Biomed Microdevices (2009) 11:1251–1257
DOI 10.1007/s10544-009-9344-2

A monolithic polymeric microdevice for pH-responsive drug delivery

Jian Chen · Michael Chu · Khajag Koulajian · Xiao Yu Wu · Adria Giacca · Yu Sun

Published online: 12 August 2009

Abstract A drug-delivery microdevice integrating pH-responsive nano-hydrogel particles functioning as intelligent nano valves is described. The polymeric microdevices are monolithic without requiring peripheral control hardware or additional components for controlling drug-release rates. pH-responsive nanoparticles were synthesized and embedded into a composite membrane. The resulting pH-responsive composite membranes were integrated with PDMS micro reservoirs via a room-temperature transfer bonding technique to form the proof-of-concept microdevices. *In vitro* release characterization of the microdevices was conducted in which the release rate of Vitamin B_{12} (VB_{12}) as a model drug increased dramatically when the local pH value was decreased from 7.4 to 4. This device concept can serve as a platform technology for intelligent drug delivery in response to various *in vivo* environmental signals.

Keywords Controlled drug delivery · Microdevices · pH-responsive hydrogel · Nano hydrogel particles

1 Introduction

Stimuli-responsive hydrogels change their behavior according to environmental conditions (Gil and Hudson 2004; Chaterji et al. 2007; Miyata et al. 2002). These properties can be utilized for the construction of smart drug-delivery systems to be responsive to temperature (Tanaka 1978; Hirokawa and Tanaka 1984; Amiya et al. 1987; Chen and Hoffman 1995), pH (Tanaka et al. 1982; Osada et al. 1992), electric field (Tanaka et al. 1980), light (Irie 1993; Suzuki and Tanaka 1990), or magnetic field (Szab et al. 1998).

Taking pH as an example. Normal organs, tissues and cellular compartments have different pH levels. Additionally, certain cancers as well as inflamed or damaged tissues exhibit a pH deviation from 7.4. Hence, pH can be a suitable target stimulus for responsive drug delivery, and pH-responsive hydrogels have been developed for controlled drug delivery (Gupta et al. 2002; Schmaljohann 2006).

pH-responsive hydrogels are composed of polymeric backbones with ionic pendant groups. Commonly studied ionic polymers for pH-responsive behavior include poly(acrylamide) (PAAm), poly(acrylic acid) (PAA),

Authors Jian Chen and Michael Chu contributed equally.

J. Chen · Y. Sun (✉)
Institute of Biomaterials and Biomedical Engineering, University of Toronto, Toronto, ON M5S 3G8, Canada
e-mail: sun@mie.utoronto.ca

M. Chu · X. Y. Wu
Department of Pharmaceutical Sciences, University of Toronto, Toronto, ON M5S 3M2, Canada

X. Y. Wu
e-mail: sxy.wu@utoronto.ca

K. Koulajian · A. Giacca
Department of Physiology, University of Toronto, Toronto, ON M5S 1A8, Canada

A. Giacca
e-mail: adria.giacca@utoronto.ca

poly(methacrylic acid) (PMAA), poly(diethylaminoethyl methacrylate) (PDEAEMA), and poly(dimethylaminoethyl methacrylate) (PDMAEMA) (Lowman 1999). In aqueous media of appropriate pH and ionic strength, the pendant groups ionize and develop fixed charges on the polymer network, generating internal electrostatic repulsive or attractive forces responsible for pH-dependent swelling or deswelling of the hydrogel, thereby controlling drug release (Kost 1999; Wu et al. 2003). The pH-responsive swelling and deswelling behavior has been used to induce controlled release of model compounds (e.g., caffeine (Nakamae et al. 1997) and indomethacin (Dong and Hoffman 1991)).

