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Sustaining guest molecules on bio-surfaces by grafting the surfaces with cyclodextrins

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ABSTRACT

Grafting cyclodextrins (CDs) onto materials is an interesting approach to provide the remarkable ability of CDs to form an inclusion complex with various guest molecules onto other (bio)materials. Here we show the design, synthesis and characterization of "a ready to graft β-CD", which is a cyclodextrinyl methacrylate (M-β-CD), which will easily attach covalently to thiol-containing materials via a simple Michael thiol-ene click reaction under a biologically mild condition. Reacting M-β-CD with soluble keratin, human hair and a mucin layer as model biomaterials, in the presence of either tris (2-carboxyethyl) phosphine or vitamin C, resulted in β-CD-grafted keratin that displayed a controlled release of 8-anilino-1-naphthalenesulfonic acid, and both β-CD-grafted-hair strands and β-CD-grafted-mucin layers that effectively retained curcumin. Although this was demonstrated with β-CD on these three biomaterials, this platform could theoretically equally be used with other CDs to give a different range of guest material complexation, and be able to be applied to other thiol-containing materials.

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1. Introduction

Cyclodextrins (CDs), cone-shaped cyclic oligosaccharides with a hydrophilic outer surface and internal hydrophobic cavity, are typically produced enzymatically from starch. The ring structure is made of six, seven or eight glucopyranose units (α-, β-, and γ-CDs, respectively) that can entrap a diverse range of compounds through host–guest interactions (Bekers, Uijtendaal, Beijnen, Bult, & Underberg, 1991; Uekama & Otagiri, 1987). This host–guest complexation can help protect the entrapped guest from environmental threats, such as oxidizing agents (Pourmokhtar & Jacobson, 2005; Reichenbach & Min, 1997) and light (Caddeo, Manconi, Valenti, Pini, & Sinico, 2007; Chen et al., 1996), as well as increasing its apparent aqueous solubility (Lin, Chean, Ng, Chan, & Ho, 2000; Lockwood, O'Malley, & Mosher, 2003). Usually, a sustained release of the guest molecule from the CD cavity is observed (Kamada et al., 2002; Marques Fernandes, Ramos, Celta Falcão, & Baptista Veiga, 2003). We envisioned that if CD can be directly grafted onto biological surfaces or biomaterials under a biological friendly condition, it would create an easy strategy for drug localization/retention in biological systems. For this new way of using CDs, a CD-grafting platform that is not only effective but also produces no toxic byproduct and requires no toxic catalyst is needed.

Recently, a number of CD-containing polymers that combine the advantageous properties of the polymer with the ability of CD to form an inclusion complex with other components have been prepared by cross-linking/polymerizing CD derivatives or grafting CD moieties onto the polymer chains (Huh, Tomita, Lee, Ooya, & Yui, 2002; Wenz, 1994). The CD-containing polymers not only showed great potential as delivery carriers for drugs and genes (Rajewski & Stella, 1996; Wong, Sevimli, Zareie, Davis, & Bulmus, 2010), but have also been used as the stationary phase in chromatographic separations (Huang, Wu, Liu, Liu, & Zhang, 2013; Martel et al., 2001). The ability to form an inclusion complex with various guest molecules has also been employed as a driving force for the self-assembling of a number of nanostructures (Bertrand, Stenzel, Fleury, & Bernard, 2012; Kulkarni, DeFrees, Hyun, & Thompson, 2012). Indeed, CD has been grafted onto many well-designed molecular assembles to create various stimuli-responsive functions via the respective host–guest complexation (Du, Liao, Khatib, Stoddart, & Zink, 2009; Xue et al., 2011; Yan et al., 2010). Surface functionalization with CD has also resulted in a fairly diverse array of advanced devices, such as medical polyester vascular prostheses functionalized with CD that then allows the sustained-elution of

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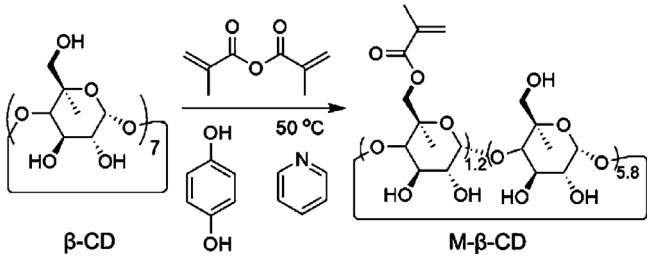
several antibiotics (i.e. sustained drug release) (Jean-Baptiste et al., 2014).

