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Reduction of cytotoxicity of natural rubber latex film by coating with PMMA-chitosan nanoparticles

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ABSTRACT

Poly(methyl methacrylate) (PMMA) latex stabilized by chitosan (CS) oligomer was synthesized via the miniemulsion polymerization. By using 1% CS solution (in 0.1 M acetic acid), the spherical PMMA-CS particles with an average size of 380 nm were obtained. The positive zeta potentials at pH 2–7 confirmed the presence of CS as the outermost layer of the latex particles. Therefore, these particles directly interacted with the indigenous non-rubbers at the surface of sulphur prevulcanized natural rubber (SPNR) film. The deposition of PMMA-CS particles caused an increase in surface roughness of the coated SPNR film as a function of latex concentration and immersion time. The simple coating of the rubber substrate with PMMA-CS particles effectively reduced the *in vitro* cytotoxicity on L-929 cells. This study would be, therefore, helpful for development of latex gloves designed for hypersensitive users.

1. Introduction

In order to facilitate donning and to prevent the inner part of natural rubber (NR) latex gloves from adhering to itself or to skin, talc or corn starch is generally sprinkled onto the gloves. However, the lubricant powder is not appropriate for the gloves used in electronic and biomedical applications. Moreover, it might cause allergic problem, bad smell and/or serve as a carrier for germs (Grant, Davies, Jones, Espiner, & Eltringham, 1976; Lundberg, Wrangsjo, & Johansson, 1997). The deposition of poly(methyl methacrylate) (PMMA) nanoparticles onto NR or sulphur prevulcanized (SP) NR film was, therefore, developed (Anancharungsuk, Polpanich, Jangpatarapongsa, & Tangboriboonrat, 2010a; Anancharungsuk, Taweepreda, Wirasate, Thonggoom, & Tangboriboonrat, 2010b; Sruanganurak & Tangboriboonrat, 2007; Sruanganurak, Sanguansap, & Tangboriboonrat, 2006). Results showed that the negatively charged PMMA particles were firmly attached onto the rubber film grafted with polyacrylamide (NR-g-PAAm or SPNR-g-PAAm). Consequently, the surface hardness or roughness of the modified rubber film increased and, hence, the surface friction decreased. Due to the reduction of direct contact between the rubber film and skin, the allergenic problem possibly caused from the non-rubber substances and/or the residue vulcanizing ingredients would be minimized. The coated SPNR-g-PAAm film was then extracted by a culture medium for the *in vitro* cytotoxicity test on L-929 fibroblasts. The results indicated no cytotoxicity potential at the extract concentrations of $\leq$12.5% for 24 h at 37 °C. In order to reduce the preparation steps and to avoid using PAAm, PMMA-polyethyleneimine (PEI) core–shell nanoparticles were synthesized and directly deposited onto the non-grafted SPNR sheet (Sunitaboon, Duangphet, & Tangboriboonrat, 2009). The driving force was the electrostatic interaction between the negative charges derived from indigenous non-rubbers (i.e., proteins and phospholipids) at the surface of rubber sheet and positive charges of PEI (Ho, 1989). However, PEI especially with high molecular weight (MW) has high cytotoxic on many cell lines which limited its use in *in vivo* bio-related applications (Wang et al., 2012).

The cationic polymer widely used in biomedical applications is chitosan (CS), a linear copolymer of D-glucosamine and *N*-acetyl-D-glucosamine linked in β $(1\rightarrow 4)$, obtained from deacetylation of chitin from shrimp and crab shell. Most of the specific properties of CS, e.g., biocompatibility, biodegradability, non-toxicity and antimicrobial activity against fungi, bacteria and viruses, related to its cationic nature. It was reported that positive charges of CS responsible for interacting with sialic groups of mucus promote a structural reorganization of the tight junction-associated

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proteins and, hence, provide its inherent bioadhesive nature (Bravo-Osuna, Ponchel, & Vauthier, 2007). The cationic CS also interacts with negatively charged molecules including nucleic acids and proteins (Lloyd, Kennedy, Methacanon, & Paterson, 1998). *O*-butyrylchitosan was grafted onto NR film surface by UV-induced immobilization to improve the hydrophilicity of rubber film (Heping, Zongqiang, Hongchao, & Yongyue, 2012). The CS was previously used as a stabilizer of colloidal particles, e.g., gold, CS-Fe^0, poly(butyl cyanoacrylate) (PBCA) and polystyrene (PS) (Huang & Yang, 2004; Marie, Landfester, & Antonietti, 2002; Tielong, Bing, Na, Zhaohui, & Xinhua, 2009; Yang, Ge, Jiang, & Yang, 2000). Both low and high MW CS could stabilize PS nanoparticles in the miniemulsion polymerization process. Since CS chain bears protonated amine groups in acidic medium, the particle stability can be induced by an electrosteric stabilization. When CS was adsorbed onto lecithin stabilized nanocapsules, its conformation was appeared as moderate due to the degree of deacetylation (DD) and MW. Compared to high MW CS at a given DD, the spatial conformation of low MW CS was easily re-structured by hiding the hydrophobic part from water contact leading to the more hydrophilic surface exposure to an environment (Santander-Ortega, Peula-Garcia, Goycoolea, & Ortega-Vinuesa, 2011). In addition, lower MW CS could compactly attach onto an oil droplet and, hence, improved the emulsifying activity and stability (Li & Xia, 2011).

