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Development of anti-biofouling interface on hydroxyapatite surface by coating zwitterionic MPC polymer containing calcium-binding moieties to prevent oral bacterial adhesion ☆

Sunah Kang [a,1], Myoungjin Lee [b,1], Minji Kang [a], Minwoo Noh [a], Joohee Jeon [a], Yan Lee [a,*], Ji-Hun Seo [b,*]

[a] Department of Chemistry, College of Natural Sciences, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 151-747, Republic of Korea
[b] Department of Materials Science and Engineering, Korea University, 145 Anam-ro, Seongbuk-gu, Seoul 136-701, Republic of Korea

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ABSTRACT

The purpose of the present study is to synthesize a 2-methacryloyloxyethyl phosphorylcholine (MPC) polymer capable of being immobilized on the tooth surface to prevent oral bacterial adhesion. The strategy is to develop an MPC-based polymer with Ca^{2+}-binding moieties, *i.e.*, phosphomonoester groups, for stronger binding with hydroxyapatite (HA) of the tooth surface. To this end, a 2-methacryloyloxyethyl phosphate (MOEP) monomer was synthesized and copolymerized with MPC by free radical polymerization. The coating efficiency of the synthesized polymer, MPC-*ran*-MOEP (abbreviated as PMP) with varied composition, onto a HA surface was estimated by means of contact angle measurement and X-ray photoelectron spectroscopy. The anti-biofouling nature of PMP-coated HA surfaces was estimated by analyzing protein adsorption, cell adhesion, and *Streptococcus mutans* adhesion. As a result, HA surface coated with a copolymer containing around 50% MPC (PMP50) showed the best performance in preventing protein adsorption and the downstream cell and bacterial adhesion.

Statement of Significance

Preparation of anti-biofouling surface on the tooth enamel is the key technique to prevent dental and periodontal diseases, which are closely related with the biofilm formation that induced by the adsorption of salivary proteins and the adhesion of oral bacteria on the tooth surface. In this research, a PMP copolymer with an optimized ratio of zwitterionic and Ca^{2+}-binding moieties could form a highly effective and robust anti-biofouling surface on HA surfaces by a simple coating method. The PMP-coated surface with high stability can provide a new strategy for an anti-adsorptive and anti-bacterial platform in dentistry and related fields.

1. Introduction

Dental and periodontal diseases are significantly affected by the fouling of the tooth surface [1–3]. The main component of tooth enamel, that is hydroxyapatite (HA), could decompose into Ca^{2+} and PO_4^{3-} during the formation and growth of oral biofilm, and this has been thought to be the beginning of dental caries and periodontal diseases [4]. The formation of a biofilm on tooth surfaces is initiated by the adsorption of organic macromolecules such as salivary proteins [5]. A thin protein layer, called pellicle, is formed as a result on the tooth surface, and a specific part, *i.e.*, a ligand, of proline-rich proteins or salivary mucins on the pellicle recruits *Streptococcus mutans* [6,7]. Then, other oral bacteria may co-aggregate to produce a biofilm on the tooth surface. Oral bacteria then metabolize nutrients in the oral cavity to generate organic acids that cause HA decomposition [4,8]. Therefore, it is expected that the initiation of dental caries and periodontal disease could be inhibited by blocking protein adsorption and the subsequent bacterial adhesion on the tooth surface.

There are several physicochemical factors controlling the attachment of proteins on biological surfaces. Surface charges, polarity, degree of hydration, or even surface geometry can

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* Corresponding authors.
E-mail addresses: gacn@snu.ac.kr (Y. Lee), seojh79@korea.ac.kr (J.-H. Seo).

[1] These authors contributed equally.

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significantly affect the adsorption of proteins or conformational changes [9,10]. Previous studies suggested that the preparation of electrically neutral and hydrophilic flat surface could be one of the most effective solutions to prevent protein adsorption and the resultant bacterial adhesion on biological surfaces [11–13].

To this end, various hydrophilic polymers, such as poly(ethylene glycol), poly(2-hydroxyethyl methacrylate), and poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC), etc., have been adopted to prepare anti-fouling surfaces [14–19]. Among these hydrophilic polymers, PMPC has been known to have an optimized molecular structure for developing a long-term anti-fouling interface that is safely applicable *in vivo* [20,21]. Therefore, 2-methacryloyloxyethyl phosphorylcholine (MPC)-based polymer coating on tooth surface is anticipated to effectively prevent the attachment of proteins and bacteria. Several papers have already reported that the introduction of MPC-based polymers is very effective in preventing bacterial adhesion on the surface of various materials [22–24]. The main problem in the MPC-based polymeric coating for developing anti-bacterial surfaces is the extremely hydrophilic nature of the polymer that makes it hard to be immobilized on the materials surfaces in aqueous media. In particular, realizing a stable coating of MPC-based polymers on the tooth surface to resist the physical stress during the masticatory movement or the dissolution by excessive saliva remains a challenging goal in the field of the dentistry.

The purpose of the present study is to synthesize an MPC-based polymer capable of being immobilized on the tooth surface to prevent the attachment of proteins and oral bacteria. The underlying concept is to develop an MPC-based polymer with Ca^{2+}-binding moieties, *i.e.,* phosphomonoester groups, for stronger binding with HA of the tooth surface. To this end, the 2-methacryloyloxyethyl phosphate (MOEP) monomer was synthesized and copolymerized with MPC by free radical polymerization. The coating effect of the polymer against the attachment of oral proteins and bacteria was investigated on the enamel-mimic HA surface.