Nevertheless, although the grafting of CD onto polymers or other materials can be performed via several different methodologies (Benito et al., 2004; Gu & Shamsi, 2011; Huh et al., 2002), the subsequent removal of the biologically toxic chemical reagents used in these multi-step-processes is required (Pun et al., 2004; Sajomsang et al., 2012; Guo et al., 2005), which then prevents the direct grafting of CD onto biological surfaces. Instead, we propose a CD derivative that would allow an easy and direct high efficiency grafting of CD onto biological sites in situ under a biologically mild condition with no toxic byproduct. In this paper, we show the design, synthesis and characterization of the methacrylate-linked β-CD (cyclodextrinyl methacrylate; M-β-CD), which can be easily grafted onto thiol-containing materials in an aqueous medium under a biologically mild condition with excellent click efficiency via a Michael thiol-ene reaction. It should be noted here that although a methacrylate-linked CD has been reported previously, the acrylate moieties on the material was used for the radical polymerization purpose (Hussain, Dickens, & Bowen, 2005; Saito & Yamaguchi, 2003) or for Michael thiol-ene addition in the preparation of photoresponsive microgels (Zhang et al., 2012).

The Michael thiol-ene reaction is a metal-free and mild conditioned click reaction that is accordingly already widely employed in various biological fields (Boyer & Davis, 2009; Gu, Chen, & Stenzel, 2009; Jones et al., 2009). The reaction can proceed under a mild base (Kumari, Malvi, Ganai, Pillai, & Sen Gupta, 2011; Wong et al., 2010) or nucleophilic catalyst (Chan, Hoyle, & Lowe, 2009) or even in the absence of catalyst in strongly polar solvents, such as water (Kakwere & Perrier, 2009). That M-β-CD is a good choice for biological functionalization is because a number of biomaterials contain proteins with cysteine residues (thiol source), thus fitting well with our platform. We also demonstrated that thiol moieties could be generated by reducing cystine disulfide bridges in biomaterial (mucin protein) with either the well-known tris (2-carboxyethyl) phosphine (TCEP) or the non-toxic vitamin C. The use of acrylate containing polymer to attach to keratin via thiol-ene click reaction has also been demonstrated previously (Slavin, Khoshdel, & Haddleton, 2012). Here, as proof of concept examples, three biomaterials (hair strands, soluble keratin and mucin surface) were grafted with the synthesized M-β-CD, and their drug retention ability was demonstrated using curcumin and 1-anilinonapthalene-8-sulfonic acid (1,8-ANS) as model payload compounds.

2. Materials and methods

The β-CD (Wako, Japan), methacrylic anhydride (Sigma Aldrich, Germany), hydroquinone (Acros Organics, Belgium), pyridine (Carlo Erba, Italy), triethylamine (TEA, Carlo Erba, Italy), tris(2-carboxyethyl) phosphine hydrochloride (TCEP–HCl, Acros Organics), hydrolyzed keratin (MW_{avg} = 125 kDa, Croda, UK), L(+)-ascorbic acid (Vitamin C, Wako), 1-anilinonapthalene-8-sulfonic acid (1,8-ANS, Sigma Aldrich), curcumin (Acros Organics) and ethanol (Merck, Germany) were used directly after purchase. Other chemicals were prepared prior to experiments as following; commercial grade dichloromethane (RCI Labscan, Thailand) was fractionally distilled; human hair samples were from the same Thai female and were cleaned with 1% (w/v) sodium lauryl sulfate surfactant and excess water. Cellulose tubing membrane (*MWCO* = 10322, flat width 76 mm, 17.9 mL/cm volume capacity, Sigma Aldrich) was used as the dialysis membrane for the purification of the β-CD-grafted keratin. All ^{1}H NMR spectra were obtained on a Varian Mercury NMR spectrometer, operated at 400 MHz for ^{1}H nuclei (Agilent). Attenuated total reflectance–Fourier transform infrared (ATR-FTIR) spectra were recorded in the mid-infrared region (4000–650 cm^{-1}) using a Nicolet 6700 FT-IR spectrometer (Thermo Electron Corporation). Electrospray ionization (positive ion mode)-mass spectra (EMS$^+$–MS) were acquired using a microTOF-Q II 10335 mass spectrometer (Bruker quadrupole–time-of-flight analyzer), equipped with an atmospheric pressure ionization source operating in the nebulizer assisted electrospray (positive ion) mode. Fluorescence spectra were obtained from a Varian Cary Eclipse spectrofluorometer (Agilent, Santa Clara, CA, USA) with the excitation and emission monochrometer band pass set at 5 nm and 3 nm, respectively, using a 1.0 cm^2 quartz fluorescence cell. Confocal laser fluorescence microscope (CLFM) used to capture the fluorescence signals of curcumin in the hair samples and mucosa plates was a Nikon Digital Eclipse C1–Si instrument equipped with Plan Apochromat VC 100×, BDLaser (408 nm, Melles Griot, Carlsbad, CA, USA), a Nikon TE2000-U microscope, a 32-channel-PMT-spectral-detector and Nikon-C1-Si software.



Scheme 1. Grafting of methacryloyl moieties onto β-CD.

2.1. Synthesis and characterization of β-cyclodextrinyl methacrylate (M-β-CD) (Scheme 1)

Prior to the reaction, a solution of β-CD (1.0 g, 0.88 mmol) and hydroquinone (0.010 g, 0.091 mmol) were prepared in pyridine (20 mL). Subsequently, methacrylic anhydride (390 μL, 2.64 mmol) was added into the acquired mixture gently. The reaction was incubated at 50 °C for 24 h prior to the removal of pyridine via a low pressure distillation. The mixture of M-β-CD and unmodified β-CD was obtained as some white powder by reprecipitation in dichloromethane (40 mL) and then subjected to ^{1}H NMR and ATR–FT-IR analysis. Similar reactions but using different amounts of methacrylic anhydride (130 and 260 μL) were also carried out. The numbers of methacryloyl moieties on the CD rings of the products were estimated from their ESI+ mass spectrum.

