were used to form polyampholyte polymers. Both polymer systems were physically adsorbed to gold surfaces, to allow for the direct quantification of nonspecific protein adsorption using a SPR biosensor. Both systems had nonfouling properties, thereby opening an avenue for applying this approach to additional substrates beyond gold and silicon.

Our group has recently focused on applying nonfouling polymer brushes as a background substrate for biosensor applications in complex media. It is known that nonfouling polymer brushes have a thickness dependent performance,[46–48] and therefore studies were completed to optimize the formation of TMA : CAA statistical copolymer brushes to maximize their nonfouling characteristics.[49] Under the optimal conditions, the nonspecific adsorption from 100% fetal bovine serum was measured to be 4.3 ± 1.7 ng/cm^2. This confirms that these polymer brush coatings are appropriate for sensing applications in complex media. A unique advantage of the TMA : CAA polyampholyte mixture for biosensing applications is the fact that the carboxylic acid groups on the CAA monomers are pH sensitive. Under acidic pH conditions, the carboxylic acid groups are protonated, making them available for protein attachment using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide/*n*-hydroxysuccinimide (EDC/NHS) conjugation chemistry. It was demonstrated that at the optimal thickness for nonfouling, over 160 ng/cm^2 of fibrinogen could be covalently attached to the TMA : CAA polyampholyte coating.[49] Therefore, TMA : CAA polyampholytes polymer brushes show great promise as a nonfouling sensor platform with the capability to specifically immobilize detection molecules.

The pH responsive properties of TMA : CAA polyampholyte polymer brushes have also been used as a unique bacteria catch and release surface.[50] Under acidic conditions (pH 4.5), the TMA : CAA polymer brushes are positively charged, and they attract *Staphylococcus epidermidis* (*S. epidermidis*), promoting the adhesion of the bacteria. Under neutral and basic conditions; however, the TMA : CAA polymer brush is neutral and nonfouling, and there is minimal *S. epidermidis* adhesion. This is not unexpected, given the nonfouling characteristics discussed

above. The unique aspect of this system is the fact that when the TMA : CAA coating with adherent *S. epidermidis* was exposed to a basic pH buffer rinse (pH 10.0), the bacteria were released from the surface. It was hypothesized that the "catch and release" behaviors were due to the pH sensitive nonfouling properties of the polyampholyte polymer brush coating.

MEMBRANE APPLICATIONS

The nonfouling and bacteria-resistant properties of polyampholyte polymer brushes have led to their adaptation for membrane applications. Much of the recent work in the area of membrane applications is focused on minimizing protein adsorption and releasing adsorbed foulants, to improve the performance of the membranes.[51–55] The pH and temperature-sensitive properties of polyampholytes allow for controlled adsorption and release of biomolecules depending on the ionic strength of the surrounding media. However, charge distribution and density play key roles in the uptake and release mechanism due to the electrostatic nature of protein–polyampholyte interactions and must be carefully controlled in the final product to achieve maximal performance.

Leal Denis et al. investigated a polyampholyte formed from MAA, 2-methylimidazole (2MI), and ethylene glycol diglycidyl ether (EDGE) that demonstrated albumin adsorption and release properties.[56] The albumin adsorption was examined when the polymer was in an overall positively, negatively, or neutral charged configuration. As controls, adsorption to the individual homopolymers cross-linked with EDGE was also examined. The interactions between albumin and the poly(EDGE-MAA-2MI) were found to be dominated by electrostatic interactions and they occurred at the highest levels under relatively low ionic strength ($I < 10$ mM). The adsorption isotherms were found to be in good agreement with both the Langmuir equation and the McGhee–von Hippel equation, and a representative adsorption isotherm is shown in Figure 4(a). The albumin adsorption reached its maximum loading value of 0.742 g/g under basic pH conditions. The desorption of albumin from the membrane was heavily dependent upon the ionic strength of the release solvent, with release levels approaching 100% under ionic strengths greater than or equal to 0.2*M* as shown in Figure 4(b). Denaday et al. have also used poly(EDGE-MAA-2MI), as an immobilization support for soybean seed coat peroxidase in a flow-based reactor to improve the lifetime of the peroxidase enzyme.[57] Similar work with other polyampholytes has been performed with albumin adsorption to a chitosan-based polyampholyte,[32] albumin adsorption to a temperature and pH responsive gel,[54] lysozyme adsorption/release,[53] and in protein separation applications.[51,55]

