International Journal of Pharmaceutics 358 (2008) 36–43

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Boronate-containing polymers form affinity complexes with mucin and enable tight and reversible occlusion of mucosal lumen by poly(vinyl alcohol) gel

Alexander E. Ivanov[a,b,*], Lars Nilsson[c], Igor Yu. Galaev[a], Bo Mattiasson[a]

[a] Department of Biotechnology, Lund University, P.O. Box 124, SE-22100 Lund Sweden
[b] Protista Biotechnology AB, IDEON, SE-22370 Lund, Sweden
[c] Department of Food Technology, Lund University, P.O. Box 124, SE-22100 Lund Sweden

ARTICLE INFO

Article history:
Received 21 November 2007
Received in revised form 8 February 2008
Accepted 11 February 2008
Available online 10 March 2008

Keywords:
Boronic acid
Controlled release
Drug delivery
Mucoadhesive
Smart material

ABSTRACT

Copolymers of *N*-acryloyl-*m*-aminophenylboronic acid (NAAPBA) with *N*,*N*-dimethylacrylamide (DMAA) formed insoluble interpolymer complexes with mucin from porcine stomach at pH 9.0. The complex formation based on boronate–sugar interactions took place between the similarly charged macromolecules and resulted in coacervate particles formation, which depended both on pH and ionic strength of the solution. The coacervation rate displayed a maximum at the intermediate DMAA-NAAPBA copolymer: mucin weight ratio, that is a pattern typical of interpolymer complex formation. The effective hydrodynamic particle diameter of the coacervates monotonously grew from 155 ± 20 nm up to 730 ± 120 nm in 2 days in 0.1 M sodium bicarbonate buffer solution, pH 9.0. Electrophoretic mobility of the resultant nanoparticles was intermediate between those of individual polymers, whereas the particles zeta-potential was -7.5 ± 0.4 mV in the above buffer solution. Pre-treatment of the inner mucosal epithelium of excised male pig urethras with 5% (w/v) solutions of acrylamide-NAAPBA or DMAA-NAAPBA copolymers at pH 8.8 allowed for tight occlusion of the lumen by poly(vinyl alcohol) – borax gel injected via a two-way catheter. Leakage of 0.15 M NaCl solution through the thus occluded organs could be prevented, while the leakage through the organs occluded by the gel without the pre-treatment was unavoidable. The gel plug could be quickly dissolved on demand after injection of 5% (w/v) aqueous fructose solution into the lumen. The described technique may be useful for temporal occlusion of mucosal lumens in living organisms. In contrast to the conventional mucoadhesive polymers like polyacrylic acid or chitosan, the boronate-containing copolymers display their mucoadhesivity at weakly alkaline pH of 8–9 and physiological ionic strength.

1. Introduction

The gels of synthetic and natural polymers are increasingly used in medicine as tissue sealants, implants and drug delivery agents. Fibrin, gelatin and poly(cyanoacrylates) have been widely studied and used as tissue sealants in a number of surgical procedures (Morikawa, 2001). Poly(vinyl alcohol) (PVA) and cellulose triacetate were successfully used for filling large and giant aneurism cavities and suggested as a new method of endovascular treatment (Piotin et al., 2001; Mawad et al., 2002). Many synthetic non-specific bioadhesives based on polyacrylic acid (Smart et al., 1984; Ahn et al., 2002) hydroxyalkyl cellulose (Taylan et al., 1996) or chitosan (van der Lubben et al., 2001) displayed satisfactory performance as materials for transmucosal drug delivery or vaccination. The polymer-based hydrogels are well suited for bioadhesion due to their flexibility and nonabrasive characteristics in the partially swollen state, which reduced damaging attrition to the epithelial tissues in contact (Ahn et al., 2002). In particular, PVA membranes and hydrogels were used to prevent abnormal joining of anatomic structures after abdominal and pelvic surgery (Weis et al., 2004). PVA hydrogels enforced with cellulose fibers were proposed as a material for cardiovascular soft tissue replacement applications (Millon and Wan, 2006).