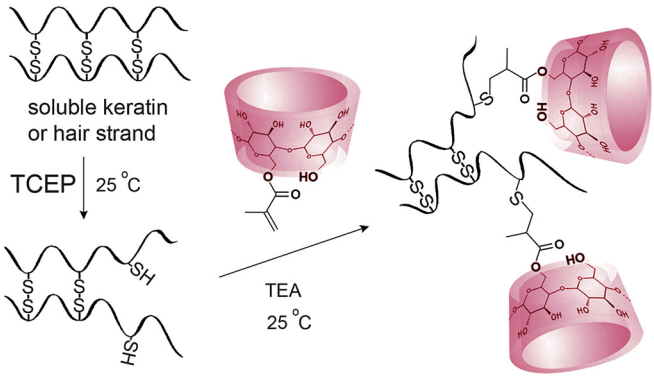
^{1}H NMR (D_2O, 400 MHz, δ ppm): 5.79 and 6.18 (CH_2=C in methacryloyl, s), 5.09 (C(1)H of β-CD, d, J = 3.5 Hz), 3.98 (C(3)H of β-CD, t, J = 9.5 Hz), 3.89–3.86 (C(5,6)H of β-CD, m), 3.72–3.47 (C(2,4)H of β-CD, m) and 1.96 (CH_3 in methacryloyl, s).

ATR–FTIR (cm^{-1}): 3289 (O—H stretching), 2923 (C—H stretching), 1712 (C=O stretching of ester), 1586 (C=C stretching) and 1151 (C—O stretching).

ESI$^+$–MS (*m*/*z*): CD, calcd. for M·Na$^+$ 1157.974, found 1157.4; CD-mono-methacrylate, calcd. for M·Na$^+$ 1225.974, found 1225.4; CD-di-methacrylate, calcd. for M·Na$^+$ 1293.974, found 1293.4; CD-tri-methacrylate, calcd. for M·H_2O·Na$^+$ 1379.974, found 1379.5.

2.2. Inclusion property of M-β-CD

To investigate the inclusion ability of the M-β-CD, comparing to unmodified β-CD, fluorescence spectroscopy was performed using 1,8-ANS as the model guest molecule. In the reaction, 1,8-ANS (3×10^{-5} M) was incubated with M-β-CD (10^{-4} M) for 40 min at room temperature (RT). Subsequently, fluorescence spectrophotometric monitoring was performed with excitation



Scheme 2. Grafting of β-CD moieties onto soluble keratin or hair strand via a thiol-ene click reaction using M-β-CD reagent.

wavelength at 370 nm and the emission collected from 400 to 700 nm at 21 ± 2 °C.

2.3. β-CD-grafted keratin (Scheme 2)

An aqueous solution of hydrolyzed keratin (2 mL, 20% w/v) was mixed with a TCEP-HCl solution (10 mg in 500 μL water) for 2 h at RT. Then, M-β-CD (60 mg, dissolved in 5 mL hot water) and TEA (4 drops) were added to the mixture and the mixture was left overnight at RT before being excessively dialyzed against deionized water. The obtained yellowish solution was freeze-dried (obtaining 263 mg product, ≈ 60–70% yield) and the dry product was subjected to ^{1}H NMR analysis. To evaluate the drug retention property, the 1,8-ANS aqueous solution (2.95×10^{-5} M) was stirred with the test material (0.17 mg/mL native β-CD, 1.7 mg/mL keratin and 1.9 mg/mL β-CD-grafted keratin) for 30 min and kept to equilibrate for another 10 min at RT before being subjected to fluorescence emission monitoring over the temperature range of 20–70 °C (scan rate of 10 °C/min) using a thermal controlled fluorescence spectrophotometer set at an excitation wavelength of 370 nm and the emission scanned from 400 to 650 nm.

