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Modulating intestinal mucus barrier for nanoparticles penetration by surfactants

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ABSTRACT

Improving peroral delivery efficiency is always a persistent goal for both small-molecule and macromolecular drug development. However, intestinal mucus barrier which greatly impedes drug-loaded nanoparticles penetration is commonly overlooked. Therefore, in this study, taking fluorescent labeled PLGA (poly (lactic-co-glycolic acid)) nanoparticles as a tool, the influence of anionic and nonionic surfactants on mucus penetration ability of nanoparticles and their mucus barrier regulating ability were studied. The movement of PLGA nanoparticles in mucus was tracked by multiple particles tracking method (MPT). Alteration of mucus properties by addition of surfactants was evaluated by rheology and morphology study. Rat intestinal villus penetration study was used to further evaluate penetration enhancement of nanoparticles. The effective diffusivities of the nanoparticles in surfactants pretreated mucus were increased by 2–3 times and the mucus barrier regulating capacity was also surfactant type dependent. Sodium dodecyl sulfate (SDS) increased the complex viscosity and viscoelastic properties of mucus, but poloxamer presented a decreased trend. Tween 80 maintained the rheological property of the mucus. With the mucus barrier regulated by surfactants, the penetration of nanoparticles in intestinal villus was obviously increased. In summary, the mucus penetration ability of nanoparticles could be enhanced by altering mucus microenvironment with surfactants. Tween 80 which largely retains the original mucus rheology and morphology properties may be a promising candidate for facilitating nanoparticle penetration through the mucus barrier with good safety profile.

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1. Introduction

The safety and effective delivery of drugs to body circulation by oral administration is fraught with many challenges, which is not only hampered by epithelial barrier but also mucus barrier [1]. With the presence of mucus layer, most foreign particles in the intestinal tract will be trapped by mucus to prevent them from contacting with epithelia cells. Meanwhile, the mucus barrier could significantly reduce the oral delivery efficiency of many well design nanocarriers by weakening their move ability in mucus [2].

Permeation barrier properties of the tenacious mucus comes from its microenvironment and structure. Mucus hydrogel is mainly composed of water, 2%–5% (w/v) mucin, small amount of lipids [3]. The networks formed by entangled mucins are the main structure of mucus layer and provide various properties to mucus. Mucins are glycoproteins which are composed of peptide chain modified by hundreds of O-linked and N-linked oligosaccharides. Negative charge of glycosylation groups in mucins, hydrophobic surface of non-glycosylated protein chain and network formed by mucin fibers constitute the structure foundation for penetration barrier [2].

Compared to the mucus penetration nanoparticles strategy, application of mucus modulating agents to overcome the mucus barrier by co-administration or co-formulation with drug carrier is easier to satisfy the industrial manufacture requirements with better applicability [4]. In order to modulate the mucus barrier, mucolytic agents and mucus production inhibitors are commonly applied, but their application usually raises the safety concern [5]. This is because mucus also plays a significant role in avoiding the damage of gastrointestinal environment including digestive enzymes, colonized bacterial, acid-base environment and other harmful antigens and microbes on epithelium under the mucus [1]. Therefore, developing and screening potential mucus penetration modulators which are general regarded as safe (GRAS) ingredients without influencing properties of mucus are economical and of special importance. Moreover, studying the interaction between pharmaceutical excipients and mucus will facilitate the design of drug carriers with mucus penetration ability and increase the accuracy of *in vivo* model prediction for nanoparticles based drug delivery system.

It has been reported that surfactants show promising potential to regulate mucus barriers, which has been widely used in pharmaceutical industry as surface tension regulator, solubility and penetration enhancing agents and stabilizers of protein and nanoparticles [6,7]. It is reported that the human cervicovaginal mucus pretreated by Pluronic F127 significantly increased the penetration of polystyrene nanoparticles without changing the pore size of mucus [8]. However, the treatment by nonionic surfactant nonoxynol-9 reduced the pore size of mucus and increased the barrier properties for 200/500 nm particles [9]. This indicated that the mucus modulating property of surfactants is not only affected by mucus source but also greatly dependent on molecular structure of surfactants. For oral administration, although surfactants have shown drug permeability enhancing ability by altering the structure and microenvironment of intestinal epithelium

[10], the interactions between surfactants and intestinal mucus, and the relationship between surfactant structure and its function are still unclear. It is also unknown how will the surfactants structure influence their mucus penetration enhancing capacity if this is the case. In addition, the mucus modulation function study of surfactants will provide valuable information for mucus penetration enhancing drug carrier design.

