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Ionic Fluoropolyphosphazenes as Potential Adhesive Agents for Dental Restoration Applications

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Abstract

Clinically, the use of photo-activated polymer-based dental composites is the preferred method to restore carious teeth due to their esthetics and ease of application. However, the longevity of these resin-based composite tooth restorations can be compromised by the sensitivity of the bonded interface between the composite and the tooth surface. The objective of the current study was to modify the tooth surface with novel fluorinated polyphosphazenes (PPZs), thereby improving the stability of the interface. Binding isotherms of PPZs with collagen (CLG) and hydroxyapatite (HA), two of the primary components of teeth, were established and indicate significant and stable adsorption to the surfaces of these materials. PPZs were also shown to protect CLG against acidic dissolution in a model system. A composite material consisting of the fluorinated polymer and CLG demonstrated three-dimensional stability and significant hydrophobicity. Additionally, no hemolytic activity was observed when evaluated using a porcine Red Blood Cells (RBC) assay. Bovine dentin treated with PPZs demonstrated increased contact angle (hydrophobicity) compared with control samples and resisted fluid penetration when assessed using a dye penetration study. Finally, microhardness evaluation of bovine dentin treated with PPZs and exposed to an acidic challenge showed that treated dentin resisted demineralization. The hardness of the untreated control was significantly reduced after exposure when compared with the PPZ-treated samples. This study represents a novel approach to overcoming the current limitations of composite restorations. These results are promising to improve the longevity of composite dental restorations and may have wider use in sealants, varnishes and other dental applications.

Lay Summary

Tooth decay remains a prevalent problem worldwide. Polymer-based composites are the most frequently used tooth restorative used in the clinic. The longevity of these fillings is limited due to conditions in the mouth that can weaken the adhesive used to bond the composite to the natural tooth. The current study uses novel polyphosphazenes (PPZs), hybrid organic-inorganic macromolecules with tunable hydrophilic-

hydrophobic properties to coat the tooth surface to achieve better compatibility with the adhesive, thereby improving the longevity of the restoration. Results indicate that PPZs have significant and stable adsorption onto teeth, which may lead to a more stable bonded interface.

Future Works

Future works will validate the potential of ionic fluoropolyphosphazenes as bonding agents for existing composite restoration materials. Bactericidal properties of polyphosphazene coatings, as well as their potential utility as topical sealants will be also explored.

Keywords Polyphosphazenes – Fluoropolymers – Coatings – Dental Adhesives – Dentin - Bonding

Declarations

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Introduction

Dental caries remains a prevalent problem worldwide [1]. The longevity of tooth cavity restorations is limited, with half of all restorations failing in less than 10 years, mainly due to secondary caries and fracture [2-6]. Failed restoration replacement accounts for 50-70% of all restorations performed [7]. This is costly, considering that the annual cost for carious tooth restorations in the U.S. was approximately $46 billion in 2005 [8]. Furthermore, the need is rapidly increasing as baby boomers enter into retirement, with increases in life expectancy as well as tooth retention in seniors [8]. In particular, the need for Class V restorations has increased [9]. Gingival recession exposes more roots, which have a higher solubility and are less resistant to biofilm acids than tooth enamel [10]. In addition, reduced saliva flow tends to increase biofilm/plaque buildup [11]. Indeed, root caries increased from 7% among young people to 56% in seniors [12]. Class V restorations currently have a high failure rate, which further increases with patient age [13]. The challenge is especially severe in subgingival restorative margins with pockets for bacterial growth, which is difficult to clean and can promote periodontitis [13].

Due to their esthetics and direct-filling capabilities, resin-based dental composites (referred to as "composites" subsequently) are the principal material for cavity restorations [2, 3]. Improvements in filler particles and matrix polymers have significantly enhanced the composite properties [14]. Composites are bonded to the tooth via bonding agents. Advantages of using adhesive bonding agents when placing resin composites include: enhancing retention and stability and the reduction of microleakage, postoperative sensitivity, recurrent caries and pulp pathology. However, the bonded interface is widely considered the weak link of composite restorations [15, 16].

Traditional dental adhesives, also called three-step formulations, consist of an acid, primer and an adhesive. The acid is used to condition the surface by dissolving mineral crystals in dentin to exposure hydrated collagen fibrils. The primer contains hydrophilic monomers (traditionally methacrylate-based) that can infiltrate the demineralized dentin. The adhesive, containing mixtures of methacrylate monomers, is applied in the third step and infiltrates the primer treated surface to create a mechanical adhesion to the conditioned dentin surface. Recent advances in dentin bonding have consolidated the three-step method into two or single step methods where the acid etchant, primer and adhesive are contained in one or two bottles to ease the clinical application.

The hydrophilic monomers present in the primer and adhesive are necessary to infiltrate the demineralized dentin surface and create the mechanical interlocking with the collagen fibrils. However, significant water sorption can occur after polymerization which can lead to water diffusion through the bonded interface [17, 18]. As this mechanism progresses, the water may solubilize the hydrophilic resin monomers, exposing the collagen matrix and causing microgaps to form. Another factor when bonding agents contain hydrophilic monomers is phase separation. A common dental bonding system may contain two monomers, BisGMA (bisphenol A-glycidyl methacrylate) and HEMA (hydroxyethyl methacrylate). BisGMA is a critical component at the bonded interface, since it has superior mechanical properties and is responsible for

structural integrity and durability. HEMA has high water solubility and acts as a diluent to facilitate the penetration of BisGMA into the conditioned dentin surface. In the hydrated demineralized dentin matrix, however, phase separation of BisGMA from HEMA leads to limited infiltration into the treated surface and may reduce the durability of the composite restoration [19]. Additionally, this phase separation may reduce the polymerization of the adhesive and lead to increased water permeability at the bonded interface [20].

Polyphosphazenes - hybrid organic-inorganic macromolecules containing phosphorus and nitrogen backbone and organic pendant groups - display significant potential as materials for life sciences applications [21-23]. In particular, fluoroelastomer based on poly[di(trifluoroethoxy)phosphazene], PTFP [24-27] gained broad recognition as a resilient and comfortable soft denture liner (Novus®) [28-33]. Among many distinct advantages of this material, such as excellent shock absorbing and molding properties, its resistance to biofilm formation and excellent adhesive strength to polyacrylates are also noted [28, 29]. The latter properties render this material an attractive candidate for dental restoration applications. However, PTFP is an inherently hydrophobic water-insoluble polymer and, as such, cannot be expected to promote required binding to proteins, such as the main organic constituent of dentin – collagen (CLG). We have previously developed fluorophosphazenes containing carboxylic [34] or sulfonic acid [35] functionalities. The key feature of these novel fluoropolymers is that despite a high content of fluorinated moieties (up to 80 % mol), they display excellent solubility in aqueous solutions. Furthermore, due to their anionic nature, these macromolecules are capable of effective interactions with polyelectrolytes of the opposite charge, which makes them ideally suitable for creating nanocoatings using layer-by-layer assembly technique [35-37]. The resulting nanofilms are biocompatible and display high hydrophobicity and self-healing behavior [35-37].