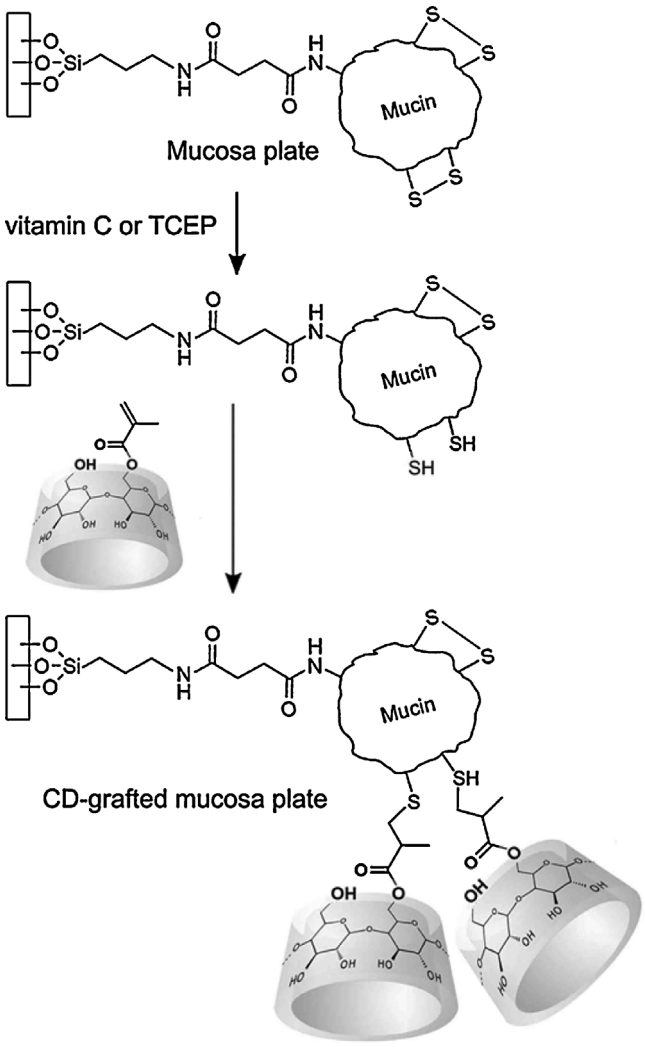
2.4. β-CD-grafted human hair (Scheme 2)

Strands of thoroughly washed human hair were soaked in TCEP-HCl solution (20 mg in 1.0 mL water) for 2 h at RT. Then, M-β-CD (60 mg, dissolved in 5 mL hot water) and TEA (4 drops) were added to the mixture and left overnight at RT. The obtained β-CD-clicked hair strands were washed under running water. Similar process was carried out using unmodified β-CD in place of the M-β-CD (to obtain control hair sample). The treated hair strands were then subjected to curcumin retention study.

The treated hair strands were incubated with curcumin (10 mL, 300 ppm) in 5% (v/v) EtOH for 1 h at RT. Then the hair samples were washed with 10% (v/v) EtOH in water. Subsequently, confocal laser scanning fluorescence microscopic (CLSM) analysis was performed with excitation wavelength at 405 nm and fluorescence detection at 410–600 nm. The obtained spectrum of each pixel was unmixed into curcumin and human hair auto-fluorescence components using chemometric analysis based on the spectral database constructed from the fluorescence spectra of pure curcumin and the original hair sample. Images were then constructed using the obtained unmixed signals.

2.5. β-CD-grafted mucin (Scheme 3)

Before the synthesis, mucin was covalently grafted onto glass slides (1.8 cm × 1.8 cm) as previously described (Tachaprutinun, Pan-In, & Wanichwecharungruang, 2013). Subsequently, M-β-CD



Scheme 3. Synthesis of the β-CD-grafted mucosa plate.

was incubated with the obtained mucin-grafted slides in a presence of 1) vitamin C (150 mg in 5 mL water) or 2) TCEP (100 mg in 5 mL water)/TEA (2% w/v), for 1–3 h. Subsequently, without washing, the plate was immersed into the M-β-CD solution (60 mg in 5 mL water) and let stand at RT for 1–3 h before being rinsed under running water. Finally, the retention of curcumin was evaluated using CLSM as previously described for the detection of curcumin on hair strands (Section 2.4).

3. Results and discussions

3.1. Synthesis of M-β-CD

Methacrylate moieties were successfully grafted onto β-CD via esterification at the primary hydroxyl groups of the β-CD using methacrylic anhydride (MA), with pyridine as both a catalyst and solvent and hydroquinone as a radical scavenger to prevent the polymerization of methacrylate moieties (Saito & Yamaguchi, 2003). Varying the molar ratio of β-CD:methacrylic anhydride revealed that a 1:2 ratio gave the highest yield (66.7%) of monomethacrylate-linked β-CDs (Table S1 in electronic Supplementary data or SD).

The obtained M-β-CD, a white powder that consisted of mono-, di- and tri-methacrylate-linked β-CDs, could be dissolved in hot water. Successful esterification was verified through ATR–FT-IR

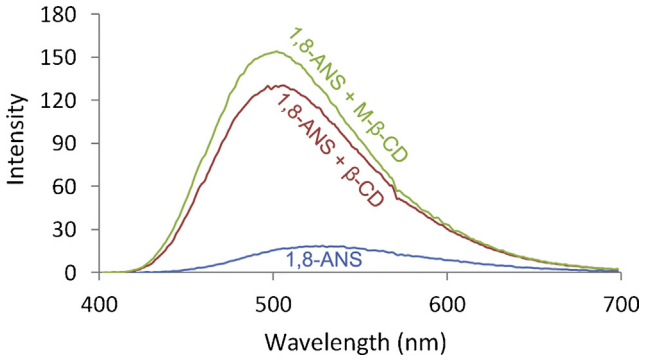


Fig. 1. Fluorescence spectra of 3.00×10^{-5} M 1,8-ANS alone or with 10^{-4} M β-CD or 10^{-4} M M-β-CD in water.