In the present study, the PMMA particles stabilized by CS oligomer (PMMA-CS) were synthesized via the miniemulsion polymerization and were directly deposited onto NR or SPNR substrate. By considering both cationic character and biocompatibility of CS, the rubber film coated with PMMA-CS particles should provide more advantages including the reduction of the cytotoxicity of rubber film, when compared to the use of bare PMMA particles in the previous work (Anancharungsuk et al., 2010a). The effects of polymerization time and initiator concentration on % conversion were investigated. The average size, size distribution and zeta potential of PMMA-CS particles were determined. The latex particles were then deposited onto the unmodified NR or SPNR latex film. The surface of the rubber film coated with PMMA-CS particles was examined by scanning electron microscopy (SEM) and atomic force microscopy (AFM). The *in vitro* cytotoxicity on L-929 fibroblasts of the culture medium used for extracting the coated NR or SPNR film was tested. The proliferation and morphology of L-929 fibroblasts before and after incubating with the extract were investigated under optical microscope (OM).

2. Materials and methods

2.1. Materials

NR (Pan Asia Bio Tech Co., Ltd., Thailand) and SPNR latexes with average particle size of 0.8 μm (kindly provided by Loykulnant, National Metal and Materials Technology Center, Thailand) were filtered through a 250 mesh aluminum. Total solid content and dry rubber content of SPNR were 61.3 ± 0.1 and 60.5 ± 0.2, respectively. Methyl methacrylate (MMA) monomer (Fluka, Purum) was purified by passing through a column packed with neutral and basic-aluminum oxide (Fluka, Purum). Acetic acid (0.1 M) (Merck, GR) was used for dissolving CS (Taming-enterprises, Oligomer 97% DD). 2,2′-Azobisisobutyronitrile (AIBN) (Fluka, Purum) and hexadecane (Fluka, Purum) were used as received.

2.2. Synthesis of PMMA-CS latex

PMMA-CS latex was synthesized via the miniemulsion polymerization process. MMA monomer (2 g) containing AIBN (0.01–0.08 g) and hexadecane (0.25 g) was mixed with 0.25–1% w/v CS solution (24 g). After stirring for 1 h, the mixture was ultrasonicated (Branson, W 450 Digital) at 90% amplitude in an ice bath for 2 min. The temperature was then raised to 80 °C and the polymerization was allowed to proceed for 2 h. The effects of polymerization time and AIBN concentration on % conversion were investigated. After polymerization, the PMMA-CS was purified by repeatedly centrifugation until the conductivity of supernatant was close to that of the deionized water.

The average size, size distribution and zeta potential of PMMA-CS particles were measured by using dynamic light scattering apparatus (Malvern, Nano ZS). TEM (Joel, JEM-2100) was used for morphological study after dropping latex onto a carbon coated copper grid. In order to observe its fine structure, PMMA-CS particle was stained with 2% phosphotungstic acid.

2.3. Deposition of PMMA-CS particles onto NR or SPNR film

NR or SPNR latex was casted onto a glass plate and allowed to dry at room temperature. The rubber film (2 cm × 5 cm × 0.1 cm) was subsequently adhered to a glass slide without using any adhesive. The film surface was cleaned by immersing a sample, while sonicating, in methanol and then Milli-Q water for 15 min in each step. The dry rubber strip was immersed into PMMA-CS latex (0.5–2.0% TSC) at pH 2–5 for 5–60 min. After washing with Milli-Q water via a series of three rinsing baths and dried at room temperature, the specimen was characterized by SEM (Hitachi, S-2500) and AFM (Digital Instrument, Nanoscope IIIa).

