ChemComm

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Journal:	*ChemComm*
Manuscript ID:	CC-COM-11-2013-048878.R1
Article Type:	Communication
Date Submitted by the Author:	09-Jan-2014
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Cite this: DOI: 10.1039/c0xx00000x

www.rsc.org/xxxxxx

ARTICLE TYPE

Zwitteration of dextran: a facile route to integrate antifouling, switchability and optical transparency into natural polymers

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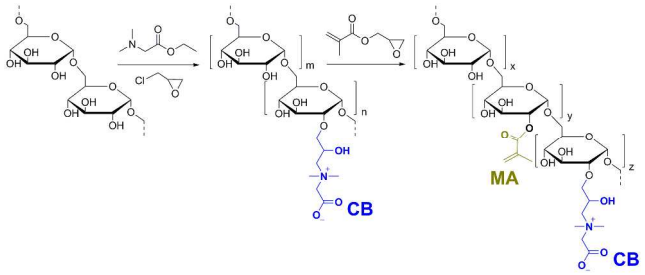
Received (in XXX, XXX) Xth XXXXXXXXX 20XX, Accepted Xth XXXXXXXXX 20XX

DOI: 10.1039/b000000x

This paper reports a facile method of zwitteration of dextran with carboxybetaine (CB), which integrating superior antifouling property, switchability between zwitterionic and cationic forms, as well as enhanced optical transparency into one polysaccharide material. This study provides a new avenue of generating multifunctional zwitterionic CB in situ.

Polysaccharides are most abundant and most commonly used natural polymers, which have been used in many biotech and biomedical applications, including coatings,[1] biosensing,[2] tissue engineering,[3] drug delivery,[4] and bioseparation/purification.[5] Polysaccharide-based materials have attracted a great attention due to their ability of resisting proteins,[6] mammalian cells and microbes,[7] biocompatibility,[8] biodegradability,[9] capability of further functionalization for biosensing and drug delivery,[10] as well as design flexibility for a broad range of applications. Despite intense interests in polysaccharide materials, there are several challenges to be addressed to fully realize the potential of polysaccharide materials in biotech and biomedical applications. Firstly, antifouling properties of natural polysaccharides are unsatisfactory in applications dealing with the complex medium.[11] For example, antifouling surface from dextran-derivatives in biosensing is not effective in resisting protein fouling from blood sample.[12] Agarose-based affinity protein purification system is troubled by non-specific protein adsorption.[13] Secondly, natural polysaccharides do not carry both antifouling property and functionality to conjugate other moieties (such as capture ligand and cell adhesion molecule), which are needed in affinity bioseparation, biosensing, tissue engineering and drug delivery. In most cases, functional groups such as tetrazole[14] and carboxylate[15] groups have to be incorporated into polysaccharides. Excessive unreacted functional groups cause non-specific protein adsorption, thus either reducing the sensitivity of the biosensor or leading to low purity in bioseparation. Thirdly, natural polysaccharides can resist bacterial attachment but cannot kill a small amount of attached microbes.[16] Microorganisms can be introduced into patients during surgical procedures, and colonized microorganisms on the surface of the implanted material/device will trigger inflammation and immune response.[17] Therefore, it is highly desired to have a material integrating all desired properties including excellent antifouling property to prolong the lifetime of implanted materials, antimicrobial property to eliminate surgical infection and chronic inflammation, and functionality for conjugating bioactive moieties to promote tissue integration.

The objective of this work is to develop a versatile and high performance zwitterionic polysaccharide platform and understand the structure-property-function relationships of zwitterionic polysaccharides, so that this platform can be readily adapted to address these key challenges for biomedical and biotech applications. Herein we report a facile method of zwitteration of dextran with carboxybetaine (CB). The integrated zwitterionic polysaccharide consists of a degradable polysaccharide backbone and multifunctional zwitterionic side chains. Polysaccharides can obtain excellent antifouling property, sensitivity to environmental stimuli, functional groups for bioconjugation and antimicrobial property via zwitterionic side chains, while zwitterionic materials can obtain biodegradability from the polysaccharide backbone. To test our hypotheses, the impact of the CB substitution on the switchability, hydrophilicity, antifouling and optical transparency of dextran was studied in this work.



Scheme 1 Synthetic route of CB-Dex-MA.

As shown in Scheme 1, CB-Dex was synthesized via one pot reaction. The molecular weight and the degree of substitution were characterized by GPC (Fig. S1) and ^{1}H NMR spectroscopy (Fig. S2), respectively. Zwitterionic CB side chains were introduced onto dextran backbone using the rational design approach. Three methacrylate (MA) modified dextran derivatives, with different degree of CB substituent, from 0 % (Dex-MA), 35 % (CB-L-Dex-MA), to 158 % (CB-H-Dex-MA) were employed in this study. All samples were kept at a similar MA ratio around 25 % (one MA unit per four glucose units).

