Synthesis of articular cartilage-inspired branched polyelectrolyte polymer for enhanced lubrication

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Abstract: The superlubrication property of articular cartilage is attributed to the hydration lubrication mechanism. This involves the complexation of biomacromolecules with phosphatidylcholine lipids to form a lubricating boundary layer on the surface. Based on this mechanism, a branched polyelectrolyte polymer – PEI-PMPC was synthesised in this study to enhance lubrication via tert-butyl hydroperoxide-initiated grafting polymerisation of 2-methacryloyloxyethyl phosphorylcholine (MPC) onto polyethylenimine (PEI) with various molecular weights. Following the characterisation of the polymer using nuclear magnetic resonance, a series of tribological tests were performed under different normal loads and different scan rates. The results showed that the PEI-PMPC polyelectrolyte polymer in aqueous solution could effectively reduce the friction coefficient and wear generation between the polyethylene ball and the silica wafer. Additionally, an improvement in the lubrication performance was detected for the PEI-PMPC polyelectrolyte polymer when high molecular weight PEI was used during the reaction, which was attributed to the formation of the hydration shells surrounding the zwitterionic charges of PMPC and the viscosity of the polymer. In conclusion, the PEI-PMPC polyelectrolyte polymer developed herein was characterised by enhanced lubrication, and may be potentially used for biomedical applications such as intra-articular injection to restore joint lubrication.

1 Introduction

Articular cartilage, which consists of various biomacromolecules such as hyaluronic acid (HA), aggrecan, and lubricin, can massively reduce interfacial friction owing to the presence of a surface boundary layer. Specifically, these biomacromolecules complex with the phosphatidylcholine (PC) lipid to enhance lubrication by hydration lubrication mechanism, which is achieved via exposing the highly hydrated phospholipid head group on the superficial surface of the boundary layer [1, 2]. As the water molecule has an inherent electric dipole, the zwitterionic phospholipid group can form a tenacious hydration shell surrounding the charged. On the one hand, because the water molecules in the hydration shell have lower Gibbs free energy compared to free water molecules, they are difficult to be squeezed out; this enables the hydration shells between two sliding surfaces to undergo physiological pressure in the major joints (e.g. hips and knees) without deformation. On the other hand, the rapid exchange of water molecules in the hydration shell with free water molecules causes the hydration shells to respond in a fluid-like manner when being sheared. This results in a greatly reduced coefficient of friction (COF) at the joint interface, typically as low as 0.001–0.01 [3]. This lubrication mechanism, proposed by Kleen et al. as hydration lubrication, has been accepted as the primary rationale for the superlubrication property of articular cartilage [4–6].

The HA, as a viscosupplement, has been commonly used in clinics for enhancing joint lubrication via intra-articular injection [7]. However, the shear-thinning behaviour of HA under reciprocating sliding across the articular cartilage greatly compromises its lubrication property, because the viscosity of HA can decrease dramatically under high shear rate and become comparable to that of water [8]. Consequently, it is necessary to design and develop a novel biomimetic articular cartilage-inspired lubricant to effectively restore joint lubrication. Considerable efforts have been made to investigate the potential application of various polymers for lubrication enhancement [9, 10].

Poly (2-methacryloyloxyethyl phosphorylcholine) (PMPC) is a biocompatible polymer with the same zwitterionic phosphocholine groups (N+(CH3)3 and PO4−) as PC lipids. Therefore, it has been widely used to improve the lubrication performance of biomedical materials via surface modification [11, 12]; further, its use is considered to be more suitable than the use of superlubricated two-dimensional materials [13]. The phospholipid groups on each branch of PMPC can attract up to 15 or more water molecules within the hydration shell. This property enables it to be recognised as one of the most effective polyelectrolytes for hydration lubrication [14]. Additionally, the branched polyethyleneimine (PEI) has an amino group at every end of the branch, which has been previously used as a typical gene carrier with an efficient transfection in many cell types [15, 16]. In this study, considering the hydration lubrication of articular cartilage, we have successfully linked PMPC to branched PEI through tert-butyl hydroperoxide (TBHP)-initiated grafting polymerisation to synthesise polyelectrolyte polymer (PEI-PMPC) for enhanced lubrication. The lubrication property of PEI-PMPC aqueous solution was examined by a series of tribological tests, and it is anticipated that the branched PEI-PMPC polyelectrolyte polymer can be used as an effective lubricant additive in aqueous conditions.

