Using Biomimetic Polymers in Place of Noncollagenous Proteins to Achieve Functional Remineralization of Dentin Tissues


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Supporting Information

ABSTRACT: In calcified tissues such as bones and teeth, mineralization is regulated by an extracellular matrix that includes noncollagenous proteins (NCP). This natural process has been adapted or mimicked to restore tissues following physical damage or demineralization by using polyanionic acids in place of NCPs, but the remineralized tissues fail to fully recover their mechanical properties. Here, we show that pretreatment with certain amphiphilic peptoids, a class of peptide-like polymers consisting of N-substituted glycines that have defined monomer sequences, enhances ordering and mineralization of collagen and induces functional remineralization of dentin lesions in vitro. In the vicinity of dentin tubules, the newly formed apatite nanocrystals are coaligned with the c-axis parallel to the tubular periphery, and recovery of tissue ultrastructure is accompanied by development of high mechanical strength. The observed effects are highly sequence-dependent with alternating polar and nonpolar groups leading to positive outcomes, whereas diblock sequences have no effect. The observations suggest aromatic groups interact with the collagen while the hydrophilic side chains bind the mineralizing constituents and highlight the potential of synthetic sequence-defined biomimetic polymers to serve as NCP mimics in tissue remineralization.

KEYWORDS: peptoids, dentin remineralization, in situ AFM, nanoindentation, TEM, SEM

INTRODUCTION

One of the salient features of biomineralization is that soluble proteins act in concert with an insoluble macromolecular scaffold to guide mineral formation. In some cases, such as tooth enamel, the scaffold is sacrificial, whereas in others, including bone and dentin, it becomes an intrinsic part of the resulting composite and contributes to tissue function. One of the major challenges for the successful repair of mineralized tissues is that an intimate spatial and temporal relationship between mineral, scaffold, and soluble matrix is critical to achieving the biomineral–matrix ultrastructure necessary for that function, but that relationship is poorly understood and difficult to recreate. Repair of dental caries provides a case in point. As caries penetrate the outer enamel, which contains little or no organic matrix, they begin to demineralize the underlying dentin, which consists of hydroxyapatite (HAP) nanocrystals located within collagen fibrils (intrafibrillar) and between collagen fibrils (extrafibrillar). The bacterial attack is also accompanied by the release of bacterial and endogenous proteases in dentin, which leads to hydrolysis of the collagen scaffold as well as the NCPs. When remineralization is attempted, the absence of NCPs inhibits the development of intrafibrillar HAP. Instead calcium phosphate precipitates on the lesion surface, and mineralization does not infiltrate the涂层。
individual collagen fibrils leading to low mechanical performance, e.g., low elastic modulus and hardness, of dentin.\textsuperscript{4,5} Recently, some acellular, biomimetic remineralization approaches using polyanionic polymers to mimic the activity of NCPs by regulating mineralization have shown promise for improved treatments.\textsuperscript{6−15} For example, the polymer-induced liquid precursor (PILP) process, which uses poly-L-aspartic acid (pAsp) to aid the delivery of mineralizing constituents in the form of nanoclusters into collagen fibrils and form apatite after deposition of amorphous calcium phosphate,\textsuperscript{6−9} promotes intrafibrillar mineralization critical to restoration of mechanical properties.\textsuperscript{4} However, use of these polyanionic polymers only mimics biomineralization to a limited degree. Dentin, like other calcified collagenous tissues, contains numerous matrix−mineral interfaces that are “bridged” and integrated by NCPs, which commonly have both conserved motifs that mediate matrix assembly\textsuperscript{16−19} and anionic functional groups that interact with the highly charged mineral surfaces. Moreover, \textasciitilde70% of the mineral content of dentin is extrafibrillar, and the amount of extrafibrillar mineral produced through the PILP process appears to be minor. Consequently, although polyanionic polymers can aid in forming intrafibrillar mineral, the extent, organization, and interaction of the HAP crystals and the collagen scaffold is inadequate for complete recovery of structure and function.