It is expected that hydroxyl and carboxylate groups generated from the reaction can undergo cyclization to form cationic lactone ring structure under acidic condition, while ring opens under neutral or basic conditions. Thus the material can switch between two different states (zwitterionic and cationic ring form) and achieve two different functions (antifouling and antimicrobial). A similar ring structure was observed in a previously study by Dr. Jiang and co-workers.[18] To confirm our hypothesis, heteronuclear multiple-bond correlation (gHMBC) 2D-NMR spectrum, which provides two- and three-bond correlations between ^{1}H and ^{13}C, was used to verify the ring structure formation of CB-Dex (Fig. S3) in TFA-d. CB side chains of dextran formed six-membered lactone ring structure and showed well resolved correlations in

the 2D NMR spectrum. The cross-peak in dotted circle shows the two bond correlation between the resonances of methylene proton adjacent to carboxylate and the resonances of carbonyl carbon. It changes from a single peak into a doublet of doublet as the evidence of ring structure formation. ^{1}H NMR data was collected at different time points to study the dynamic ring formation process (Fig. 1B). The conversion ratio was calculated based on the integral ratio from the methyl (-CH_3) protons in each form. As shown in Fig. 1C, about 90% of CB-Dex side chains were converted into the six-membered ring form in TFA within 2 hours. The ring open kinetics (Fig. 1D) was studied by dissolving polymer in their cationic forms in pure D_2O. Calculations were performed with the same method as ring formation, and the final conversion was 86% for CB-Dex in 10 hours. Unmodified dextran was used as a control material. No ring formation was observed with unmodified dextran under acidic condition.

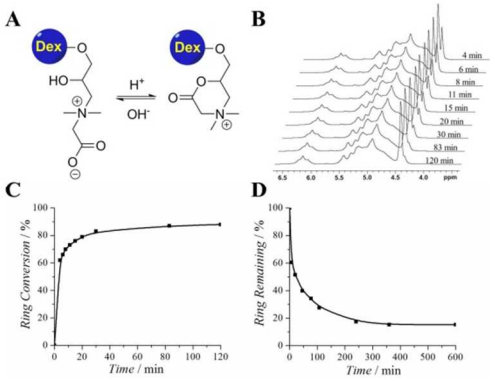


Fig. 1 A) CB-H-Dex structural switch between zwitterionic form and cationic form. B) and C) the lactone ring formation of CB-H-Dex from zwitterionic form to its cationic ring form in TFA-d. D) the lactone ring open from cationic ring form to its zwitterionic form in D_2O.

Zwitterionic coatings can reduce bacterial attachment and delay biofilm formation on surfaces, but they cannot kill attached microorganisms. It is known that biofilm will cause the drop of local pH in vitro and in vivo. It will be ideal if a surface can switch from an antifouling surface to an antimicrobial surface in response to environmental pH drop. In our previous studies,[19] two switchable antimicrobial/antifouling materials were developed and they can switch to an antimicrobial material from a zwitterionic material under acidic conditions. It can kill 99.5% of attached bacteria in its cationic antimicrobial form and then release 95% of killed cells at their zwitterionic antifouling state. It is expected that switchable CB-Dex would have same antimicrobial/antifouling functions.

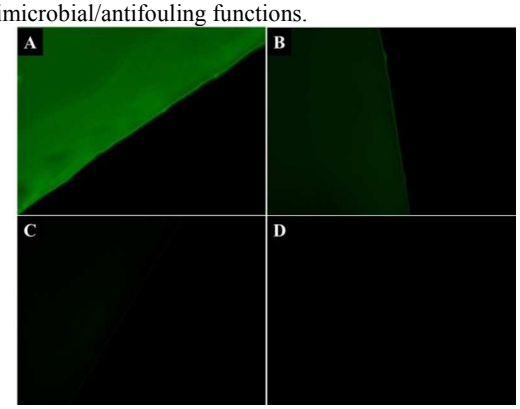


Fig. 2 Protein (FITC-Fg) fouling test on hydrogels visualized under fluorescence microscope at the same excitation light intensity and exposure time. A) Dex-MA, B) CB-L-Dex, C) CB-H-Dex, and D) Control hydrogel surface with no protein contact.