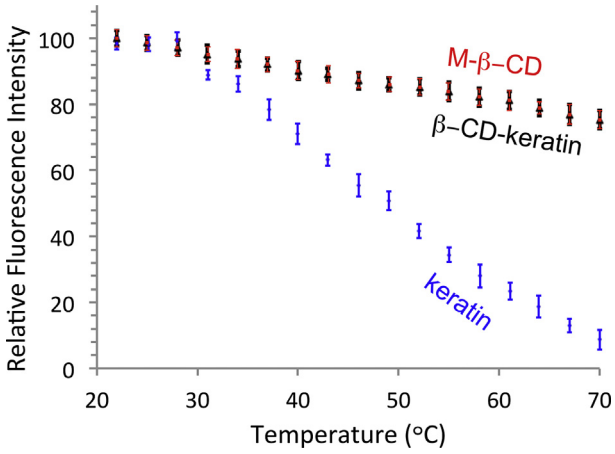


Fig. 2. Relative fluorescence intensity of 2.95×10^{-5} M 1,8-ANS in the presence of excess M-β-CD, β-CD-grafted keratin and keratin at various temperatures. Relative fluorescence intensities are shown as the means + 1 SD, obtained from three independent experiments.

analysis of the product, which showed new peaks at 1712 and 1586 cm^{-1} assigned to the ester and vinyl functionalities, respectively (Fig. S1, SD). The ^1H NMR spectrum of the product also showed the singlet signal of the methacryloyl moiety (CH_2=C) at 5.79 and 6.18 ppm, the singlet signal of the methacryloyl terminal methyl group at 1.90 ppm and β-CD protons at 5.04 (C(1)H), 3.93–3.78 (C(3,5,6)H) and 3.63–3.52 (C(2,4)H)) ppm (Fig. S2, SD). ESI^+–MS of the product showed peaks at 1157.4 (Na + CD), 1225.4 (Na + CD-mono-methacrylate), 1293.4 (Na + CD-di-methacrylate) and 1379.4 (Na + CD-tri-methacrylate + H_2O) with an intensity ratio of approximately 0.100:0.667:0.167:0.0667, indicating an average number of methacryloyl moiety on each ring of 1.20 (Fig. S3, SD).

3.2. Inclusion ability of M-β-CD

The obtained M-β-CD was tested for its inclusion ability using 1,8-ANS as a model guest molecule. The choice of 1,8-ANS was based upon the fact that it has previously been used as a fluorescence probe for exploring hydrophobic regions, including in the cavity of β-CD where it showed a strong fluorescence in the hydrophobic environment of the CD cavity but not in water (Wagner & Fitzpatrick, 2000).

As expected, with no β-CD the aqueous solution of 1,8-ANS gave a weak fluorescence as it was quenched by the surrounding water molecules. However, a stronger fluorescence was observed in the presence of either β-CD or M-β-CD (Fig. 1), which indicated that they both could effectively form an inclusion complex with 1,8-ANS. The fact that the fluorescence signal of 1,8-ANS in the presence of β-CD was comparable to that in the presence of M-β-CD (at the same molar concentration) indicates that functionalization of β-CD with methacrylate did not significantly affect its complexation ability with 1,8-ANS.

3.3. M-β-CD-grafted keratin

The click-ability of the synthesized M-β-CD via a thiol-ene reaction was first evaluated on keratin, a protein with a large amount of cysteine residues. Keratin is a biocompatible natural polymer with applications in cosmetics, wound dressing and tissue engineering (Tachibana, Furuta, Takeshima, Tanabe, & Yamauchi, 2002). Endowment of this protein with an additional drug retention property, such as grafting with β-CD moieties, should open up more advanced applications for this natural polymer.

Most of the cysteine residues in keratin normally exist as disulfide bridges (cystine) and so prior to the click reaction the cystine disulfide bridges were reduced into cysteine residues by tris(2-carboxyethyl) phosphine (TCEP), an oxidizing agent. The thiol containing protein was subsequently clicked with M-β-CD without removing the TCEP (Scheme 2). The nucleophilic TCEP acted as a catalyst for the thiol-ene reaction. Because the TCEP used in this reaction was in an acidic form (TCEP–HCl), triethylamine (TEA) was added to minimize the by-products that would otherwise be formed under acidic conditions (Li et al., 2010). In addition, TEA helps facilitate the thiol-ene reaction through proton abstraction from thiols.

Successful grafting of M-β-CD onto keratin was confirmed through the appearance of all the proton peaks of the CD rings (1.90 and 3.5–4.0 ppm) and the absence of the proton resonances of the vinyl groups (5.8 and 6.2 ppm, Fig. S4, SD) in the ^1H NMR spectrum of the product. Note that the ^1H NMR spectrum of keratin obtained from the same β-CD-grafting process except that β-CD was used in place of M-β-CD, gave no signal from the protons of the β-CD ring. Considering ^1H NMR result, there are 11 CD units/keratin chain or ~10% w/w, and the efficiency of the reaction is as high as 85% (SD), thus indicating a favorable grafting reaction (see SD for the estimations of CD substitution on keratin and reaction efficiency of M-β-CD).