2.4. In vitro cytotoxicity test

The colony-forming technique was used to examine the *in vitro* cytotoxicity of L-929 cells according to ISO 10993 part 5 (ISO 10993, 1999) and the methods previously described (Anancharungsuk et al., 2010a). The sample (0.1 g) of the unmodified rubber film or that coated with PMMA-CS particles was separately immersed into a completed medium (1 mL), i.e., RPMI-1640 supplemented with HEPES (25 mM), D-glucose (1.8 mg/mL), glutamine (2 mM), gentamicin (40 mg/mL) and 10% heat-inactivated Fetal calf serum (FCS). After incubating at 37 °C under 5% CO_2 atmosphere for 3 h and 9 h, the extract (designated 100%) was serially diluted with the completed medium to attain 50%, 25% and 12.5%. The culture medium of L-929 cells seeded at density of 100 cells/well in a 24-well plate was replaced with the diluted extract. The medium without the extract (1 mL) acted as negative control. At day 6, the number of living cell colonies on each well were counted under OM (Olympus, CKX41) and % growth inhibition was determined.

3. Results and discussion

3.1. Analysis of PMMA-CS latex particles

The percent conversions of MMA monomer in the presence of CS (1.0%) as function of AIBN concentrations (0.5%, 1%, 2% and 4%) and polymerization times were examined (supplementary data, Fig. S1). It was clearly observed in all curves that the percent conversions of MMA rapidly increased with increasing polymerization times in the initial period. With 0.5% and 1% of AIBN, the plateau values of 70% conversion were reached within 40 min whereas 2% and 4% of AIBN provided the constant conversion of 80% and 90%, respectively, within 30 min. An explanation was based on the mechanism of miniemulsion polymerization whose polymerization rate (R_p) is influenced by the number of radical in monomer droplet or initiator concentration in early stage of nucleation (Odian, 2004). It was found that R_p of PMMA-CS latexes obtained from the initial slopes of the conversion–time curves was

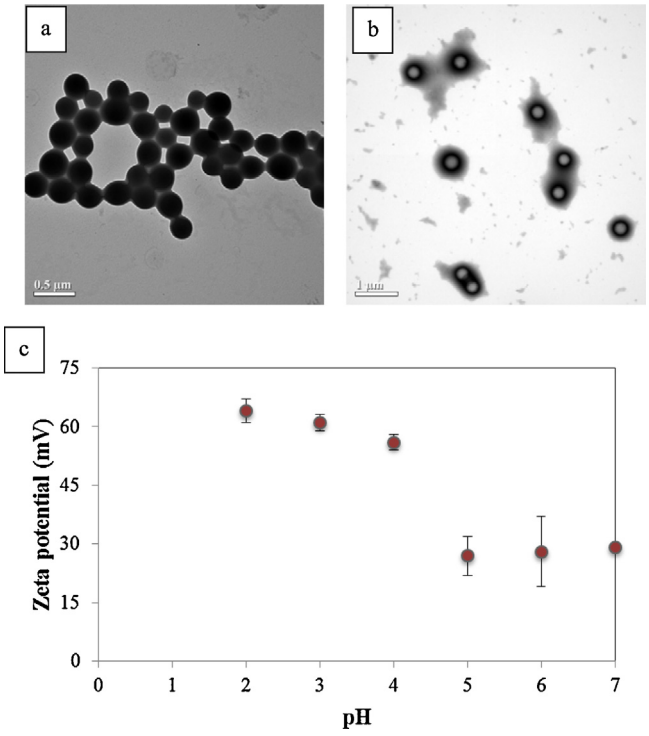

Fig. 1. TEM micrographs of PMMA-CS latex particles using 1% CS as surfactant (a) before and (b) after being stained with 2% phosphotungstic acid and (c) zeta potentials of PMMA-CS latex particles as a function of pH.

directly proportional to AIBN concentrations or the amount of oligoradicals in monomer droplet (Odian, 2004).

The effect of CS concentrations on the hydrodynamic diameter (D_h) of PMMA-CS latex particles was then studied. As expected, the D_h values were inversely proportional to the CS concentrations, i.e., the D_h decreased from 450 nm to 380 nm when the CS increased from 0.25% to 1%. This confirmed the role of CS as a surfactant which reduced the interfacial tension between water and oil phases (Zanetti-Ramos et al., 2006). However, it should be mentioned that when the concentration of CS was <1%, the PMMA-CS latex was not stable. As previously reported, the emulsifying stability index of CS significantly decreased when using CS concentration of <0.75% for stabilization of oil droplet (Li & Xia, 2011). Therefore, 1% CS solution was selected for the synthesis of PMMA-CS latex particles which were subsequently deposited onto the rubber substrate.