2. Materials and methods

2.1. Materials

The 2-Methacryloyloxyethyl phosphorylcholine (MPC) monomer was purchased from KCI (Gyeonggi-Do, Korea). Dimethyl chlorophosphate (DCP), 2-hydroxyethyl methacrylate (HEMA), α, α′-azobisisobutyronitrile (AIBN), K_2HPO_4, $CaCl_2$, mucin (from porcine stomach), α-amylase (from porcine pancreas) and hydroxyapatite (HA) powder were purchased from Sigma-Aldrich (St. Louis, MO, USA). Bromotrimethylsilane (TMSBr) was purchased from TCI (Japan). Pyridine, tetrahydrofuran (THF), ethanol, $NaHCO_3$, KCl, and chloroform were purchased from Daejung (Seoul, Korea). NaCl were purchased from Samchun (Seoul, Korea). Dulbecco's phosphate buffered saline (DPBS, without calcium chloride and magnesium chloride), Dulbecco's modified eagle's media (DMEM), bovine serum albumin (BSA), and fetal bovine serum (FBS) were purchased from WelGENE Inc. (Daegu, Korea). A micro-BCA protein assay reagent kit was purchased from Pierce Chemical (Rockford, IL, USA). A cell counting kit-8 (CCK-8) was purchased from Dojindo (Tokyo, Japan). A brain heart infusion (BHI) broth powder was purchased from KisanBio (Seoul, Korea). *Streptococcus mutans* (ATCC25175) was received from Korean Collection for Type Cultures (KCTC, Korea).

2.2. Synthesis of MOEP monomer

Methacryloyloxyethyl phosphate (MOEP) was synthesized according to the previously reported procedure with a slight modification (Fig. S1, supporting information) [25]. Briefly, DCP (0.040 mol) was dissolved in 15 mL of chloroform with pyridine (0.040 mol). A HEMA solution (0.010 mol) in 15 mL of was mixed with the DCP solution. The reaction mixture was stirred for 2 h on ice, and further stirred for 3 h at ambient temperature. The reaction mixture was then washed with 0.01 M HCl (×5), and the organic layer was evaporated using a rotary evaporator. Dimethyl methacyloyloxyethyl phosphate (DMOEP) was obtained as a colorless oil. DMOEP (1.3 mmol) was dissolved in anhydrous chloroform (40 mL), and TMSBr (5.2 mmol) was slowly added into the solution. After stirring for 3 h on ice under an inert atmosphere, the solvent was removed by evaporation. Then, 100 mL of H_2O/THF (v/v = 1/7) was added to the reaction mixture. The reaction mixture was stirred overnight at ambient temperature. Demethylated MOEP was obtained as a sticky and transparent oil after evaporation without further purification. The total yield was 90.0%.

2.3. Synthesis of PMPC, PMOEP, and PMPC-ran-MOEP (PMP)

Random copolymers with various MPC and MOEP contents were synthesized by free radical polymerization. MPC and MOEP monomers were dissolved in ethanol at a monomer concentration of 0.5 M. AIBN (1.0 mol%) was added to the solution. Polymerization proceeded for 3 h at 60 °C under an argon atmosphere. The resulting polymeric solution were dialyzed against deionized water though a membrane (Spectrum Laboratories, MWCO: 1000 Da) to remove impurities. After lyophilization, the polymers were obtained as white powder. The yield was generally over 60%.

2.4. Gel permeation chromatography

The molecular weight distributions of synthesized polymers were examined by gel permeation chromatography (GPC; Superdex GPC column, GE Healthcare, UK). Sodium phosphate buffer (50 mM, pH 7.4, 150 mM NaCl) was used as the eluent at a flow rate of 0.7 mL/min. Poly(ethylene glycol) (PEG) polymers were used as standards.

2.5. Preparation of hydroxyapatite (HA) disks

HA disks were prepared by spark plasma sintering (SPS). The HA powder was put into a graphite mold and then pressed in Spark plasma instrument at a pressure of 612 kg/f (60 MPa) and at 1100 °C for 10 min under vacuum condition. After cooling, the disks were washed by sonication in water for 30 min. After drying, white HA disks with a diameter of 12 mm and a thickness of 2 mm were obtained. Surface roughness of the HA disks was analyzed using a confocal laser scanning microscope (VK8700, KEYENCE, Japan) (Fig. S5). The roughness averages (R_a) of prepared disks are similar to each other.

2.6. Surface coating of HA disks with polymers

Each polymer was dissolved in deionized water at a concentration of 0.010 wt% and 1.0 wt% (only for bacterial adhesion test). The HA disk was immersed into each solution for 10 min and thoroughly washed with deionized water for 1 min followed by drying overnight at 50 °C *in vacuo*. In order to examine the stability of the coating, sonication was conducted for 10 min for the detachment of weakly bound polymers on the HA disks.

2.7. Measurement of water contact angle

The dynamic water contact angles were measured to examine the hydrophilicity of the HA disk surface. Advancing and receding

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