2 Materials and methods

2.1 Materials and reagents

PMPC monomer was supplied by Joy-Nature Corp. (Nanjing, China). PEI with various molecular weights (1.8, 10, and 25 k)
was purchased from Aladdin Corp. (Shanghai, China), TBHP was purchased from J&K Scientific Ltd. (Beijing, China), and silica wafer (SiO₂, 10 mm × 10 mm) was purchased from Haisi Corp. (Linyi, China).

2.2 Synthesis of PEI-PMPC polymer

PMPC was linked to the amino groups on the side chains of PEI through TBHP-initiated grafting polymerisation based on a previous protocol [17], as shown in Fig. 1a. Briefly, PEI (25 k, 0.53 g, 0.021 mmol) and MPC (0.345 g, 1.17 mmol) were added in a round-bottom flask and completely dissolved in 15 ml deionised water. Subsequently, TBHP (10 μl) was added to the solution and the solution was degassed with nitrogen for 30 min. To enable the reaction to proceed, the temperature of the solution was increased to 80°C by placing the flask in an oil bath. Thereafter, the water in the solution was evaporated under reduced pressure and the dried product was re-dissolved in 50 ml methanol. Then 10% HCl aqueous solution (5 ml) was slowly added to the solution with constant stirring to precipitate the polymer product. The precipitate was collected via centrifugation (8000 rpm, 30 min) and re-dissolved in 30 ml deionised water. After the pH value of the solution was adjusted to 8.0 with Na₂CO₃, the solution was dialysed against deionised water for 48 h using a dialysis tube with a molecular weight cut-off of 8000–12,000 Da. Finally, the water in the solution was removed by freeze-drying to obtain the yellowish solid product of PEI-PMPC. The three PEI-PMPC polymers synthesised using different molecular weights of PEI were labelled as PEI-PMPC₂₅k, PEI-PMPC₁₀k, and PEI-PMPC₁.₈k, respectively; the molar ratio of PEI to MPC in the reaction remained unchanged. The ¹H nuclear magnetic resonance (NMR) spectrum of PEI-PMPC was characterised using an NMR spectrometer (JNM-ECS400, JEOL, Japan).

2.3 Tribological test

The lubrication property of the PEI-PMPC polyelectrolyte polymer in aqueous solution was investigated using a UMT-3 universal materials tester (Bruker, Billerica, MA, USA) in reciprocating mode. The upper and lower friction tribopairs were polyethylene (PE) ball (diameter: 8 mm) and SiO₂ wafer. The tribological test was performed at different loads (1, 3, and 5 N) and frequencies (1, 3, and 5 Hz; sliding distance: 8 mm) at 25°C. Each test was carried out for a duration of 5 min. Deionised water and various aqueous solutions (10 mg/ml) of PMPC, PEI-PMPC₂₅k, PEI-PMPC₁₀k, and PEI-PMPC₁.₈k were used as the lubricant. The curve of COF versus time was recorded for each test, and four independent tests were performed to ensure repeatability. Following the tribological test (load: 3 N; frequency: 5 Hz), a three-dimensional white light interferometer (NeXView, Zygo, USA) was used to examine the surface topography of the wear areas on the SiO₂ wafer.

2.4 Calculation of the maximum contact pressure

In the tribological test, the apparent maximum contact pressure between the PE ball and the SiO₂ wafer was calculated by Hertz...
equation for ball-on-flat configuration [18]

\[ P = \frac{1}{\pi} \sqrt{\frac{6F}{((1 - \mu_1^2/E_1) + (1 - \mu_2^2/E_2))R^2}} \]  

(1)

Here \( P \) is the contact pressure (MPa), \( F \) is the applied load (1, 3, and 5 N), \( \mu_1 \) and \( E_1 \) are the Poisson’s ratio and elastic modulus of PE (0.4, 1.0 GPa), and \( \mu_2 \) and \( E_2 \) are the Poisson’s ratio and elastic modulus of SiO\(_2\) (0.22, 72 GPa), respectively, and \( R \) (4 mm) is the radius of the PE ball [19, 20]. Therefore, \( P \) was calculated as 24.2 MPa (1 N), 34.9 MPa (3 N), and 41.4 MPa (5 N).