Other investigators have focused on applying polyampholyte chemistries as surface coatings on existing membrane separation platforms, as an alternative to a standalone polyampholyte membrane, to take advantage of the benefits of polyampholytes. For example, Zhao et al. modified a polypropylene membrane to reduce the membrane biofouling during filtration.[58] In this study, TM and SA monomers were grafted to the surface through UV-induced polymerization in five different molar ratios. The first membrane performance metric that was evaluated was the relative flux recovery rate after filtering solutions of either bovine serum albumin (BSA) or lysozyme (LYZ). It was demonstrated that the flux recovery was >90% following the filtration of both proteins when the surface grafting was composed of a 1 : 1 ratio of the TM and SA monomers. However, when one of the monomers was enriched, the flux recovery dropped to levels as low as 12%. The second membrane performance metric that was evaluated was the membrane resistance to *Escherichia coli* (*E. coli*) adhesion. All the membranes with an SA composition that was equal to or greater than the TM composition showed resistance to *E. coli* adhesion following 48 h of exposure. The authors concluded that electrostatic interactions between the three probe molecules (BSA, LYZ, *E. coli*) and the varying surface modifications dictate the binding interactions that were seen. In addition, the polyampholyte coating with a 1 : 1 ratio of TM : SA had resistance to fouling from all three probes. These conclusions also support those drawn regarding polymer brush coatings in general, as discussed above.

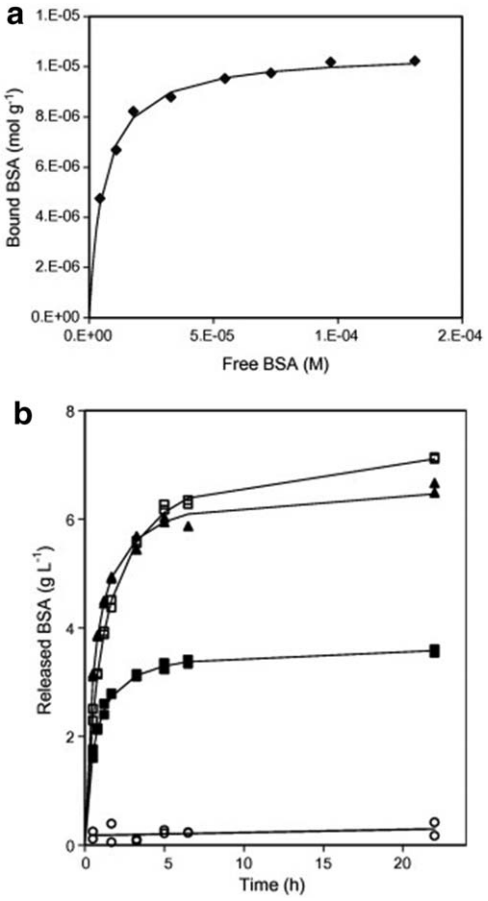

Figure 4. Bovine serum albumin adsorption (a) and desorption (b) from a poly(EDGE-MAA-2MI) membrane. The figure is reprinted from Ref. 56 with permission. Copyright 2008 Elsevier.

DRUG DELIVERY APPLICATIONS

Much of the recent work investigating polyampholytes as drug delivery systems has focused on the development of polymer microgels of varying compositions.[59–61] The justification for