The present paper investigates the potential of ionic polyphosphazenes as adhesive agents for dental restoration applications. Poly[(trifluoroethoxy)(carboxylatophenoxy)] containing various contents of fluorinated groups – FP20 (20 % mol) and FP77 (77 % mol), as well as their non-fluorinated analog – poly[di(carboxylatophenoxy)phosphazene, PCPP (Fig. 1) are shown to effectively bind and modify biologically relevant properties of main components of dentin – CLG and hydroxyapatite, HA. *In vitro* experiments conducted using bovine dentin demonstrate that polyphosphazene treatment leads to an increase in hydrophobicity and improved stability of dentin surface. Microhardness and dye penetration studies indicate that the polyphosphazene coatings can act as an effective barrier in the oral environment against water infiltration and acidic attack – the main causes of dental caries and restoration failure.

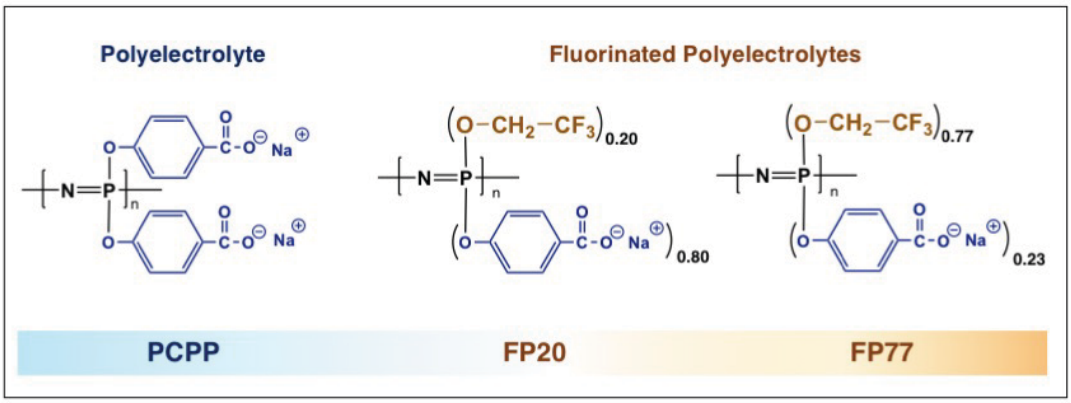

Fig. 1 Schematic presentation of polyphosphazene polyelectrolyte (PCPP) and its fluorinated mixed copolymers (FP20 and FP77)

Materials and Methods

Materials

PCPP (molecular weight 800 kg/mol), FP20 (20% mol trifluoroethoxy/80% mol carboxylatophenoxy groups; molecular weight 140 kg/mol), and FP77 (77% mol trifluoroethoxy/80% mol carboxylatophenoxy groups; molecular weight 200 kg/mol) were synthesized as reported previously [38, 39, 34]. The selection of these specific polymers was based on the desire to assess the outcomes in polymers with high and low concentrations of fluorinated groups. Additionally, these polymers were used in previous unrelated studies. Type I insoluble collagen (CLG) (lyophilized, from bovine flexor tendon) and type I CLG soluble in acetic acid (lyophilized, from calf skin) (VWR, West Chester, PA), hydroxyapatite, HA (≥90% as $Ca_3(PO_4)_2$) (Sigma-Aldrich, Milwaukee, WI), and phosphate buffered saline (PBS, 1X, pH 7.4) (ThermoFisher Scientific, Waltham, MA) were used as received. Freshly-extracted bovine incisors (Old Line Custom Meat Company, Baltimore MD) were cleaned and stored in 0.1% thymol until use.

Polymer Adsorption on CLG and HA

CLG (4 mg) was dispersed in solution of PCPP (0.5 mL; 0.05 to 1 mg/mL) or fluorinated polymer (0.5 mL; 0.05 to 2 mg/mL) in PBS. Each mixture was vortexed and left for four days while agitated (2100 Platform Shaker, New Brunswick Scientific, Enfield, CT). The samples were then centrifuged and the supernatant was analyzed by UV-spectrophotometry at 235 nm (Thermo Scientific NanoDrop 2000, Waltham, MA) to determine polymer concentration in solution. The amount of adsorbed polymer was calculated as the difference between initial and remaining polymer concentration in solution. Adsorption of PCPP or FP77 on HA was conducted similarly using 4 mg HA and 0.5 mL polymer solutions in PBS.

Acidic Dissolution of CLG and Stabilizing Effects of FP77 and PCPP

To evaluate protective effect of polymers on the dissolution of CLG in acidic environment, dispersed CLG systems were treated with solutions of PCPP or FP77. CLG (1 mg) was placed in an Eppendorf tube, to which polymer solution in PBS (1 mL of 0.1, 1, or 2 mg/mL) was added. A control sample was prepared by dispersing CLG (1 mg) in PBS (1 mL). Acetic acid was then added to 0.1 N concentration. The mixtures were stirred for one hour, centrifuged, and the supernatant was analyzed by UV-spectrophotometry to determine the amount of CLG released. To determine the percentage of CLG dissolved, peak absorbance at 208 nm was compared with that of the completely dissolved sample.

Modification of Bovine Dentin Surfaces – Water Contact Angle Studies

Bovine dentin disks were obtained from the coronal buccal surfaces and polished with 1200 and 2000-grit silicon carbide paper. PCPP and FP77 were dissolved in distilled water and FP20 was dissolved in ethanol at a concentration of 10 mg/mL. Prior to application of the polyphosphazenes, dentin disks were conditioned by application of 37% phosphoric acid gel for 15 s to etch the surface and remove the smear layer, which is composed of organic and inorganic debris on the dentin surface left as a result of cutting. The dentin was rinsed with distilled water and air dried for 10 s. Two coatings of each polyphosphazene were applied to the etched dentin surface using a microbrush and subsequently air dried. Treated dentin surfaces were subjected to one of the following test conditions: (1) immersion in saliva-like solution (50 mmol/L 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) buffer, 8.7 mmol/L $CaCl_2$, 8.7 mmol/L KH_2PO_4, 0.05ppm NaF, adjusted to pH 7 with KOH) at 37 °C for 3 days without agitation; (2) exposed to ultrasonication for 15 min; (3) dry. Water contact angle on etched dentin and treated dentin surfaces was then evaluated (DSA100, KRUSS, Matthews NC). Two drops of deionized water were dispensed on the tested surfaces and their dynamic wetting was tracked for 30 s.