To improve this biomimetic strategy for functional remineralization, we began with the hypothesis that the function of NCPs is dependent upon their inherent sequence specificity, which allows scaffold- and mineral-directed motifs to be placed in an arrangement necessary to control both mineral
formation and organization of the mineralizing matrix. To test this hypothesis, we then designed a small library of synthetic sequence-defined polymers presenting a mix of polar and nonpolar side chains with the expectation that the polar groups would act like the polyionic polymers in complexing the inorganic ions while the nonpolar side-chains would mimic the phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Trp) residues in NCP sequences that are believed to contribute matrix–matrix interactions.20–23 As our surrogate for NCPs, we chose peptoids that are N-substituted glycines able to form biomimetic structures across a wide range of length scales.24–26 The peptoid backbone is identical to that of a peptide, but its side chains are attached to the nitrogen rather than the \( \alpha \)-carbon, eliminating inter- or intrachain hydrogen bonding and main-chain chirality, thus resulting in lower structural complexity and more facile synthesis. Peptoids also exhibit potent biological functionalities and are biocompatible.27–29 In a recent study,30,31 dramatic modifications of \( \text{CaCO}_3 \) crystal growth rate and morphology by peptoids highlighted the potential of using these polymers for biomimetic approaches to controlling mineralization and remineralization. Here, we showed the potency to guide and modulate HAP mineralization on collagen substrates. Peptoids are potent and versatile for re-creating ultrastructure and restoring the mechanical properties of tissues, useful for dentin remineralization given the fact that dentin does not undergo physiological remodeling and has limited self-regenerative ability.

**EXPERIMENTAL SECTION**

**Peptoid Synthesis.** Peptoids were synthesized on polystyrene Rink amide resin (0.57 mmol/g) using either a Prelude or AAPPTec automated peptide synthesizer and the solid-phase submonomer peptoid synthesis procedure according to previously described methods.32,33 The peptoids were cleaved from the resin using 95:2.5:2.5 TFA/H\(_2\)O/TIPS (v/v/v). The volatiles were removed using a Biotage V-10 evaporator to afford the crude peptoids, which were redissolved in a mixture of acetonitrile and water (1:1, v/v) and purified by reverse-phase HPLC using a C18 semipreparative column (5 \( \mu \)m, 250 mm \( \times \) 21.2 mm, C18 Vydac column) with a flow rate of 15 mL/min. The collected fractions were concentrated and lyophilized to

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**Figure 1.** Turbidity measurements showing the incubation time for nucleation of calcium phosphate from aqueous solutions ([Ca\(^{2+}\)] = 6.75 mM, [HPO\(_4\)\(^{2-}\)] = 3.15 mM in tris buffffer at 37 °C with Na\(_3\) added as a biocide). (a) The addition of peptoid C792-3 at the concentrations shown. The results are compared to the turbidity data for peptoid-free solutions (control) and solutions containing 3.7 \( \mu \)M of 27 kDa pAsp. (b) Inhibitory peptoid CC81-3, which delayed (blue and green curves, 2 and 4 \( \mu \)M, respectively) and completely inhibited (brown curve, 8 \( \mu \)M) nucleation of calcium phosphates.
afford the purified peptoids as white, fluffy powders. Analytical HPLC analyses were performed on a 5 μm, 150 mm × 4.6 mm C18 Vydac column at 60 °C with a flow rate of 1.0 mL/min. HPLC traces were monitored at 214 nm.

We synthesized peptoids exhibiting two basic designs, one of which consisted of alternating hydrophobic and hydrophilic groups, and the other was based on a diblock sequence (Scheme 1). Each contains carboxylic acids (N,N-carboxymethylglycine) as hydrophilic monomers and chloro- or dichloro-substituted 2-phenylglycine units (either N-(2-(4-chlorophenyl))ethyl)glycine, or N-(2-(2,4-dichlorophenyl))ethyl)-glycine) as hydrophobic monomers and were differentiated through variations in the number and/or position of the hydrophilic and hydrophobic groups.