Since transient swelling-deswelling behavior of the stimuli-responsive hydrogel is limited by the diffusion of stimuli into the hydrogel matrix and by the absorption and expulsion of the solvent (usually water), stimuli-responsive micro and nano hydrogels have been developed for controlled drug delivery with shorter response time (Beebe et al. 2000; Baldi et al. 2003; Zhang and Wu 2004; Ichikawa and Fukumori 2000). For example, a closed-loop insulin delivery system utilizing micro-hydrogel via microfabrication was developed (Baldi et al. 2003). In this design, a thin hydrogel poly(3-methacrylamido phenylboronic acid-*co*-acrylamide)(PMPBA-AAm) was confined in a cavity that allowed an external glucose solution to diffuse through a stiff porous membrane. The hydrogels volume change produced deflections of a diaphragm that opened and closed an intake orifice of a valve to deliver insulin. In order to obtain fast response and enhanced mechanical strength/integrity of stimulus-responsive polymeric hydrogels, prototypes of nano-hydrogel composite membranes containing poly(N-isopropylacrylamide-*co*-MAA) (PNIPAm-MAA) nanoparticles were developed, which responded to temperature and pH changes. The much smaller sizes of hydrogel particles (hundreds of nanometers) led to faster responses than bulk hydrogels. The membranes also possessed significantly enhanced mechanical strength/integrity (Yam and Wu 1999; Zhang and Wu 2004).

An alternative method for achieving pulsatile release of drugs is based on microfabrication technologies (Santini et al. 1999; Staples et al. 2006; Li et al. 2005, 2004; Maloney et al. 2005; Prescott et al. 2006; Grayson et al. 2003; Ryu et al. 2007; Intra et al. 2008). In the first implantable drug-delivery device, electrical pulses were employed to dissolve a gold membrane via electrochemical dissolution, allowing the diffusion of drugs out of silicon-made reservoirs on demand (Santini et al. 1999; Staples et al. 2006; Li et al. 2005, 2004). In contrast to the electrochemical dissolution approach, electrothermal activation to open reservoirs for controlled drug release has also been demonstrated (Maloney et al. 2005; Prescott et al. 2006). For the construction of implantable drug-delivery microdevices, biodegradable membranes have also been proposed for multi-dose drug delivery (Grayson et al. 2003; Ryu et al. 2007; Intra et al. 2008). The first biodegradable polymeric microchip was formed by compression-molding polylactic acid (PLA) (Grayson et al. 2003). Individual membrane recipes were prepared by using various ratios of lactic acid/glycolic acid and different molecular-weight polymers for controlled drug release. An advantage of biodegradable polymeric microchips is the elimination of a second surgery for device removal.