Adhesion of polymeric gels to the adjacent biological tissues can be enhanced by incorporation of specific cell adhesion peptides into the polymers. The conjugation of ArgGlyAsp (RGD) cell adhesion peptides with a vinyl alcohol copolymer allowed

* Corresponding author at: Department of Biotechnology, Center for Chemistry and Chemical Engineering, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden. Tel.: +46 46 222 3915; fax: +46 46 222 4713.
E-mail address: alexander.Ivanov@biotek.lu.se (A.E. Ivanov).

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.02.017

for attachment of endothelial cells to the polymer clots formed within the aneurism cavities (Ohyama et al., 2004). The RGD-grafted polymers assisted reorganizing the cavities better than the non-grafted polymers. Another type of bioaffinity adhesion is presented by sugar-specific proteins, lectins, situated on bioadhesive microparticles and liposomes. These particles were shown to provide highly selective targeting of human or mouse epithelial cells (Lehr, 2000; Ann Clark et al., 2000). Sugar-specific interactions offer wide prospects for directed drug delivery and tissue sealing because both the epithelial and endothelial glycocalix contain many mucins or mucin-like glycoproteins exhibiting numerous oligosaccharides (Lasky, 1995). Thus, sugar-specific bioadhesivity was imparted to microfabricated poly(methyl metacrylate) (PMMA) microdevices (150 μm × 150 μm) by means of chemical attachment of tomato lectin capable of targeting cells in the gastrointestinal tract. The *in vitro* studies performed with Caco-2 cell cultures demonstrated a several fold increase in quantity of the cell-bound microdevices compared with the PMMA microdevices containing no lectins (Tao et al., 2003). These findings confirm a high potential of sugar-specific interactions for chemical design of new bioadhesive materials and devices.

Incorporation of lectins into macro dosage forms and tissue sealants, as a mean to increase their bioadhesivity, can face, however, some limitations. The surface area of a macro dosage is many times larger than the surface of a particular cell in contact. Real mucosal surfaces containing different cell types as well as insoluble layer of mucus would exhibit many different end-group saccharides besides those complementary to the lectin. Broadly speaking, the overall strength of bioadhesion can be expected to suffer from the high selectivity of the saccharide–lectin interactions. High cost of lectins is also a limiting factor.

One might anticipate synthetic polymers with lectin-like binding specificity to carbohydrates as ideal mucoadhesives. Such polymers might be used as components of macro dosage forms with increased and/or controlled mucoadhesivity. Lectin-like binding activity is a known feature of water-soluble polymers containing phenylboronate (PBA) functional groups (Uchimura et al., 2001; Winblade et al., 2002; Kuzimenkova et al., 2006). Ability of these polymers to coat cell surfaces and to block cell–cell adhesion was confirmed in an *in vitro* model relevant to peritoneal adhesion formation using a monolayer of RM4 mesothelial cells (Winblade et al., 2002). The high viability of cells demonstrated in these experiments has proven non-toxicity of the boronate-containing copolymers (BCC). Non-toxicity and mitogenic properties of DMAA-NAAPBA copolymers have been independently demonstrated by Uchimura et al. (2001).

Recently, we have observed a spontaneous sugar-specific adsorption of water-soluble BCC onto the cross-linked polysaccharide gel (Kuzimenkova et al., 2006). Further, the BCC spontaneously formed insoluble complexes with mucin from porcine stomach in aqueous solution, due to boronate–sugar interactions (Ivanov et al., 2006). It seems challenging to investigate whether BCC can enhance mucoadhesivity of polymeric gels, in particular, those intended to plug the corporal cavities and lumen-containing organs. A temporal plugging of animal organs followed by restoration of their patency may have applications in surgery (Schmitt, 1994; Naughton et al., 2004). Dissolution of the plugs, even by means of organic solvents like dimethylsulfoxide, still faces, however, serious difficulties (Naughton et al., 2004). The goals of the present study are to investigate the complex formation between the BCC and mucin and to evaluate possibility of tight occlusion of a mucosal lumen by means of PVA gel. Another goal of the study is to show a possibility of the gel-plug dissolution under mild, physiologically acceptable conditions.