Modification of Bovine Dentin Surfaces – Dye Penetration Studies

The permeability of polyphosphazene-coated dentin by assessing the penetration of a water-based dye through the coated dentin. Bovine incisors were stored in 0.1% thymol solution at 4 °C before use. Coronal dentin slabs were cut using a low speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA). Each crown was split through its mid-dentin region resulting in two dentin-faced slabs lined by either enamel or the pulp chamber. All surfaces of dentin slabs were covered with two layers of acid resistant nail varnish with the exception of the dentin surface that was to be treated with the polyphosphazene coating. Dentin slabs were conditioned by application of 37% phosphoric acid gel for 15 s to etch the surface and remove the smear layer. The dentin was rinsed with distilled water and air dried for 10 s. Two coatings of each polyphosphazene were applied to the etched dentin surface using a microbrush and subsequently air dried. Dentin samples were then exposed to saliva-like solution for 1 or 3 days without agitation (pH 7.0) or ultrasonically agitated for 15 min. Then, dentin slabs were immersed in a 1% (w/w) methylene blue solution (pH = 7.0) for 4 h. The samples were sectioned transversely and penetration of the blue dye was determined using a microscope.

Modification of Bovine Dentin Surfaces – Microhardness Studies

Bovine dentin disks were obtained from the coronal buccal surfaces and polished with 1200 and 2000-grit silicon carbide paper. PCPP and FP77 were dissolved in distilled water and FP20 was dissolved in ethanol at a concentration of 10 mg/mL. The dentin was rinsed with distilled water and air dried for 10 s. Two coatings of each polyphosphazene were applied to the etched dentin surface using a microbrush and subsequently air dried. Treated dentin surfaces were exposed to ultrasonication for 15 min. After exposure, the Vickers microhardness (Shimadzu Micro Hardness Testers HMV-G, Shimadzu Corporation, Kyoto, Japan) was measured on the dentin surface (n=6) and recorded. Subsequently, each dentin specimen was immersed in 5 mL of acidic saliva-like solution (8.7 mmol/L $CaCl_2$, 8.7 mmol/L KH_2PO_4, 0.05ppm NaF, 75 mmol/L acetic acid, pH 4.0 - adjusted with KOH) for 48 h to simulate acidic challenges experienced in the oral environment. After 48 h, dentin disks were rinsed with distilled water, dried and Vickers microhardness measurements were repeated and compared with the hardness before acid exposure.

Results

Adsorption of Polyphosphazenes on Hydroxyapatite (HA) and Collagen (CLG)

We first investigated the ability of ionic polyphosphazenes to interact with main components of dentin – HA and CLG focusing on potential differences in the behavior of highly fluorinated polymer (FP77) and its conventional counterpart – PCPP (Fig. 1). Adsorption of polyphosphazenes on HA was studied in PBS by monitoring the decrease in UV absorbance of polymers after incubating them with insoluble inorganic material. Polyphosphazenes showed significant adsorption on HA, however the maximum amount of deposited fluorinated polyacid was approximately 3-fold larger than for homopolymer - PCPP (Fig. 2a). The saturation was achieved at a somewhat lower concentration of PCPP in solution - 0.16 mg/mL, as opposed to 0.33 mg/mL for FP77. To further elucidate potential differences in interactions of FP77 and PCPP with HA, the results were re-plotted as the amount of adsorbed carboxylic acid groups versus their concentration in solution (Fig. 2b). As seen from the Figure, the maximum amount of deposited acidic groups, as well as concentration of ionic groups in solution, at which the saturation occurs, are similar for both polymers – 0.32 mg/mg and about 0.9 mEq/L correspondingly. This suggests the dominating role of ionic groups in the adsorption of polyphosphazene polyacids.

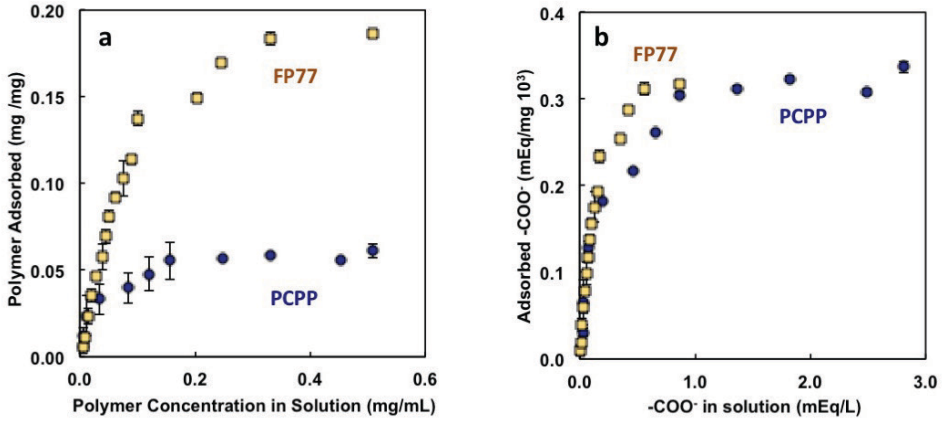

Fig. 2 (a) PCPP or FP77 adsorbed on HA versus their concentration in solution; (b) concentration of carboxylic acid groups adsorbed on HA versus their concentration in solution (PBS, pH 7.4, 4 mg HA, 0.05 to 0.3 mg/ml, adsorbed polymer values are shown in mg of polymer per mg of HA)

Dental adhesives typically rely on bonding to the CLG matrix of dentin, therefore interactions of polyphosphazenes with CLG were also studied. PCPP and fluoropolymers showed significant adsorption on CLG in aqueous solutions at neutral pH (Fig. 3a), however the values of deposited polymers were somewhat lower compared to the values for HA (Fig. 2a). The maximum amount of FP77 adsorbed on CLG was 0.13 mg/mg, as opposed to 0.19 mg/mg of fluorinated polymer deposited on the mineral material. Similarly to adsorption on HA, the maximum amount of deposited FP77 was approximately 2.5 fold larger than for PCPP (Fig. 3a) and the saturation was achieved at a significantly lower concentration of PCPP in solution (0.06 mg/mL) compared to FP77 (0.6 mg/mL). However, contrary to the results observed for HA, in which the maximum amount of deposited carboxylic acid groups was practically similar for both polymers, more ionic groups were adsorbed on CLG in case of PCPP.

