Biomaterials Science

pH and Glucose Responsive Nanofibers for the Reversible Capture and Release of Lectins

Journal:	*Biomaterials Science*
Manuscript ID:	BM-ART-07-2014-000269.R1
Article Type:	Paper
Date Submitted by the Author:	30-Aug-2014

Complete List of Authors:	Wang, Yinan; University of Alberta, Chemical and Materials Engineering Kotsuchibashi, Yohei; National Institute of Materials Science, Biomaterials Unit Uto, Koichiro; National Institute for Materials Science (NIMS), Ebara, Mitsuhiro; National Institute of Materials Science, Biomaterials Unit Aoyagi, Takao; National Institute for Materials Science (NIMS), Biomaterials Center Liu, Yang; University of Alberta, Narain, Ravin; University of Alberta, Chemical and Materials Engineering

pH and Glucose Responsive Nanofibers for the Reversible Capture and Release of Lectins

Yinan Wang[1, 4], Yohei Kotsuchibashi[2], Koichiro Uto[3], Mitsuhiro Ebara[3], Takao Aoyagi[3], Yang Liu[4], Ravin Narain[1]*

[1]Department of Chemical and Materials Engineering, University of Alberta, 116 St and 85 Ave, Edmonton, AB T6G 2G6, Canada. E-mail: narain@ualberta.ca; Tel: +1 (780) 492 1736; Fax: +1 (780) 492 2881

[2]International Center for Young Scientists (ICYS), National Institute for Materials Science (NIMS), 1-1 Namiki, Tsukuba, Ibaraki 305-0044, Japan

[3]Biomaterials Unit, International Center for Materials Nanoarchitectonics (WPI-MANA), National Institute for Materials Science (NIMS), Tsukuba, Ibaraki 305-0044, Japan

[4]Department of Civil and Environmental Engineering, University of Alberta, 116 St and 85 Ave, Edmonton, AB T6G 2G6, Canada

Abstract

A dual pH and glucose responsive boronic acid containing nanofiber has been constructed for the reversible capture and release of lectins. The effects of surface groups and pH values on selective lectin capture have been investigated by fluorescence microscopy. Compared to the pristine nanofibrous membrane, glucose and galactose functionalized nanofiber surfaces showed significantly higher capture of ConA and Jacalin, under alkaline conditions. On the other hand, treatment of the modified nanofibers with acidic solution resulted in the detachment of both the lectins and the glycopolymers from the nanofiber surface. As expected, once the glycopolymers are displaced, no lectins were adhered to the nanofiber surface under alkaline conditions. These functional nanofibers can therefore be easily modified and hence can be used for the quick removal of selective proteins or toxins from solution.

Keywords: Electrospinning, 3-acrylamidophenylboronic acid, photochemical crosslink, carbohydrate-lectin interactions

1. Introduction

Over the past decades, nanofibers of various polymers or inorganic materials with diameters ranged from a few tens of nanometers to a few micrometers have been produced by the relatively simple and low-cost electrospinning technique.[1] The obtained nanofibrous mats have shown great success in various fields, include tissue engineering,[2] drug delivery,[3] membrane filtration,[4, 5] and sensors,[6, 7] due to their extremely large surface area and high porosity.[1, 5] Among the numerous electrospun materials, glycopolymers are believed to be one of the most attractive, not only because of the important roles that carbohydrate-protein interactions play in many biological processes,[8, 9] but also due to the multiple copies of sugar residues attached to the polymer backbones that can enhance the binding affinity with proteins by the "glycoside cluster effect".[10]

However, the electrospun nanofibers based on the bulk glycopolymers remain a challenge, since most of the synthetic glycopolymers are water soluble, which lead to the instability of their nanofibers in aqueous conditions. Moreover, nanofibers made from glycopolymers (homopolymers) often suffer from the lower efficacy towards molecular recognition processes due to the low availability of the sugar residues on the nanofiber surfaces.[11] To solve these problems, researchers have grafted sugar residues on water-insoluble electrospun nanofibrous surface[12, 13] or performed the copolymerization of sugar monomers with water insoluble monomers to obtain water insoluble copolymers which formed water insoluble electrospun nanofibrous mats after electrospinning.[11, 14]

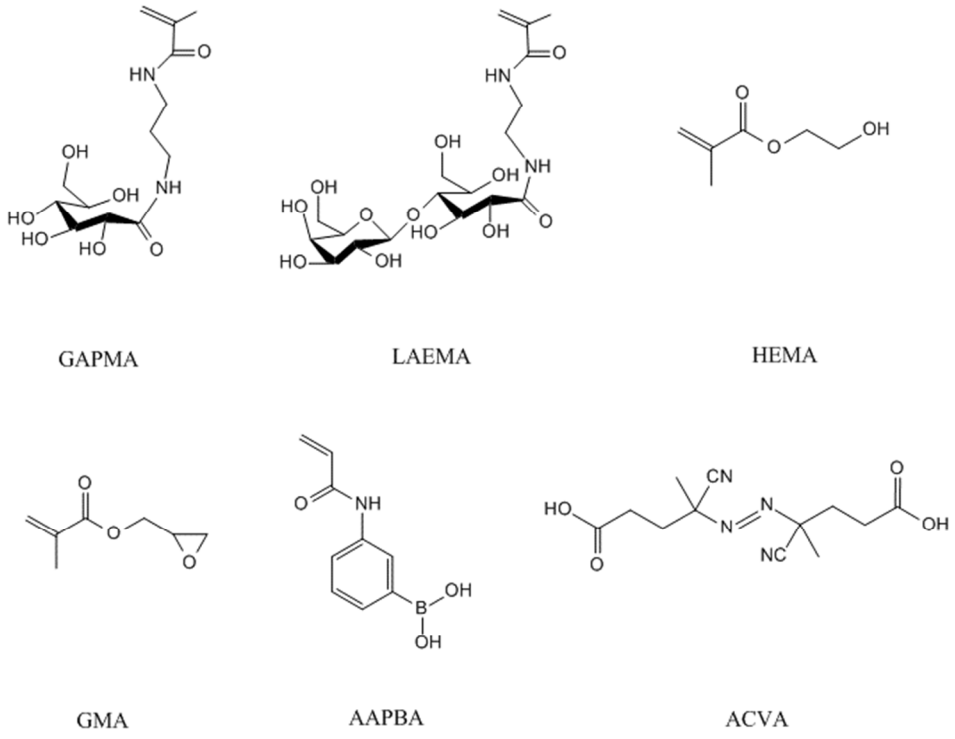
Recently, it was also noticed that the increased surface area on electrospun nanofibers can enhance the sensitivity of the stimuli-responsive materials to the external stimuli, and therefore resulting in dynamically and reversibly tunable "smart" nanofibrous structure that can be

potentially used for delivery of drugs or cells.[3, 15] Boronic acids and their ester derivatives is a class of important stimuli-responsive materials, which can reversibly interact with diols at a pH value higher than their pKa.[16, 17] This unique property have made these materials attractive in a wide ranges of biomedical fields, such as control release of insulin,[18-20] capture and release of circulating tumor cells (CTCs),[21] glucose sensing,[22-24] and tissue engineering.[25] However, electrospun nanofibers containing boronic acids are not very common[26] due to the cost associated in making those nanofibers. We propose here a low cost version of boronic acid based photo-crosslinked nanofibers by using a copolymer derived from 3-acrylamidophenylboronic acid (AAPBA) and 2-hydroxyethyl methacrylate (HEMA) and the subsequent modification of the nanofibers with glycopolymers allowed the selective binding of specific lectins.

2. Materials and Method

2.1.Materials

All chemicals were purchased from Sigma-Aldrich Chemicals (Oakville, ON, Canada) and the organic solvents were from Wako Pure Chemical Industries, Ltd (Japan). The glycomonomers were synthesized as previously described[27-30] (The structures of the monomers and initiator (4,4'-azobis(4-cyanovaleric acid) (ACVA)) are shown in Scheme 1.



Scheme 1. Chemical structures of the monomers and ACVA.

