International Journal of Sciences & Applied Research

www.ijsar.in

RESEARCH PROGRESS OF ZWITTERIONIC POLYMERS IN CARIES PREVENTION AND TREATMENT

Chunmei He[1], Wu Zhang[2]*

[1]School of Stomatology, Jinan University, Guangzhou 510630, China
[2*]Hospital of Stomatology, the First Affiliated Hospital of Jinan University, Guangzhou 510630, China
[1]Email:1173959052@qq.com
[2]*Email:zhangw1216@163.com

ABSTRACT

Caries is caused by bacteria attached to the plaque on the surface of teeth that produce acid from dietary sugars as raw materials, leading to the demineralization of tooth hard tissues and even the destruction of tooth tissue structure. Based on the formation of dental plaque biofilm and its cariogenic mechanism, new materials that can inhibit the formation of caries pathogen biofilm, reduce demineralization and induce remineralization of dental hard tissues have become a research hotspot. Zwitterionic polymers (ZPs) have unique structures and properties that can resist adhesion and sterilization of proteins and bacteria, thus resisting biofilm and acting as templates for functionalization to regulate mineralization, while maintaining good biocompatibility. Therefore, this review will focus on its structural characteristics and its application in the prevention and treatment of dental caries.

Keywords： Zwitterionic polymers, Dental caries, Biofilm, Rremineralization

INTRODUCTION

According to the World Health Organisation, dental caries is one of the top three diseases that threaten human health[1, 2]. Most carious lesions, if not recovered in the early stages of demineralisation, will gradually deteriorate, resulting in serious complications such as cavities and even pulpitis and periapical inflammation[3]. Conventional caries treatments are generally invasive. It consists of preparing cavities in intact dental tissues and restoring them with conventional materials such as silver amalgam and composite resin. However, due to the difference in properties between the dental tissues and the artificial materials, the long-term performance of the restored teeth is far from expected and the incidence of secondary caries is high.

Caries is caused by acid production by bacteria attached to plaque on the tooth surface using dietary sugars as a raw material, and the balance between demineralisation and remineralisation on the tooth surface is disrupted, leading to loss of tooth hard tissue. Based on the formation of plaque biofilm and its cariogenic mechanism, the strategy for the prevention and restoration of early caries is aimed at: inhibiting biofilm formation and inducing remineralisation. Commercial products (e.g., toothpastes and mouthwashes) containing antibiotics, chlorhexidine, and other chemicals are commonly used to prevent the formation of biofilms of caries pathogens. However, bacterial resistance limits the long-term use of antibiotics, and long-term use of CHX can result in side effects such as staining of tooth surfaces and altered tongue taste sensation[4].fluoride[5] and some based on calcium phosphate[6] Reagents such as casein phosphopeptide - Amorphous calcium phosphate induces mineral deposition on the tooth surface but does not integrate into the enamel microstructure[7].Most of the current anti-caries agents are only monofunctional. Therefore, novel materials that can simultaneously resist biofilm formation and promote early caries remineralisation have attracted great attention. Zwitterionic polymers are polymeric materials that contain the same number of anionic and cationic groups on the side chain of the same monomer[8].Recent studies have revealed that ZPs have begun to attract great attention in caries control due to their ability to resist adhesion of proteins and bacteria, prevent biofilm formation and act as functionalized templates to regulate mineralisation. In this review, we briefly introduce the structure and properties of ZPs and the trend of material application in caries control.

1. Structure and properties of zwitterionic polymers

1.1 Structure of zwitterionic polymers

Zwitterionic polymers are polymeric materials that contain the same number of anionic and cationic groups on the side chain of the same monomer, and these materials are characterised by high dipole moments and highly charged groups, but are still electrically neutral overall. There are four main types of cationic groups in zwitterionic polymers: quaternary ammonium cations, quaternary phosphonium cations, pyridinium ions, and imidazolium ions; and three main types of anionic groups: sulfonate negative ions, carboxylate negative ions, and phosphate negative ions. Different zwitterionic ions can be constructed by combining anionic and cationic groups, among which 2-methacryloyloxyethyl phosphorylcholine (MPC), methacryloyloxyethyl sulfobetaine (SBMA), and carboxybetaine methyl methacrylate (CBMA) obtained by combining quaternary ammonium cations with different types of anions have been the most widely used[9].