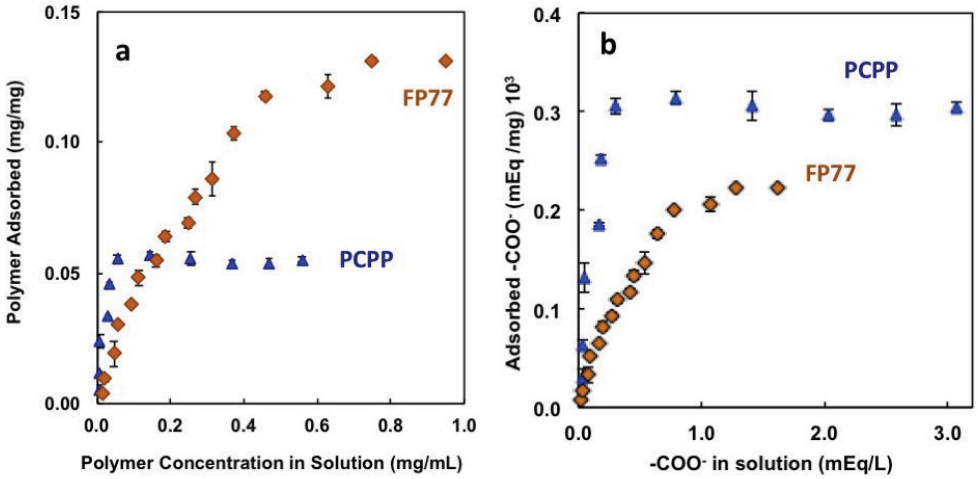

Fig. 3 (a) PCPP or FP77 adsorbed on CLG versus their concentration in solution; (b) concentration of carboxylic acid groups adsorbed on CLG versus their concentration in solution (PBS, pH 7.4, 4 mg CLG, 0.05 to 0.3 mg/ml, adsorbed polymer values are shown in mg of polymer per mg of CLG)

Polyphosphazene Coatings Inhibit CLG Dissolution in Acidic Environment

In the next set of experiments, we explored whether polyphosphazenes deposited on substrates, such as CLG, can provide coatings, which are sufficiently dense and uniform to enable protection against harsh environments. This was carried out by investigating the effect of polyphosphazenes on low pH digestion of CLG. Dispersed acid dissolvable CLG samples were treated with either PCPP or FP77 and then incubated in 0.1 N acetic acid for one hour. Both polymers were efficient in protecting CLG against dissolution in a dose dependent manner (Fig. 4a). Whereas unprotected CLG was completely dissolved under the conditions of the experiment, polyphosphazene coatings were able to protect up to 70% of the protein.

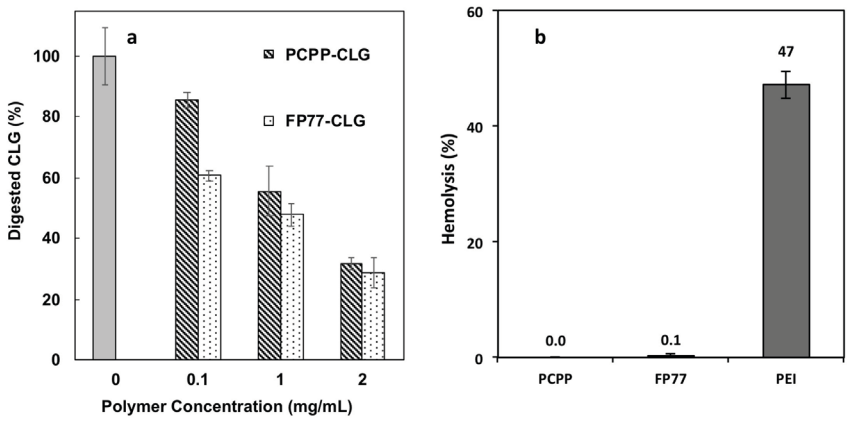

Fig. 4 (a) Acidic dissolution of CLG and stabilizing effect of FP77 and PCPP (0.1 N acetic acid); (b) hemolytic activity of FP77, PCPP and PEI (for comparative purpose) against porcine red blood cells

Hemolytic Activity of FP77 and PCPP

In vitro evaluation of polymer compatibility with blood components is a necessary part of early preclinical screening [40]. Hemocompatibility of FP77 and PCPP was evaluated using established porcine Red Blood Cells (RBC) assay [41-43]. No hemolytic activity was observed for polyphosphazenes, while cationic polyethylene imine (PEI), which was used for comparative purposes, showed about 50% lysis (Fig. 4b).

Fabrication of Hydrophobic FP77-CLG Composite Material

The ability of fluorinated polyphosphazene to serve as a bonding material for CLG was illustrated by fabricating composite protein-fluoropolymer material. FP77 was dissolved in water, mixed with dispersed CLG, cast in a mold, and lyophilized. Fig. 5a shows a solution cast composite FP77-CLG material of cylindrical shape demonstrating excellent bonding properties of the fluoropolymer. The composite was further "mineralized" with calcium ions through the treatment with aqueous solution of calcium chloride. Typically, curing of polyphosphazene polyacids with multivalent cations results in a formation of soft, ionically cross-linked hydrogels [34, 44, 45]. Notably, CLG loaded material based on fluoropolymer showed no swelling and no change in shape when exposed to aqueous solution (Fig. 5b). The lack of swelling observed is potentially related to ionotropic cross-linking of PCPP and its copolymers with calcium.

Fig. 5 (a) Images of FP77, CLG and the resulting composite material – FP77-CLG as solids and (b) after dissolution or immersion in aqueous solution

Modification of Bovine Dentin Surfaces – Water Contact Angle Studies

Typical dental adhesive systems consist of mixtures of hydrophilic and hydrophobic methacrylate-based resins. Hydrophilic monomers present in the primer and adhesive are necessary to infiltrate the demineralized dentin surface and create the mechanical interlocking with the collagen fibrils. However, significant water sorption can occur after polymerization which can lead to water diffusion through the bonded interface. As

this mechanism progresses, the water may solubilize the hydrophilic resin monomers, exposing the collagen matrix and causing microgaps to form. These microgaps can facilitate the penetration of acid-producing bacteria into the gap and, ultimately result in the failure of the composite restoration. Figure 6 shows the water contact angle (WCA) on treated and untreated, etched dentin surfaces as a function of time. Treatment of etched dentin surfaces with either FP20 or FP77 significantly increased the water contact angle and, therefore, hydrophobicity of the surface. The contact angle was reduced significantly over time for the untreated etched dentin, however double coating of the polyphosphazenes at 10 mg/mL resulted in consistently higher WCA. In fact, at 10 mg/mL, the contact angle is stable for 30 seconds, indicating that hydrophobicity was maintained and water sorption is reduced. Simulated salivary conditions and mechanical agitation via ultrasonication show little effect on the WCA. This behavior reflects the stability of the polyphosphazene layer on demineralized dentin.