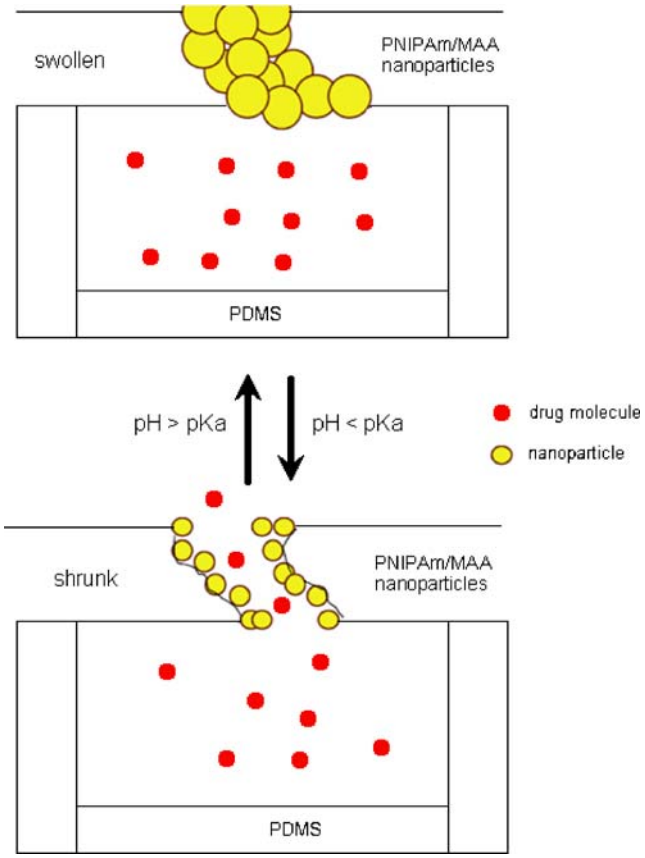

Fig. 1 Illustration of the mechanism for pH-responsive drug release out of the microdevice. *Top*: nanoparticles are in the swollen state when the surrounding pH value is higher than pKa (acid dissociation constant) of the nanoparticles. *Bottom*: Nanoparticles are in the shrunk state when the surrounding pH value is lower than pKa. Resulting volumetric swelling and shrinking of the nanoparticles control drug-release rates

In addition, the lack of electronics reduces size and other manufacturing restrictions.

In these implantable drug-delivery microdevices (Santini et al. 1999; Staples et al. 2006; Li et al. 2005, 2004; Maloney et al. 2005; Prescott et al. 2006; Grayson et al. 2003; Ryu et al. 2007; Intra et al. 2008), although drugs can be released on demand, the microdevices are not capable of regulating drug-delivery rate according to local micro environmental signals such as pH and glucose changes.

In this study, we have developed the first pH-responsive drug-delivery microdevice targeting short-term implantation use in rodents. The proof-of-concept devices integrate pH-sensitive composite membranes embedded with responsive hydrogel nanoparticles with microfabricated reservoirs. The polymeric microdevices are monolithic without requiring peripheral control hardware or additional components for controlling drug-release rates.

Compared to normal physiological pH (7.4), the device controls the drug-delivery rate to provide higher release rates at lower pH values such as found in tumor tissue (Martin and Jain 1994). As shown in Fig. 1, the patterned PDMS structure forms a drug reservoir and provides physical support for the thin nano-hydrogel embedded composite membrane. The embedded hydrogel nanoparticles in the composite membrane detect environmental pH changes as intelligent nano valves. Corresponding volumetric swelling and shrinking response of the nanoparticles controls drug-release rates.

2 Materials and methods

2.1 pH-responsive nanoparticle synthesis and characterization

For nanoparticle synthesis, N-Isopropylacrylamide (NIPAm, 99%), methacrylic acid (MAA), N,N-bisacrylamide (BIS), and potassium persulfate (KPS) were from Sigma. Sodium Lauryl Sulfate (SDS) was from Fisher. Ethylcellulose powder (viscosity 45) for composite membrane fabrication was from Dow.

The pH-responsive poly(N-isopropylacrylamide-*co*-methacrylic acid) (PNIPAm-MAA) nanoparticles with 1:1 molar ratio of NIPAm to MAA were prepared by an aqueous dispersion polymerization process using N'N'-methylenebisacrylamide (BIS) as the crosslinking agent, potassium persulfate as the initiator (KPS), and sodium dodecyl sulfate (SDS) as the stabilizer (Wu and Lee 1993). Monomer mixtures (763.83 mg of NIPAm and 0.57 ml of MAA) were dissolved in 100 ml distilled water. After the incorporation of 133.22 mg of BIS, 11.54 mg of SDS was added to the reaction mixture. The reaction mixture was purged with nitrogen for 0.5 h and then polymerization was initiated by the addition of 56.77 mg of KPS. The polymerization process was conducted under a nitrogen blanket at 70°C for 4 hours at 200 rpm. Typically, nanoparticles in aqueous solution with a polymer concentration of ~1.5 wt% were prepared. More concentrated samples were obtained through centrifugation.

Synthesized nanoparticles were dispersed in 10 mg/ml concentration with distilled water. A volume of 50 μl of solution was taken and placed into glass light scattering test tube with 700 μl of phosphate buffer solution (PBS) at different pH values. Particle sizer measurements were performed with a NICOMP 380 particle sizer.