1.2 Properties of zwitterionic polymers

1.2.1 Anti-biofilm

Zwitterionic polymers are made by polymerisation of monomers containing both anionic and cationic groups, which are generally electrically neutral; and achieve anti-bioadhesion[10] and charge-switching antimicrobial properties through superhydration and antipolylyte effects[11]. (1) Superhydration: strong hydration is considered to be the main source of resistance to non-specific protein adsorption[12]. Both zwitterionic and nonionic PEG materials exhibit strong hydration[13], and polyethylene glycol (PEG) has been the ‘gold standard’ for antifouling polymers. However, PEG has poor stability, rapidly autoxidises and degrades at room temperature, and loses its resistance to protein adsorption when the temperature rises to 35°C[14]. Zwitterionic polymers can form a more solid hydration layer through electrostatic interaction (the hydration layer formed by electrostatic interaction is stronger than that by hydrogen bonding)[15], and therefore can effectively reduce protein adsorption. (2) Anti-polyelectrolyte effect: Ampholytic ionic polymers in low molecular salt solution, the interaction between the structural chains with the same charge decreases and chain elongation occurs, while the interaction with water molecules ions is enhanced to achieve entropic equilibrium, which results in the formation of a dense hydrated film on its surface. When proteins approach the surface of the material and destroy the hydrated layer, a strong repulsive force will be generated, resulting in the formation of a strong spatial site resistance effect to achieve non-specific resistance to the adsorption of proteins, bacterial adherence and the formation of biofilm. (3) Charge switching: Zwitterionic ions have both cationic and anionic groups, allowing the zeta potential to be completely reversed from below zero to above zero in response to changes in environmental conditions. Zwitterionic ions used for self-targeting platforms (e.g., CBMA) in the vicinity of biofilms or in acidic environments promote cationisation of quaternary amino groups in CBMA, which take on a positive charge on the surface, attracting negatively charged bacteria through the electrostatic double layer and producing targeted penetration and accumulation in biofilms, thereby providing a bactericidal effect.

1.2.2 Remineralisation template

Because zwitterionic polymers contain both positively and negatively charged groups, their structure is similar to that of collagen and non-collagenous proteins, and they can play a positive role in promoting mimetic mineralisation by recruiting precursor ions (Ca^{2+} and $PO4^{3-}$), lowering interfacial energies during heterogeneous nucleation, stabilising amorphous calcium phosphates (ACPs), and mediating the transition from ACPs to oriented apatite crystals.

2. Material applications of zwitterionic polymers

2.1 Resins

Composite resins are the most common dental materials used in the restoration of dental defects; however, oral biofilms tend to form on the resin surface, leading to secondary caries and damaging the composite material. Zhang et al [16] added MPC to composite resins and found that protein adsorption, bacterial attachment, and biofilm colony-forming unit counts on the surface of the resins were greatly reduced from 0% ~3%. Inspired by this, Zhang et al.[17] further investigated combining the antimicrobial effect of QAM with the protein rejection property of ZPs, and found that compared with the use of MPC or dimethylaminodecyl methacrylate (DMAHDM) alone, the simultaneous addition of 3% MPC and 1.5% DMAHDM reduced the plaque biofilm by more than three orders of magnitude. Several studies have shown that saliva-derived protein films on cationic antimicrobial surfaces reduce the original bactericidal efficacy[18, 19], and since MPC-containing resins can greatly reduce protein adsorption, there will be much less protein on the resin, and therefore more direct contact between the resin and the bacteria, resulting in improved contact killing efficacy. In addition, Lee et al.[20] doped MPC (1.5-10wt%) into commercial SPRG-filled resin matrix composites and they found that the amount of ions released from the SPRG-filled composite resin increased with the increase of MPC content, which could be attributed to the catalytic effect of MPC with negatively polarised groups on the surface. Meanwhile, 3 wt% MPC exhibited significant resistance to protein adsorption ($P<0.01$) and inhibited the formation of biofilm by inhibiting a wide range of bacteria compared to other test and control samples.5 wt% MPC significantly improved the acid-neutralising properties of the composite resin filled with SPRG, which, due to the ions released from SPRG filler, rapidly neutralised the acid produced by lactic acid solution, and could increase the pH to the critical pH of 5.5, thus preventing enamel demineralisation and inhibiting secondary caries.