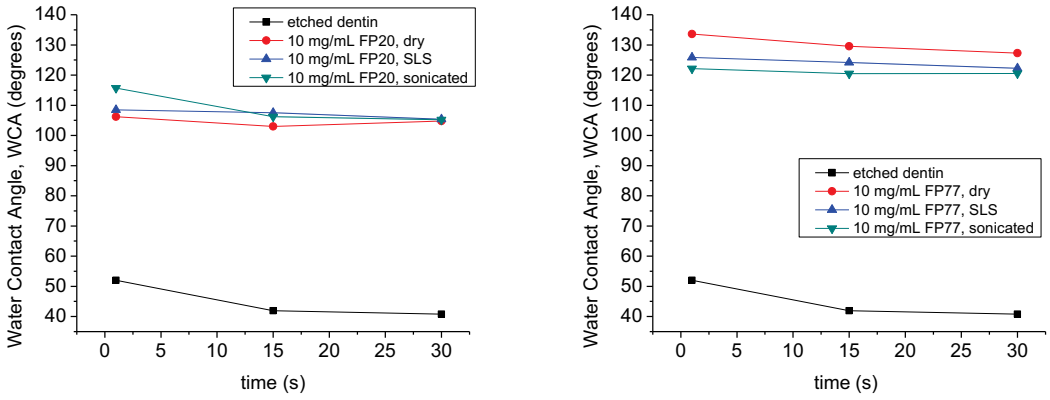

Fig. 6 Water contact angle (WCA) of acid-etched bovine dentin before and after mechanical and saliva-mediated challenges for dentin treated with (a) FP20 and (b) FP77 polyphosphazenes

Modification of Bovine Dentin Surfaces – Dye Penetration Studies

Following WCA measurements, we evaluated methylene blue dye penetration through polyphosphazene-coated dentin and control buffer-treated dentin samples. The dye significantly penetrated through the surface of control dentin compared to polyphosphazene-coated dentin. The dye penetration was hindered significantly in dentin coated with PCPP, FP20 and FP77 (Fig. 7). It should also be noted that dentin tubules are wider in bovine teeth than in human teeth [46], indicating that the polyphosphazene coatings are effective even in more challenging environments where dentin morphology would normally result in increased water

penetration. After challenge by ultrasonication and immersion in artificial saliva (SLS), the polyphosphazene-coated dentin maintained its resistance to dye penetration.

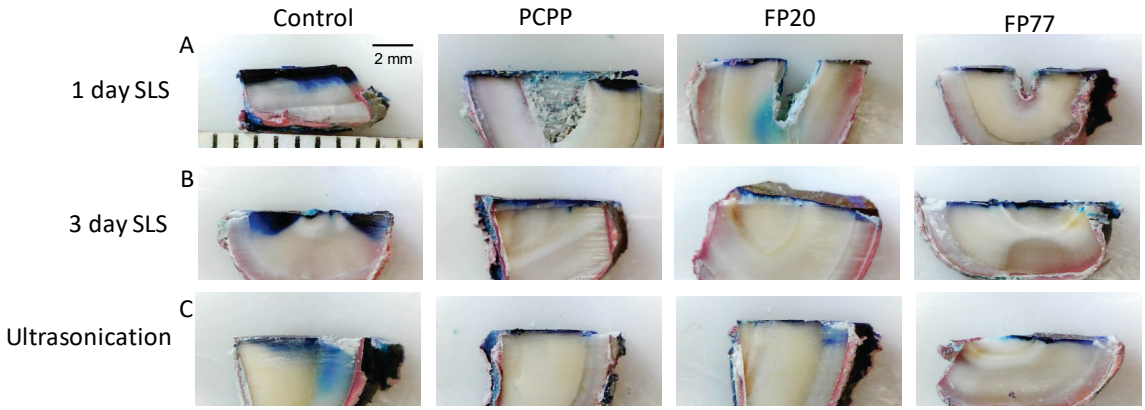

Fig. 7 Dye penetration in control (acid-etched) dentin and dentin treated with polyphosphazenes (PCPP, FP20, FP77) after (A) 1 day in saliva-like solution, (B) 3 days in saliva-like solution and (C) 15 min of mechanical agitation via ultrasonication. Significantly more dye permeation is seen in the untreated control dentin.

Modification of Bovine Dentin Surfaces – Microhardness Studies

Based on the results of the dye penetration tests, we assessed whether the application of different polyphosphazene coatings could protect exposed dentin against demineralization from an acidic challenge. As stated previously, acid attack by oral bacteria is a primary cause of dental restorative failure. Demineralization by acid attack has been shown to produce a decrease in the hardness of dental tissue. In this microhardness, dentin disk specimens were first exposed to a mechanical challenge similar to previous experiments via ultrasonication for 15 min and then exposed to an acidic form of saliva-like solution for 48 h. As seen in Fig. 8, dentin samples left untreated, exhibited an almost 50% loss in microhardness. Meanwhile, all dentin samples treated with either PCPP, FP20 or FP77 showed a significant amount of protection from acidic demineralization when compared with the control. Dentin samples treated with PCPP maintained 87% of their original hardness, whereas dentin treated with FP20 and FP77 maintained 77% and 81% respectively.

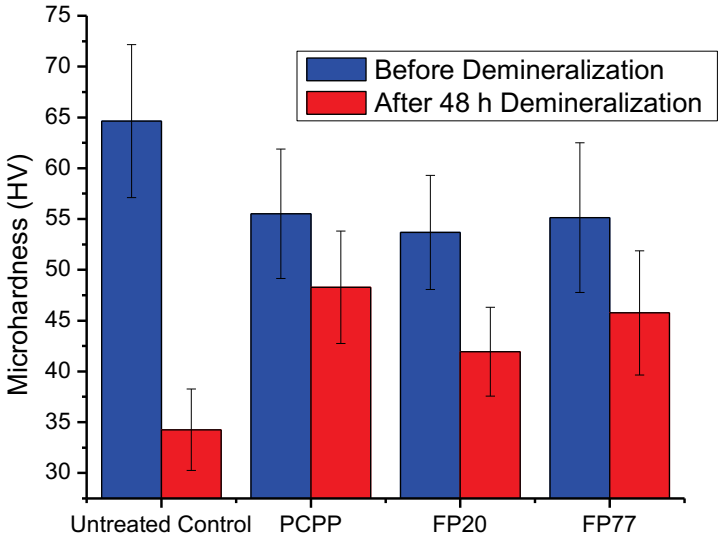

Fig. 8 Vickers microhardness in control (untreated) dentin and dentin treated with polyphosphazenes (PCPP, FP20, FP77) before and after exposure to an acidic demineralization solution for 48 h

Discussion

PTFP fluoropolyphosphazene has a long history as a biocompatible and biofilm resistant material for dental prosthetic devices [28-32], but its potential utility as dental adhesive has not been explored, presumably due to its highly hydrophobic nature and expected poor adhesion to proteins and inorganic materials [47]. Concurrently, PCPP – a water-soluble ionic polyphosphazene, has shown excellent avidity to inorganic bone constituent – HA and their composite materials were prepared and investigated for hard tissue replacement applications, primarily in orthopedics [48-50]. Development of polyphosphazene copolymers, which integrate both of those ionic and fluorinated functionalities in a single macromolecule [34, 35, 37, 36], opened a potential pathway to combining excellent biocompatibility of PTFP with strong avidity of PCPP to biological materials. In order to evaluate the potential of such hybrid polymers as dental adhesives, we first investigated fundamental aspects of their interactions with main dentin components – HA and CLG, and then demonstrated their ability to form coatings with advanced biologically relevant characteristics.