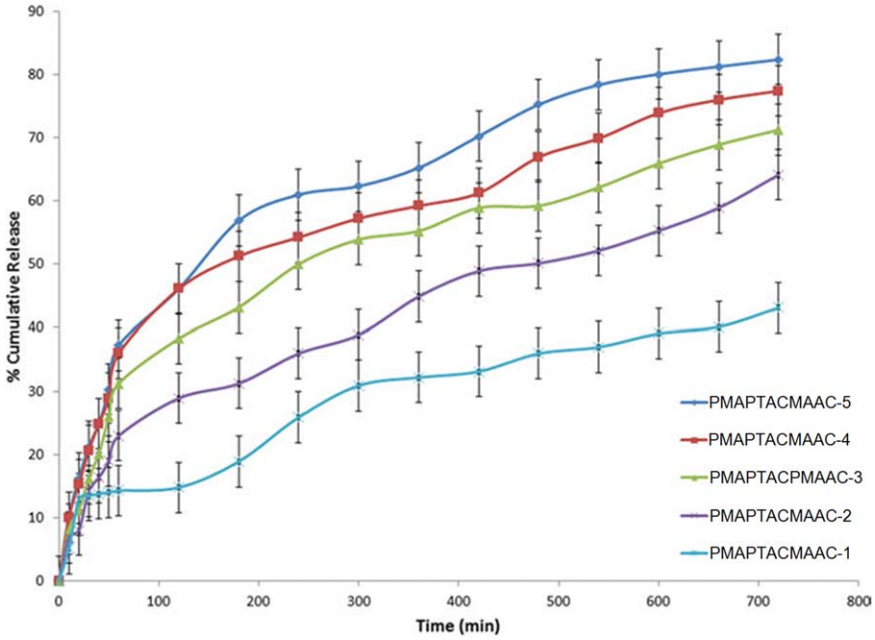

Figure 5. Cumulative release of IND from poly(MAPTAC-*co*-MAA) hydrogels with different compositions. The figure is reprinted from Ref. 67 with permission. Copyright 2012 Wiley Periodicals. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

investigating these microgel systems is that fact that they have pH and/or temperature sensitive swelling behaviors as shown schematically in Figure 2. By taking advantage of this characteristic, it is possible to load drug molecules into the hydrogel network and control their release by providing external stimuli. However, the polymerization technique and resulting structure play important roles in the responsive nature of the resulting drug delivery vehicle.

In one example, Ng and Ng investigated the release of three model molecules from poly(2-hydroxyethyl methacrylate) (pHEMA) coated with varying combinations of AA and DEAEM copolymers.[62] The model systems included methylene blue, metanil yellow, and caffeine as cationic, anionic, and neutral molecules, respectively. The AA : DEAEM weight percentage ratio was varied from 60 : 0 to 20 : 40. Methylene blue was released with a burst profile at pH 3, with limited release at pH 10, until the amount of DEAEM was equal to or greater than the amount of AA. At that point, there was also a smaller burst release of methylene blue at pH 10. A similar response was seen for metanil yellow. However, the release only occurred at pH 10 for all of the mixtures that were tested. The neutral caffeine molecule was released at both pH 3 and 10, although the burst releases were greater at pH 3 than pH 10 for all the AA : DEAEM ratios tested. Based on these results, the authors concluded that the model drug release was controlled by electrostatic interactions. Similar release profiles and conclusions were also drawn for negatively charged methyl orange and positively charged rhodamine 6G,[36] chitosan,[63] cationic cetylpyridinium chloride,[64,65] triton X,[65] and doxorubicin.[66] Bovine serum albumin adsorption or loading and release have also been tested as a model protein-based therapeutic.[32] Finally, Popescu et al. used a pH sensitive terpolymer to develop self-assembled hydrogels encapsulating liposomal drug carriers containing calcein.[21] By controlling the amount of terpolymer, they were able to extend the release of casein by at least threefold over the liposomal carriers alone.

Based on the promising results obtained with model drug molecules, investigators have shifted their focus to the delivery and bioactivity of several clinically relevant drugs. In one example, Mishra et al. investigated the delivery of indomethacin (IND), which is a hydrophobic non-steroidal anti-inflammatory.[67] IND was loaded into poly(MAPTAC-*co*-MAA) copolymers with varying ratios of the monomers. Representative IND release profiles that were obtained in this study can be seen in Figure 5. It can be seen that as the amount of MAA was increased in the copolymer mixture, there was an improvement in the total IND release. The performance of the drug loaded poly(MAPTAC-*co*-MAA) hydrogels was demonstrated with RAW 264.7 murine macrophage cells. Cell populations were decreased by 34% after 24 h of exposure and 44% after 5 days of exposure to the drug loaded hydrogels and they were unaffected by the blank hydrogels alone. Others have also demonstrated that drug loaded polyampholyte hydrogels are effective at reducing cell numbers. In a series of investigations, Lee and colleagues have demonstrated the pH dependant release of paclitaxel (PAX) from poly(DMAEM-*co*-MAA) hydrogels. These systems performed significantly better than PAX alone based on the IC_{50} levels that were determined for two types of drug-resistant cell lines, MCF7/ADR and MT3/ADR,[68] and Caco-2 cells.[69]