Protein fouling on the surfaces of devices in the complex medium can cause the failure, affect the service life or decrease the sensitivity of the devices. One of main reasons for the underperformance of polysaccharides is their unsatisfactory capability to resist protein adsorption from the complex medium. We hypothesized that zwitterionic CB side chains can dramatically reduce non-specific protein adsorption on polysaccharide materials. Protein adsorption studies were carried out on the hydrogel surfaces and visualized with fluorescence microscopy. Three types of samples were compared. Hydrogels of Dex-MA without CB side chains were used as controls in this study. After equilibrium in PBS, hydrogel samples were briefly rinsed with DI-water, and submerged in 0.1 mg/mL fluorescein isothiocyanate-labelled fibrinogen (FITC-Fg) solution for 30 minutes. Hydrogels with no contact of FITC-Fg was used as the control. The exposure time of the microscope was adjusted with the samples with no contract of FITC-Fg, until a completely dark background was obtained. All images of different samples were obtained from the fluorescence microscope at the same excitation light intensity and exposure time thereafter. As shown in Fig. 2, among all samples tested, Dex-MA hydrogels show the highest fluorescence intensity, which indicated the highest protein adsorption. The one with highest CB ratio (CB-H-Dex) shows the lowest amount of adsorbed protein, while dextran hydrogel with low CB substitution (CB-L-Dex) show the medium fluorescence intensity. Image-J software was utilized to quantify the fluorescence intensity values of each image. Compared to Dex-MA hydrogel, CB-L-Dex and CB-H-Dex hydrogels showed 26.6 % and 4.6 % of fluorescent signal intensities (Table S1) respectively. Hydrogel samples with different degrees of CB substitution show similar equilibrium water content (Table S1), which indicate that no direct correlation between hydrogel water content and the degree of CB substitution. So that the difference in protein adsorption on hydrogel surfaces was not because of water content but rather CB functional groups.

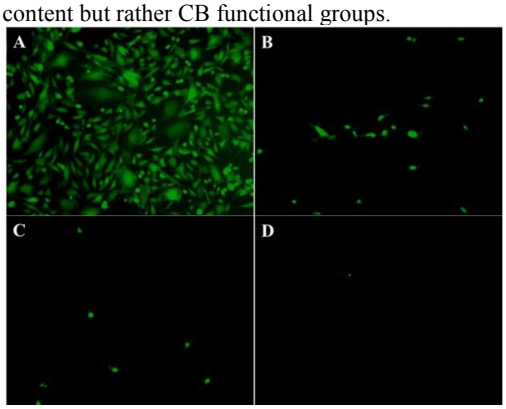


Fig. 3 BAECs attachment test on A) TCPS, B) Dex-MA hydrogel, C) CB-L-Dex hydrogel, D) CB-H-Dex hydrogel surfaces.

For implantable materials, protein adsorption on surfaces from blood and body fluid can trigger the cell attachment, which can further trigger foreign body reaction and lead to chronic inflammation or isolation of implanted materials.[20] The foreign body reaction can be minimized if the surface implanted materials can effectively resist protein adsorption and cell attachment. To further test antifouling properties of CB-Dex hydrogels, cell adhesion studies were performed with bovine aorta endothelial cells (BAECs). After incubated at 37 ^{o}C for 24 hours, the control tissue culture polystyrene (TCPS) surface turned out full coverage of BAECs. However, there was almost no cell adhesion on CB-H-Dex surface (Fig. 3). These results demonstrated that we have successfully created a zwitterionic CB-Dex hydrogel that highly resist cell adhesion. It is expected that the CB-H-Dex coating can prolong the service life of

implanted materials by minimizing protein adsorption and cell attachment.

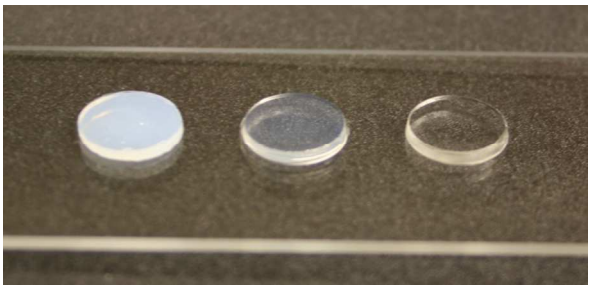


Fig. 4 Digital image of dextran hydrogels. Left to right: Dex-MA, CB-L-Dex, CB-H-Dex.