Therefore, in this paper, using PLGA nanoparticles as a model, influence of different type of surfactants on mucus permeation efficiency of nanoparticles and the interaction between surfactants and mucus were explored. Here, surfactants with different surface charge and HLB (hydrophilic-lipophilic balance) values, including sodium dodecyl sulfate (SDS, anionic, HLB 40, CMC: 8.2 mmol/l), poloxamer 188 (nonionic, HLB 29, CMC: 0.48 mmol/l), poloxamer 407 (nonionic, HLB 22, CMC: 2.8 μ mol/l) and Tween 80 (nonionic, HLB 15, CMC: 0.015 mmol/l) were selected in this study. Permeation process of the nanoparticles was tracked and calculated to further understand the interaction between surfactants and mucin. The interaction mechanism between surfactants and mucus, and the network structure change of mucus in the presence of surfactants were investigated by rheology study and morphology observation. After penetrating through the mucus barrier layer, penetration of nanoparticle in intestinal villus was also observed by confocal microscopy.

2. Material and methods

2.1. Materials

Porcine original mucus were obtained from intestine of freshly slaughtered pigs and mucus was gently scraped from the intestinal wall (Shenyang, Liaoning). In order to minimize the influence of mucus variability on the result, mucus sample was collected and stored at -20°C before use to make sure the mucus sample used for each compound was from the same source [11,12]. Poloxamer 407 and 188 (P407, P188) were obtained as gifts from BASF (Germany). SDS was bought from Biotopped Co., Ltd.(China). PLGA (Resomer[®] RG 503) was purchased from Evonik Industries (Germany). Poly(vinyl alcohol) (PVA 205) was obtained from Kuraray China Co., Ltd.(China). Coumarin 6 were purchased from J&K chemical Ltd.(China). Tween 80 was obtained from Tianjin Bodi Chemical Co., Ltd. (Tianjin, China). DAPI (4',6-diamidino-2-phenylindole) staining solution and antifade mounting medium were bought from Beyotime Institute of Biotechnology (China).

2.2. Preparation of coumarin 6 labeled PLGA nanoparticles

PLGA nanoparticles containing coumarin 6 were prepared by O/W emulsion solvent evaporation method [13,14]. In brief, 20 mg PLGA were dissolved in 1 ml dichloromethane containing 150 μ g coumarin 6 as oil phase in dark environment. Outer water phase, 8 ml 2% PVA solution were added to the oil phase and then the mixture was sonicated by ultrasonic homogenizer for 60 s, 100 W to prepare emulsions (SCIENTZ IID, Scientz Biotechnology, Ningbo, China). The O/W emulsions were

then added to 20 ml 1% PVA solution and hardened by evaporating dichloromethane under stirring for 3 h (84–1A, Shanghai Sile Instrument Co., Ltd., China). The nanoparticles were washed for 2 times by centrifugation and resuspending in the same volume of deionized water (HC-2062, USTC Zonkia Scientific Instruments Co., Ltd., Anhui, China) [15]. The PLGA nanoparticles suspension were used for following study.

2.3. Movement of PLGA nanoparticles in intestinal mucus

Multiple particles tracking method (MPT) was applied to track the brownian movement of nanoparticles in mucus sample and further evaluate the mucus barrier properties for nanoparticle penetration in the presence of different surfactants [16]. Briefly, probe coumarin 6 labeled PLGA nanoparticles were gently added into mucus samples in microwells and incubate for 30 min at 37 °C. Inverted fluorescence microscope IX 71 with CCD imaging system DP 70 and 40X objective (OLYMPUS, Japan) was used to record the trajectory of coumarin 6 labeled PLGA nanoparticles at a frame rate of 15 fps for 10 s and three experiments were performed for each sample. For sample preparation, mucus samples were evenly mixed with 1% (w/w) surfactants solution including P407, P188, SDS and Tween80 at a weight ratio of 4:1. As the reference sample, native mucus was mixed with distilled water in parallel to eliminate the influence of dilution process. 400 µl mucus was evenly added into 24-well cell culture plates and 10 µl nanoparticles were gently added to the mucus. Trajectory of nanoparticles were tracked by particle tracker plugin in ImageJ software [17,18]. The position value of trajectory was analyzed and time-averaged mean square displacement (MSD) and effective diffusivities (D_{eff}) were calculated according to the following equations: $\text{MSD} = [x(t + \tau) - x(t)]^2 + [y(t + \tau) - y(t)]^2$, $D_{\text{eff}} = \text{MSD}/(4\tau)$, where x, y is coordinates of nanoparticles in mucus and τ represents time scale [16].