Georgiou and Patrickios have demonstrated that polyampholyte star conetworks are capable of adsorbing DNA through electrostatic interactions.[17] Based on their initial studies, they have proposed that these materials could be used as a pH sensitive DNA delivery vehicle. Similarly, in one of the more interesting drug delivery applications, Yoshihara et al. have begun to

investigate the impact of polyampholyte polymers on the delivery and release of plasmid DNA vectors complexed with a polyethyleneimine carrier.[70] In this study, a poly ethylene glycol (PEG) polymer backbone was modified to contain pendant amino and carboxyl side chains with a range of NH_2 : COOH ratios. It was determined that the inclusion of the polyampholyte PEG molecules improved the transcriptional efficiency *in vitro*, in both B16 and Chinese hamster ovary (CHO) cell lines. The most significant improvement was seen for modified PEG polymers that had a ratio of 32 : 68 of NH_2 : COOH side chains. Monte Carlo simulations supported the authors conclusion that the polyampholyte PEG polymers loosened the electrostatic interactions between the negatively charged DNA and positively charged polyethyleneimine carrier. This conclusion was based on both the simulations and the performance of the polyampholyte PEG molecule as a function of the modification ratio. More importantly, it demonstrates the potential for polyampholyte polymers to have an impact in the field of gene delivery.

TISSUE ENGINEERING APPLICATIONS

The characteristics of polyampholyte materials that make them attractive for drug delivery options have also led to their adaptation as tissue engineering scaffolds. In addition to their high levels of hydration and general biocompatibility, it is possible to tailor the mechanical properties of polyampholyte polymer hydrogels through monomer selection criteria. Furthermore, polyampholyte hydrogels have also been demonstrated to have resistance to nonspecific protein adsorption.[71,72] It has been hypothesized that this property will lead to an improved wound healing response, by reducing the foreign body reaction to the implanted biomaterial.

In the first study of its kind, Chen and Jiang synthesized a number of polyampholyte hydrogels from different cationic and anionic monomer subunits, and the nonspecific adsorption of both IgG and fibrinogen (FBG) to the polyampholyte hydrogels was compared to known nonfouling compounds.[71] It was demonstrated that both the IgG and FBG adsorption levels were very low on gels prepared from overall neutral co-polymer systems, while significant adsorption occurred on gels formed from positively charged monomers alone. It was also noted that by lowering the ionic strength of the protein adsorption solution, the protein adsorption levels were increased slightly on negatively charged hydrogels (~5% increase) and greatly on positively charged hydrogels (~50%). In a more recent study, Dobbins et al. further examined the properties of hydrogels synthesized with a 1 : 1 molar ratio of TM and SA and various amounts of TEGDMA as a cross-linking agent.[72] In this study, it was demonstrated that the mechanical properties of the hydrogel could be easily adjusted without impacting the nonfouling properties. The results of these two studies suggest that polyampholyte hydrogels are nonfouling and it is possible to tailor additional hydrogel properties through either monomer selection criteria or polymerization procedures.

Very recently, our group has taken the concept of protein resistant polyampholyte hydrogels for tissue engineering a step further by demonstrating the unique multi-functional properties of