Turbdity Measurements. To screen these candidate peptoids and assess their effectiveness in modulating calcium phosphate nucleation and growth in solution, we performed turbidity measurements commonly used in the life sciences for drug compound solubility, bacterial or fungal growth studies, protein aggregation, hydrophobic groups.

variations in the number and/or position of the hydrophilic and hydrophobic sequences to make various micromolar concentrations of each peptoid and assess their effects on mineralization of dentin lesions with only calcium–phosphate mixing. These experiments on the freshly prepared monolayer were performed using a NanoScope 8 atomic force microscope (Digital Instruments J scanner, Bruker) with hybrid probes consisting of silicon tips on silicon nitride cantilevers (SNL-10 triangular lever, k = 0.35 N/m, tip radius < 10 nm; resonance frequency = 65 kHz in air; Bruker). The drive amplitude was ~20 nm (in fluid), and the signal-to-noise ratio was maintained above 10. The scanning speed was 1 Hz. The amplitude set point was carefully tuned to minimize the average loading force (~50 pN) during in situ imaging.

The effects of various peptoid concentrations on calcium phosphate nucleation were investigated using in situ AFM on the freshly prepared monolayered collagen substrate in solutions at a fixed supersaturation, σ, of 3.34. The supersaturation is defined as $\sigma = \ln ([Ca^{2+}]^{3}([PO_{4}^{3-}]^{3}(OH^{-})^{7}/K_{sp})$, where $[Ca^{2+}]$ denotes the molar concentration of calcium ions, $[PO_{4}^{3-}]$ denotes the molar concentration of phosphate ions, and $K_{sp}$ denotes the equilibrium solubility constant at 25 °C. This supersaturated solution (σ = 3.34) was prepared by mixing 13.5 mM of CaCl$_2$·2H$_2$O with 6.3 mM of K$_2$HPO$_4$ in equal volumes to achieve $[Ca^{2+}] = 6.75$ mM and $[PO_{4}^{3-}] = 3.15$ mM.

Preparation of each collagen substrate by assembly into a monolayer was performed immediately before the calcium phosphate nucleation experiments. The collagen substrate was briefly cleaned by flushing 1 mL of deionized ($\geq$18 MQ) water to remove unbound collagen. Calcium and phosphate solutions were prepared immediately before use from reagent-grade calcium chloride (CaCl$_2$·2H$_2$O) and potassium phosphate dibasic (K$_2$HPO$_4$) in Tris-buffered saline (TBS, pH 7.4). Peptoids were introduced into the calcium solutions at various concentrations (100, 200, and 500 nM and 2 μM).

Transmission Electron Microscopy. Transmission electron microscopy (TEM) of 70 nm ultrathin sections prepared from two specimens per group were carried out to determine ultrastructures of mineralized lesion specimens, and selected area electron diffraction (SAED) was used to identify the nature and crystallinity of the mineral formed by remineralization treatments. Sections from either artifcial human dentin lesions. Peptoid polymers were added at various micromolar concentrations to calcium chloride solution or preincubated on dentin lesions before mineralization. An equal volume of dipotassium phosphate solution was added to the calcium–phosphate mix, resulting in a calcium-to-phosphate ratio of 2.14. Solutions were prepared from reagent-grade calcium chloride dihydrate (CaCl$_2$·2H$_2$O) and potassium phosphate dibasic (K$_2$HPO$_4$) dissolved in Tris-buffered saline (TBS, pH 7.4) and filtered to remove insoluble chemicals. Remineralization of dentin lesions with only peptoids was carried out for 14 days at 37 °C with constant solution stirring.

For testing whether peptoids counteracted or complemented poly-L- aspartic acid used in the PILP process, inhibitory peptoids were introduced (1) via preincubation on dentin lesions at fixed 2 μM for various times (2) or mixing at 2 μM with poly-L-aspartic acid in calcium solution. Each specimen was then remineralized following a process similar to the PILP process for a period of 14 days at 37 °C.

Atomic Force Microscopy. Monolayer Collagen Assembly. The collagen (brand name: Purecol) was obtained from Advance Biomatrix Corporation. This collagen solution contains 3.1 mg/mL of collagen (purified bovine type I (97%) and type III collagen (3%)) at pH 2.0 and was diluted in a phosphate buffer containing 300 mM KCl and 10 mM Na$_2$HPO$_4$ at pH 7.4. The initial collagen concentration was fixed at 12 μg/mL in all cases to exclude the possibility of collagen assembly in liquid crystalline phase, which is known to occur in tissues and at high collagen concentrations (>20 mg/mL).