2.2 Bonding agents

Restoration and attachment of teeth involves bonding using an adhesive and primer system. The adhesive is not only the connection between the tooth structure and the restorative composite, it also acts as a barrier to protect the demineralised collagen scaffold from attack by oral bacteria, enzymes and acidic fluids. Biofilm-generated acids can lower the local pH to the cariogenic range of 4 to 5 and the adhesive dissolves and breaks down, leading to demineralisation of tooth structure and secondary caries formation[20, 21]. Therefore, antimicrobial adhesives may be an effective way to prevent secondary caries. Oral bacteria attach to teeth and restorations via surface-adsorbed salivary proteins, which are prerequisites for bacterial attachment and biofilm growth[22, 23].Zhang et al [24]. studied the protein adsorption and biofilm growth characteristics of anti-protein adsorption dental adhesives for the first time, and verified that the incorporation of MPC with a mass fraction of 7.5% into the primer and adhesive could achieve a strong protein rejection without affecting the dentin bond strength. This new approach successfully reduced protein adsorption to the adhesive resin by an order of magnitude, while reducing the CFU of the oral biofilm by an order of magnitude. It is important to note that MPC-containing resins have no bactericidal ability, although they can reduce bacterial adhesion. A further study by Zhang et al[25] combined the antimicrobial agents dimethylaminohexyloxy methacrylate (DMAHDM) and MPC into dental resins, 7.5% MPC and 5% DMAHDM doped into primers and adhesives to obtain protein repelling and antimicrobial activity with no adverse effect on dentin shear bond strength.Xie et al.[26] developed a novel adhesive by incorporating MPC and DMAHDM doped into NACP rechargeable adhesive, the novel bioactive adhesive showed strong protein repulsion and greatly reduced bacterial attachment and viability, with a reduction in biofilm CFUs of nearly four orders of magnitude compared to the commercial control. And the pH of the bioactive binder with biofilm cultures on the microscopic surface of dental plaque was maintained above 6, whereas the cariogenic pH of the commercial control binder was 4. And the ionic concentration increased significantly when the level of NACP filler was increased from 20% to 40% ($p < 0.05$). These results suggest that NACP-containing binders have reservoirs of Ca and P ions nearby to facilitate remineralisation. Thus, novel adhesives with triple benefits are expected to protect tooth structure and inhibit biofilm and caries formation, allowing remineralisation of early lesions. Orthodontic adhesives used to attach brackets directly to the enamel are also highly susceptible to plaque build-up[27], leading to damage to the surrounding enamel structure, which manifests itself as white spot lesions[28]. For this reason, Park[29] evaluated the antimicrobial and remineralisation effects of mixing MPC and MNB with orthodontic binders in different ratios. They demonstrated that MPC protein rejection and antibacterial effects improved with the addition of MBN. Similarly, the antimineralisation effect of MBN itself increased with the addition of MPC. This combination could be an option for the prevention of WSL.

2.3 Fluoride Varnishes and Sealants

Fluoride varnishes and sealants prevent caries by forming an impermeable mechanical barrier, but they do not have the ability to inhibit salivary protein adsorption and inhibit plaque formation on their own.Kwon et al.[30] found that light-curing fluoride varnish doped with 3% MPC significantly reduced protein and bacterial adherence, and reduced the depth of demineralisation of enamel from 46.44 μm to 23.62 μm.A subsequent study ZPs in the form of CBMA, MPC and SBMA were incorporated into light-curing fluoride varnish (LV) at 3% mass fraction each, and the three materials were found to have almost the same effect on resistance to protein adsorption, biofilm formation and prevention of enamel demineralisation[31].Lee et al.[32] investigated a novel bioactive resin-based sealant with 3% MPC and 12.5% bioactive glass BAG (RBS), and found that it was comparable to the commercial RBS. (RBS) and found that RBS with MPC and BAG + MPC significantly reduced protein and bacterial adhesion and inhibited multispecies biofilm attachment compared to commercial and experimental controls. This new RBS has great potential to prevent secondary caries by promoting enamel remineralisation and inhibiting biofilm adhesion.