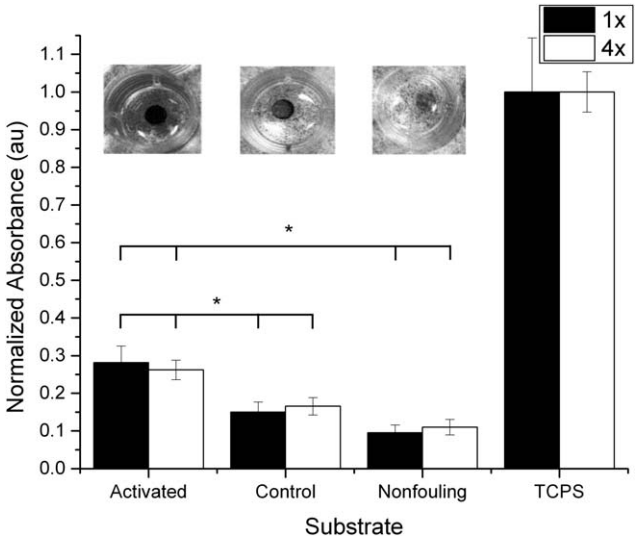

Figure 6. Enzyme-linked immunosorbent assay results showing the relative levels of FBG adsorption or conjugation to a TMA : CAA polyampholyte hydrogel material. The figure is reprinted from Ref. 73 with permission. Copyright 2013 American Chemical Society.

TMA : CAA hydrogels.[73] These hydrogels had resistance to nonspecific protein adsorption that was on par with the nonfouling controls as seen for other polyampholyte hydrogels and they resisted the attachment of MC3T3-E1 cells. However, as shown in Figure 6, the TMA : CAA hydrogels are also capable of covalently attaching proteins through EDC/NHS chemistry. This property is similar to that demonstrated and discussed earlier for polymer brushes composed of the same monomer subunits.[49] When the TMA : CAA hydrogels had conjugated FBG present they were able to support the attachment of MC3T3-E1 cells to a greater extent than FBG-coated tissue culture polystyrene. These results suggest that polyampholyte polymers that contain CAA show great promise for tissue engineering.

Other recent work has been focused on using synthetic and naturally derived hydrogel chemistries for tissue engineering, including hemicellulose-*g*-polyacrylamide gels,[74] pig skin gelatin and poly(vinyl alcohol),[75] gelatin/zeolite scaffolds,[76] and chitosan-hydroxyapatite,[77] among others. In one of the only studies with an *in vivo* component, Weng et al. synthesized hydrogels composed of *N*-carboxyethyl chitosan (CEC) with oxidized dextran (Odex) as the cross-linking agent.[78] The gelation speed and mechanical properties were examined as a function of the degree of oxidation of dextran. It was found that the gelation time decreased strongly with an increase in the extent of oxidation of dextran. The ratio of Odex to CEC was also found to influence swelling ratio, with a minimum swelling ratio of 23.2 occurring at an Odex content of 60%. The swelling was minimized when the theoretical ratio of CHO-groups on the Odex to NH_2-groups on the CEC was close to 1. Ratios greater or lower than this were determined to have a lower extent of cross-linking, which resulted in greater swelling. Cytotoxicity and cell viability assays were performed using 3 : 7, 5 : 5, and 7 : 3 Odex : CEC hydrogels, with no significant difference in the results for the gels and a control after 30 days. Encapsulation studies were also performed and

initially the cells did not adhere well to the hydrogel. However, after 1 week of incubation cell adhesion was observed. Finally, the effect of the hydrogel on wound healing was also examined in a murine model. Wound healing in the presence of the hydrogel was quantified by the granulation tissue formation and re-epithelialization/epidermal hyperplasia versus a PBS control. $84.9 \pm 9.0\%$ granulation tissue formation was observed in the wound bed treated with a hydrogel, while the PBS control only exhibited $46.3 \pm 8.3\%$ granulation tissue formation. Additionally, the application of a hydrogel to the wound bed resulted in 100% re-epithelialization, while only $54.3 \pm 8.4\%$ re-epithelialization occurred in the control wound bed. Again, this success suggests that polyampholytes have strong potential to serve as tissue engineering scaffolds.

FUTURE DIRECTIONS

Polyampholyte polymers have great potential for many biomedical applications because of the ability to tailor the mechanical properties, protein adsorption, protein conjugation, and pH responsiveness of the underlying copolymer through the selection of the charged monomer subunits and control over the polymerization reaction conditions. As discussed throughout this review, these advantages have been widely demonstrated in *in vitro* test environments. However, very few studies have taken polyampholytes beyond the laboratory setting and into real world *in vivo* applications that have been proposed by many. Future investigations by our group and others will certainly trend in this direction if polyampholytes are to be fully embraced by the biomedical community.

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