TEM micrograph of the PMMA-CS latex particles with using 1% CS solution is displayed in Fig. 1(a). The spherical particles having an average size and polydispersity index of 338 nm and 1.04, respectively, were observed. After staining with 2% phosphotungstic acid which preferentially reacted with amine groups of CS, Fig. 1(b) revealed the core–shell morphology of the prepared PMMA-CS (Chaleawlert-umpon & Pimpha, 2009). It was believed that the surface of PMMA core (light) was firmly attached with CS shell (dark) via the hydrogen abstraction mechanism during the polymerization (Marie, Landfester, & Antonietti, 2002). The presence of CS as the outermost layer of PMMA-CS particles was confirmed by the positive zeta potentials (+64 mV to +29 mV) at pH 2–7 as presented in Fig. 1(c) (Kanjanathaworn, Kaewsaneha, Polpanich, Jangpatarapongsa, & Tangboriboonrat, 2012).

3.2. Characterization of coated NR or SPNR film

The deposition of PMMA-CS particles onto the surface of NR or SPNR film was investigated at pH of the as-prepared PMMA-CS (pH 3) and at pH > isoelectric point (pI) of NR latex (pH 5) (Sanguansap, Suteewong, Saendee, Buranabunya, & Tangboriboonrat, 2005). Fig. 2(a) and (b) show SEM micrographs of NR films after immersing in the PMMA-CS latexes at pH 3 and 5, respectively.

Both SEM images supported the existence of PMMA-CS particles on the NR films. At pH 3 where PMMA-CS particle and NR substrate possessed positive charges, the particle deposition caused from a hydrophobic interaction (Pavinatto, Caseli, & Oliveira, 2011). At pH 5, the electrostatic interaction between $-NH_3^+$ along the CS chain and $-COO^-$ on NR was a key as a driving force. However, the latex particles did not adsorb as a monolayer and entirely cover the rubber surface in both cases. The explanation involved the non-uniform charge distribution of non-rubber substances (protein–lipid) on NR film surface. It was clearly evident that proteins covalently bound to NR particle surface in the form of cluster (Tangboriboonrat, Tiyapiboonchaiya, & Lerthititrakul, 1998). After latex film formation, the low chain mobility of NR brought about their non-uniform distribution (Ho & Khew, 2000). Moreover, the CS chains possibly formed the bridging among several latex particles.

As previously reported, the —COOH groups of the non-rubber substances on the surface of NR particles at pH < pI could form hydrogen bonding with poly(ethylene oxide) (PEO) chain adsorbed on polychloroprene particles (Tangboriboonrat & Buranabunya, 2001). This principle was, therefore, adapted for the deposition of PMMA-CS particles onto NR or SPNR film via the complex formation between —OH and $-NH_3^+$ groups of CS and —COOH groups on the rubber substrate. The SEM images showing PMMA-CS particles deposited onto NR and SPNR films at pH 2 are presented in Fig. 3(b) and (d) whereas the unmodified NR and SPNR films are displayed in Fig. 3(a) and (c), respectively.

The micrographs in Fig. 3(b) indicated that PMMA-CS particles were partially embedded into NR film surface. It was explained that the stiff CS chains (glass transition temperature; $T_g = 140\,^\circ C$) around the particles could intervene into the tiny holes caused from the rearrangement of random coil NR molecules (Brydson, 1978; Dong, Ruan, Wang, Zhao, & Bi, 2004). Then, the hydrogen bonding between the functional groups on CS and NR was formed. These results were different from those reported in the previous works using NR-*g*-PAAm or SPNR-*g*-PAAm film as substrate. In that case, PMMA particles stabilized by $-SO_4^{2-}$ were regularly coated onto the hairy PAAm grafted rubber film via the electrostatic interaction (Anancharungsuk et al., 2010a, 2010b; Sruanganurak et al., 2006; Sruanganurak & Tangboriboonrat, 2007). In addition, our results were differed from the deposition of hairy PMMA-PEI particles onto the non-grafted SPNR film surface (Sunitaboon, Duangphet, & Tangboriboonrat, 2009). The characteristics of polymer shell, i.e., functional group and chain stiffness, were important factors to predict the mechanism for the deposition of the particles onto the rubber film. Although both CS and PEI ($T_g = -43\,^\circ C$) (Pangon, Tashiro, & Chirachanchai, 2011) composed of $-NH_2$ groups along their chains, the higher stiffness of CS chain brought about the embedment of PMMA-CS particles into the soft NR substrate. The stronger attractive force originated from the hydrogen bonding occurred between CS and NR compared to the electrostatic interaction of PEI also encouraged the partial embedding of PMMA-CS particles into the NR film (Intermolecular force, http://en.wikipedia.org/wiki/Intermolecular_force, 2012). However, it was noticed that PMMA-CS particles did not bury into SPNR film surface as shown in Fig. 3(d) and the number of PMMA-CS particles per surface area was 4.5×10^7 particles/cm^2. The explanation concerned the difficulty of CS chains for penetration into the crosslinked film although Fig. 3(c) revealed many pores on the SPNR surface possibly caused from the detachment of residue vulcanizing ingredients (Ho & Khew, 1999).