2.4. Particle size analysis

The size and zeta potential of the nanoparticles were characterized by dynamic light scattering technology by Nano ZS90 (Malvern Instruments, Worcestershire, UK) at 25 °C at a scattering angle of 90°.

2.5. Morphology observation of mucus

Morphology study of original mucus and mucus treated by surfactants was performed by macroscopic observation, optical microscope and atomic force microscope (AFM). In order to better distinguish the difference of mucus samples for macroscopic observation, native mucus and mucus treated by surfactants were centrifuged at 2000 r/min for 10 min. Optical microscope observation was performed by BI-2000 Image Analysis System (Chengdu Techman Software Co., Ltd) at X40 objective. Atomic force microscope (Agilent Technologies, USA) was used to study the morphology of mucus at micrometer scale. The samples were added to a clean mica plate and dried at room temperature. Then the samples were tested by tapping mode.

2.6. Rheological measurements

Rheology properties of mucus were determined by plate-plate model at 37 °C using controlled stress rheometer AR2000 (TA Instruments, USA) and frequency sweep of oscillation tests were performed at 2% strain that was within the linear viscoelastic regime (LVR). Volume of the sample was fixed at 0.314 ml. Mucus samples preparation process was the same with “2.3 Movement of PLGA nanoparticles in intestinal mucus”.

Complex viscosity and viscoelastic parameters including complex modulus, elastic and viscous modulus, damping factor were used to evaluate the alteration of rheology of mucus by addition of surfactants. Complex modulus (G^*) was computed based on $G^* = G' + iG''$. Damping factor ($\tan\delta$) which was calculated by $\tan\delta = G''/G'$ [19].

2.7. Intestinal villus penetration study

Rat intestinal villus penetration study was used to further evaluate the influence of surfactants on the penetration of nanoparticles. All animal experiments followed the Principles of Laboratory Animal Care and approved by Shenyang Pharmaceutical University Ethics Committee. Male Wister rats weighing 180–230 g were fasted overnight and was anesthetized by intraperitoneal injection of 5% chloral hydrate. Abdominal cavity was opened and two ends of ileum (about 5 cm) were ligated. 0.3 ml 1% surfactant were injected to the ligated ileum segment as pretreatment process for 30 min and then 0.3 ml PLGA nanoparticles were administrated to the loop. After administration of PLGA nanoparticles for 30 min, the rats were euthanized with an overdose of chloral hydrate and the loops were removed. The ileum segment was gently washed by 5 ml PBS and then treated by 4% paraformaldehyde for 2 h and 30% sucrose overnight at 4 °C before frozen sections. The loops were coated by O.C.T. Compound (Tissue-Tek®, SAKURA, USA) and were rapidly frozen for section. The ileum section was stained with DAPI for nucleus dyeing and observed by multi-photon confocal microscope (CLSM, LSM 710, Zeiss, Germany) at 405 nm and 458 nm lasers source. The fluorescence intensity in intestinal villus was compared and quantified by Image J software.

2.8. Statistical analysis

The results were analyzed using ANOVA two-way and data are presented as mean value \pm SD ($n \geq 3$). Probability values $P < 0.05$ were considered to be statistically significant.

3. Results and discussion

3.1. Preparation and characterization of C6 labeled PLGA nanoparticles

As a biocompatible polymer, poly (lactic-co-glycolic acid) (PLGA) was widely applied to prepare micro and nanoparticles for different administration routes due to its mature manufacturing process, good encapsulation for both hydrophobic and hydrophilic drugs and controlled release behavior [20].