The collagen solutions at desired concentration (12 μg/mL) were then applied onto a freshly cleaved muscovite mica disc (diameter = 9.9 mm, Ted Pella, Inc.) and left for a 60 min incubation at pH 7.4, which was sufficiently long for collagen assembly onto the substrate. All in situ AFM images were captured in tapping mode at room temperature (23 °C) with a NanoScope 8 atomic force microscope (Digital Instruments J scanner, Bruker) with hybrid probes consisting of silicon tips on silicon nitride cantilevers (SNL-10 triangular lever, k = 0.35 N/m, tip radius < 10 nm; resonance frequency = 65 kHz in air; Bruker). The drive amplitude was ~20 nm (in fluid), and the signal-to-noise ratio was maintained above 10. The scanning speed was 1 Hz. The amplitude set point was carefully tuned to minimize the average loading force (~50 pN) during in situ imaging.
Scanning Electron Microscopy. Selected specimens (n = 2 per group) from the artificial lesion and remineralization groups were evaluated by scanning electron microscopy to characterize structural variations of remineralized dentin lesions. Specimens were coated with a 10–20 nm thick Au thin film using a sputter coater (Denton Vacuum Inc., Model # Desk II, Moorestown, NJ) and imaged using a Hitachi S-4300 field emission gun scanning electron microscope (Hitachi High Technologies America, Pleasanton, CA) at an accelerating voltage of 10 kV and using working distances larger than 12 mm in general.

Elastic Modulus Measurements. Previous work has shown that measurement of mineral content alone does not always reflect the restoration of mechanical properties. Therefore, we relied on nanoindentation to characterize structural variations of remineralized dentin lesions. Specimens were coated with a 10–20 nm thick Au thin film using a sputter coater (Denton Vacuum Inc., Model # Desk II, Moorestown, NJ) and imaged using a Hitachi S-4300 field emission gun scanning electron microscope (Hitachi High Technologies America, Pleasanton, CA) at an accelerating voltage of 10 kV and using working distances larger than 12 mm in general.

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Reduced Elastic Modulus of Lesions from Bottom to Top (Cross-Section Measurement). Briefly, we did a series of nanoindentations along the cross sectioned lesions that extended from the most demineralized portion of the specimen through the transition zone and finished on the deeper normal dentin. This was done while the specimen was fully hydrated.

Cross sections of the lesion were prepared by removing excess moisture by blotting the dentin blocks containing the treated lesions prior to embedding them in room temperature curing epoxy (Epoxycure, Buehler, Lake Bluff, IL). The embedded blocks were cut with a slow speed saw under water (Buehler, Lake Bluff, IL) perpendicular to the treated occlusal surface to reveal the lesion profile. A thin slice obtained from the center of the specimen (∼1200 μm) was glued onto the AFM specimen discs (Ted Pella, Redding, CA) with a small amount of cyanoacrylate (QX-4, MDS Products, Laguna Hills, CA) and then polished through steps to a final polish with 0.25 μm diamond paste. Indentations in the lesion cross-section were made with a Berkovich tip (tip radius of ∼100 nm) with a loading force varied over 200–500 μN to accommodate an E_R range from 0 to over 20 GPa.
Reduced Elastic Modulus of Peritubular Mineralization. Optical microscopy and AFM contact mode imaging were used on the cross-sectioned specimens to identify the depth and region with peritubular dentin modified by the peptoid-PILP treatment. The specimen was then carefully polished down to reveal the target depth in such a way that the tubule orientation was perpendicular to the polished surface so that the indentation values would be least affected by the underlying intertubular dentin. Site-specific Es values were obtained with a 3 μm trapezoidal loading curve as a cross-sectional lesion in wet conditions. A sharp cube-corner tip (tip radius ~20 nm) was employed for improved topographic imaging immediately after indentation. Indentation depths of 64–154 nm reflecting the differences in the material were obtained with a constant loading force of 300 μN. Dentin tubule occlusion induced by peptoids was imaged and evaluated by AFM: specimens without inhibitory peptoids were polished down to the same target depth as that of peptoid-treated specimens that showed modification induced by the peptoids. Contact-mode AFM in dry conditions was used to capture images in three randomly selected areas (100 × 100 μm) of the specimens mineralized both with and without peptoid. The number of fully or partially occluded tubules and non-occluded tubules were counted to obtain the ratio of one to the other.