The optical clarity of dextran hydrogels with different degrees of CB substitution is dramatically different (Fig. 4). Dex-MA hydrogel shows white colour and is mostly opaque. When the degree of CB substitution reaches 35 %, it becomes translucent. The CB-H-Dex hydrogel with high CB content is completely transparent. It is important to have an optically transparent clear material to meet the needs of optical sensor or devices, such as contact lens, optical sensors, coatings, etc, which work in the complex fouling environments. The water content indicates that the difference in the optical transparency of hydrogels is not caused by the water contents, since the water content of all three samples are similar. It is possible that ionic interaction between the zwitterionic domains with water trapped inside of hydrogel network increase the solubility of dextran so that the matrix became more transparent with the increase of CB substitution.

To confirm our hypothesis that the zwitteration method can not only integrate multifunction into dextran, it can also benefit from dextran with better degradability. Enzyme degradation study of dextran and CB-L-Dex was carried out under the same condition. The GPC results (Fig. S4) are consistent with earlier investigations[21] that chemical modifications will decrease the rate of enzymatic hydrolysis. It shows that dextran without CB modifications degraded relatively faster compared to CB-Dextran under the same conditions. However, the major reason here must not be insolubility, since CB units can definitely increase the solubility of polymer. Instead, the charge distribution of zwitterionic structure may stabilize the ring from deformation to a certain extent.

In summary, it is demonstrated that zwitteration of dextran can be simply achieved via one pot reaction. Zwitterionic CB-Dex show superior antifouling properties, enhanced optical transparency, as well as switchability between cationic and zwitterionic states. The properties of zwitterionic polysaccharides can be tuned through controlling the ratio of substitution. Unique properties from two distinct materials (polysaccharides and zwitterionic materials) were integrated into one material without sacrificing any properties. To the best of our knowledge, such facile zwitteration method has not been reported. All the advantages, including: simple one pot synthetic pathway in aqueous solution, switchability of two distinct functions, low cost and natural abundance of raw materials, relatively easy purification steps, together with quantitative high yield make this a very promising zwitteration pathway of nature products. Through this study, we also developed an understanding of basic properties of zwitterionic polysaccharides, and this platform can be adapted to a range of applications (e.g. biosensing, drug delivery, tissue engineering, implantable medical devices, and bioseparation). This work has shed light on ingenious designing of zwitterionic material, and provided a new avenue of generating multifunctional zwitterionic CB side chains from the polysaccharide backbone.

Notes and references

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† Electronic Supplementary Information (ESI) available: [1D, 2D NMR and GPC results]. See DOI: 10.1039/b000000x/

This work is supported by National Science Foundation (NSF CMMI-1129727).

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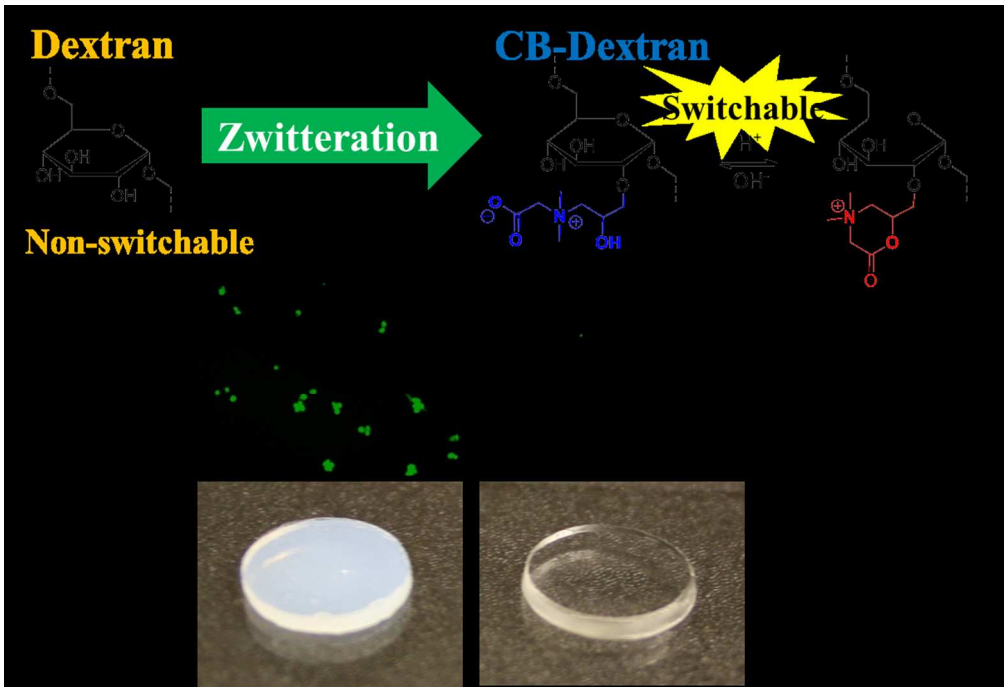

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