3 Results and discussion

3.1 Calculation of weight percentage of PMPC in PEI-PMPC

The \(^{1}H\) NMR spectra of the PEI-PMPC polyelectrolyte polymer with different molecular weights of PEI are depicted in Figs. 1b–d. The weight percentage of PMPC in PEI-PMPC is calculated based on the following analysis [17]. Each PMPC repeat unit has nine protons, with six protons in the \( d, e, \) and \( c \) positions. Therefore, the integral area of \( H_g \) equals 9/6 multiplied by the integral area of \( H_d, H_e, \) and \( H_c \). Each PEI repeat unit has four protons, and the molecular weights of the PEI repeat unit and MPC are 43 and 295, respectively.

The equation to calculate the weight percentage of PMPC in PEI-PMPC is defined as follows. The integral area required for this calculation can be obtained from the \(^{1}H\) NMR spectra.

Consequently, the weight percentage of PMPC relative to PEI-PMPC for the resulting polymers, namely, PEI-PMPC\(_{25k}\), PEI-PMPC\(_{10k}\), and PEI-PMPC\(_{1.8k}\), is estimated to be 6.14, 18.39, and 90.65%, respectively. Clearly, the weight percentage of PMPC in PEI-PMPC increases with the decrease in the molecular weight of PEI, which is related to the branching degree of the polymer. The branching degree of PEI is relatively small for low molecular weights, and thus, the content of the –NH\(_2\) group, which is grafted with the PMPC monomer in the reaction, per unit molecular weight is large. This is considered as the main reason for the high grafting degree of PEI-PMPC at low molecular weights of PEI (1.8 k) and the low grafting degree of PEI-PMPC at high molecular weights of PEI (25 k).

\[
\text{MPC\%} = \frac{m_{\text{MPC}}}{m_{\text{MPC}} + m_{\text{PEI}}} \times 100\% \\
= \frac{295}{295 + \left(\left(\frac{S_{\text{PEI},g}}{9/6}\right)\times 4\right)} \times 100\% \\
= \frac{295}{295 + \left(\left(\frac{S_{\text{PEI},d,e,c}}{9/6}\right)\times 4\right)} \times 100\%
\]  

(2)

\( \text{MPC\%}: \) weight percentage of PMPC in PEI-PMPC

\( m_{\text{MPC}}: \) the relative molecular mass of MPC in PEI-PMPC.

\( m_{\text{PEI}}: \) the relative molecular mass of PEI in PEI-PMPC.

\( S_{\text{PEI},d,e,c}: \) integral area of PEI and \( H_g \) in the \(^{1}H\) NMR spectrum of PEI-PMPC.

\( S_{\text{PEI},d,e,c}: \) integral area of \( H_d, H_e, \) and \( H_c \) in the \(^{1}H\) NMR spectrum of PEI-PMPC.

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**Fig. 2** Tribological test between the PE ball and SiO\(_2\) wafer under various experimental conditions

* a Schematic illustration showing the tribological test setup
* b COF–time curves of the tribopair between the PE ball and SiO\(_2\) wafer lubricated by deionised water, PMPC, and PEI-PMPC aqueous solutions
* c Lubrication performance of the PEI-PMPC\(_{25k}\) aqueous solution under different frequencies (load: 3 N)
* d Lubrication performance of the PEI-PMPC\(_{25k}\) aqueous solution under loads (frequency: 3 Hz)

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3.2 Tribological test

The schematic illustration of the tribological test is depicted in Fig. 2a, and the lubrication performance of the PMPC and PEI-PMPC (synthesised with various molecular weights of PEI) aqueous solutions, represented by the COF between the PE ball and the SiO2 wafer, is illustrated in Fig. 2b (contact pressure: 34.9 MPa; frequency: 3 Hz; concentration: 10 mg/ml). It is indicated from the COF–time curves that the COF of PEI-PMPC is lower than that of deionised water (0.0403) and PMPC (0.0370). Additionally, the COF–time curves demonstrate a slightly decreasing trend of COF for PEI-PMPC with the increase in the molecular weight of PEI, i.e. 0.0340 for PEI-PMPC1.8k, 0.0313 for PEI-PMPC10k, and 0.0307 for PEI-PMPC25k. This subtle difference may be attributed to the viscosity of the polymer aqueous solution, where a higher viscosity of the lubricant (obtained by the PEI-PMPC with higher molecular weight, PEI-PMPC25k) contributes to the formation of a lubrication film in the contact area, therefore enhancing the lubrication of the tribological system.