It was expected that the β-CD-grafted keratin would possess the ability to retain some guest molecules through inclusion complexation with the β-CD moieties. To evaluate this, 1,8-ANS was used as the model guest molecule. In the presence of native keratin, 1,8-ANS showed a high fluorescence emission at temperatures below 32 °C (Fig. 2), indicating that the 1,8-ANS molecules were in a hydrophobic environment. Since keratin is capable of forming a self-assembled structure through hydrophobic interactions (Ikkai & Naito, 1976; Steinert, Idler, & Zimmerman, 1976), it was likely that at temperatures below 32 °C the hydrophobic 1,8-ANS molecules associated well with keratin through hydrophobic interactions. This association with keratin helped shield the 1,8-ANS from a direct contact with water molecules and resulted in a high fluorescence emission. However, at temperatures above 32 °C, the entropy probably dominated over the keratin-1,8-ANS binding enthalpy, so dissociation of 1,8-ANS from keratin took place abruptly, sending 1,8-ANS into the surrounding water and so quenching the fluorescence emission. In contrast, when the β-CD-grafted keratin was used in place of the native keratin, no abrupt decrease in the fluorescence emission was observed over the whole tested temperature range of 20–70 °C, implying that the association of 1,8-ANS with β-CD-grafted keratin was stronger than that with native keratin. Interestingly, the change in the fluorescence emission of 1,8-ANS in the presence of β-CD-grafted keratin was similar to that in the presence of free M-β-CD (Fig. 2). This indicates that the interaction of 1,8-ANS with the β-CD-grafted keratin probably took place through

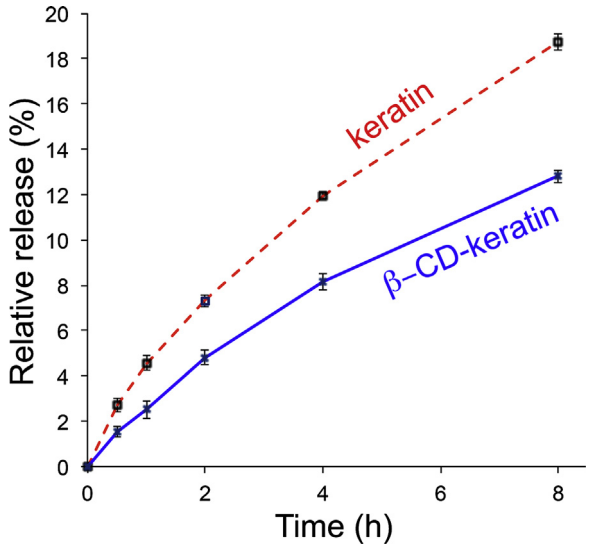


Fig. 3. Relative release of 1,8-ANS from β-CD-clicked keratin at 37 °C. An aqueous solution of β-CD-clicked keratin (11.2 mg/mL) was incubated with 1,8-ANS (0.635 mg/mL), and the release study was performed by dialyzing against water for 8 h. Data are shown as the mean ± 1 SD, derived from three independent repeats.

the inclusion complexation with the grafted β-CD moieties. Therefore, by grafting keratin with β-CD moieties the ability to retain 1,8-ANS via the formation of 1,8-ANS/β-CD inclusion complexes was endowed upon the β-CD-grafted keratin.

Difference in the ability to hold 1,8-ANS between the keratin and β-CD-grafted keratin reported above suggests that at the constant body temperature of 37 °C, the β-CD-grafted keratin should sustain the release of the model drug molecule more effectively, comparing to keratin. To demonstrate this, drug release profile from the β-CD-grafted keratin was evaluated at 37 °C for 8 h using 1,8-ANS as a guest molecule (Fig. 3). At 8 h, amounts of 1,8-ANS released from β-CD-grafted keratin and keratin were 11 and 17%, respectively. This result indicated that an incorporation of β-CD moieties into the biopolymer led to an increase in sustained release for ~35%. This result should encourage application of the M-β-CD reagent on other biomaterials to improve their sustained drug release property.

It should be noted here that the free CD, although can trap some drug molecules, its size is too small to survive renal filtration in the body. One obvious application for this M-β-CD is, therefore, in the area of drug delivery. Here drug molecules will be trapped in the CD cavities of the CD-grafted polymer. The size of the polymer can be selected to survive various filtration systems in the body, and various desired features can also be added to the polymer, e.g. putting on a targeting moieties for specific cells.