2.2. Methods

The ^{1}H NMR spectra of the monomers and polymers were recorded on a Varian 500 MHz spectrometer using D_2O or DMSO-d_6 as the solvent. The number average molecular weight (M_n) and polydispersity (M_w/M_n) were determined using polystyrene standards (M_w = 5,900-788,000 g mol^{-1}) at room temperature and a Viscotek model 250 dual detector (refractometer/viscometer in DMF eluents (containing 10mM LiBr) with flow rate of 1.0 mL/min. The capture and release of FITC-labeled lectins (Vector Laboratories, USA) on glycopolymers modified boronic acid based nanofibers were studied by fluorescence microscopy (Microscope Axio Imager.M2, Carl Zeiss, Germany) with a wide-Field fluorescence microscope excitation light source (X-cite® 120Q, Lumen Dynamic, ON, Canada).

2.2.1. Synthesis of glycopolymers

The glycopolymers were synthesized by free radical polymerization by using 4,4'-azobis(4-cyanovaleric acid) (ACVA) as the initiator. For a typical homopolymerization, a galactose containing monomer, 2-lactobionamidoethyl methacrylamide (LAEMA) (1 g, 2 mmol), was dissolved in 6 mL distilled water in a 10-mL Schlenk tube with 1 mL of ACVA (8 mg, 0.032 mmol) *N,N'*-Dimethylformamide (DMF) stock solution. The tube was then sealed and degassed by purging it with nitrogen for 30 minutes. Polymerization was carried out in an oil bath (70 ^{o}C) for 24 hours and followed by precipitation in acetone and subsequent washing with methanol to remove the monomers and residual initiator. The molecular weights and polydispersity (PDI) of the synthesized glycopolymers were determined by gel permeation chromatography (GPC) at room temperature with a Viscotek model 250 dual detector (refractometer/viscometer in aqueous eluents (0.5 M sodium acetate and 0.5 M acetic acid)). The conversion of the polymer was calculated by JNM-GSX300 ^{1}H NMR spectrometer (JEOL, Tokyo, Japan) using D_2O as the solvent.

2.2.2. Synthesis of photo-crosslinkable boronic acid based polymer

The photo-crosslinkable boronic acid based polymer was synthesized by free radical polymerization (Scheme 2, Synthesis of GMA modified photo-crosslinkable P(HEMA-st-AAPBA)). The boronic acid groups in 3-acrylamidophenylboronic acid (AAPBA) (0.2558 g, 1.34 mmol) were first protected by stirring with diols (1,4-butanediol, 0.60 g (6.55 mmol)) in 10 mL DMF in dark for 4 hr. After that, the protected AAPBA DMF solution was transferred to a 10-mL Schlenk tube and mixed with 5 mL of ACVA (20 mg, 0.07 mmol) and 2-Hydroxyethyl methacrylate (HEMA) (3.312 g, 25.45 mmol) DMF stock solution. The tube was then sealed and degassed by purging with nitrogen for 30 minutes. Polymerization was carried out in an oil bath at 70 ^{o}C for 24 hours and polymers were precipitated and purified by repeating washing with

large amount of diethyl ether. The conversion of the polymerization was determined by JNM-GSX300 ^{1}H NMR spectrometer (JEOL, Tokyo, Japan) using DMSO-d_6 as the solvent and operated at 300 MHz. The polymer's molecular weight and polydispersity were determined by gel permeation chromatography (GPC) at 40 °C (DMF including 10mM LiBr, 1 mL/min) with a TOSOH TSK-GEL a-2500 and a-4000 (Tosoh, Tokyo, Japan) and connected to a RI-2031 refractive index detector (JASCO International Co., Ltd., Tokyo, Japan).

To introduce the free double bonds to P(HEMA-st-AAPBA) chains, 0.5 g polymer was dissolved in 20 mL DMF with exceeding amount (1000 x) of glycidyl methacrylate (GMA). After the additional of small amount of triethylamine, the solution was left stirring 24 hr in dark, and precipitated and purified by repeating washing with large amount of diethyl ether. The conversion of the GMA modified P(HEMA-st-AAPBA) was again determined by JNM-GSX300 ^{1}H NMR spectrometer (JEOL, Tokyo, Japan) using DMSO-d_6 as the solvent.

2.2.3. Electrospinning of polymers and photo-crosslinking

To make the electrospun P(HEMA-GMA-st-AAPBA) NFs be easier to handle, in this study, we decided to collect the NFs on glass slides. To do this, 0.3 g P(HEMA-GMA-st-AAPBA) and 30 mg photo-initiator (Benzophenone) was dissolved in 3 ml of 1,1,1,3,3,3 hexafluoro-2-propanol (HFIP) and poured into a 5 ml plastic syringe equipped with a metal capillary needle (25 gauge). Glass slides with diameter of 15 mm were placed on a grounded aluminum foil, which was 19 cm away from the needle. The electrospinning process (Imoto IMC-19F5, Japan) was performed 1 hr at a polymer feed rate and a DC voltage of 0.5 mL/hr and 20 kV, respectively. The as-spun NFs were then photo-crosslinked by irradiated with nine UVA (350 nm) lamps in a Luzchem photoreactor for 30 min. The morphologies of the NFs were observed by SEM (Neoscope JCM-5000, JEOL, Japan) at an acceleration voltage of 10 kV.[3]

2.2.4. Lectin capture and release on photo-crosslinked NFs surface

The interactions between glycopolymers and boronic acid containing nanofibers, as well as the nanofibers pH and glucose dual responsiveness were first evaluated by fluorescent microscope (IX71, Olympus, Japan). To do this, the photo-crosslinked P(HEMA-st-AAPBA) nanofibers were placed in 24-wells plates and loaded with 0.4 mL of 0.1 M HCl to deprotect the boronic acid groups. After 15 min, nanofibers were gently washed with PBS for several times to remove the residual HCl. The nanofibers were then incubated in 10 mg/mL FITC-glycopolymers (PLAEMA (with galactose pendent groups) or PGAPMA (with glucose pendent groups)) Tris-0.1 M buffer solution (pH 9.0) for 15 min, rising with Tris-0.1 M HCl buffer (pH 9.0) and observed by fluorescent microscope. After that, the FITC-glycopolymers labeled nanofibers were incubated in 500 mg/mL glucose Tris-0.1 M buffer solution (pH 9.0) for 48 hr, rinsing with pH 9.0 Tris-HCl buffer (0.1 M) three times and imaged by fluorescent microscope again to evaluate the materials glucose responsiveness. Similarly, the materials pH responsiveness was evaluated by observing the fluorescence after the acid (0.1 M HCl) treated FITC-glycopolymers modified nanofibers rinsed with pH 9.0 Tris-HCl buffer (0.1 M) for three times. The fluorescent images were obtained from three independent repeats and for each at least 5 different areas were recorded.

For lectin capture, the deprotected nanofibers were incubated in 10 mg/mL glycopolymers (PLAEMA (with galactose pendent groups) or PGAPMA (with glucose pendent groups)) Tris-0.1 M buffer solution (pH 9.0) for 15 min to make the glycopolymers deposit on nanofibers. The free glycopolymers were washed away from the nanofiber surfaces by rising with Tris-0.1 M HCl buffer (pH 9.0), followed by immersing the nanofibers into 100 μL of FITC-lectins (Jacalin or ConA, 20 μL/mL) Tris-0.1 M HCl buffer solution (pH 9.0) for 15 min at room temperature.

The nanofibers were washed again with pH 9.0 Tris-0.1 M HCl buffer for several times before observing the florescence of the captured FITC-lectins in a fluorescent microscope.

For lectin release, the FITC-lecins/NFs loaded 24-wells plates were filled with 0.1 M HCl and incubated at room temperature in dark for 15 min. The NFs were then rinsed with pH 9.0 Tris-0.1 M HCl buffer for three times and imaged in a fluorescent microscopy. At least three lectin capture and release cycles were evaluated in the current study (Scheme 3, FITC-lectins capture and release on a photo-crosslinked polymer NF.).