The results of studies on interactions between polyphosphazenes and main components of dentin, HA and CLG, demonstrate that these biological materials can effectively adsorb polyphosphazenes on their surfaces with a trend of accumulating fluoropolymer in larger quantities. In our experiments, the adsorption of fluorinated FP77 on both substrates was up to three-fold higher than that of its conventional counterpart. Deposition of polyphosphazenes on inorganic material, HA, appears to be entirely driven by electrostatic interactions as adsorption of macromolecules correlated with concentration of polymer bound ionic moieties. This is in line with previous findings on the dominating role of ionic interactions between HA and various carboxylic acids [51, 52]. Although the trend for a greater adsorption of fluoropolymer was also observed for

protein component of dentin, CLG, the avidity of PCPP to this substrate appeared to be higher. This can be illustrated by a semi-quantitative comparison of two binding isotherms (Fig. 3b) using concentrations of carboxylic acid functions at half-saturation of CLG, which roughly corresponds to a dissociation constant for a simplified binding model [53]. The three-fold magnitude difference in such apparent constants (0.3 mEq/L for FP77 vs. 0.1 mEq/L for PCPP) indicates a lower avidity of fluoropolymer compared to polyacid homopolymer. Although, ionic mechanism of interactions between soluble CLG and polyacids has been noted previously [54], the differences in the adsorption behavior of two polymers suggests presence of other mechanisms of interactions. As was reported previously, PCPP is capable of forming hydrogen bonds at neutral pH with proteins and other macromolecules due to the presence of non-dissociated carboxylic acid groups in this high charge density polyelectrolyte [55, 56]. These intermolecular interactions are especially pronounced for glycosylated proteins [56]. Since CLG is a naturally glycosylated protein, in which sugar molecules play an important role in the formation of structural fibrils [57], it is conceivable that the same mechanism extends to interactions between polyphosphazene polyacids and CLG. It may be further suggested that lower charge density of hydrophobic FP77 displays reduced efficiency in forming hydrogen bonds.

Biologically relevant properties of polyphosphazene coatings were explored by carrying out CLG acid dissolution experiments, which showed their ability to protect the protein against acidic environment caused by oral bacteria. Compounding of CLG and FP77 was performed by blending and casting from aqueous solutions, which resulted in a composite with distinct characteristics - hydrophobicity and excellent three-dimensional stability. The experiments also revealed the ability of fluoropolymer to bind calcium ions. Since the removal of calcium constitutes an important step in the dental restoration process - dentin “etching”, fluoropolymer can potentially be considered as a new alternative for the development of “self-etching” adhesive systems.

The resistance to dye penetration by bovine dentin treated with different polyphosphazenes is relevant because dental caries progression is catalyzed by secretion of acid by oral bacteria. The acids produced by these bacteria demineralize dental hard tissues and can degrade restorative resins [58]. Additionally, the reduced permeability of polyphosphazene-coated dentin might be relevant in preventing the elution of unreacted monomers to the pulp chamber, which can provoke severe post-operative sensitivity and pulpitis [59-61]. This is one of the main limitations to treat deep cavities with resin composite restorations. Finally, the polyphosphazene treated dentin may aid in the resistance to dental resin phase separation. A common dental bonding system may contain two monomers, BisGMA (bisphenol A-glycidyl methacrylate) and HEMA (hydroxyethyl methacrylate). BisGMA is a critical component at the bonded interface, since it has superior mechanical properties and is responsible for structural integrity and durability. HEMA has high water solubility and acts as a diluent to facilitate the penetration of BisGMA into the conditioned dentin surface. In the hydrated demineralized dentin matrix, however, phase separation of BisGMA from HEMA leads to limited infiltration into the treated surface and may reduce the durability of the composite restoration

[19]. Reduced water permeability in the polyphosphazene-treated dentin may lead to a reduction in phase separation and result in a more long-lasting dental restoration.

Finally, microhardness results, in conjunction with the dye penetration study indicate that the polyphosphazene coatings can act as an effective barrier in the oral environment against water infiltration and acidic attack which can lead to dental caries and restoration failure. These results are promising to improve the longevity of composite dental restorations and may have wider use in sealants, varnishes and other dental applications. Future studies will investigate the mechanical stability of the adhesive interface, the susceptibility of these coatings to hydrolysis as well as their stability when exposed to photopolymerization conditions.

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Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

References

1. Dye BA, Thornton-Evans G. Trends in Oral Health by Poverty Status as Measured by Healthy People 2010 Objectives. Public Health Reports. 2010;125(6):817-30. doi:10.1177/003335491012500609.

2. Deligeorgi V, Mjör IA, Wilson NHF. An Overview of Reasons for the Placement and Replacement of Restorations. Primary Dental Care. 2001;os8(1):5-11. doi:10.1308/135576101771799335.

3. Demarco FF, Corrêa MB, Cenci MS, Moraes RR, Opdam NJM. Longevity of posterior composite restorations: Not only a matter of materials. Dental Materials. 2012;28(1):87-101. doi:https://doi.org/10.1016/j.dental.2011.09.003.

4. Ferracane JL. Resin composite—State of the art. Dental Materials. 2011;27(1):29-38. doi:https://doi.org/10.1016/j.dental.2010.10.020.

5. Sakaguchi RL. 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 & Science University, Portland, Oregon. Dental Materials. 2005;21(1):3-6. doi:https://doi.org/10.1016/j.dental.2004.10.008.

6. Mjör IA, Moorhead JE, Dahl JE. Reasons for replacement of restorations in permanent teeth in general dental practice. International Dental Journal. 2000;50(6):361-6. doi:https://doi.org/10.1111/j.1875-595X.2000.tb00569.x.

7. Beazoglou T, Eklund S, Heffley D, Meiers J, Brown LJ, Bailit H. Economic impact of regulating the use of amalgam restorations. Public Health Rep. 2007;122(5):657-63. doi:10.1177/003335490712200513.

8. Saunders RH, Meyerowitz C. Dental Caries in Older Adults. Dental Clinics of North America. 2005;49(2):293-308. doi:https://doi.org/10.1016/j.cden.2004.10.004.