Additionally, the lubrication performance of the PEI-PMPC25k aqueous solution under different test conditions, i.e. various frequencies of 1, 3, and 5 Hz (load: 3 N) and loads of 1, 3, and 5 N (frequency: 3 Hz) is depicted in Figs. 2c and d. It is demonstrated that the COF of PEI-PMPC25k decreases with the increase in frequency (0.049 for 1 Hz, 0.031 for 3 Hz, and 0.028 for 5 Hz) and load (0.040 for 1 N, 0.031 for 3 N, and 0.019 for 5 N). This indicates that the lubrication of the PEI-PMPC25k polyelectrolyte polymer in an aqueous solution is stable not only under different applied loads (i.e. different contact pressures) but also under different sliding speeds, as tested in this study.

The surface morphology of the wear areas on the SiO2 wafer using deionised water, PMPC, and PEI-PMPC aqueous solutions as the lubricant was examined employing a three-dimensional white light interferometer following the tribological test (load: 3 N; frequency: 5 Hz; concentration: 10 mg/ml). Figs. 3a–e illustrate the surface profiles of the SiO2 wafer across the wear area, and it is clear that the minimum depth of the wear area is 0.11 μm for PEI-PMPC, which is significantly lower that of deionised water (0.74 μm) and PMPC (0.25 μm). Similarly, the wear volume of the wear area on the SiO2 wafer is considerably reduced when the lubricant is switched from deionised water to PEI-PMPC (Fig. 3f). This result indicates that the PEI-PMPC polyelectrolyte polymer can not only decrease the COF but also reduce the wear generation between the two sliding surfaces. Additionally, the lubrication performance of PEI-PMPC polyelectrolyte polymer can be flexibly regulated by varying the molecular weight of PEI during the synthesis reaction.

3.3 Lubrication mechanism

The enhanced lubrication property of the PEI-PMPC polyelectrolyte polymer aqueous solution is attributed to the hydration lubrication mechanism, which is the same scenario dominating the superlubrication behaviour of articular cartilage [3, 21]. Although the water molecule is neutral overall, it has a large electric dipole due to the residual charges on the H and O atoms. As depicted in Fig. 4, the zwitterionic charges in PMPC can attract several water molecules to form a tenacious hydration shell surrounding the phosphorylcholine group, which is responsible for the low COF tested under aqueous conditions [22, 23]. Specifically, it is difficult for the hydration shells to overlap with each other due to the steric effect, and the lower Gibbs free energy of the water molecules in the hydration shells makes it difficult for them to deform, and consequently, the hydration shells can undergo a large normal pressure without deformation. In contrast, the rapid exchange of the water molecules in the hydration shell with the nearby free water molecules results in a fluid-like response of the hydrated shells when being sheared, and correspondingly, a reduced COF at the sliding interface. However, hydration lubrication, which is derived from a single brush-like structure of the PMPC molecule in an aqueous solution, may not be effective and durable as it is.
damaged under high pressures between two sliding surfaces [24]. In this study, the PEI-PMPC polyelectrolyte polymer with a branched framework is synthesised via TBHP-initiated grafting polymerisation. This polymer can be physically cross-linked with each other and, thus, the lubrication performance remains stable in an aqueous solution.

4 Conclusions

In this study, inspired by the hydration lubrication mechanism of articular cartilage, we successfully synthesised the branched PEI-PMPC polyelectrolyte polymer to enhance lubrication performance. PMPC was linked to various molecular weights of PEI by TBHP-initiated grafting polymerisation to regulate the weight percentage of PMPC in PEI-PMPC, while the molar ratio of the PMPC monomer to PEI in the reaction remained unchanged. The tribological test demonstrated that the COF of the PEI-PMPC aqueous solution was reduced in comparison with that of deionised water and PMPC. Moreover, the lubrication property of PEI-PMPC was further improved by increasing the molecular weight of PEI, and was maintained stable under different test conditions. This was attributed to the formation of the hydration shells surrounding the zwitterionic charges of PMPC, which could undergo high pressure without being squeezed out and respond in a fluid-like manner when sheared, and the viscosity (formation of lubrication film) of the polymer. The articular cartilage-inspired PEI-PMPC polyelectrolyte polymer may be used to restore joint lubrication via intra-articular injection, and future studies will be concentrated on the potential for biological applications of the polymer.

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6 References

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