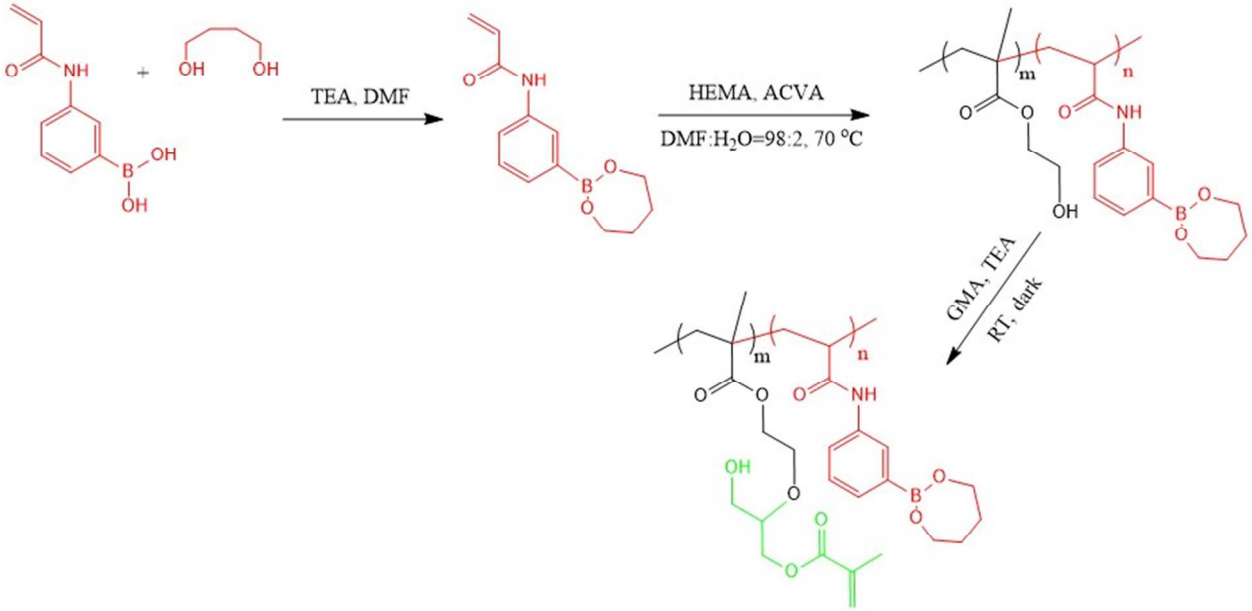
The glycopolymer modification as well as lectin capture and release efficiencies on the NF surfaces were evaluated by the % fluorescent area value, which is determined as: $\frac{\text{Fluorescent are (pixels)}}{\text{Total microscopy image area (pixels)}} \times 100\%$. The fluorescent area was measured by ImageJ, whereas the total microscopy image area were always fixed as 1443520 pixels. The % fluorescent area values were obtained from three independent repeats.

3. Results and Discussion

3.1. Polymer synthesis

3-acrylamidophenylboronic acid (AAPBA) was first protected with 1,4-butanediol in the presence of triethylamine. The protected monomer was then copolymerized with 2-hydroxyethyl methacrylate (HEMA) via conventional free radical polymerization, and the molecular weight details are shown in Table 1. Since only 1,2-, 1,3-, and 1,4- diols can form complex with boronic acids,[16] the adjacent hydroxyl groups in PHEMA should not have any interactions with boronic acids during the copolymerization.[31] The resulting copolymer was subsequently reacted with glycidyl methacrylate (GMA) so that free vinyl groups could be introduced to the copolymers.[32, 33] (Scheme 2) The 1,4-butanediol protection on boronic acid is expected to prevent any unnecessary reactions of the epoxy rings from GMA with the hydroxyl groups on boronic acid,[33]

and additionally this could interfere with the complex formation between boronic acid and carbohydrates.



Scheme 2. Synthesis of GMA modified photo-crosslinkable P(HEMA-st-AAPBA).

The ^{1}H NMR spectra of PHEMA and 1,4-butanediol protected P(HEMA-st-AAPBA) before and after the introduction of GMA is shown in Figure S1. It was found ~ 20 mol% of hydroxyl groups on PHEMA homopolymer were successfully reacted with GMA as evidenced from the =CH chemical shifts at δ= 5.5-6.5 (Figure S1a). On the other hand, in Figure S1b, the proportion of phenyl groups in P(HEMA-*st*-AAPBA) was found to be around 5 mol%, which was identical to the amount of 1,4-butanediol present in polymers, indicating all boronic acids had been successfully protected. However, from the ^{1}H NMR spectra of the GMA modified P(HEMA-*st*-AAPBA) (Figure S1b), only 10 mol% of the hydroxyl groups on copolymer chains had been successfully modified by the GMA molecules (Based on the signal to noise in the spectra (Figure S1b), the actual proportion of the GMA in polymer might be lower than 10 mol%). Although the amount of double bonds introduced to polymers were relatively low in this study, according to

Aoyagi et al. work,[3] 10 mol% of photo-crosslinkable moiety presented in electrospun nanofibers are high enough for photochemical crosslinking.

Table 1. GPC results of P(HEMA) and P(HEMA-*st*-AAPBA) synthesized by free radical polymerization

	N_{GMA}[a]	M_n (Da)	M_w/M_n
$PHEMA_{321}$	64	50,100	1.98
Diol protected $P(HEMA_{760}\text{-st-}AAPBA_{38})$	69	108,200	1.64

[a] The number of GMA in each polymer (N_{GMA}) was calculated as $N_{HEMA} \times GMA\ mol\%$

Similarly, for the glycopolymers synthesized by the conventional free radical polymerization, polymers' molecular weights and structure were characterized by GPC (Table 2) and NMR (Figure S1c), respectively. It was found both glycopolymers (PLAEMA and PGAPMA) had been successfully synthesized by free radical polymerization. The polymers' molecular weights were found over 80 kDa with very wide molecular weight distributions (PDIs are over 4.0). Based on these information, the high molecular weight glycopolymers are capable to interact on boronic acid containing nanofiber surface by boronate-diol interaction,[19] while part of the carbohydrate residues on the polymer chains are still available to capture the lectins in aqueous media.

Table 2. GPC results of PLAEMA and PGAPMA synthesized by free radical polymerization

	M_n (Da)	M_w/M_n
$PLAEMA_{231}$	108,400	4.24
$PGAPMA_{270}$	86,400	4.38

3.2. Electrospinning and photo-crosslinking of the nanofibers

The photo-crosslinkable P(HEMA$_{760}$-*st*-AAPBA$_{38}$) nanofibers were fabricated by electrospinning under optimized conditions (Figure 1a). To ensure the UV light penetrate completely through the electrospun nanofibrous mats during the photo-crosslink process, we have controlled the thickness of the electrospun nanofibrous mats by electrospinning the polymer solution for a short period (1 hr)[34, 35] with low polymer concentration (10 wt%)[36], slow pumping rate (0.5 mL/hr) and large needle-to-collector distance (19 mm)[1]. As shown in Figure 1, nanofibers, with an average diameter of ~ 400 nm, were randomly distributed and formed the continuous fibrous structure on either aluminum foil (Figure 1b) or glass surface (Figure 1c). Therefore, the morphology of the electrospun nanofibers is independent on the collector materials. On the other hand, the as-spun P(HEMA$_{760}$-*st*-AAPBA$_{38}$) nanofibrous mats showed high porosity and therefore light can easily penetrate through resulting in a bright background (Figure 1c). Therefore, we believe the nanofibrous mats should be completely crosslinked during the photo-crosslinking process.

When irradiated by the UV light, the photoinitiator distributed on surface or bulk of the nanofibers generated free radicals and chemically crosslink the alkene groups on the P(HEMA$_{760}$-st-AAPBA$_{38}$) nanofibers (Scheme 2).[3] The photo-crosslinked nanofibers were water insoluble even after incubation in PBS buffer for 24 hr (Figure 1e). Interestingly, in pH 9.0 Tris - 0.1 M HCl buffer, the nanofiber were found to be swollen (Figure 1f), which could be explained by the increasing water adsorption on nanofibers when boronic acids changed to the anionic ($B(OH)_3^-$) in basic aqueous environment.[16, 37]

The P(HEMA$_{321}$) nanofibers were electrospun and crosslinked in a similar way as the P(HEMA$_{760}$-st-AAPBA$_{38}$) nanofibers. Interestingly, after 24 hr incubation in pH 9.0 Tris - 0.1 M

HCl buffer, the photo-crosslinked P($HEMA_{321}$) nanofibers were found to be extremely swollen (Figure 1g) possibly due to the adsorption and retention of a large amount of water.