9. Amer RS, Kolker JL. Restoration of root surface caries in vulnerable elderly patients: a review of the literature. Special Care in Dentistry. 2013;33(3):141-9. doi:https://doi.org/10.1111/j.1754-4505.2012.00302.x.

10. Hoppenbrouwers PMM, Driessens FCM, Borggreven JMPM. The mineral solubility of human tooth roots. Archives of Oral Biology. 1987;32(5):319-22. doi:https://doi.org/10.1016/0003-9969(87)90085-9.

11. Griffin SO, Griffin PM, Swann JL, Zlobin N. Estimating rates of new root caries in older adults. J Dent Res. 2004;83(8):634-8. doi:10.1177/154405910408300810.

12. Curzon MEJ, Preston AJ. Risk Groups: Nursing Bottle Caries/Caries in the Elderly. Caries Research. 2004;38(suppl 1)(Suppl. 1):24-33. doi:10.1159/000074359.

13. Schätzle M, Lang NP, Ånerud Å, Boysen H, Bürgin W, Löe H. The influence of margins of restorations on the periodontal tissues over 26 years. Journal of Clinical Periodontology. 2001;28(1):57-64. doi:https://doi.org/10.1111/j.1600-051X.2001.280109.x.

14. Jokstad A, Bayne S, Blunck U, Tyas M, Wilson N. Quality of dental restorations FDI Commission Project 2–95*. International Dental Journal. 2001;51(3):117-58. doi:https://doi.org/10.1002/j.1875-595X.2001.tb00832.x.

15. Amirouche-Korichi A, Mouzali M, Watts DC. Effects of monomer ratios and highly radiopaque fillers on degree of conversion and shrinkage-strain of dental resin composites. Dental Materials. 2009;25(11):1411-8. doi:https://doi.org/10.1016/j.dental.2009.06.009.

16. Lim BS, Ferracane JL, Sakaguchi RL, Condon JR. Reduction of polymerization contraction stress for dental composites by two-step light-activation. Dental Materials. 2002;18(6):436-44. doi:https://doi.org/10.1016/S0109-5641(01)00066-5.

17. Tay FR, Pashley DH, Suh BI, Carvalho RM, Itthagarun A. Single-step adhesives are permeable membranes. J Dent. 2002;30(7):371-82. doi:https://doi.org/10.1016/S0300-5712(02)00064-7.

18. Tay FR, Pashley DH, Yoshiyama M. Two Modes of Nanoleakage Expression in Single-step Adhesives. J Dent Res. 2002;81(7):472-6. doi:10.1177/154405910208100708.

19. Kostoryz EL, Dharmala K, Ye Q, Wang Y, Huber J, Park J-G et al. Enzymatic biodegradation of HEMA/bisGMA adhesives formulated with different water content. J Biomed Mater Res, Part B. 2009;88B(2):394-401. doi:10.1002/jbm.b.31095.

20. Jacobsen T, Söderholm K-J. Some effects of water on dentin bonding. Dental Materials. 1995;11(2):132-6. doi:https://doi.org/10.1016/0109-5641(95)80048-4.

21. Allcock HR. Chemistry and Applications of Polyphosphazenes. Hoboken, NJ: Wiley; 2002.

22. Andrianov AK, editor. Polyphosphazenes for Biomedical Applications. Hoboken, New Jersey: John Wiley & Sons; 2009.

23. Andrianov AK, Allcock HR, editors. Polyphosphazenes in Biomedicine, Engineering & Pioneering Synthesis. ACS Symposium Series, vol 1298. Washington, DC: American Chemical Society; 2018.

24. Allcock HR, Steely LB, Singh A. Hydrophobic and superhydrophobic surfaces from polyphosphazenes. Polym Int. 2006;55(6):621-5. doi:10.1002/pi.2030.

25. Singh A, Steely L, Allcock HR. Poly[bis(2,2,2-trifluoroethoxy)phosphazene] Superhydrophobic Nanofibers. Langmuir. 2005;21(25):11604-7. doi:10.1021/la052110v.

26. Gleria M, Bertani R, Jaeger RD, Lora S. Fluorine containing phosphazene polymers. J Fluorine Chem. 2004;125(2):329-37. doi:https://doi.org/10.1016/j.jfluchem.2003.07.015.

27. Bates MC, Yousaf A, Sun L, Barakat M, Kueller A. Translational Research and Early Favorable Clinical Results of a Novel Polyphosphazene (Polyzene-F) Nanocoating. Regen Eng Transl Med. 2019:1-13. doi:10.1007/s40883-019-00097-3.

28. Gettleman L. Uses of Polyphosphazene in Dentistry. In: Gleria M, De Jaeger, R, editor. Applicative Aspects of Poly (organophosphazenes). Hauppauge, New York: Nova Science Publishers; 2004. p. 33-47.

29. Rathi S, Verma A. Resilient liners in prosthetic dentistry: An update. Int J Appl Dent Sci. 2018;4(3):34-8.

30. Razavi R, Khan Z, Haeberle CB, Beam D. Clinical Applications of a Polyphosphazene-Based Resilient Denture Liner. J Prosthodontics. 1993;2(4):224-7. doi:10.1111/j.1532-849X.1993.tb00414.x.

31. Saber-Sheikh K, Clarke RL, Braden M. Viscoelastic properties of some soft lining materials II—ageing characteristics. Biomaterials. 1999;20(21):2055-62. doi:https://doi.org/10.1016/S0142-9612(99)00109-X.

32. Gettleman L. Polypohosphazene Fluoroelastomer for Denture Liners and Facial Prosthetics. Phosphorus, Sulfur Silicon Relat Elem. 1999;144(1):205-8. doi:10.1080/10426509908546218.

33. Gettleman L, Farris C, LeBoeuf R, Rawls H, Dillingham E. IMPROVEMENT OF EXPERIMENTAL ELASTOMERS FOR PERMANENT SOFT DENTURE LINERS: PHYSICAL AND BIOLOGIC PROPERTIES. In: Saha S, editor. Biomedical Engineering I. Pergamon; 1982. p. 307-9.

34. Andrianov AK, Marin A, Peterson P, Chen J. Fluorinated polyphosphazene polyelectrolytes. J Appl Polym Sci. 2007;103(1):53-8.

35. Albright V, Marin A, Kaner P, Sukhishvili SA, Andrianov AK. New Family of Water-Soluble Sulfo–Fluoro Polyphosphazenes and Their Assembly within Hemocompatible Nanocoatings. ACS Appl Bio Mater. 2019;2(9):3897-906. doi:10.1021/acsabm.9b00485.