Data from SEM agreed well with the AFM images showing the three-dimensional images of the unmodified and modified

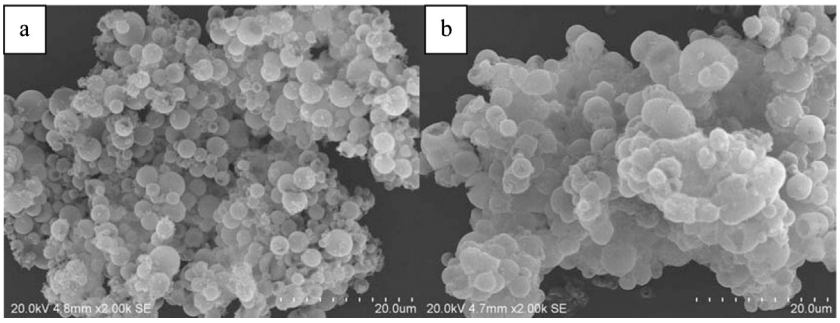

Fig. 2. SEM micrographs of NR film surface after deposition of PMMA-CS latex particles at (a) pH 3 and (b) pH 5 (0.5% latex concentration, dipping time = 60 min).

rubber film surfaces presented in Fig. 4(a)–(d). After deposition of PMMA-CS particles onto the soft and rather smooth surface of NR substrate (Fig. 4(a)), the modified NR surface composed of many nodules which slightly sticked out at the surface. Whereas the deposited particles clearly protruded from the SPNR film as displayed in Fig. 4(d).

3.3. Surface roughness of the modified NR and SPNR films

AFM in tapping mode was also used for measuring the surface roughness (Ra) of the rubber films before and after particle deposition (Sanguansap, Thonggoom, & Tangboriboonrat, 2006). The Ra of the rubber films coated with PMMA-CS particles at pH 2 as a function of latex concentrations and immersion times are plotted in Fig. 5(a) and (b), respectively.

It was observed that the Ra of NR films increased from 19 nm to 27 nm after dipping into 0.5% PMMA-CS latex. With increasing latex concentration, the Ra was constant and then slightly decreased. The partially embedded PMMA-CS particle into NR film surface was responsible for the low Ra and insignificant change-value. In the case of SPNR, the Ra of modified films linearly increased from 46 nm to 80 nm when increasing the latex concentration from 0% to 2.0%. The high Ra was due to the existence of hard PMMA-CS latex particles onto the SPNR film as displayed in Figs. 3(d) and 4(d). As expected, an increasing latex concentration or particle number also increased the Ra values of the coated SPNR.

The Ra of the modified NR and SPNR films using 0.5% PMMA-CS latex concentration plotted with the immersion time are presented in Fig. 5(b). Results showed that the Ra values of NR films increased from 18 nm to 31 nm within 15 min of immersion whereas those of SPNR increased from 46 nm to 64 nm within 10 min. Further increase in an immersion time did not significantly affect the Ra of the modified NR and SPNR films possibly because of the saturation of PMMA-CS adsorption.

3.4. In vitro cytotoxicity test on L-929 cells

The allergy induced by indigenous proteins presented in NR latex and/or by lubricant powder is claimed to be a serious health problem among the hypersensitive gloves' users (Koh, Ng, Leow, & Goh, 2005; Rolland & O'Hehir, 2008; Truscott, 2002; Yip & Cacioli, 2002). The deposition of PMMA-CS particles onto rubber film surface was proposed to be an easy method for reduction of direct contact between latex proteins and human skin. The cytotoxicity test of the NR or SPNR coated with PMMA-CS particles on L-929 fibroblast cells was investigated by using the CFA.

3.4.1. Effect of type of substrates

Two types of rubber, i.e., NR and SPNR, which were used as substrate for particle deposition, were extracted by the culture medium and the toxicity of the extract on the cells was tested. Since the