Therefore, PLGA nanoparticles were prepared as a nanoparticles model and hydrophobic fluorescence dye coumarin 6 (C6) was loaded for the ease of observation. The particle size of PLGA nanoparticles was about 241 ± 5.2 nm, PDI: 0.294 ± 0.04 and zeta potential value was -14.7 ± 0.3 mV. The release of coumarin-6 from PLGA nanoparticles in PBS buffer (non-sink condition) was under detection limit in 2 h. The similar results were also reported in published literatures [21–23]. Some literatures showed that PVA coating on nanoparticles could affect its mucus penetration ability and the nanoparticles could be more easily immobilized by mucus than that by PEG coating [24]. However, previous study indicated the residual amount of PVA was dependent on solvent type in oil phase and concentration of PVA. When dichloromethane was used as solvent and 5% (w/w) PVA solution were applied to prepare PLGA nanoparticles, 6.15% of the total PVA (w/w) added was adsorbed on the surface of PLGA nanoparticles after 2 times washing [25]. In this study, based on the assumption that 6.15% of the added PVA was remained on PLGA nanoparticle surface, the residual PVA on nanoparticle surface was about 4.1×10^{-3} % (w/w), which was greatly lower than the concentration of PVA used for coating purpose (0.01%–1%), therefore, it has limited influence on the interaction between nanoparticles and mucus. In addition, although some residual PVA might exist on the surface of nanoparticles, the nanoparticles were used as nanoprobe in parallel in all the groups to show the mucus barrier properties change after treatment by different surfactants. Thus, the effect of residual PVA has been well justified. Therefore, coumarin 6 labeled PLGA nanoparticles was used as a nanoprobe to track its trajectory in intestine mucus and understand the influence of surfactants on nanoparticles penetration ability.

3.2. Movement tracking of nanoparticles in intestinal mucus

The diffusional barrier mainly comes from micro network structure of mucus and interactions between nanoparticles and mucus components [26]. The variation of microenvironment of mucus treated by surfactants may enhance penetration of nanoparticles. Therefore, the movement trajectories of PLGA nanoparticles in the mucus pretreated by different surfactants were tracked. The applied concentration of surfactants in pharmaceutical area as emulsifier or stabilizer was 0.3%–5% for poloxamer, 0.5%–2.5% for SDS and 1%–15% for Tween 80 [27]. In preliminary experiments, the interaction strength between mucus and surfactants increased with the increase of surfactant concentration, and equilibrium was achieved when the concentration of surfactants was above 1%. Higher concentration of surfactants will also increase the risk of safety concern. Therefore, 1% concentration of surfactant was selected for the following study. Mean squared displacements (MSD) (Fig. 1) of PLGA nanoparticles showed that all of the surfactants investigated could improve the mobility of nanoparticles in mucus. The ensemble-average effective diffusivity of PLGA nanoparticles in mucus containing different kinds of surfactants at 5 s time scale was increased by SDS for 2.46 times ($0.1308 \pm 0.03 \mu\text{m}^2/\text{s}$), P407 for 2.14 times ($0.1139 \pm 0.02 \mu\text{m}^2/\text{s}$), P188 for 1.81 times ($0.0965 \pm 0.023 \mu\text{m}^2/\text{s}$) and Tween 80 for 1.82 times ($0.0967 \pm 0.011 \mu\text{m}^2/\text{s}$) compared

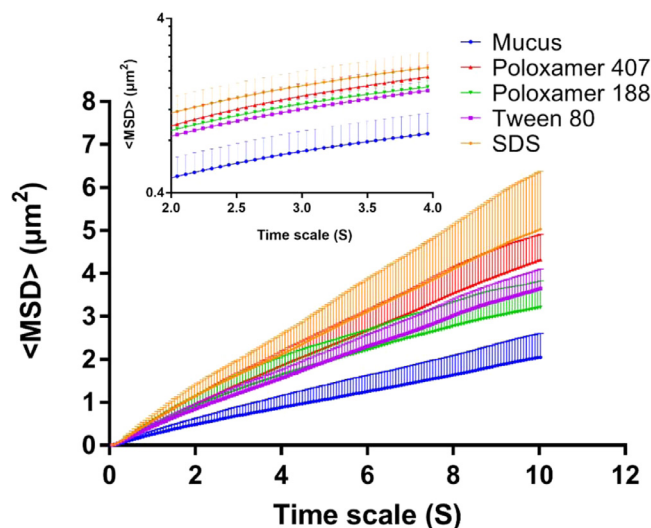


Fig. 1 – Influence of surfactants on mucus penetrations of PLGA nanoparticles.

with that in mucus without surfactants ($0.0532 \pm 0.017 \mu\text{m}^2/\text{s}$). Among them, SDS showed the highest effective diffusivity value of nanoparticles in the mucus, indicating SDS is superior to enhance the mucus penetration of PLGA nanoparticles. For nonionic surfactant, MSD of the nanoparticles in the mucus treated by P188 was lower than that of P407, and Tween 80 exhibited a better mucus penetration enhancing ability than that of P188 at long time scale.