36. Selin V, Albright V, Ankner JF, Marin A, Andrianov AK, Sukhishvili SA. Biocompatible Nanocoatings of Fluorinated Polyphosphazenes through Aqueous Assembly. ACS Appl Mater Interfaces. 2018;10(11):9756-64. doi:10.1021/acsami.8b02072.

37. Albright V, Selin V, Hlushko H, Palanisamy A, Marin A, Andrianov AK et al. Fluorinated Polyphosphazene Coatings Using Aqueous Nano-Assembly of Polyphosphazene Polyelectrolytes. Polyphosphazenes in Biomedicine, Engineering, and Pioneering Synthesis. ACS Symposium Series, vol 1298: American Chemical Society; 2018. p. 101-18.

38. Andrianov AK, Chen J, LeGolvan MP. Poly(dichlorophosphazene) as a precursor for biologically active polyphosphazenes: Synthesis, characterization, and stabilization. Macromolecules. 2004;37(2):414-20.

39. Andrianov AK, Svirkin YY, LeGolvan MP. Synthesis and biologically relevant properties of polyphosphazene polyacids. Biomacromolecules. 2004;5(5):1999-2006.

40. Dobrovolskaia MA, Clogston JD, Neun BW, Hall JB, Patri AK, McNeil SE. Method for Analysis of Nanoparticle Hemolytic Properties in Vitro. Nano Lett. 2008;8(8):2180-7. doi:10.1021/nl0805615.

41. Yessine M-A, Leroux J-C. Membrane-destabilizing polyanions: interaction with lipid bilayers and endosomal escape of biomacromolecules. Adv Drug Delivery Rev. 2004;56(7):999-1021.

42. Lackey CA, Murthy N, Press OW, Tirrell DA, Hoffman AS, Stayton PS. Hemolytic activity of pH-responsive polymer-streptavidin bioconjugates. Bioconjugate chemistry. 1999;10(3):401-5.

43. Rozema DB, Ekena K, Lewis DL, Loomis AG, Wolff JA. Endosomolysis by masking of a membrane-active agent (EMMA) for cytoplasmic release of macromolecules. Bioconjugate chemistry. 2003;14(1):51-7.

44. Andrianov AK, Chen J, Payne LG. Preparation of hydrogel microspheres by coacervation of aqueous polyphosphazene solutions. Biomaterials. 1998;19(1-3):109-15.

45. Allcock HR, Kwon S. An ionically cross-linkable polyphosphazene: Poly[bis(carboxylatophenoxy)phosphazene] and its hydrogels and membranes. Macromolecules. 1989;22(1):75-9.

46. Lopes MB, Sinhoreti MA, Gonini Júnior A, Consani S, Mccabe JF. Comparative study of tubular diameter and quantity for human and bovine dentin at different depths. Braz Dent J. 2009;20(4):279-83.

47. Allcock HR, Steely L, Singh A, Hindenlang M. Hydrophobic and superhydrophobic polyphosphazenes. J Adhes Sci Technol. 2009;23(3):435-45.

48. Greish Y, Bender J, Lakshmi S, Brown P, Allcock H, Laurencin C. Composite formation from hydroxyapatite with sodium and potassium salts of polyphosphazene. J Mater Sci: Mater Med. 2005;16(7):613-20.

49. Greish YE, Bender JD, Lakshmi S, Brown PW, Allcock HR, Laurencin CT. Formation of hydroxyapatite–polyphosphazene polymer composites at physiologic temperature. J Biomed Mater Res, Part A. 2006;77A(2):416-25. doi:10.1002/jbm.a.30145.

50. Greish YE, Brown PW, Bender JD, Allcock HR, Lakshmi S, Laurencin CT. Hydroxyapatite–Polyphosphazane Composites Prepared at Low Temperatures. J Am Ceram Soc. 2007;90(9):2728-34. doi:10.1111/j.1551-2916.2007.01780.x.

51. Yoshida Y, Van Meerbeek B, Nakayama Y, Yoshioka M, Snauwaert J, Abe Y et al. Adhesion to and Decalcification of Hydroxyapatite by Carboxylic Acids. J Dent Res. 2001;80(6):1565-9. doi:10.1177/00220345010800061701.

52. Misra DN. Adsorption of Polyacrylic Acids and Their Sodium Salts on Hydroxyapatite: Effect of Relative Molar Mass. J Colloid Interface Sci. 1996;181(1):289-96. doi:https://doi.org/10.1006/jcis.1996.0380.

53. Kuriyan J, Konforti B, Wemmer D. The molecules of life: Physical and chemical principles. New York, NY and London: Garland Science; 2012.

54. Nezu T, Winnik FM. Interaction of water-soluble collagen with poly(acrylic acid). Biomaterials. 2000;21(4):415-9. doi:https://doi.org/10.1016/S0142-9612(99)00204-5.

55. Andrianov AK, Marin A, Fuerst TR. Self-assembly of polyphosphazene immunoadjuvant with poly(ethylene oxide) enables advanced nanoscale delivery modalities and regulated pH-dependent cellular membrane activity. Heliyon. 2016;2(4):Article e00102. doi:http://dx.doi.org/10.1016/j.heliyon.2016.e00102.

56. Andrianov AK, Marin A, Fuerst TR. Molecular-Level Interactions of Polyphosphazene Immunoadjuvants and Their Potential Role in Antigen Presentation and Cell Stimulation. Biomacromolecules. 2016;17(11):3732-42. doi:10.1021/acs.biomac.6b01251.

57. Terajima M, Perdivara I, Sricholpech M, Deguchi Y, Pleshko N, Tomer KB et al. Glycosylation and Cross-linking in Bone Type I Collagen. J Biol Chem. 2014;289(33):22636-47. doi:10.1074/jbc.M113.528513.

58. Kidd EAM, Fejerskov O. What Constitutes Dental Caries? Histopathology of Carious Enamel and Dentin Related to the Action of Cariogenic Biofilms. J Dent Res. 2004;83(1_suppl):35-8. doi:10.1177/154405910408301s07.

59. Hanks CT, Strawn SE, Watahai JC, Craig RG. Cytotoxic Effects of Resin Components on Cultured Mammalian Fibroblasts. J Dent Res. 1991;70(11):1450-5. doi:10.1177/00220345910700111201.

60. Ratanasathien S, Wataha JC, Hanks CT, Dennison JB. Cytotoxic Interactive Effects of Dentin Bonding Components on Mouse Fibroblasts. J Dent Res. 1995;74(9):1602-6. doi:10.1177/00220345950740091601.

61. de Souza Costa CA, Vaerten MA, Edwards CA, Hanks CT. Cytotoxic effects of current dental adhesive systems on immortalized odontoblast cell line MDPC-23. Dent Mater. 1999;15(6):434-41. doi:https://doi.org/10.1016/S0109-5641(99)00071-8.