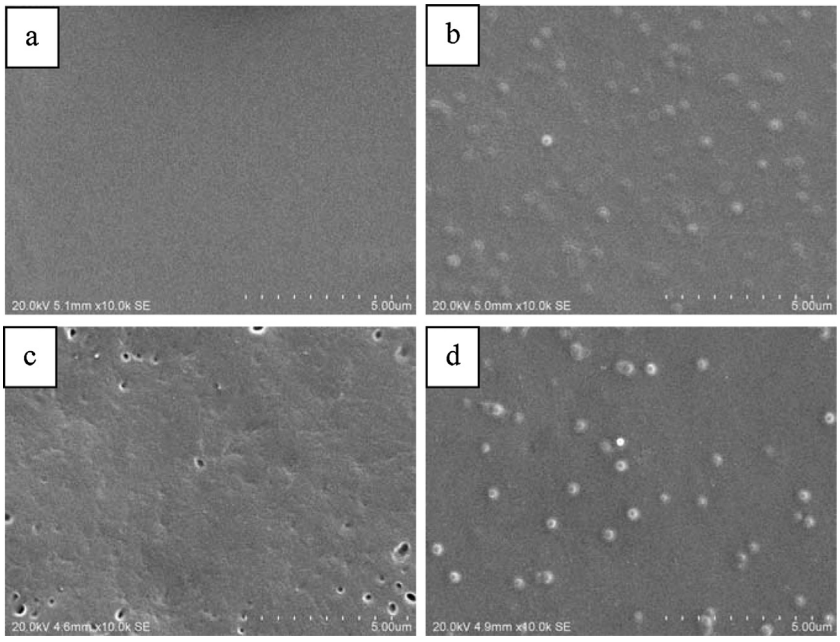

Fig. 3. SEM micrographs of (a) NR, (b) modified NR, (c) SPNR and (d) modified SPNR film surfaces (pH 2, 0.5% latex concentration and dipping time of 60 min).

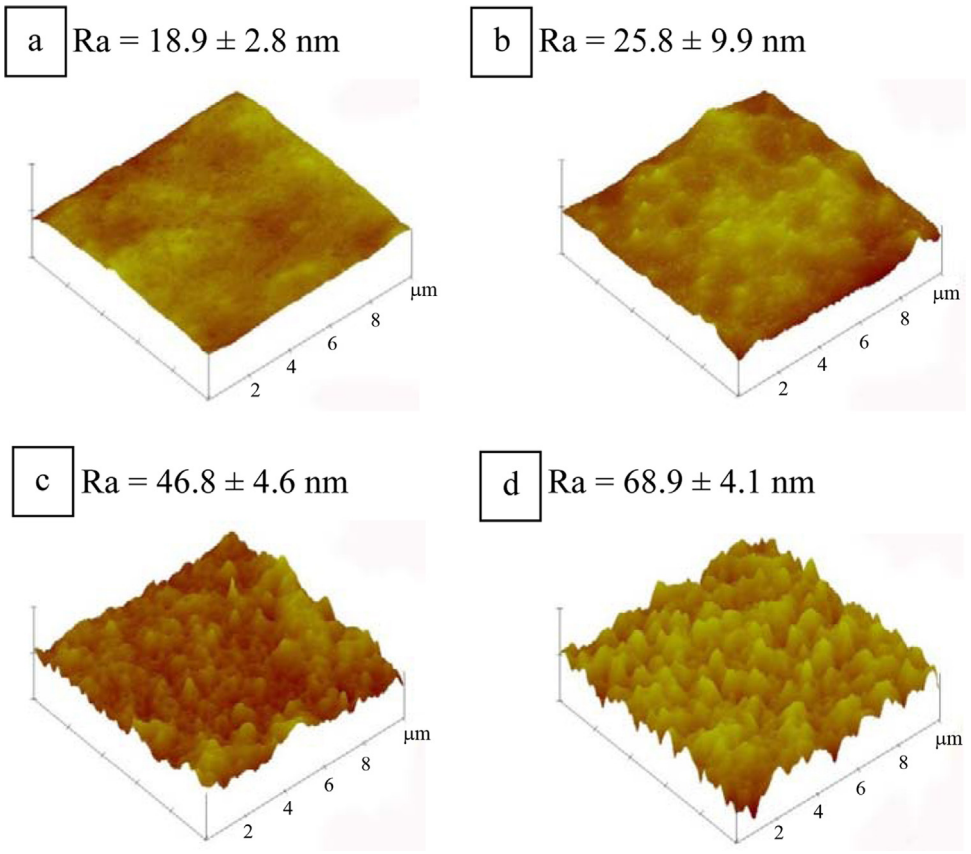

Fig. 4. AFM images of (a) unmodified NR, (b) modified NR, (c) unmodified SPNR and (d) modified SPNR.