2.4 Polymer Coating

Recently, Dong et al.[33] prepared an amphiphilic/phosphonate copolymer p (DEMMP- co - MPC) based on amphiphilic polymers utilising the dual functions of anti-bacterial adhesion and inducing biomineralisation, and in vitro and in vivo experiments consistently demonstrated that amphiphilic coatings can induce remineralisation of enamel and dentin surfaces, and also extensive remineralisation of dentin tubules to the original dentin similar to the original dentin. Moreover, both antifouling properties and remineralisation capacity were positively correlated with the amount of the ampholytic ion pMPC in the coating copolymer. These findings provide new ideas for the treatment of dentin hypersensitivity and caries with amphiphilic pMPC-based materials.

2.5 Nanocomposites

He et al.[34] prepared pCBAA/ACP nanocomposites. pCBAA showed high resistance to bacterial adhesion and biofilm formation in physiological PH environment due to electrostatic-induced hydration. pCBAA was cationised as the PH changed from neutral to acidic and its positively charged quaternary ammonium interacted with the negatively charged cell membranes of the bacteria, leading to cell membrane disruption and cytoplasmic leakage thereby killing the bacteria. Furthermore, this nanocomposite showed excellent results in promoting remineralisation of demineralised enamel and occlusion of exposed dentin tubules in vivo and in vitro compared to fluoride. Their study provides a theoretical and experimental basis for pCBAA/ACP nanocomposites as a potential bifunctional agent for caries prevention.

3. Limitations of zwitterionic polymers in dental material applications

Most of the previous studies applied ZPs as additives to dental materials, but the anti-biofilm effect was not significant when applied alone, and if more zwitterionic polymers were added to the materials to achieve better stain resistance, this had a detrimental effect on the flexural strength, modulus of elasticity, and hardness of the materials themselves, which is very unfavourable for permanent restorations. Therefore, it is often necessary to combine other antimicrobial components, and the addition of antimicrobial agents will lead to a reduction in the biocompatibility of the material and the development of bacterial resistance. The preventive demineralisation and remineralisation efficacy of the material is synergistically achieved by the addition of exogenous remineralisation agents, and little research has been carried out on the remineralisation template function of ZPs themselves.

4. Summary and outlook

Zwitterionic polymers are expected to reduce dental hard tissue demineralisation, secondary caries, and promote remineralisation of early demineralised dental tissues due to their unique structure and properties, which can be resistant to biofilms and act as functionalised templates to regulate mineralisation. Most of the current research will be zwitterionic ionic polymers in the form of direct addition to dental materials, the most widely used MPC alone can effectively reduce the surface protein adsorption, and with antibacterial monomers such as DMAHDM can play a synergistic antimicrobial effect, through the addition of exogenous remineralising agents (bioactive glass, calcium phosphate, etc.) can be used to inhibit the remineralisation of dental hard tissues by promoting the release of ions and adjusting the pH value of the local microenvironment, and to inhibit the remineralisation of dental hard tissues. PH value of the microenvironment, inhibit enamel demineralisation and remineralise early lesions. Several recent studies have begun to develop new bioactive materials using the dual functions of ZPs' own anti-bacterial adhesion and biomimetic mineralisation templates with excellent results[33, 34], offering promising alternative therapies for the prevention and repair of early caries. Since oral biofilm is a complex environment, if the environmental smart response (e.g., PH, enzyme, etc.) can be further utilised to design strategies for adaptive transformation of bactericidal / anti-adhesion functions of amphiphilic polymeric materials, efficient anti-biofilm will be achieved while reducing the use of antimicrobial drugs and the production of drug-resistant bacteria, and improving the biosafety of the materials. In the future, new intelligent anti-biofilms and remineralised novel bifunctional amphiphilic polymer composites need to be developed to promote their development in the direction of caries control.