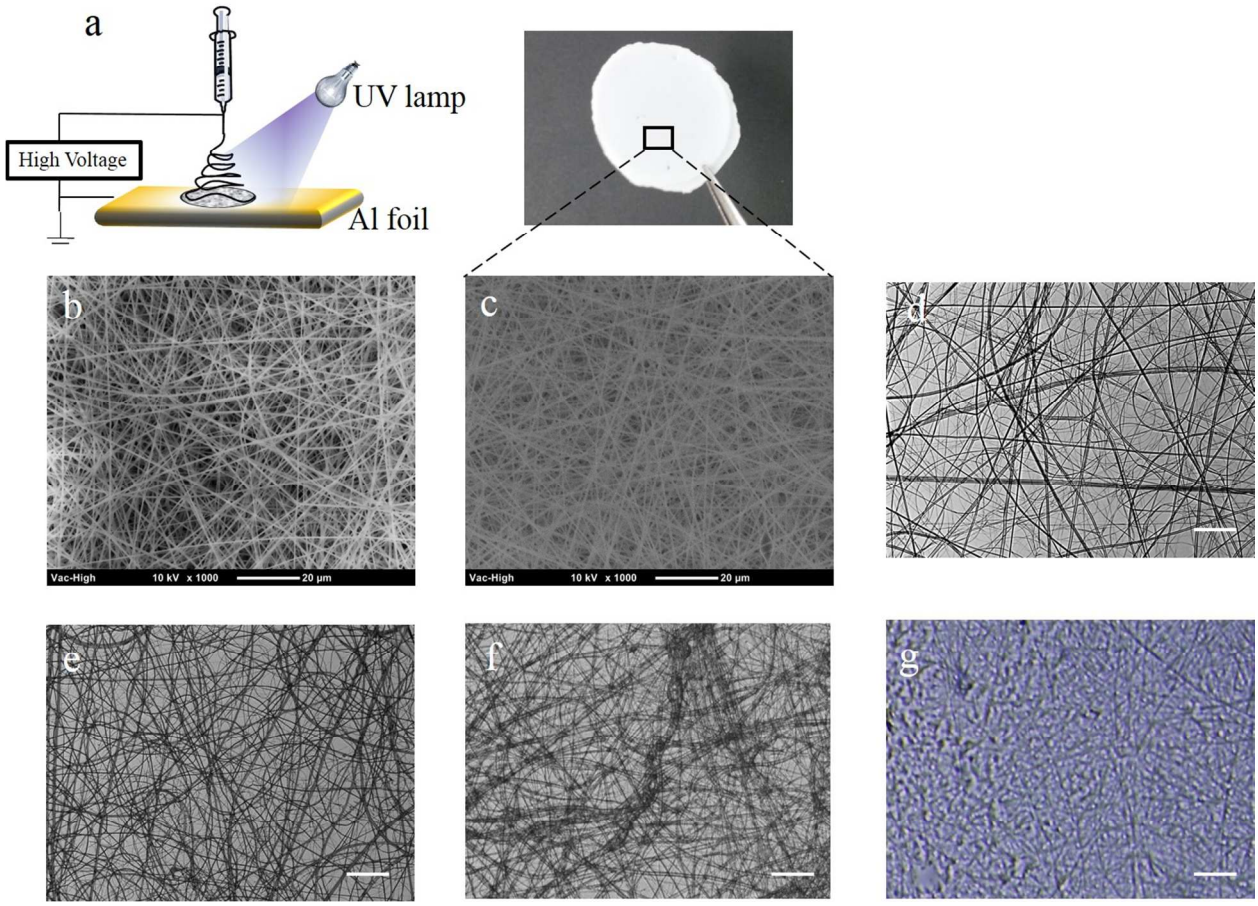


Figure 1. a) Fabrication of photo-crosslinkable polymer nanofibers by electrospinning. SEM images of P($HEMA_{760}$-st-$AAPBA_{38}$) nanofibers deposited on different substrates b) aluminum foil, c) glass slides. d) Optical microscopy images of the P($HEMA_{760}$-st-$AAPBA_{38}$) nanofibers before photo-crosslink, and photo-crosslinked P($HEMA_{760}$-st-$AAPBA_{38}$) nanofibers after incubated in PBS (e) and pH 9.0 Tris - 0.1 M HCl buffer (f) for 24 hr. Optical microscopy images of the photo-crosslinked P($HEMA_{321}$) nanofibers after incubated in pH 9.0 Tris - 0.1 M HCl buffer for 24 hr (g). Scale bar = 20 µm.

3.3. pH and glucose dual responsiveness of the boronic acid containing nanofiber surface

Since the boronate-diol interaction only occurs at basic conditions,[16, 19, 37], the photo-crosslinked P($HEMA_{760}$-st-$AAPBA_{38}$) can be deprotected by incubation in a 24-wells plate that is loaded with 0.4 mL of 0.1 M HCl. On the other hand, the binding affinity between the boronic acid and 1,4-diol is much weaker than 1,2 and 1,3-diols.[16, 37] Therefore, in the present study, if there are residual 1,4-butanediol left on nanofiber surface after the acid treatment, it should be completely replaced by the FITC-glycopolymers and showed images with the fluorescent signals uniformly distributed along the nanofibers (Figure 2). During the HCl deprotection and FITC-glycopolymer modification, we believe chemistry was occurred not only on the surface but also in the bulk of the nanofibrous mats as evidenced by the swelling (Figure 1e) and FITC staining (Figure 2 and 3) in the bulk of the mats.

The responsive nature of the resulting glycopolymer modified nanofiber to glucose was then studied. Immersion in glucose solution resulted in the displacement of the glycopolymer from the nanofiber surfaces, however, long incubation (48 hrs) was required for the complete displacement of the glycopolymers as shown in Figure 2a and b. Interestingly, compared to the FITC-PGAPMA modified NFs, the one modified by FITC-PLAEMA showed larger areas of fluorescence and residue of fluorescence could be spotted on NFs surface even after 48 hr incubation in high glucose solution, (Figure 2c). These observations could be explained by the higher associate constant (pKa) between galactose and boronic acids.[38]

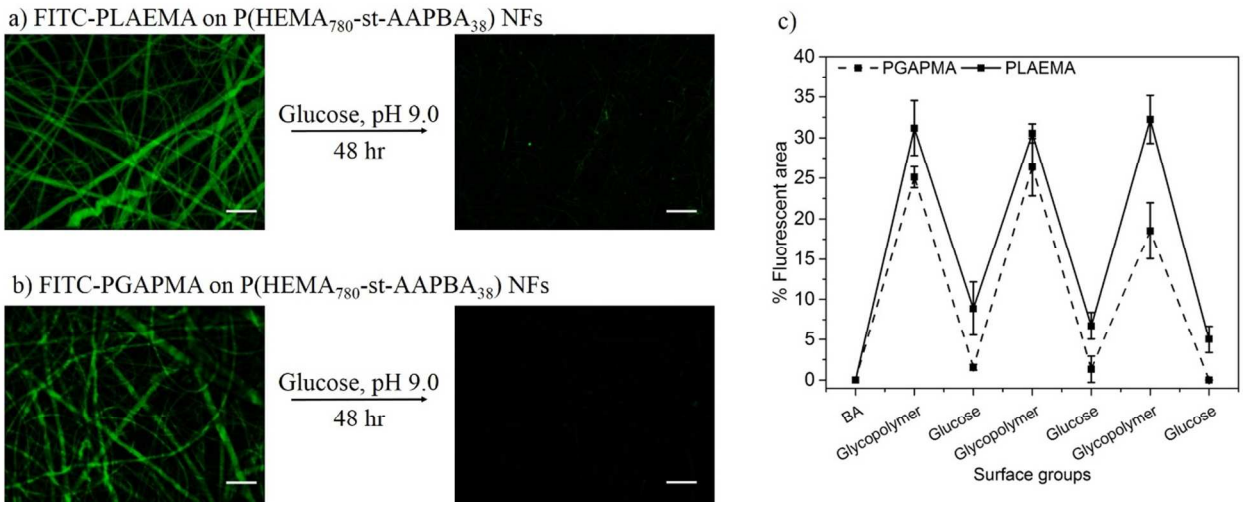


Figure 2. FITC-PLAEMA (a) and FITC-PGAPMA (b) modified photo-crosslinked P($HEMA_{760}$-st-$AAPBA_{38}$) nanofibers surfaces. Fluorescence could be removed after incubating glycopolymers modified NF in 500 mg/mL glucose solution (pH 9.0) for 48 hr. (c) Reversible % fluorescent areas changes when FITC-PLAEMA (solid line) and FITC-PGAPMA (dash line) modified NFs were incubated in 500 mg/mL glucose and 10 mg/mL FITC glycopolymer solutions (pH 9.0) for 48 hr and 15 min, respectively. Scale bar = 20 μm.