3.3. Morphologic observation of mucus

To clarify whether the improvement of mucus penetration ability of PLGA nanoparticles by surfactants is related to microstructure change of mucus, morphology of various mucus samples was observed at different scales. Since the mucus was too viscous to distinguish the change of appearance and all the samples showed similar hydrogel state, the centrifugal supernatant of different mucus samples was compared to understand the influence of different surfactants on the appearance of mucus. As shown in Fig. 2A, the supernatant of SDS treated mucus turned into a relative viscous translucent liquid, while the supernatant of P407, P188 and Tween 80 treated mucus didn't show distinguished difference with the original mucus.

The variation of mucus microstructure was further investigated with optical microscope. As shown in Fig. 2B, based on the images observed in micrometer scale, morphology change of the mucus treated by SDS, P188 and P407 was found, with aggregation or increased gap among the components, while mucus treated by Tween 80 didn't show obvious difference in appearance compared with the original mucus. Furthermore, the morphology was investigated in the nanometer scale ($10 \mu\text{m}$ range) using AFM. As shown in Fig. 2C, aggregation was observed on the mucus surface treated by SDS compared with the original mucus, while the addition of P407, P188 and Tween 80 increased the pore size of the mucus network to

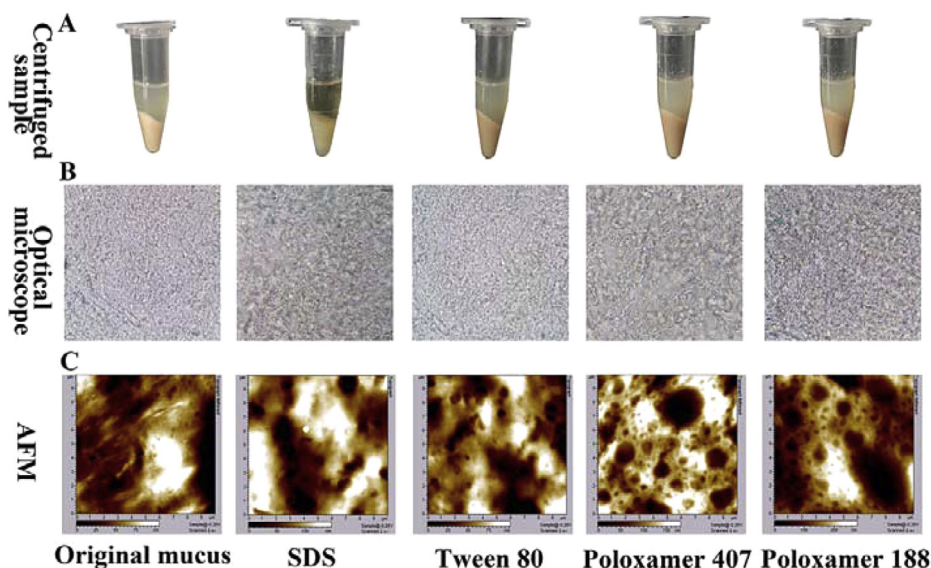


Fig. 2 – The morphology study of mucus at different scales. (A) Macroscopic observation (B) Optical microscope observation and (C) Atomic force microscope images.

various degree, forming a honeycombed passages in the mucus.

Since morphology study can only provide limited intuitive results and the observation process by AFM might potentially influence the mucus morphology, rheology study was further carried out to understand the interaction mechanism between surfactants and mucus.

3.4. Influence of surfactants on the rheological properties of mucus

The rheology property of mucus is a crucial parameter for mucus barriers, which can also reflect mucus microstructure change. In this paper, the variation of complex viscosity and viscoelastic parameters of mucus induced by surfactants were studied to better understand the way of surfactants to increase mucus penetration ability of PLGA nanoparticles and modify the mucus barrier properties.

To evaluate the influence of surfactants on the overall viscosity of mucus, change of complex viscosity of mucus was determined. Complex viscosity of original mucus and mucus containing different surfactants were tested under oscillation model at a fixed strain of 2%. The results, as shown in Fig. 3, showed that the addition of surfactants modulated the complex viscosity of mucus with different extent. SDS showed a viscosity increase effect on mucus. In contrast, Tween 80 and poloxamer series surfactants decreased the viscosity of mucus. Among these, P407 exhibited a stronger ability to decrease mucus complex viscosity. No statistical difference between P188 and Tween80 was found. Viscoelastic property of mucus were further studied to understand the interaction between mucus and surfactants.