2.2 Composite membrane synthesis and characterization

To form a useful material for device construction, the nanoparticles were embedded into a composite membrane. Casting of composite membranes started with dissolving 0.65 g of ethylcellulose powder in 15 ml of anhydrous alcohol at room temperature (22°C). Ethylcellulose solution was mixed with dispersed nanoparticle solution for another 2 h. Ethylcellulose/nanoparticle solution was then poured into a 10 cm Teflon plate and placed in a vacuum tank for 15 minutes for degassing. Solution was allowed to dry for 24 hours in a sealed desiccator to slow evaporation and prevent contamination. SEM analysis of the membrane was performed to obtain visual characterization of the nanoparticle and membrane.

Permeability testing of nano-hydrogel composite membranes was conducted in side-by-side diffusion cells using VB_{12} as a model drug ($M_w = 1355$). Multiple 2 cm diameter circular samples were soaked in pH 7.4 phosphate buffer solution for 24 h before testing. Side-by-side water jacketed diffusion cells housed both the receptor and donor cells for permeability testing shown in Fig. 2. The volume of each cell was 3 ml and the area for permeation was 0.63 cm^2. Polyethylene tubes connected to a peristaltic pump allowed for continuous flow from the receptor cell to a cuvette. The cuvette was placed in a UV spectrometer for kinetic measurements

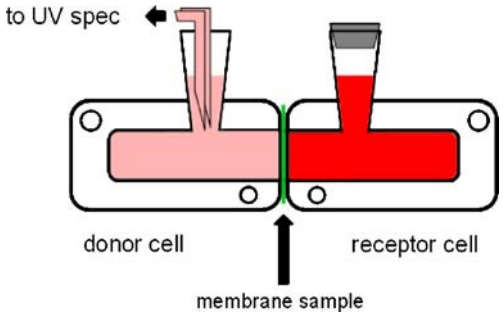

Fig. 2 A schematic view of a side-by-side diffusion cell

of VB_{12} diffusion. Donor cell was filled with 1 mg/ml VB_{12} solution. Data was collected over 4 h periods at 10 min intervals at different pH values.

Solute permeability $P = DK/h$ was calculated according to the following equation $M_t = PSC_d(t - t_L)$ based on Fick's first law of diffusion with assumptions including: (1) steady state is reached in the membrane after a lag time, t_L; (2) the area for permeation, S, and solute concentration in the donor cell, C_d, are constant; (3) sink condition is maintained at the receptor side; where M_t is the mass of a drug permeated till time t; D and K are, respectively, the diffusion coefficient and partition coefficient of the drug; h is the thickness of the membrane. The P value was calculated from the slope of the curve of M_t vs. t at the steady state.

2.3 Microdevice fabrication and characterization

The integration of nano-hydrogel composite membranes and PDMS drug reservoirs must ensure the physical and functional integrity of the composite membranes without exposing the hydrogel nanoparticles to harsh processing conditions, such as UV, plasma, high temperatures or wet chemical etchant.

The PDMS reservoir was formed via standard soft lithography using SU-8 as the mould master. The thickness of the SU-8 master was approximately 1 mm, obtained by spinning two layers of SU-8 2100. After the PDMS drug reservoir was peeled off from the substrate, room-temperature transfer bonding was used to integrate nano-hydrogel embedded composite membranes. A thin layer of PDMS was spun on a substrate as a bonding adhesive layer (Fig. 3(a)). Through microcontact printing, the adhesive layer was transferred to the PDMS drug reservoir (Fig. 3(b) and (c)). The drug reservoir was then bonded to a nano-hydrogel