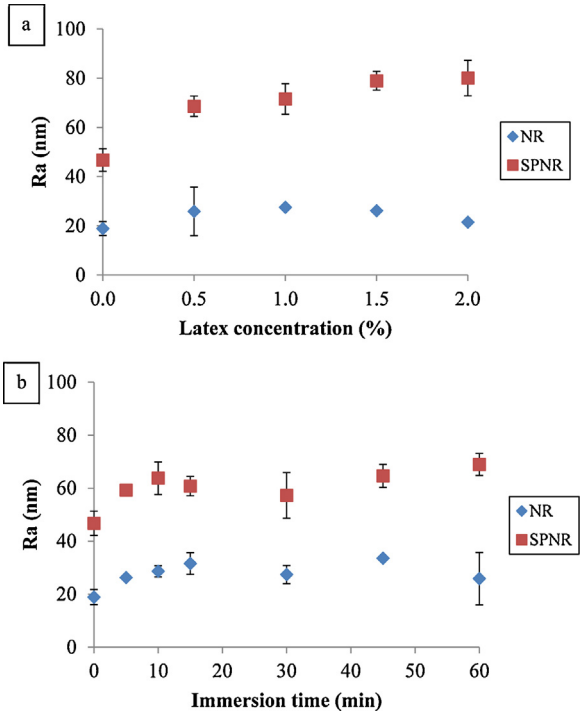

Fig. 5. Surface mean roughness (Ra) of NR and SPNR films coated with PMMA-CS latex particles as function of (a) latex concentration and (b) immersion times at pH 2.

previous work reported that the SPNR-*g*-PAAm/PMMA extracted for 3 h and 6 h showed less toxicity to the cells compared with the untreated SPNR (Anancharungsuk et al., 2010a), the unmodified and modified rubber surfaces were, therefore, extracted for 3 h in this study. The cells were cultured under 100% concentration of the extract and the result was compared to the negative control (culture medium without extract) and the positive control (unmodified NR or SPNR film). The % growth inhibition of L-929 cells as a function of times used for immersing NR or SPNR substrate in PMMA-CS latex are presented (supplementary data, Fig. S2). Results showed that the % growth inhibition caused from the unmodified NR was higher than that caused from SPNR. The lower protein contents due to either leaching out or being locked by crosslinked polymer chains might be responsible for the lower cytotoxicity of SPNR (Yip & Cacioli, 2002). Although it was reported that chemical agents used in the prevulcanization process showed toxicity on human skin (Hamann et al., 1998; Schlogl, Aust, Schaller, Holzner, & Kern, 2010; Truscott, 2002; Yip & Cacioli, 2002), this effect could not be detected.

It was observed that the growth inhibitions of 40% when testing the unmodified and modified NR with immersion time of 5–60 min were similar. The results agreed with the SEM image in Fig. 3(b) that the PMMA-CS particles were partially embedded into NR surface and, hence, most of cells contacted with NR film in all cases. On the contrary, PMMA-CS particles protruded from the SPNR surface reduced the direct contact between SPNR film and the cells resulting in the significant decrease of growth inhibition of the coated SPNR as also observed when the SPNR-*g*-PAAm was coated with PMMA particles (Anancharungsuk et al., 2010a). It was worth noting that coating SPNR with PMMA-CS particles provided lower % growth inhibition of L-929 cells compared to the bare PMMA particles coated onto the SPNR-*g*-PAAm. The dissociation energy of

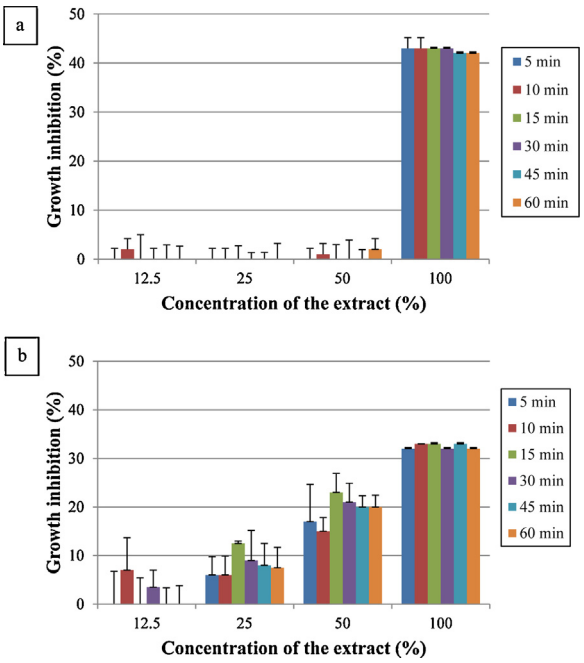

Fig. 6. Percentage growth inhibition of L-929 fibroblasts at various concentrations of the extract of (a) the modified NR and (b) the modified SPNR films with the immersion time used for immersing NR or SPNR substrate in PMMA-CS latex of ( ) 5 min, ( ) 10 min, ( ) 15 min, ( ) 30 min, ( ) 45 min and ( ) 60 min (extraction time of 9 h).