The change of mucus viscoelastic properties in terms of elastic, viscous modulus, complex modulus and damping fac-

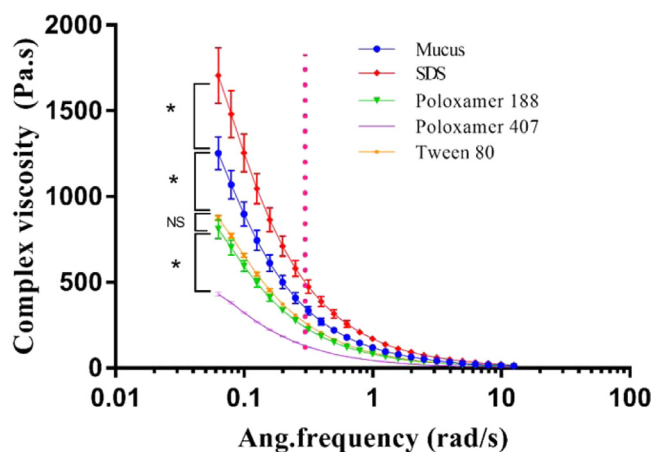


Fig. 3 – Complex viscosity of the original and surfactants treated samples.

tor were studied. Fig. 4A showed the influence of different surfactants on elastic modulus (G') of mucus. The addition of anionic surfactants, SDS, enhanced the elastic property of mucus. For nonionic surfactant, Tween 80, P188 and P407 reduced the elastic modulus (G') of mucus, and the value can be decreased to a lower degree by P407. Similar trend was also observed in the results of viscous modulus (G'') and complex modulus (G^*) (Fig. 4B and C). The comparison of damping factor ($\tan \delta$) of mucus containing different surfactants showed that all the mucus sample exhibited a lower damping factor ($\tan \delta < 1$), indicating the mucus tended to exhibit higher elastic properties rather than the viscous property (Fig. 4D). Among all the surfactants investigated, the damping factor is similar at frequency less than 1rad/s. With the in-

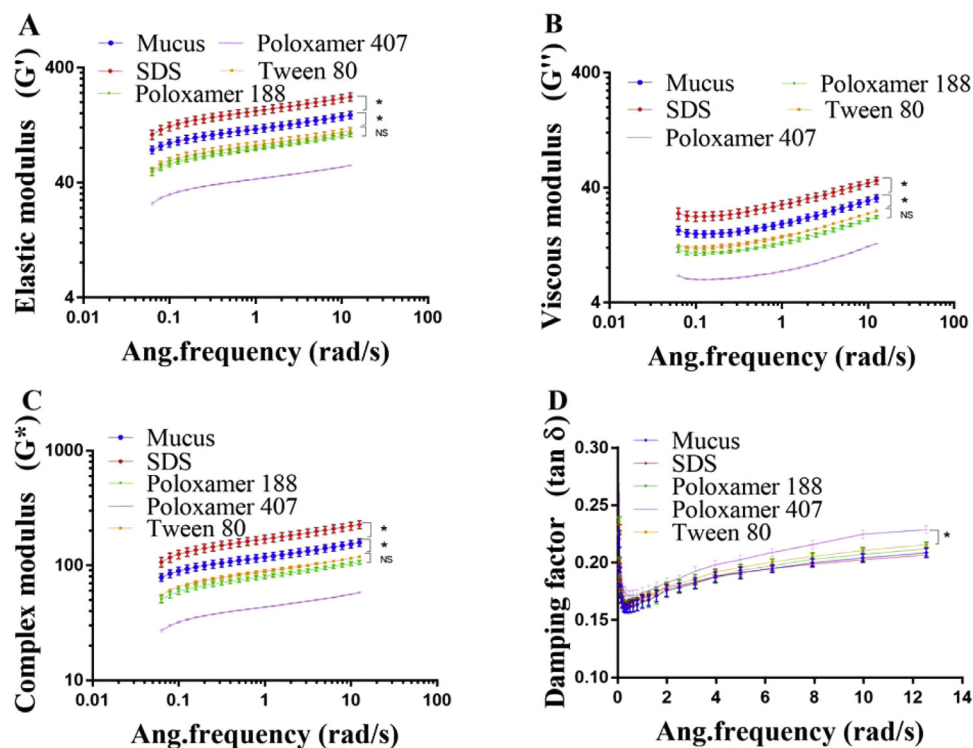


Fig. 4 – Viscoelastic modulus of the original and surfactants treated mucus samples: (A) elastic modulus (G'), (B) viscous modulus (G''), (C) complex modulus (G^*) and (D) damping factor ($\tan \delta$).

crease of frequency, P407 exhibited a stronger ability to increase the damping factor of mucus in comparison with other surfactants.