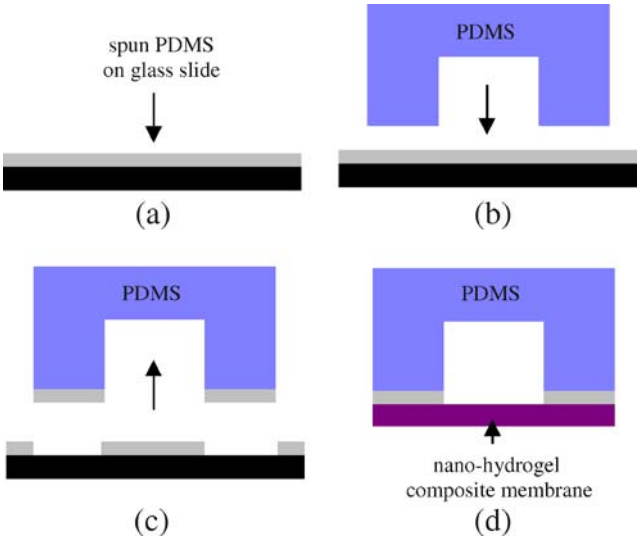

Fig. 3 Microfabrication steps for the integration of nano-hydrogel composite membranes with patterned PDMS structures

composite membrane and left to cure at room temperature (Fig. 3(d)).

In vitro testing was conducted on the micro devices filled with 5 mg/ml VB_{12} solution. Device-containing chambers were soaked in a water bath at 37°C and solutions around micro devices were measured by a UV spectrometer. Data was collected over 8 h periods with a change in pH from 7.4 to 4 triggered by adding dilute acetic acid. After each change in pH, the devices were washed several times with distilled water before re-testing.

To verify the suitability of the microdevices for short-term implantation use in animals, *in vivo* biocompatibility testing was conducted in rats. The rats were randomly divided into 2 groups and underwent implant surgery under isoflurane anesthesia. The sham group (n = 6) underwent surgery without an implant while in the device group (n = 6), rats were implanted subcutaneously in the interscapular tissue with ethylene oxide sterilized empty micro devices for two weeks.

3 Results and discussion

3.1 Nanoparticle and membrane characterization

Figure 4(a) shows that the diameters of the nanoparticles decrease dramatically from 490 ± 41.6 nm (pH 7.4) to 327 ± 29.3 nm (pH 4). Figure 4(b) shows a

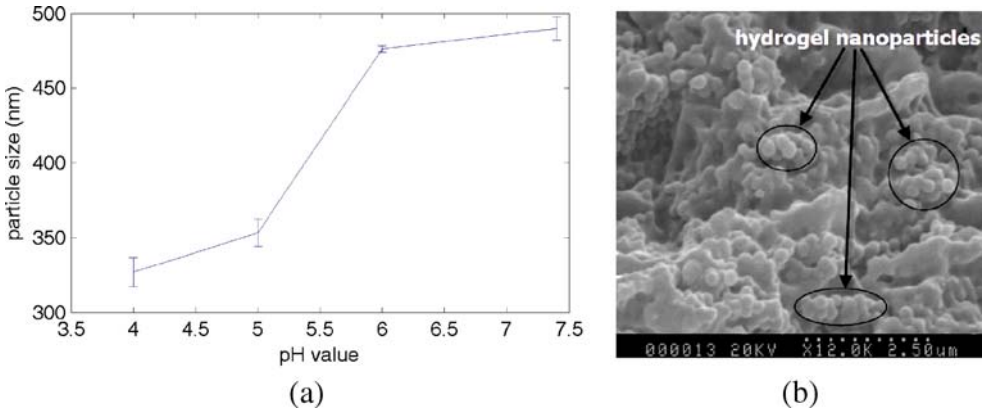

Fig. 4 (**a**) Size transition of poly(NIPAm-MAA) nanoparticles resulting from varied pH values at 37°C. (**b**) SEM picture of a cross section of 40% nanoparticle-loaded ethylcellulose membrane. *Circled* are nanoparticles into membrane channels in ethylcellulose matrix

scanning electron microscopy picture of a composite membrane after freezing and cross-sectional fractioning. The membranes have sufficient mechanical strength and suitable for physical handling.