3.4. Applications of the M-β-CD on human hairs

To further demonstrate that the M-β-CD could easily install a drug retention property to selected biomaterials, human hair strands were treated with the M-β-CD to form the β-CD-grafted hair. Note that attempts to use ATR–FTIR to acquire the information of the linked CD moieties on the hair surface failed. This is because the technique detects information at the depth of approximately 8–10 nm from the surface. The grafting reaction was on the surface which was less than 8–10 nm depth. We, therefore, evaluated the success of grafting through an ability of the β-CD-grafted hair to retain curcumin. Since curcumin possesses the autofluorescence property and ability to form an inclusion complex with β-CD (Patro et al., 2013), the compound was chosen as a model guest molecule

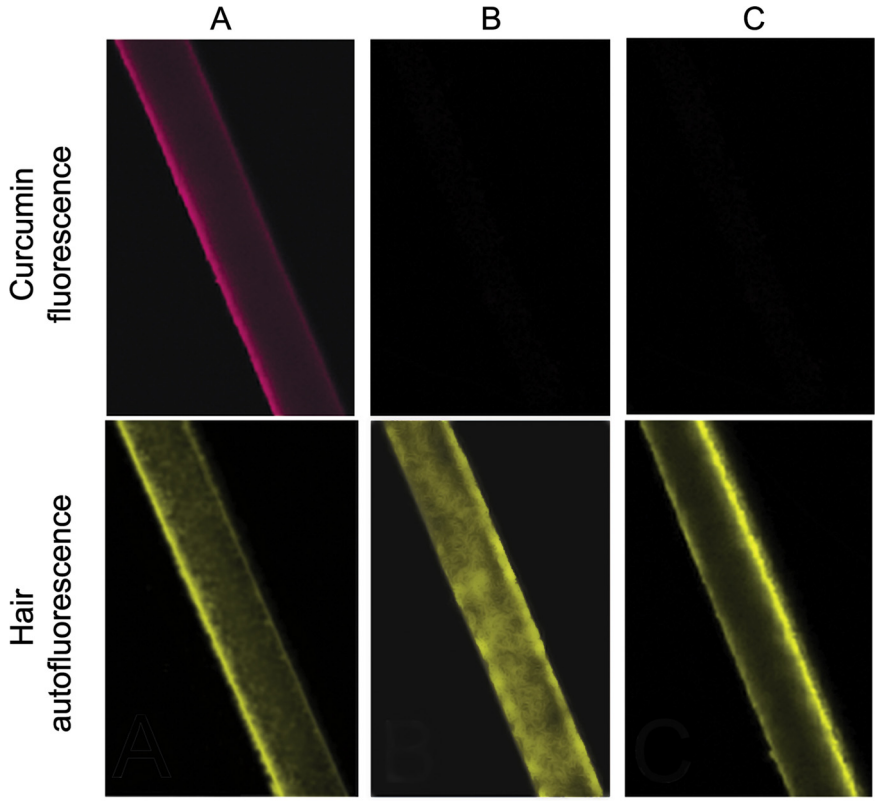


Fig. 4. Confocal fluorescence images of curcumin incubated with different hair samples: (A) hair treated with M-β-CD, (B) hair treated with β-CD and (C) original untreated hair. Curcumin fluorescence is shown in pink while the hair autofluorescence is shown in yellow. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

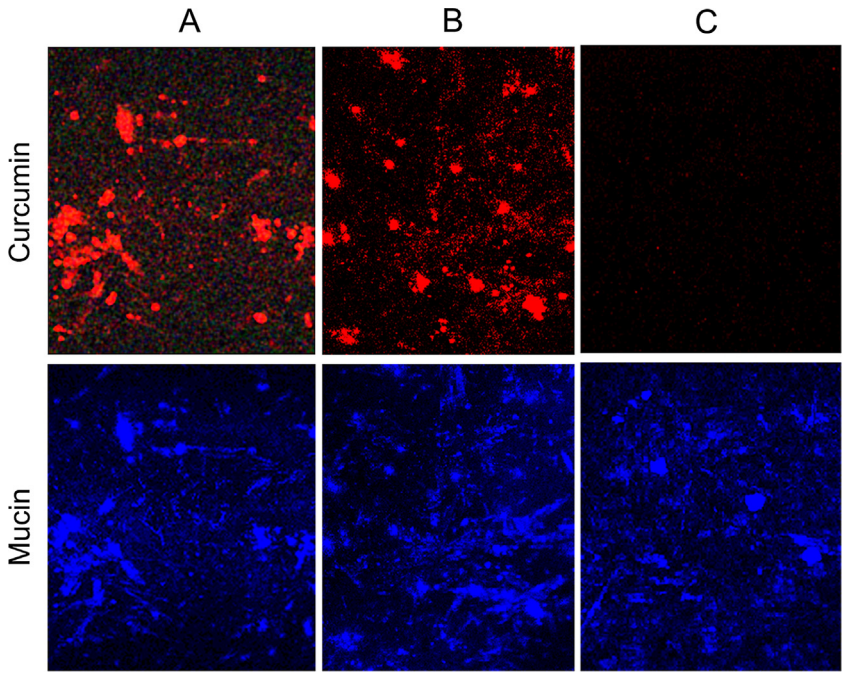


Fig. 5. Confocal fluorescent images showing curcumin fluorescence signal (red, top) and mucin autofluorescence signal (blue, bottom) of the different mucosa plates after being incubated with curcumin solution and thoroughly washed. (A) CD-grafted mucosa plate prepared using TCEP/TEA and M-β-CD. (B) CD-grafted mucosa plate prepared using vitamin C and M-β-CD. (C) Unfunctionalized mucosa plate prepared using TCEP/TEA and β-CD. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

to demonstrate the drug retaining property of the β-CD-grafted hair.