The observed aggregation of mucus induced by SDS (Fig. 2) is also in good agreement with the increase of its viscoelastic properties, implying that SDS may induce denaturation and aggregation process [28]. The denaturation process may be due to the insertion of SDS in mucin which may change the molecular conformation. SDS, as a linear anionic surfactant, has shown a strong potential to bind to the protein molecular. It has been reported that longer hydrophobic alkyl group and ionic group will lead to stronger interactions with proteins [29–31]. Thus, the anionic head group of SDS, which provides high hydrophilicity (HLB 40) and hydrocarbon chains, might be the essential component to provide the high strength of interaction. Although both the hydrophilic head of SDS and mucin are negatively charged, the electrostatic repulsion among them were not strong enough to prevent their associations which are mainly driven by hydrophobic interaction [29]. Thus, the binding of SDS to mucin will weaken the interaction among mucin and decrease the hydrophobic property of mucus and then increase the mucin–water interaction [29–31]. The extended conformational structure and the improved water solubility of mucin fibers eventually facilitate the aggregation of mucin, leading to increased complex viscosity and viscoelastic properties of mucus.

As liner nonionic surfactants composed of polyoxyethylene and polyoxypropylene segment, poloxamer, showed a good ability to decrease the viscoelastic properties of mu-

cus, indicating the mucus network structure strength was reduced (Fig. 4). However, a report indicated that in human cervicovaginal mucus (CVM) pretreated by P407, the penetration ability of nanoparticles increased, but the pore structure of mucus was not changed [32]. This may be due to the mucus source difference, and the contents of native intestinal mucus was more complex, which contains not only mucin but also DNA, proteins and lipids mixture [18,33]. The lipids existed in the intestinal mucus, which greatly influence the mucus properties, might also be sensitive to surfactants. Although P188 and P407 own similar structure, P407 showed a greater ability to reduce the mucus rheology properties. This can probably be attributed to the variation of P407 and P188 in HLB value, ratio of polyoxyethylene to polyoxypropylene segment and molecular weight, which may influence the interaction process between surfactant and mucus components (P188: HLB: 29; MW:7.7–9.5 kDa; ratio: 2.96. P407: HLB: 22; MW: 9.8–14.6 kDa; ratio: 1.80.). For poloxamer, higher ratio of hydrophilic parts and HLB value have a higher tendency to adsorb on the surface of mucus membrane rather than insertion [34,35]. As the most hydrophilic surfactant among poloxamers, P188 may tend to adsorb on the mucus surface, with slight change of mucus structure. In contrast, the higher molecular weight of P407, which can provide better steric stabilization, may contribute to the decrease of the viscoelastic properties of mucus. For branched nonionic surfactant Tween 80, alteration of mucus rheology was comparable to that of P188, which was consistent with the limited change of mucus morphology (Fig. 2).

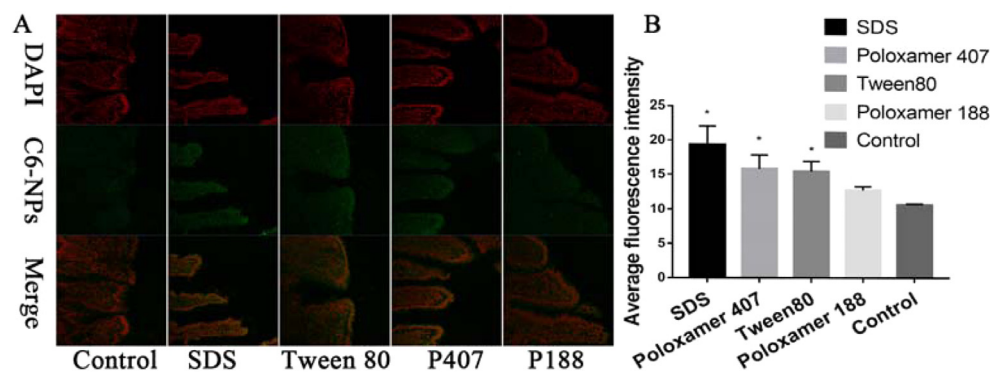


Fig. 5 – Intestinal villus penetration study. (A) CLSM images of intestinal villi of rat ileum segment after administration of coumarin 6 labeled PLGA nanoparticles suspension, (coumarin 6 and DAPI exhibited green and red color, respectively). (B) Average fluorescence intensity of coumarin 6 labeled PLGA nanoparticles in intestinal villus ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.5. Intestinal villus penetration study

The nanoparticles which penetrated through mucus barrier would reach intestinal epithelium [36,37].