2. Experimental

2.1. Materials

Sodium hydrogen carbonate, disodium hydrogen phosphate, di-sodium tetraborate (borax) and sodium hydroxide, were products of Merck KGaA (Darmstadt, Germany). Acrylamide (AA), *N*,*N*-dimethylacrylamide (DMAA) and neutral aluminium oxide, type 507C, were products of Aldrich (Steinheim, Germany). 2,2′-Azobis(2-methylpropionitrile) (AMPN) was purchased from ACROS (Geel, Belgium). 1,4-Dioxane-D8 99 atom% D and deuterium oxide 99.95 atom% D were from Dr.Glaser AG (Basel, Switzerland). *N*-Acryloyl-*m*-aminophenylboronic acid (NAAPBA) was prepared as described by Ivanov et al. (2004). PVA, Mowiol 20–98, M_w = 125000 g mol^{-1}, was purchased from Clariant GmbH (Frankfurt, Germany). Freshly excised male pig urethras were excessively rinsed with 0.15 M NaCl containing 0.02% (w/v) sodium azide to remove blood both from the outside of the organ and from the lumen. The rinsed organs were frozen and kept at −18 °C.

2.2. Synthesis of DMAA-NAAPBA and AA-NAAPBA copolymers

Synthesis of the copolymers was performed according to the earlier described method of Ivanov et al. (2004), the molar proportion of the monomers being varied from 97.5:2.5 to 90:10. Briefly, DMAA (18–19.5 mmol), NAAPBA (0.5–2 mmol) and AMPN (10 mg) were dissolved in 20 mL of ethanol. Free radical polymerization was started by heating the reaction mixture to 70 °C under nitrogen bubbling and carried out for 6 h. The thus obtained solution of copolymer was added drop-wise to 200 mL diethyl ether for precipitation of the copolymer and its separation from the monomers. The precipitate was collected by filtration on Munktell No. 3 filter paper, washed with diethyl ether and dried in air and under vacuum. The yields of the copolymers were in the range of 75–85%. Synthesis of the AA-NAAPBA copolymer was performed as described by Kuzimenkova et al. (2006). Designations and characteristics of the copolymers are listed in Table 1.

2.3. Molecular weight determination

Weight-average molecular weight (M_w) of DMAA-NAAPBA copolymers with boronate units molar percentage up to 8.8 was calculated from their intrinsic viscosity using the formula for poly-DMAA: $[\eta] = 17.5 \times 10^{-5} \times M_w^{0.68}$ (Polymer Handbook, 1989). An Ubbelohde viscosimeter was used to measure intrinsic viscosity of the copolymer in methanol at 25 °C.

2.4. Turbidity of the mucin-copolymer coacervates

DMAA-NAAPBA copolymers were dissolved in 0.1 M sodium bicarbonate buffer solution (pH 9.0) at concentration of 8 mg mL^{-1}. Mucin from porcine stomach was dissolved in the buffer solution at concentration of 4 mg mL^{-1}. The solutions of polymers were filtered through a Minisart® filter with pore size of 0.45 μm. The solution of mucin (0.5 mL) was combined with the solution of the copoly-

Table 1
Characteristics of polymers

Polymer sample	NAAPBA mol% taken for synthesis	NAAPBA mol% in the copolymer	M_w (g mol^{-1})
polyDMAA	0	0	7000
DMAA-NAAPBA(2.5)	2.5	2.7	N.D.
DMAA-NAAPBA(5)	5	5.2	22000
DMAA-NAAPBA(10)	10	8.8	19000
AA-NAAPBA(15)	15	13	6700

N.D. = not determined.