3.2 Permeability testing of composite membranes

Figure 5 shows a representative set of permeability testing data, which proves the pH responsiveness of the nano-hydrogel composite membranes. After the addition of dilute acid to decrease the pH of the side-by-side diffusion cell from 7.4 to 4, VB_{12} amount diffused into the receptor cell increased steadily with a calculated permeability of $1.17 \times 10^{-5} \pm 1.46 \times 10^{-6}$cm/s.

Nanoparticle percentages ranging from 10% to 40% were embedded into the ethylcellulose membranes. At 30% and lower, there was no obvious response to pH, possibly due to the insufficient amount of nanoparticles to create nano-channels for drug diffusion. At 40%, pH response was strong (Fig. 5). Even higher percentages (>40%) resulted in uneven ethylcellulose membranes.

Alcohol-based ethylcellulose was chosen as the base polymer since it showed highly satisfactory film formation at room temperature, and membrane synthesis was relatively fast. Furthermore, the membranes remained mechanically strong after the addition of nanoparticles. Cellulose acetate, despite being a similar base polymer, required a much longer synthesis time (24 hours vs. ~3.5 days), and the resulting membrane was poor in quality (e.g., surface uniformity). Aquacoat, an aqueous suspension of ethylcellulose, was also tested for membrane synthesis. The resulting membranes were weak in mechanical strength and remained weak with

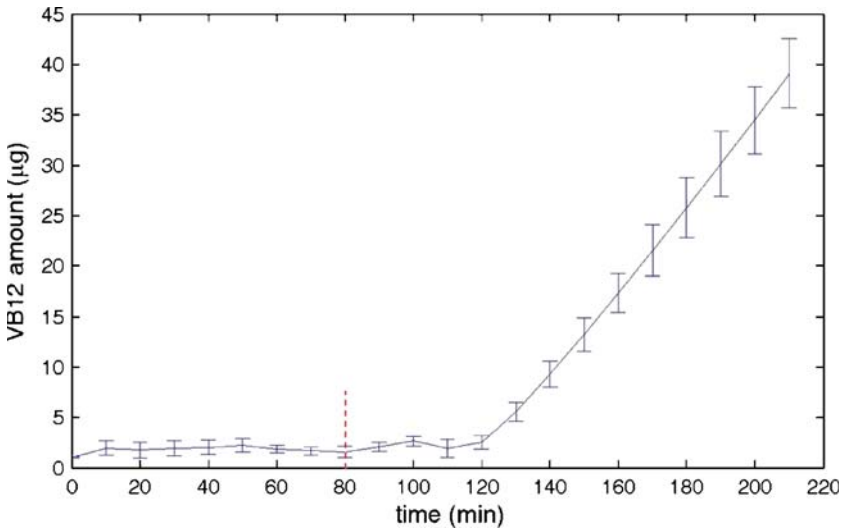

Fig. 5 pH-dependent permeation of VB_{12} through ethylcellulose membranes (n = 3) that contain 40% w/w nanoparticles. Dilute acetic acid was added at 80 min to decrease pH from 7.4 to 4. *Error bars* represent standard deviations

Fig. 6 Prototype microdevices for pH-responsive drug release

the addition of plasticizers, making physical handling of the membranes difficult.

3.3 Microdevice characterization

Figure 6 shows prototypes of the pH responsive drug-delivery microdevices. The drug reservoirs were constructed with PDMS due to its mechanical stability, the feasibility of precise patterning using microfabrication, and its biocompatibility and insusceptibility to protein adsorption and fouling. Other biocompatible materials such as PEVA, poly(ethylene-*co*-vinyl acetate) can also be valid options. VB_{12} filling and refilling were conducted through the backside of the PDMS reservoirs with a small-gauge syringe needle, made possible by the self-sealing property of PDMS.

Figure 7 shows VB_{12} release profiles of two-cycle tests of two microdevices. After the addition of dilute acid to decrease the pH of the surrounding medium from 7.4 to 4, VB_{12} amount in the surrounding medium increased steadily. The results proved the release concept and the capability of the microdevices for pH-responsive drug delivery.