The ability to retain curcumin by (a) hair treated with M-β-CD, (b) control hair that was treated with β-CD in place of M-β-CD and (c) untreated hair, was compared. All three hair samples were incubated with curcumin, washed and subjected to CLSM analysis. The original hair (untreated hair) and the control hair (hair treated with β-CD instead of M-β-CD) showed no curcumin fluorescence signal after washing. However, the hair treated with M-β-CD showed an obvious curcumin fluorescence signal along the hair strand (Fig. 4). This indicated successful grafting with the M-β-CD. The obtained β-CD-grafted hair could retain curcumin effectively, unlike the other two control hair types. This curcumin retention probably occurred through an inclusion of the curcumin molecules into the cavities of the β-CD moieties on the hair strand.

3.5. Application of the M-β-CD onto mucin layers

Finally, the ability of the M-β-CD to impart a sustained drug release character to a biomaterial was evaluated on the biopolymer mucin. Mucin is a key mucoprotein component in most gel-like secretions produced by epithelial tissues of many organs, such as in the gastrointestinal and nasal-bronchial tracts. An increased ability of the mucin to retain drug molecules should result in higher drug localization at the corressponding area (Svensson & Arnebrant, 2010), and accordingly lead to a higher target site drug absorption. The mucin used here was first linked onto a glass slide to form a mucosa plate (Tachaprutinun et al., 2013). The mucosa plate was then treated with M-β-CD under both the TCEP/TEA containing and vitamin C containing conditions. The β-CD-grafted mucosa plates obtained were then evaluated for their ability to retain curcumin. After incubation with curcumin and thorough washing, both the β-CD-grafted mucosa plates prepared with the TCEP and with the vitamin C, showed a clear curcumin fluorescence emission. The original mucosa plate (no CD grafting) showed no curcumin fluorescence signal (Fig. 5). This indicated the successful β-CD grafting under both conditions (TCEP and vitamin C). The result confirms that vitamin C (ascorbic acid) could be used to successfully reduce the cystine disulfide bridges in the mucin layer into thiol moieties. In fact, the curcumin retention on the plate could be observed by eye because of the yellow color of curcumin (Fig. S6 in SD). Because vitamin C is nontoxic, this platform should offer an easy method for the in situ functionalization of biomaterials with CD. Any mucin coated bio-surfaces could be covalently installed with β-CD and a higher concentration of the selected (i.e. capable of complexing with β-CD) drug molecules could be localized there via the drug/β-CD inclusion complexation. It should be noted here that the two control plates (plates treated with TCEP/TEA/unmodified CD and plates treated with vitamin C/unmodified β-CD) also showed no curcumin fluorescence signal. This implied that the CD grafting was successful only when M-β-CD was used, unmodified β-CD could not be directly grafted on the mucin under this condition.

4. Conclusion

A simple platform to install a drug retention property to (bio)polymers or materials was developed based upon covalently grafting β-CD onto the thiol containing materials (such as biopolymers), to allow the β-CD to form an inclusion complex with the guest molecules (drugs), and so imparted an enhanced drug retention property to the material. Here we demonstrated a simple one step reaction between the β-CD and methacrylic anhydride to functionalize the β-CD with methacrylate moieties, yielding M-β-CD. The inclusion complexation property of the β-CD was still preserved in the M-β-CD, at least for the evaluated guest molecules of 1,8-ANS and curcumin. Using the M-β-CD, keratin could easily be grafted with β-CD moieties under a biologically mild condition, resulting in the formation of β-CD-grafted keratin with the ability to retain 1,8-ANS (as a model guest molecule). Another creative way of using this M-β-CD is for hair treatment. The grafting of hair strands with β-CD moieties was easily achieved via the thiol-ene reaction using the M-β-CD. The resulting β-CD-grafted

hair strands could effectively retain the curcumin guest molecules. The last application of the M-β-CD evaluated in this study was the installation of β-CD moieties onto mucin. The β-CD-grafted mucosa plates showed a strong retention of curcumin on their surface even after thorough rinsing. Since the grafting reaction could be done successfully in water, using the non-toxic vitamin C as the reducing agent in the thiol generating step, this β-CD grafting platform may find application in real biological sites, such as the stomach surface.

Supporting information

Average number of methacrylate moiety on CD ring obtained under various ratios of reactants, ATR–FT-IR spectra of β-CD and M-β-CD, ^{1}H NMR spectrum of M-β-CD, ESI$^+$–MS spectra of M-β-CDs prepared at various β-CD to methacrylic anhydride ratios, ^{1}H NMR spectra of keratin and β-CD-grafted keratin, estimation of CD substitution degree on keratin, estimation of reaction efficiency of the M-β-CD on keratin, pictures showing the retention of curcumin on the β-CD-grafted mucosa plate.

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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.2014.11.051.

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