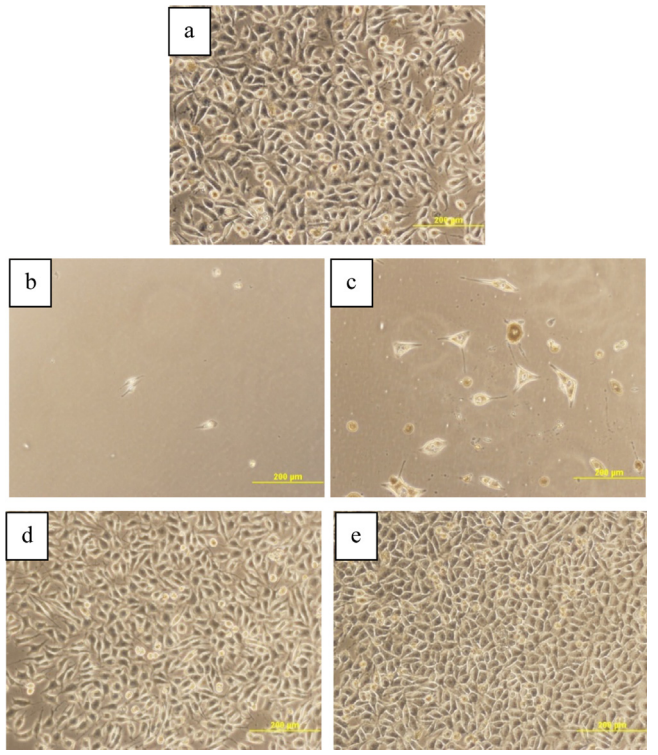

Fig. 7. Morphologies of L-929 cells incubated with (a) negative control, (b) positive control of NR, (c) positive control of SPNR, (d) modified NR and (e) modified SPNR (12.5% extract concentration at day 6 after culturing).

hydrogen bonding (12–16 kcal/mol) between PMMA-CS particles and SPNR film which is higher than that of the electrostatic interaction (0.5–2 kcal/mol) between PMMA and SPNR-g-PAAm surface might effectively suppress the detachment of those allergens to the extract at a certain incubation time (Intermolecular force, http://en.wikipedia.org/wiki/Intermolecular_force, 2012). Since the hydrogen bonding is pH-insensitive, the interaction of PMMA-CS with SPNR film could not be disturbed at any incubation pH including pH of the RPMI medium.

3.4.2. *Effect of the extract concentrations*

Since it was reported that the growth of L-929 cells was completely inhibited when using the medium extracted from the PMMA particles coated NR-*g*-PAAm for ≥9 h (Anancharungsuk et al., 2010a), the effect of the extract concentrations on growth inhibition of the cells cultured for 9 h was studied in this work. The number of cell colonies at day 6 was counted and % growth inhibition at various concentrations of the extract of the modified NR and modified SPNR films are presented in Fig. 6(a) and (b), respectively.

In the case of NR, Fig. 6(a) shows that at ≤50% of the extract, the growth inhibition of cells was close to the negative control (0%). After coating with PMMA-CS particles, 100% extract caused 40% growth inhibition irrespective to the immersion time. For the modified SPNR in Fig. 6(b), the % growth inhibition of cells was directly proportional to the extract concentration for all immersion times. The highest growth inhibition of 30% was induced by 100% extract whereas the lowest growth inhibition (0%) was found at 12.5% extract concentration. It was, therefore, assumed that the cytotoxicity of L-929 could be reduced by coating NR and SPNR film with PMMA-CS particles.

3.4.3. *Morphological studies of L-929 fibroblasts*

The morphologies of L-929 fibroblast cells incubated with 12.5% of the medium extracted from NR and SPNR coated with PMMA-CS particles compared with the positive and the negative controls are presented in Fig. 7. It was observed that L-929 cells of the negative control in Fig. 7(a) were spindle in shape and adherent growing as a confluent monolayer. On the other hand, when using the unmodified NR and SPNR, the loss of spindle shape and detachment of the plate were observed in Fig. 7(b) and (c), respectively. The extract of PMMA-CS coated NR and SPNR did not significantly induce cell morphological change as presented in Fig. 7(d) and (e), i.e., the cells spread and exhibited a spindle to elongated shape similar to the case of negative control. The results confirmed that coating NR and SPNR film with PMMA-CS particles reduced the cytotoxicity of L-929 cells.

4. Conclusion

The cationic PMMA-CS particles, synthesized via the miniemulsion polymerization, were successfully deposited onto the unmodified NR and SPNR film surfaces in one step. The driving force was the hydrogen bonding formed between —COOH groups of proteins on rubber surface and —OH and $—NH_3^+$ groups of CS chain on PMMA-CS particles at pH 2.0. The presence of PMMA-CS particles led to an increase in surface roughness of the SPNR film. Coating NR or SPNR film with PMMA-CS resulted in the effective reduction of cytotoxicity of L-929 fibroblast.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.carbpol.2012.12.078.

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