REFERENCE

[1] SELWITZ R H, ISMAIL A I, PITTS N B. Dental caries [J]. The Lancet, 2007, 369(9555): 51-9.
[2] JAMES S L, ABATE D, ABATE K H, et al. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017 [J]. The Lancet, 2018, 392(10159): 1789-858.
[3] BOWEN W H, BURNE R A, WU H, et al. Oral Biofilms: Pathogens, Matrix, and Polymicrobial Interactions in Microenvironments [J]. Trends Microbiol, 2018, 26(3): 229-42.
[4] RASZEWSKI Z, NOWAKOWSKA D, WIECKIEWICZ W, et al. The Effect of Chlorhexidine Disinfectant Gels with Anti-Discoloration Systems on Color and Mechanical Properties of PMMA Resin for Dental Applications [J]. Polymers (Basel), 2021, 13(11).
[5] MEMARPOUR M, SOLTANIMEHR E, SATTARAHMADY N. Efficacy of calcium- and fluoride-containing materials for the remineralization of primary teeth with early enamel lesion [J]. Microsc Res Tech, 2015, 78(9): 801-6.
[6] ZHANG Y Y, WONG H M, MCGRATH C P J, et al. In vitro and in vivo evaluation of electrophoresis-aided casein phosphopeptide-amorphous calcium phosphate remineralisation system on pH-cycling and acid-etching demineralised enamel [J]. Sci Rep, 2018, 8(1): 8904.
[7] PETZOLD M. The influence of different fluoride compounds and treatment conditions on dental enamel: a descriptive in vitro study of the CaF(2) precipitation and microstructure [J]. Caries Res, 2001, 35 Suppl 1: 45-51.
[8] LASCHEWSKY A, ROSENHAHN A. Molecular Design of Zwitterionic Polymer Interfaces: Searching for the Difference [J]. Langmuir, 2019, 35(5): 1056-71.
[9] MI L, JIANG S. Integrated antimicrobial and nonfouling zwitterionic polymers [J]. Angew Chem Int Ed Engl, 2014, 53(7): 1746-54.
[10] ERFANI A, SEABERG J, AICHELE C P, et al. Interactions between Biomolecules and Zwitterionic Moieties: A Review [J]. Biomacromolecules, 2020, 21(7): 2557-73.
[11] WANG D Y, SU L, YANG G, et al. Self-targeting of zwitterion-based platforms for nano-antimicrobials and nanocarriers [J]. J Mater Chem B, 2022, 10(14): 2316-22.
[12] HE M, GAO K, ZHOU L, et al. Zwitterionic materials for antifouling membrane surface construction [J]. Acta Biomater, 2016, 40: 142-52.
[13] CHOI W, JIN J, PARK S, et al. Quantitative Interpretation of Hydration Dynamics Enabled the Fabrication of a Zwitterionic Antifouling Surface [J]. ACS Appl Mater Interfaces, 2020, 12(7): 7951-65.
[14] MANGAL U, KWON J S, CHOI S H. Bio-Interactive Zwitterionic Dental Biomaterials for Improving Biofilm Resistance: Characteristics and Applications [J]. Int J Mol Sci, 2020, 21(23).
[15] WU J, LIN W, WANG Z, et al. Investigation of the hydration of nonfouling material poly(sulfobetaine methacrylate) by low-field nuclear magnetic resonance [J]. Langmuir, 2012, 28(19): 7436-41.