The pH responsiveness of the photo-crosslinked P($HEMA_{760}$-st-$AAPBA_{38}$) nanofibers was then studied as shown in Figure 3. Before fluorescence microscopy observations, the acid treated nanofibers were washed by Tris - 0.1 M HCl buffer (pH 9.0) to prevent the fluorescence quenching at the acidic condition.[39] The results clearly indicated the FITC-glycopolymers were able to adsorb on the boronic acid containing nanofiber surfaces at basic condition (pH 9.0), whereas rapid dissociation of glycopolymers occurred when the nanofibers were rinsed with 0.1 M HCl.

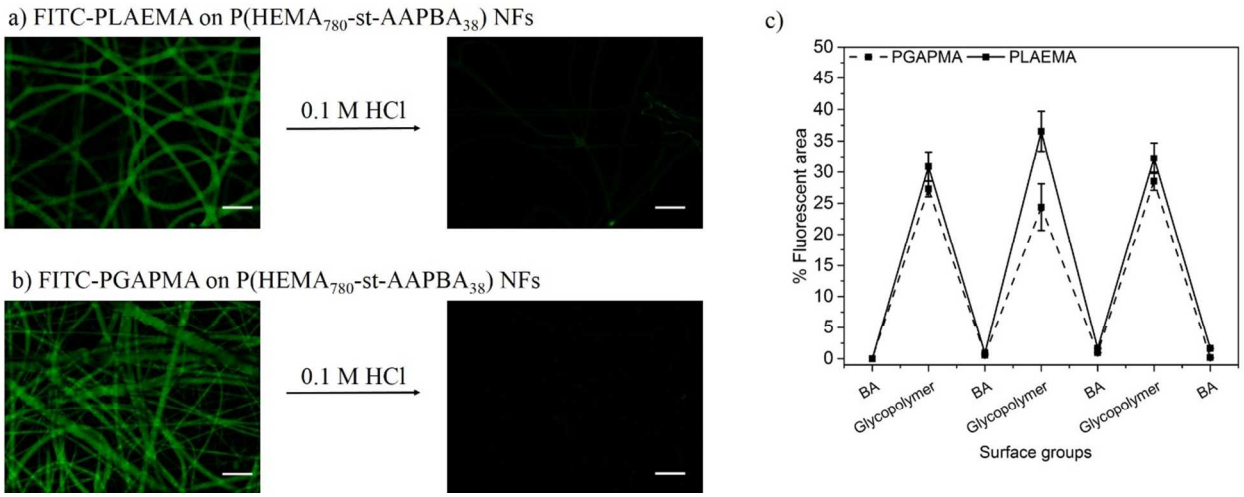


Figure 3. FITC-PLAEMA (a) and FITC-PGAPMA (b) modified photo-crosslinked P($HEMA_{760}$-st-$AAPBA_{38}$) nanofibers surfaces. Fluorescence could be removed after rinsing the glycopolymers modified NFs with 0.1 M HCl. (c) Reversible % fluorescent areas changes when FITC-PLAEMA (solid line) and FITC-PGAPMA (dash line) modified NFs were incubated in 10 mg/mL FITC glycopolymer solutions (pH 9.0) for 15 min, followed by rinsing with 0.1 M HCl. Scale bar = 20 μm.

3.4. Lectins binding on different surfaces

The adsorption of FITC labeled lectins on photo-crosslinked nanofibrous membranes deposited glass slides were first studied by fluorescence microscopy. The glass slides were treated with an acidic solution to deprotect the boronic acid groups in the electrospun nanofibers. After rinsing with DI water and incubation with glycopolymers in Tris-0.1 M HCl buffer solution (pH 9.0) for 15 min, the resulting glycopolymer modified nanofibers were incubated with FITC labeled lectins in Tris-0.1 M HCl buffer solution (pH 9.0) for another 15 min and washed with Tris-0.1 M HCl buffer (pH 9.0) again to remove the free FITC-lectins. The results are shown in Figure 4.

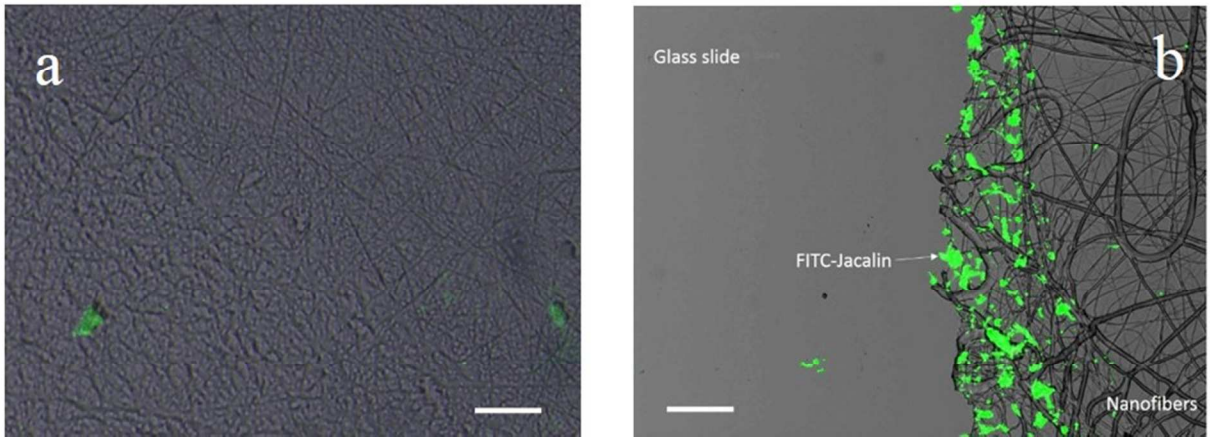


Figure 4. FITC-Jacalin adhered on photo-crosslinked a) P($HEMA_{321}$) and b) P($HEMA_{760}$-st-$AAPBA_{38}$) nanofibers surfaces. At the edge of the nanofibrous mat on a glass slide, most of the FITC-Jacalin adsorbed on PLAEMA modified photo-crosslinked P($HEMA_{760}$-st-$AAPBA_{38}$) nanofibers, whereas negligible the FITC-Jacalin could be spotted on glass slides. Scale bar = 20 μm.

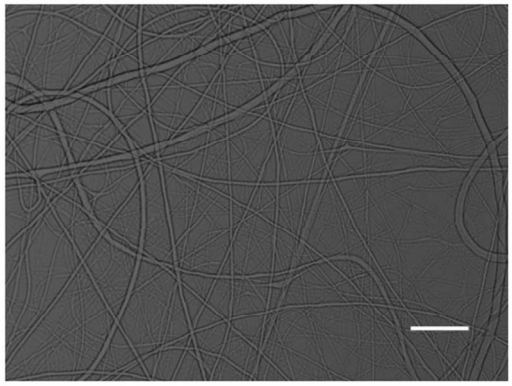
It was found that the FITC-Jacalin (galactose specific lectin) interaction on photo-crosslinked P($HEMA_{321}$) nanofiber (Figure 4a) surfaces was negligible as compared to that on PLAEMA modified photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers surface (Figure 4b). As compared to the results from Wang et al. no lectin adsorption could be observed on glycopolymer free nanofibers,[11] our results might suggest neither glycopolymers nor lectin can strongly adsorb on the photo-crosslinked P($HEMA_{321}$) homopolymer nanofibrous surfaces, whereas significantly larger amount of FITC-Jacalin could be captured on glycopolymers (PLAEMA) modified photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers surface through the lectin-carbohydrate interactions (Figure 4).