For the same PLGA nanoparticles, the amount variation of nanoparticles in intestinal villus would depend on the penetration ability of nanoparticles in the mucus layer treated by surfactants. The penetration of PLGA nanoparticles labeled by coumarin 6 in villus was observed by confocal microscope and the improvement of mucus penetration induced by surfactants was evaluated intuitively. Fig. 5 shows that, except for P188, the coumarin 6 fluorescence intensity in the intestinal villus was obviously increased after pretreatment with surfactants, but no significant difference was found between SDS, P407 or Tween 80 groups.

Taking into consideration of rheology and mucus penetration results, the mechanism of improving mucus penetration of nanoparticles by surfactant was predicted and discussed. The steric barrier of mucus network and interaction between the mucus substances and nanoparticles greatly limit the penetration movement of nanoparticles in mucus. As common components used in drug delivery system, surfactants could modify the penetration ability of mucus by decreasing the interaction strength, changing the mucus structure and increasing hydrophilicity of the microenvironment. The adsorption of nanoparticles to mucin will also be reduced because of the adsorbed surfactant layers. The interaction between SDS and mucin could change the mucin conformation, and adsorption of SDS on mucin and lipids will increase hydrophilicity of the microenvironment, this would also facilitate the penetration ability of nanoparticles. However, the increased viscosity and viscoelastic properties induced by aggregate of mucin would also show adverse impact on penetration of nanoparticles. For nonionic surfactant P407/188 and Tween 80, they could reduce the interaction between mucin fibers and increase the network pores, which can eventually facilitate nanoparticles penetration by increasing the pore size of mucus. Compared with P188, the better mucus penetration enhancing ability of P407 may attribute

to its stronger ability to decrease the viscoelastic properties and therefore morphology change of the mucus. Although modification of mucus structure induced by Tween 80 was similar with P188, it showed better promotion effect on the penetration of nanoparticles. It is predicted that branched hydrophobic segment may promote the adsorption of surfactant on mucin and increase the nanoparticle penetration, although the change of morphology and rheology of mucus is not as obvious as P407 and SDS treated mucus.

The intestinal villus penetration enhancing effect was in good agreement with the mucus penetration ability of different surfactants (Fig. 5). The binding of surfactants to mucin will increase the mesh space of mucus and modify the mucus microenvironment to increase the penetration possibilities of nanoparticles. The arrangement of surfactant on the surface of the lipid and mucin will form a hydrophilic layer to greatly reduce the interactions. Thus, more nanoparticles will contact with intestinal villus and increase its penetration possibility. Although M cells in intestinal loop models will potentially affect the uptake of hydrophobic nanoparticles, the low number ratio of M cells in total intestinal epithelial cells (1 in 10^7) and higher affinity to hydrophobic nanoparticles limit its influence on the uptake of hydrophilic nanoparticles [38,39]. In our study, the hydrophilic surface of PLGA nanoparticles will reduce the influence of M cells. The slices also showed nanoparticles were evenly distributed in the villus, nanoparticles uptake by M cells were not found. In addition, the membrane permeability which was enhanced by surfactants may also facility the nanoparticle penetration [10].

4. Conclusions

Mucus, as viscoelastic hydrogel, showed inevitable penetration barrier for peroral delivery of nanoparticles. Mucus penetration enhancing ability of surfactants for PLGA nanoparticles were investigated and compared in this paper. The results showed that both ionic and nonionic surfactant can increase the mucus permeation ability of PLGA nanopar-

ticles. Although all surfactants can increase the hydrophilicity of mucus microenvironment, mechanism of different surfactants to facilitate the movement of nanoparticles is structure dependent. As foremost consideration of its application in drug administration, safety of surfactants which would not change the mucus properties is important. Thus, Tween 80 which partly retains the original mucus rheology and morphology properties, may be a promising candidate for facilitating nanoparticle penetration through the mucus barrier.

Conflicts of interest

The authors declare that there is no conflicts of interest.

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