## RESULTS AND DISCUSSION

### Screening Peptoids Using Turbidity Test.

Peptoids with a diblock structure (e.g., C792-3 or C792-5, Scheme 1) produced minor inhibition (C792-3) or a slight acceleration of nucleation (C792-5), displaying turbidity curves very similar to those obtained without the addition of peptoid (Figure 1a and Figure S1). For that result to be placed in context, poly-L-aspartic acid used in the PILP process was found to strongly inhibit nucleation, producing a nearly flat curve (Figure 1a and Figure S2). In contrast to the diblock peptoids, those with hydrophobic and hydrophilic side groups alternately arranged along the backbone (e.g., CC81-3, Scheme 1) exhibited significant inhibition of calcium phosphate mineralization with the incubation time increasing as the peptoid concentration increased (Figure 1b and Figure S2). In fact, no nucleation was detected for times exceeding 16 h at a CC81-3 concentration of 8 μM (Figure 1b). Inhibition of calcium phosphate nucleation is characteristic of natural NCPs and thus thought to be a key factor in collagen biomimeralization. We therefore chose inhibition or nucleation lag time as a criterion for selecting peptoids to be used in observations of collagen assembly and mineralization as well as dentin remineralization.

### Peptoids Potential to Improve Biomimetic Mineralization.

We used in situ AFM to observe the assembly of collagen on mica into ordered matrices in which the collagen molecules are coaligned and exhibit the 67 nm periodicity of native collagen fibrils (Figure 2). After assembly in Tris buffer (Figure 2a, left), the addition of inhibitory peptoids appeared to induce improved order (within 10 min) as measured by the degree of coverage and continuity of the gap and overlap zones, with higher peptoid concentration leading to further improvements and the creation of a nearly uniform and continuous sheet of ordered collagen (compare Figure 2a left vs right).

Mineralization of the ordered collagen matrices was then induced by injecting 2 mL of calcium phosphate solution ([Ca++] = 6.75 mM, [HPO4−2] = 3.15 mM in tris buffer) into the AFM fluid cell, as described previously. Under these conditions, the initial phase to form is amorphous calcium phosphate (ACP), which then transforms to octacalcium phosphate (OCP) and finally to HAP. The results show that the addition of inhibitory peptoids to the collagen matrices impacts both the nucleation of ACP and its transformation to the crystalline phase (Figure 2b–e). For peptoid concentrations of 500 nM or less, both the incubation time for nucleation and the time to transformation were decreased with the size of the effects growing as the peptoid concentration was increased (Figure 2b,c,e). However, at higher concentrations (2 μM), both nucleation and transformation were inhibited (Figure 2d,e). The findings show that these peptoid designs also produce interactions with the inorganic constituents that impact the mineralization kinetics.

### Dentin Remineralization Using Peptoids As Modulators.

Given their ability to promote remineralization of artificial human dentin lesions produced using acetic acid demineralization of human tooth as described previously, we tested their ability to promote remineralization of artificial human dentin lesions produced using acetic acid demineralization of human tooth as described previously (Figure 3a,b). In contrast to the results with either the peptoid-free control or the diblock peptoid sequences, when dentin lesions were first incubated in solution containing inhibitory peptoids (30 μL of 2 mM peptoid solution until dry) prior to mineralization in peptoid-free solution (14 days in 40 mL of solution at 37 °C with [Ca++] = 4.5 mM, [HPO4−2] = 2.1 mM in tris buffer with NaN3 added as a biocide), a uniform calcium phosphate mineral layer formed on top of the lesion (Figure 3b–d). This mineral layer closely contacted the underlying dentin lesion without gaps at the interfaces (Figure 3c,d), forming a potentially protective layer on the demineralized lesion. Dentin tubules were also filled with calcium phosphate precipitates (Figure 3e), and this further adhered the surface mineral layer to the dentin lesion.