[16] ZHANG N, CHEN C, MELO M A, et al. A novel protein-repellent dental composite containing 2-methacryloyloxyethyl phosphorylcholine [J]. Int J Oral Sci, 2015, 7(2): 103-9.
[17] ZHANG N, ZHANG K, MELO M A, et al. Effects of Long-Term Water-Aging on Novel Anti-Biofilm and Protein-Repellent Dental Composite [J]. Int J Mol Sci, 2017, 18(1).
[18] MULLER R, EIDT A, HILLER K A, et al. Influences of protein films on antibacterial or bacteria-repellent surface coatings in a model system using silicon wafers [J]. Biomaterials, 2009, 30(28): 4921-9.
[19] LI F, WEIR M D, FOUAD A F, et al. Effect of salivary pellicle on antibacterial activity of novel antibacterial dental adhesives using a dental plaque microcosm biofilm model [J]. Dent Mater, 2014, 30(2): 182-91.
[20] LEE M J, KWON J S, KIM J Y, et al. Bioactive resin-based composite with surface pre-reacted glass-ionomer filler and zwitterionic material to prevent the formation of multi-species biofilm [J]. Dent Mater, 2019, 35(9): 1331-41.
[21] SAKAGUCHI R L. Review of the current status and challenges for dental posterior restorative composites: clinical, chemistry, and physical behavior considerations. Summary of discussion from the Portland Composites Symposium (POCOS) June 17-19, 2004, Oregon Health and Science University, Portland, Oregon [J]. Dent Mater, 2005, 21(1): 3-6.
[22] COCCO A R, CUEVAS-SUAREZ C E, LIU Y, et al. Anti-biofilm activity of a novel pit and fissure self-adhesive sealant modified with metallic monomers [J]. Biofouling, 2020, 36(3): 245-55.
[23] LEE M J, KIM M J, OH S H, et al. Novel Dental Poly (Methyl Methacrylate) Containing Phytoncide for Antifungal Effect and Inhibition of Oral Multispecies Biofilm [J]. Materials (Basel), 2020, 13(2).
[24] ZHANG N, MELO M A, BAI Y, et al. Novel protein-repellent dental adhesive containing 2-methacryloyloxyethyl phosphorylcholine [J]. J Dent, 2014, 42(10): 1284-91.
[25] ZHANG N, WEIR M D, ROMBERG E, et al. Development of novel dental adhesive with double benefits of protein-repellent and antibacterial capabilities [J]. Dent Mater, 2015, 31(7): 845-54.
[26] XIE X, WANG L, XING D, et al. Novel dental adhesive with triple benefits of calcium phosphate recharge, protein-repellent and antibacterial functions [J]. Dent Mater, 2017, 33(5): 553-63.
[27] AHN S-J, KHO H-S, KIM K-K, et al. Adhesion of oral streptococci to experimental bracket pellicles from glandular saliva [J]. American Journal of Orthodontics and Dentofacial Orthopedics, 2003, 124(2): 198-205.
[28] CHAPMAN J A, ROBERTS W E, ECKERT G J, et al. Risk factors for incidence and severity of white spot lesions during treatment with fixed orthodontic appliances [J]. Am J Orthod Dentofacial Orthop, 2010, 138(2): 188-94.
[29] PARK S Y, YOO K H, YOON S Y, et al. Synergetic Effect of 2-Methacryloyloxyethyl Phosphorylcholine and Mesoporous Bioactive Glass Nanoparticles on Antibacterial and Anti-Demineralisation Properties in Orthodontic Bonding Agents [J]. Nanomaterials (Basel), 2020, 10(7).
[30] KWON J S, LEE M J, KIM J Y, et al. Novel anti-biofouling light-curable fluoride varnish containing 2-methacryloyloxyethyl phosphorylcholine to prevent enamel demineralization [J]. Sci Rep, 2019, 9(1): 1432.
[31] KANG S, LEE M, KANG M, et al. Development of anti-biofouling interface on hydroxyapatite surface by coating zwitterionic MPC polymer containing calcium-binding moieties to prevent oral bacterial adhesion [J]. Acta Biomater, 2016, 40: 70-7.
[32] LEE M J, KIM J Y, SEO J Y, et al. Resin-Based Sealant with Bioactive Glass and Zwitterionic Material for Remineralisation and Multi-Species Biofilm Inhibition [J]. Nanomaterials (Basel), 2020, 10(8).

[33] DONG X, PENG W, SUN J, et al. Zwitterionic/phosphonate copolymer coatings endow excellent antifouling properties and robust re-mineralization ability of dentine substrates [J]. J Mater Chem B, 2022, 10(31): 5976-88.
[34] HE J, YANG J, LI M, et al. Polyzwitterion Manipulates Remineralization and Antibiofilm Functions against Dental Demineralization [J]. ACS Nano, 2022, 16(2): 3119-34.