Incubation of the pristine and glycopolymers modified photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers in FITC-lectins Tris-0.1 M HCl buffer solutions (pH 9.0) for 15 min (Figure 3) showed that the lectins can only interact with the glycopolymers functionalized

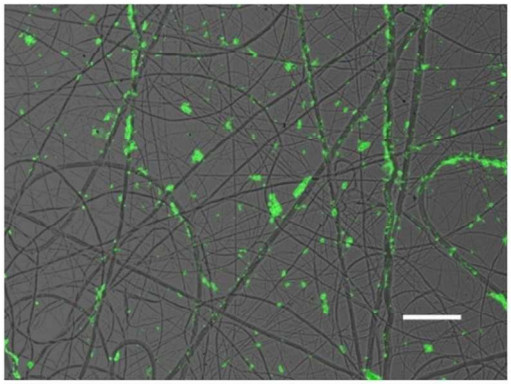
nanofibers surface, and not on the boronic acid modified nanofiber surfaces (Figure 5a and e). Nanofibers with PLAEMA (galactose containing glycopolymer) and PGAPMA (glucose containing polymer) modification can capture Jacalin (Figure 5b) and ConA (Figure 5d), respectively. No fluorescent signals could be observed when FITC-ConA was incubated with the PLAEMA modified photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers (Figure 5c and e). From these observations, the nanofibers could be used to selectively capture different lectins when surface functionalized with different glycopolymers.

On the other hand, compared to the FITC-glycopolymers modified photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers (Figure 2c and 3c), less fluorescence could be observed when FITC-lectins were captured on the glycopolymers modified P($HEMA_{780}$-st-$AAPBA_{38}$) nanofiber surfaces (Figure 5e). These observations might be explained by lower concentration of FITC-lectins (20 μL/mL) used for lectin capture assay or weaker affinity between carbohydrates and lectins in aqueous environment.[40] Interestingly, unlike the FITC-glycopolymer that uniformly distributed along the photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers (Figure 2 and 3), FITC-lectins were found to be aggregated when adsorbed on the glycopolymers modified nanofiber surfaces (Figure 5). We believed the latter case could be explained by the electrical double layer depressing and absolute zeta potential decreasing of the proteins in the Tris-buffered saline.[41]

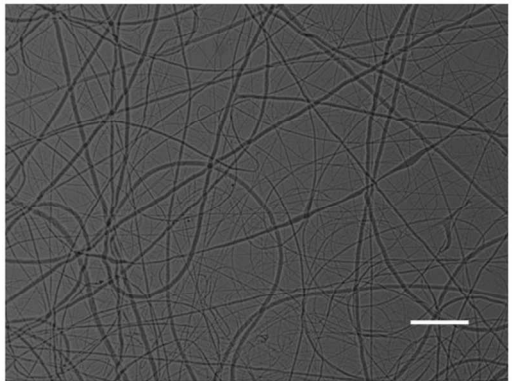
a) Jacalin on P(HEMA$_{780}$-st-AAPBA$_{38}$) NFs



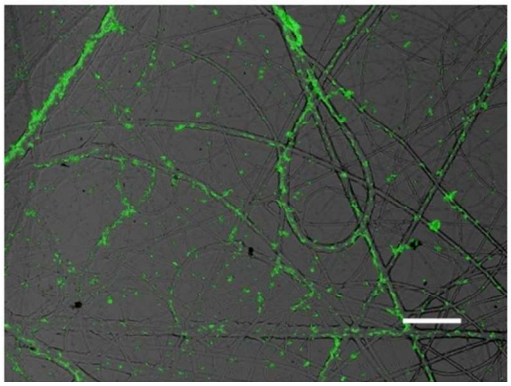
b) Jacalin on PLAEMA/P(HEMA$_{780}$-st-AAPBA$_{38}$) NFs



c) ConA on PLAEMA/P(HEMA$_{780}$-st-AAPBA$_{38}$) NFs



d) ConA on PGAPMA/P(HEMA$_{780}$-st-AAPBA$_{38}$) NFs



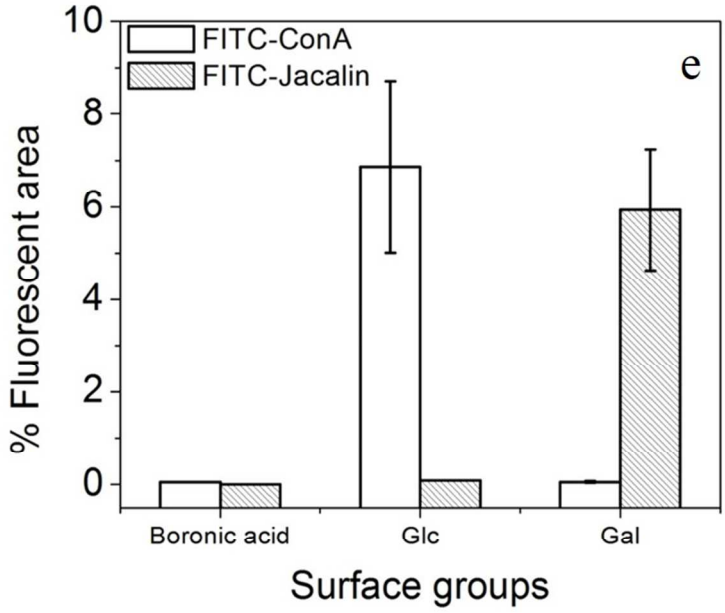
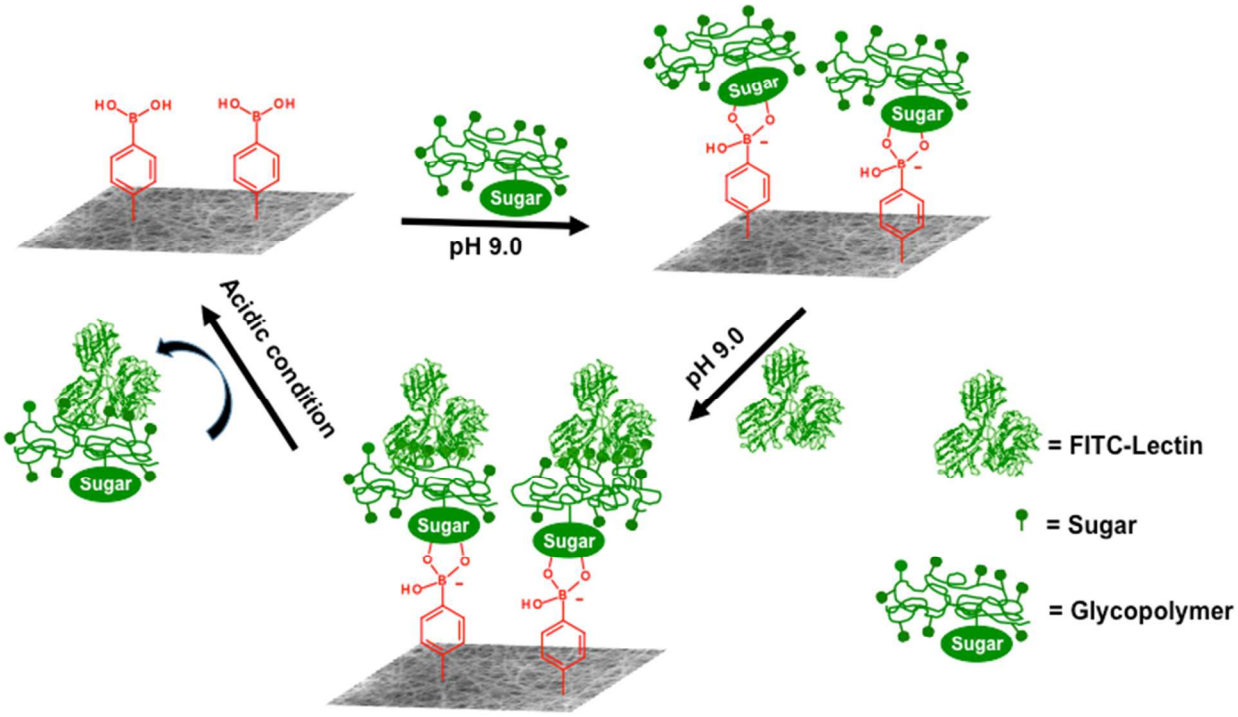


Figure 5. Fluorescent microscopy images for FITC-Jacalin adsorption on pristine (a) and PLAEMA modified photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers (b), and FITC-ConA on PLAEMA (c) and PGAPMA (d) modified photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers. Scale bar = 20 μm. (e) % Fluorescent areas of FITC-ConA or Jacalin on different nanofibers surfaces.