Despite the encouraging results, the newly formed mineral layer did not permeate into the lesion matrix. Because previous work using the PILP process via introduction of pAsp to remineralize artificial dentin lesions led to extensive intrabfrillar mineralization in collagen matrices, we evaluated the potential synergistic effects of peptoids and pAsp for improving dentin remineralization. The incubation step was carried out with a mixture of inhibitory peptoid and pAsp solutions (2 mM peptoid and 100 μg/mL of 27 kDa pAsp for 1 h, 1 day, or 5 days), and remineralization was then performed in pAsp-containing solution (14 days in 40 mL of 100 μg/mL 27 kDa pAsp at 37 °C with [Ca++] = 4.5 mM, [HPO4−2] = 2.1 mM in tris buffer with NaN3 added as a biocide).

The results show that the combination of pretreatment by inhibitory peptoids and mineralization via the PILP process produces remarkable recovery of dentin ultrastructure to the point that the remineralized lesion resembles normal dentin (Figure 4a–d) with the extent of remineralization depending on incubation time (Figure S3). In all cases for which dentin lesions (Figure 4a,b) were preincubated with inhibitory peptoids, a collar of mineralization with substantial thickness was grown at the peritubular region of each dentin tubule akin to normal peritubular dentin (Figure 4d), and the zone of mineralization extended into the lesion matrix. In contrast, PILP treatment alone induced mineralization in the collagen matrix but did not re-establish the peritubular dentin (Figure 4c). Longer peptoid preincubation promoted greater regrowth of peritubular dentin (see Figure S3). Mixing peptoids with pAsp in the PILP remineralization solution, i.e., no preincubation, did not produce obvious peritubular mineralization (Figure S3).
mineral layers to clinical treatment, we performed AFM-based nanoindentation on the polished cross sectional surfaces of remineralized coronal dentin lesions. Whereas normal peritubular dentin has a very constant $E_R$ of approximately 30 GPa,6,53–55 approximately one-third of the depth of the lesion from the surface, the peritubular mineral of occluded tubules produced using pretreatment with inhibitory peptoids and PILP-based remineralization exhibited remarkably higher values of the reduced elastic modulus ($E_R$) ranging from 40 to $55$ GPa (Figure 4l). Examination of indentations obtained using an identical loading force for each measurement clearly shows the enhancement of the elastic modulus (Figure 4k). The indentations on the hard peritubular mineral collar are much smaller and shallower than those in the remineralized intertubular dentin regardless of position, an outcome consistent with the exceptionally high values of $E_R$. Similar measurements on the intertubular dentin matrix show it also recovered more than 60% of its reduced elastic modulus (Figure S4). The peritubular mineral growth induced by the inhibitory peptoid completely or partially filled at least 46% of tubules, much more structural recovery compared to lesions remineralized with only the PILP process (see Figure S5). The main reason for the larger $E_R$ values is the higher mineral content that results from incubation of the dentin with peptoid solution, as seen in Figure 4d. The alignment of the HAP platelets perpendicular to or at a high angle to the direction of the indentation force may also be a contributing factor.

The findings reported above show that the use of peptoids designed to interact with both the collagen matrix and mineralizing constituents enhances the in vitro assembly of collagen, impacts subsequent scaffold mineralization, and has a dramatic effect on remineralization of dentin lesions. The improvement of collagen assembly upon introduction of the inhibitory peptoid (compare Figure 2a left vs right) may be attributed to the interactions between the aromatic side groups of peptoids and the proline (Pro) and hydroxyproline (Hyp) residues of collagen monomers. Previous work showed that protons donated by Pro and Hyp residues could interact with acceptors such as electron-rich aromatic groups, leading to CH⋯π interactions.20–23 Other studies showed that π–σ interactions between peptoids lead to their self-assembly into highly ordered structures.24,25 Thus, the high content of both Pro and Hyp on the surface of the collagen triple helix can be expected to promote extensive interactions with the phenethyl side groups of the peptoids and enhance the intercollagen interactions that promote coalignment.