The slight lag time for diffusion could be due to the chain of chemical events. The hydrogen ions from acid need to come in contact with the embedded nanoparticles, which requires diffusion into the membrane. Although the nanoparticle shrinkage time is short, drug diffusion takes time to permeate the composite membrane. By adjusting nanoparticle percentages and the thickness of the composite membranes, the response time could be further shortened. The slight drop of VB_{12} after the surrounding pH decreased from 7.4 to 4 could be attributed to osmotic pressures and hydrogen ion gradients across the membrane which could have brought a small amount of VB_{12} together with water into the microdevices, as nanoparticles began to shrink.

3.4 Biocompatibility testing of microdevices

Microdevices after implantation were retrieved. They demonstrated satisfactory mechanical strength for implantation. The total white blood cell (WBC) at the end of the experiment for the "device implantation" ($5.35 \times 10^9/\text{L} \pm 1.78 \times 10^9$) group was slightly but not significantly increased compared to the "sham" group ($3.78 \times 10^9/\text{L} \pm 1.32 \times 10^9$) (Fig. 8). This may indicate the presence of some inflammation due to the foreign

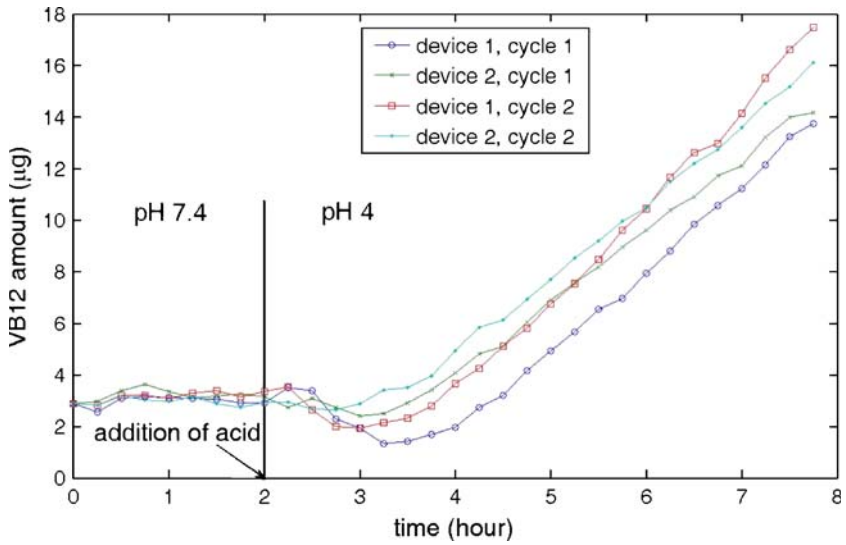

Fig. 7 Testing results of pH-responsive release. Data show two cycles of *in vitro* testing of VB_{12} permeation on two microdevices. Dilute acetic acid added at 2 h

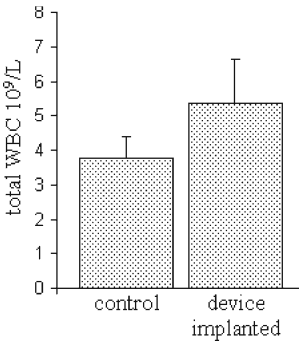

Fig. 8 Total white blood cell (WBC) numbers for the control group (no device implanted, n = 6) and the device implantation group (n = 6)

implant. The count of specific inflammatory cells shows mostly an increase in the number of lymphocytes, which may be an indication of chronic inflammation. This, however, was not significant.

4 Conclusion

In summary, this paper demonstrated the first monothlic pH-responsive drug-delivery microdevices. The microdevices integrated pH-responsive nanoparticles as intelligent nano-valves that were embedded into composite membranes. *In vitro* release characterization proved the concept and the capability of the microdevices for pH-responsive drug delivery. *In vivo* biocompatibility testing verified the suitability of the devices for short-term implantation in rodents.

Acknowledgement This work was supported by a NSERC-CIHR Collaborative Health Research Grant (CHRP#482996).

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