3.5. Reversibly capture and release lectins on glycopolymers modified nanofibers surfaces

The reversible capture and release of lectins on glycopolymers modified photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers was studied (Scheme 3) and the results are shown in Figure 6. It was found fluorescently labeled Jacalin and ConA could interact with PLAEMA (Figure 6a) and PGAPMA (Figure 6b) modified photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers at basic condition. After incubation with 0.1 M HCl for 15 min and rinsed with pH 9 buffer solution, no fluorescence was observed even after re-incubating the nanofibers with lectins (Figure 6a and b). Considering that negligible amount of lectins could interact with pristine photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers (Figure 5a), these results suggest that the glycopolymers from the nanofiber surfaces was removed during the acid solution treatment.



Scheme 3. FITC-lectins capture and release on a photo-crosslinked polymer NF.

Figure 6c shows the % fluorescent area changes on the photo-crosslinked P(HEMA$_{780}$-st-AAPBA$_{38}$) nanofibers in response to alterations of surface groups between carbohydrates and boronic acid. When glycopolymer (PLAEMA) modified nanofibers were incubated with FITC-Jacalin for 15 min, the lectin was captured by the galactose groups on nanofibers and showed ~ 6% coverage with fluorescent signals in microscope images (Figure 6a and c). Once the nanofibers were immersed to 0.1 M HCl for 15 min and rinsed with pH 9 buffer solution, the fluorescent areas were reduced to ~ 0%, indicating PLAEMA and glycopolymer-lectin conjugates were removed from the nanofibers surfaces, and the surface groups were restored to boronic acids (Figure 6a and c). These observations could be repeated by alternatively incubating the photo-crosslinked PLAEMA modified P(HEMA$_{780}$-st-AAPBA$_{38}$) nanofibers in FITC-Jacalin and acidic solutions (Figure 6C), suggesting our materials could be used to reversible capture

and release Jacalin. We also evaluated the FITC-ConA capture and release on glucose containing glycopolymer (PGAPMA) modified nanofibers. The results were similar to the FITC-Jacalin capture and release on PLAEMA modified nanofibers surface (Figure 6c), suggesting using different glycopolymers to modify the nanofiber surface, our photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers could be used to reversible capture and release various lectins. Moreover, the nanofiber fabricated in the present study could also be used for the reversible capture of virus or even bacteria from contaminated water. Compared to other platforms such as carbon nanotubes,[42] surfaces[43] and nanoparticles,[44, 45] for pathogen capture/detection, we believe our nanofibers are easier for handling, reusable and less expense.

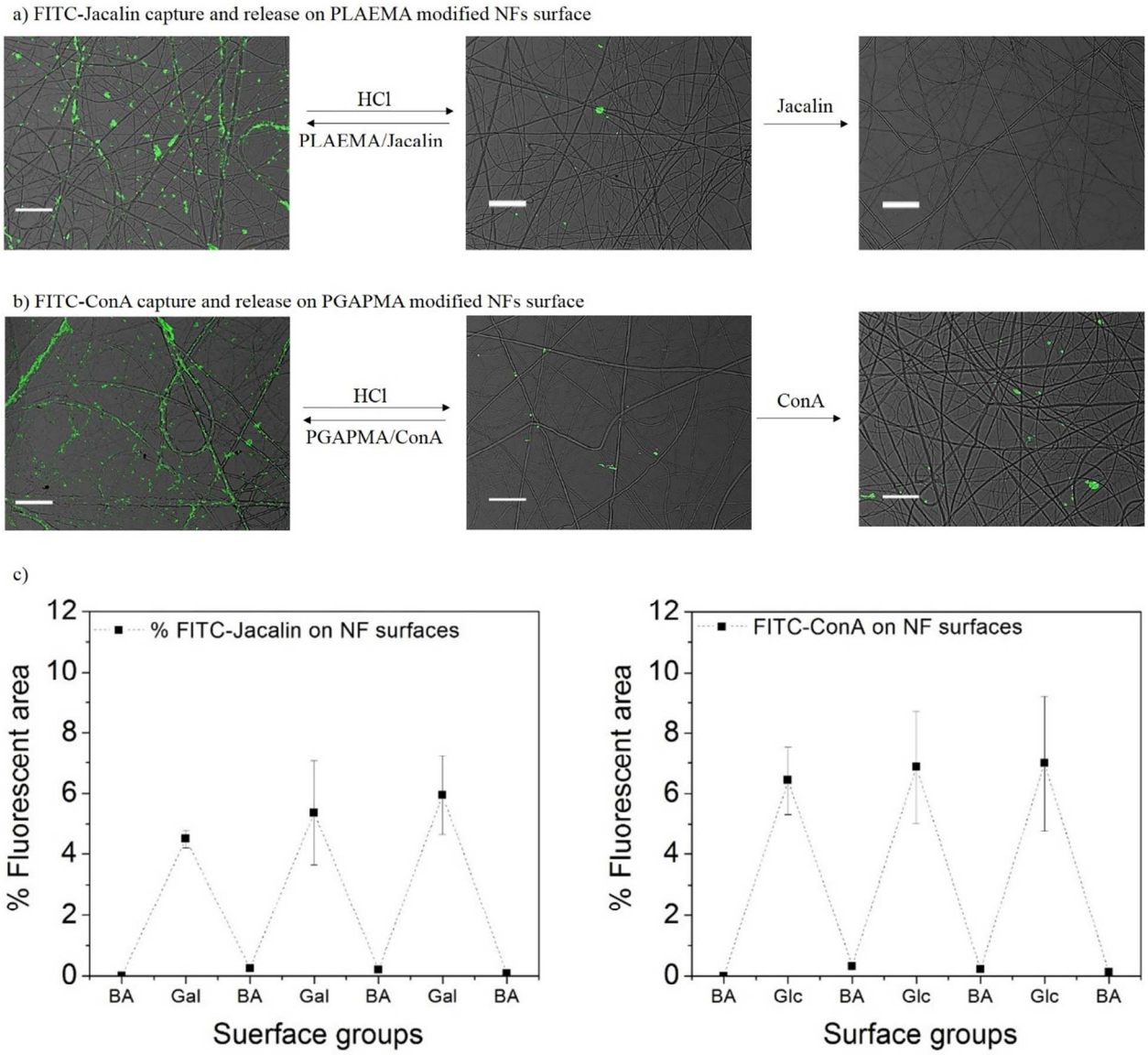


Figure 6. Fluorescent microscopy images for FITC-Jacalin (a) and FITC-ConA reversibly captured and released from the photo-crosslinked P($HEMA_{780}$-st-$AAPBA_{38}$) nanofibers. Scale bar = 20 μm. (c) % Fluorescent areas of FITC-ConA and FITC-Jacalin on nanofibers surface in response to alternations of surface groups between carbohydrates and boronic acid.

4. Conclusions

This study presents the first example of using boronic acid containing photochemically crosslinked polymer nanofiber membrane for the reversible capture and release of lectins. The

pH and glucose dual responsive behavior, as well as the adsorption of lectins (FITC-Jacalin and FITC-ConA) on different surfaces (pristine, galactose and glucose containing polymers modified nanofibers) were studied by fluorescence microscopy. The FITC labeled glycopolymers could adsorb on nanofiber surface under basic condition (pH 9.0) and are released at either high glucose concentration or under acidic conditions. FITC-Jacalin and FITC-ConA were successfully captured on galactose and glucose containing polymers modified nanofiber surface, respectively, whereas no lectin adsorption can be observed on the pristine nanofibers. Immersion of the FITC-lectin conjugated nanofibers in acidic solution for 15 min resulted in the rapid release of both the lectins and the glycopolymers from the nanofibers surfaces. Therefore, such type of nanofibers can find applications in the quick removal of specific proteins or toxins in solution.