The impact on mineral formation is also understandable given the large number of carboxylic groups, which are well-known to complex Ca and comprise a large fraction of the amino acid side chains in natural biomineral-associated proteins. The observed change from acceleration to inhibition of both nucleation and phase transformation as the concentration of inhibitory peptoids was increased is reminiscent of the biomimetic activity reported for carboxyl-rich synthetic peptides,56 proteins extracted from natural biominerals,57 and even certain peptoids,58 all of which accelerated the growth of calcite crystals at nM concentrations but inhibited growth at higher concentrations with the strongest inhibitors being the most potent accelerants. There as well, a mix of hydrophobic and hydrophilic groups was found to be important to achieve the effect and, though the mechanism remains unclear, ion binding by the carboxyl

Close examination of the regrown peritubular minerals (Figure 4e,f) showed that those formed through the PILP process after pretreatment with inhibitory peptoids were composed of lamellae of fine-crystalline apatite nanocrystals stacked along the tubular periphery (Figure 4e) with better apparent alignment than those of normal dentin (Figure 4f). Indeed, diffraction data showed that their c-axes were mostly parallel to the long axis of the tubules (Figure 4h). Thus, treatment of the dentin lesions with inhibitory peptoids led to a high level of control over HAP nanocrystal formation and alignment.

Such distinctive ultrastructure of peritubular mineral layers is likely to produce exceptional mechanical properties. To determine the mechanical properties and evaluate the prospective application of these peptoid-induced peritubular
residues and hydrophobic interactions by the nonpolar groups were implicated.

The high degree of nanocrystal alignment and packing in the peritubular region combined with the enhanced mechanical performance, which were absent when polyanionic polymers alone were used, suggests that the integration of both functions, i.e., collagen binding and mineral ion complexation, into a single polymer is a critical design element. Moreover, the

Figure 4. Peptoid-induced peritubular mineralization and change in mechanical properties. (a−d) SEM images of (a) normal dentin, (b) artificial dentin lesions, (c) lesion remineralized via PILP process (40 mL of solution at 37 °C with [Ca²⁺] = 4.5 mM, [HPO₄²⁻] = 2.1 mM in tris buffer with NaN₃ added as a biocide), and (d) via PILP process with preincubation in peptoid CC81-3 solution (2 μM peptoid and 100 μg/mL of 27 kDa pAsp for 5 days). The addition of the incubation step with peptoid CC81-3 induced peritubular mineralization (compare insets in (c) and (d)), resembling normal peritubular dentin. (e, f) TEM images with SAED data (g, h) showing (e, g) peritubular minerals generated with the addition of peptoid CC81-3 compared with (i, h) those of normal peritubular dentin. Tb: dentin tubule. Peptoid-induced peritubular minerals consist of coaligned apatite nanocrystals (double-head arrow guiding the view of alignment). (i−k) AFM images showing peritubular remineralization. (i) Dentin tubule prior to remineralization. (j) Dentin tubules 30−50% occluded by peritubular mineralization when the dentin lesion was incubated with 2 μM peptoid prior to PILP remineralization. (k) Series of nanoindentations made across the occlusal surface of a remineralized dentin lesion in both the peritubular and intertubular regions following peptoid-induced remineralization. (l) Reduced elastic modulus ($E_R$) determined from the nanoindentations in (k). Values within the peptoid-induced peritubular mineral from 40 to 55 GPa, which are markedly higher than the values measured either across the intertubular region or in normal peritubular dentin (28.6 GPa). AFM image shows triangular indents across the occlusal surface of a remineralized dentin lesion using peptoid+PILP process. The vertical scales of all AFM images are in nm (i−k).
lack of any effect when diblock peptoids with the same constituent monomers are employed shows that polymer sequence is also a critical design element. Presumably, the alternating hydrophobic–hydrophilic design maximizes the exposure of the phenethyl side groups to the collagen and of the carboxyl groups to the solvated ions when the peptoids and collagen helices are themselves coalesged, providing a ready template to promote coagliment of the resulting HAP nanocrystals.

CONCLUSIONS

The peptoids chosen for this study were based on highly simplified designs with little of the compositional or structural complexity found in NCPs, yet they exhibit a remarkable level of control over dentin lesion remineralization. Given the large diversity of potential side chain chemistry (e.g., over 100 peptoid monomers are commercially available) and the huge number of possible combinations of monomers, even for the short 12-mer designs used here, the results presented here highlight the tremendous potential offered by synthetic sequence-defined polymers like peptoids as substitutes for NCPs in medical applications of tissue mineralization.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsbiomater.7b00378.

Details of turbidity measurements from exemplar peptoids, effect of peptoid preincubation, reduced elastic modulus profile, and AFM topographic image of remineralized dentin (PDF)

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Notes

The authors declare no competing financial interest.

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