Acknowledgments

This research was supported by research grants from the Natural Sciences and Engineering Research Council (NSERC) of Canada, the Canada Foundation for Innovation (CFI), and National Institute for Materials Science (NIMS) internship program.

Notes and References

† Electronic Supplementary Information (ESI) available: ^{1}H NMR spectra of P($HEMA_{321}$) and 1,4-butanediol protected P($HEMA_{780}$-st-$AAPBA_{38}$) before and after the introduction of GMA.

1. D. Li and Y. Xia, *Adv. Mater.* , 2004, **16**, 1151-1170.
2. H. Yoshimoto, Y. M. Shin, H. Terai and J. P. Vacanti, *Biomaterials*, 2003, **24**, 2077-2082.
3. Y.-J. Kim, M. Ebara and T. Aoyagi, *Angew. Chem. Int. Ed.* , 2012, **51**, 10537-10541.
4. K. Namekawa, M. T. Schreiber, T. Aoyagi and M. Ebara, *Biomater Sci-Uk*, 2014, **2**, 674-679.
5. X. Wang, X. Chen, K. Yoon, D. Fang, B. S. Hsiao and B. Chu, *Environ. Sci. Technol.* , 2005, **39**, 7684-7691.

6. J. Shin, S.-J. Choi, I. Lee, D.-Y. Youn, C. O. Park, J.-H. Lee, H. L. Tuller and I.-D. Kim, *Adv. Funct. Mater.* , 2013, **23**, 2357-2367.
7. D.-J. Yang, I. Kamienchick, D. Y. Youn, A. Rothschild and I.-D. Kim, *Adv. Funct. Mater.* , 2010, **20**, 4258-4264.
8. D. R. Bundle and N. M. Young, *Curr. Opin. Struct. Biol.* , 1992, **2**, 666-673.
9. N. Sharon, *FEBS Lett.* , 1987, **217**, 145-157.
10. Y. C. Lee and R. T. Lee, *Acc. Chem. Res.* , 1995, **28**, 321-327.
11. L. Wang, G. R. Williams, H.-l. Nie, J. Quan and L.-m. Zhu, *Polym. Chem.*, 2014, **5**, 3009-3017.
12. S. Patel, K. Kurpinski, R. Quigley, H. Gao, B. S. Hsiao, M.-M. Poo and S. Li, *Nano Lett.* , 2007, **7**, 2122-2128.
13. T. Uyar, R. Havelund, J. Hacaloglu, F. Besenbacher and P. Kingshott, *ACS Nano*, 2010, **4**, 5121-5130.
14. Q. Yang, J. Wu, J.-J. Li, M.-X. Hu and Z.-K. Xu, *Macromol. Rapid Commun.* , 2006, **27**, 1942-1948.
15. Y.-J. Kim, M. Ebara and T. Aoyagi, *Adv. Funct. Mater.* , 2013, **23**, 5753-5761.
16. S. D. Bull, M. G. Davidson, J. M. H. van den Elsen, J. S. Fossey, A. T. A. Jenkins, Y.-B. Jiang, Y. Kubo, F. Marken, K. Sakurai, J. Zhao and T. D. James, *Acc. Chem. Res.* , 2012, **46**, 312-326.
17. S. L. Wiskur, J. J. Lavigne, H. Ait-Haddou, V. Lynch, Y. H. Chiu, J. W. Canary and E. V. Anslyn, *Org. Lett.* , 2001, **3**, 1311-1314.
18. Q. Guo, Z. Wu, X. Zhang, L. Sun and C. Li, *Soft Matter*, 2014, **10**, 911-920.
19. Y. Kotsuchibashi, R. V. C. Agustin, J.-Y. Lu, D. G. Hall and R. Narain, *ACS Macro Letters*, 2013, **2**, 260-264.
20. A. Matsumoto, K. Yamamoto, R. Yoshida, K. Kataoka, T. Aoyagi and Y. Miyahara, *Chem. Commun.* , 2010, **46**, 2203-2205.
21. H. Liu, Y. Li, K. Sun, J. Fan, P. Zhang, J. Meng, S. Wang and L. Jiang, *J. Am. Chem. Soc.* , 2013, **135**, 7603-7609.
22. T. Hoare and R. Pelton, *Macromolecules*, 2007, **40**, 670-678.
23. M. B. Lerner, N. Kybert, R. Mendoza, R. Villechenon, M. A. Bonilla Lopez and A. T. Charlie Johnson, *Appl. Phys. Lett.* , 2013, **102**, -.
24. C. Zhang, M. D. Losego and P. V. Braun, *Chem. Mater.* , 2013, **25**, 3239-3250.
25. T. Aoki, Y. Nagao, K. Sanui, N. Ogata, A. Kikuchi, Y. Sakurai, K. Kataoka and T. Okano, *J. Biomater. Sci., Polym. Ed.* , 1997, **9**, 1-14.
26. E. Baştürk and M. V. Kahraman, *Polym. Compos.* , 2012, **33**, 829-837.
27. M. Benaglia, E. Rizzardo, A. Alberti and M. and Guerra, *Macromolecules*, 2005, **38**, 3129-3140.
28. Z. Deng, H. Bouchékif, K. Babooram, A. Housni, N. Choytun and R. Narain, *J. Polym. Sci., Part A: Polym. Chem.* , 2008, **46**, 4984-4996.
29. Z. Deng, M. Ahmed and R. Narain, *J. Polym. Sci., Part A: Polym. Chem.* , 2009, **47**, 614-627.
30. Z. Deng, S. Li, X. Jiang and R. Narain, *Macromolecules*, 2009, **42**, 6393-6405.
31. Y.-J. Lee, S. A. Pruzinsky and P. V. Braun, *Langmuir*, 2004, **20**, 3096-3106.
32. W. N. E. van Dijk-Wolthuis, O. Franssen, H. Talsma, M. J. van Steenbergen, J. J. Kettenes-van den Bosch and W. E. Hennink, *Macromolecules*, 1995, **28**, 6317-6322.

33. A. V. Reis, A. R. Fajardo, I. T. A. Schuquel, M. R. Guilherme, G. J. Vidotti, A. F. Rubira and E. C. Muniz, *J. Org. Chem.* , 2009, **74**, 3750-3757.
34. G. Kim, H. Yoon and Y. Park, *Appl. Phys. A* 2010, **100**, 1197-1204.
35. J. Lee, Y. H. Jeong and D.-W. Cho, *Macromol. Mater. Eng.* , 2014, n/a-n/a.
36. S. V. Fridrikh, J. H. Yu, M. P. Brenner and G. C. Rutledge, *Phys. Rev. Lett.* , 2003, **90**, 144502.
37. D. G. Hall, in *Boronic Acids*, Wiley-VCH Verlag GmbH & Co. KGaA, 2006, pp. 1-99.
38. J. P. Lorand and J. O. Edwards, *J. Org. Chem.*, 1959, **24**, 769-774.
39. D. Fangkai, M. Yunhao, Z. Fang, Y. Changmin and W. Shuizhu, *Nanotechnology*, 2013, **24**, 365101.
40. C. Ke, H. Destecroix, M. P. Crump and A. P. Davis, *Nat Chem*, 2012, **4**, 718-723.
41. P. Attard, in *Adv. Chem. Phys.* , John Wiley & Sons, Inc., 2007, pp. 1-159.
42. L. Gu, T. Elkin, X. Jiang, H. Li, Y. Lin, L. Qu, T.-R. J. Tzeng, R. Joseph and Y.-P. Sun, *Chem. Commun.* , 2005, 874-876.
43. Q. Yang, M. Strathmann, A. Rumpf, G. Schaule and M. Ulbricht, *ACS Appl. Mat. Interfaces* 2010, **2**, 3555-3562.
44. G. Pasparakis and C. Alexander, *Angew. Chem. Int. Ed.* , 2008, **47**, 4847-4850.
45. R. Ribeiro-Viana, M. Sánchez-Navarro, J. Luczkowiak, J. R. Koeppe, R. Delgado, J. Rojo and B. G. Davis, *Nat Commun*, 2012, **3**, 1303.