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Is there value in chemical modification of fish scale surfaces?

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ABSTRACT: Fish scales are an abundant biowaste apparently unused, except for isolating major components as feedstocks, sacrificing the useful properties inherent to scales. We modified scale surfaces using hydrophilic and hydrophobic (meth)acrylates and tetraethylorthosilicate with in situ polymerization, and partial degradation of the biomineral or collagen layer. Chemical changes were assessed qualitatively by Fourier-transform infrared spectroscopy and quantitatively by nanomechanical analysis. No modification was selective but they were "preferential". Hydrophobic modifications were inefficient and reduced scale modulus. Inorganic and hydrophilic modifications were efficient and increased modulus. On adding sodium-citrate-modified scales to a weak alginate hydrogel, rheology showed an order of magnitude increase in storage modulus compared to alginate with no or unmodified scale reinforcement. Fish scales can be a useful new reinforcement. This work highlights simple pathways to manipulate surface composition and modulus of waste fish scale to enhance composite properties. © 2015 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2016**, *133*, 42868.

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INTRODUCTION

Nature routinely uses hierarchical structures to give materials exceptional abilities, such as the superhydrohobicity of the lotus leaf,[1] the exceptional strength of nanostructured cellulosic fibrils,[2] or the toughness of shell nacre.[3] Scientists are studying how these hierarchical designs impart superior properties to materials. The desire to replicate these designs gave rise to the science of biomimetics. Nevertheless, our current level of synthetic and nanomanufacturing skills makes replication challenging and expensive. With current manufacturing capabilities, only high-value applications are likely to be able to employ such complex biomimetic materials in the near future. For low-cost materials, a better strategy is to find ways to employ natural materials in composites so that the composites can benefit from their properties.

Fish scales are an abundant and growing waste resource and they also have a hierarchical structure. Annual harvests are ~140–176 million tonnes/year (2005); so assuming a 1% dry mass for scales of food fish, 1.4 million tonnes of waste scales, or more than 400,000 tonnes/year of scales from farmed fish, are available annually.[4] Despite their abundance, there seem to be no uses for intact scales, though some scales are used industrially, by harvesting their major components,[5–7] i.e., hydroxyapatite (HAP) from the biomineral layer and collagen, to be used individually. The recovery of individual components from biowaste is valuable and consistent with a "bio-refinery" approach to using biomass, but sacrifices the beneficial mechanical properties built into the scale's design.

Prior studies have investigated the hierarchical design of individual scales,[8,9] the mechanical properties of individual scales,[6,9,10] mathematical analyses to understand[11] and simulate their mechanical properties,[12] and the arrangement of scales to see how they work together as an assembly.[13] Much of the above work seems intended to aid the efforts of scientists and engineers to understand, reproduce, and perhaps exceed scale properties using synthetic materials, e.g., for body armor or other armors. That is why the bony scales of the alligator gar[14] and the ctenoid scales of arapaima, a South American fish

Additional Supporting Information may be found in the online version of this article.

whose scales resist predation by piranha,[15,16] are most studied.[12,14]

Structurally, fish scales possess an outer (top) surface that is a calcium-deficient hydroxyapatite (HAP) layer, also called the biomineral layer, because it contains organic components in addition to HAP. Its composition is similar to dentin.[17] The scale's inner layer is flexible, consisting of smaller layers of collagen fibers[18] arranged at 90° to one another. The thickness of a scale may vary, but the alternating collagen layers are each ~50 μm thick.

Understanding scale design has fundamental value, but neither gar nor arapaima scales are an abundant resource, and so their scales are not valued as a material resource themselves. Nevertheless, studies on the Arapaima prove valuable mechanical properties. Recent studies on the ctenoid scales of the striped bass (*Morone saxatilis*)[6] prove useful properties in abundant food fish scales too, evaluating failure modes and overall scale toughness, and showing those scales resisted penetration more effectively than either polystyrene or polycarbonate. A key mechanism used by ctenoid scales to dissipate energy without failure was shown to be the ability of the stacked collagen layers to slide past one another. One study of cycloid scales was also found that used X-ray microscopy to study the collagen fiber structure of the red seabream (*Pagrus major*), harvested from the Sea of Japan.[19]

Few studies sought to use intact scales as materials. One prior study looked at unmodified scales as reinforcement in epoxy but found only modest improvement in tensile and flexural strengths.[20] Although they reported H-bonding between the epoxy and the scale, this may have been insufficient for effective load transfer. Another prior study used scales as a support for TiO_2 nanoparticles for photocatalysts,[21] but only as a support and not to benefit from the properties of the scales themselves. Our long-term interest is to find uses for fish scale waste that can take advantage of the properties of the scales from food fish.

In an effort to explore the value of whole fish scales as a material resource, we studied the cycloid scales of the lake whitefish (*Coregonus clupeaformis*), in the family of Salmonidae, which are abundant and extensively harvested throughout Canada and the northern United States. Here we provide the first study of the nanomechanical properties of the relatively unstudied cycloid type of fish scale, and the first investigation of the ability to chemically alter the surfaces of fish scale, and the effects of those changes on the surface composition and nanomechanical properties. These studies may point the way to applications for fish scales beyond simply isolating scale components. Potential applications for scales include use as packaging additives and composite reinforcements, possibly including hydrogels. As these applications, and many others, benefit from modifying the interface, our studies looked at Fourier-transform infrared spectroscopy (FTIR) to identify key changes from modifications and those effects on the nanomechanical and moisture properties of the scales, and effects on rheology when a surface-modified scale was added to a hydrogel.

EXPERIMENTAL

Materials

Whitefish scales were obtained from processing facilities that harvested fish taken from Lake Superior, Huron, and Michigan. Hydrochloric acid (HCl_{conc}, 37%), sodium hydroxide (NaOH), tetraethyl orthosilicate (TEOS, 98%), methyl methacrylate (MMA, 99%), oligo (ethylene glycol) methyl ether acrylate (OEGA, *Mn* = 480g/mol), tert-butyl methacrylate (MA, 98%), sodium citrate (≥99.9%), sodium alginate, calcium carbonate ($CaCO_3$, ≥99.9%), and D-(+)-gluconic acid δ-lactone (GDL, ≥99.0%), and 2,2′-azoisobutyronitrile (AIBN, 98%) were purchased from Sigma-Aldrich. Monomers MA, MMA, and OEGA were purified by passing through a neutral aluminum oxide column to removed inhibitor. AIBN was recrystallized from ethanol. Clorox® was purchased from a local store. The MSDS reports pH 10 and composition as 5–10% NaOCl. The other chemicals were used as received.

Fish Scale Modifications

Figure 1 illustrates the expected scale surfaces from the different scale treatments. The designation given to all samples is shown

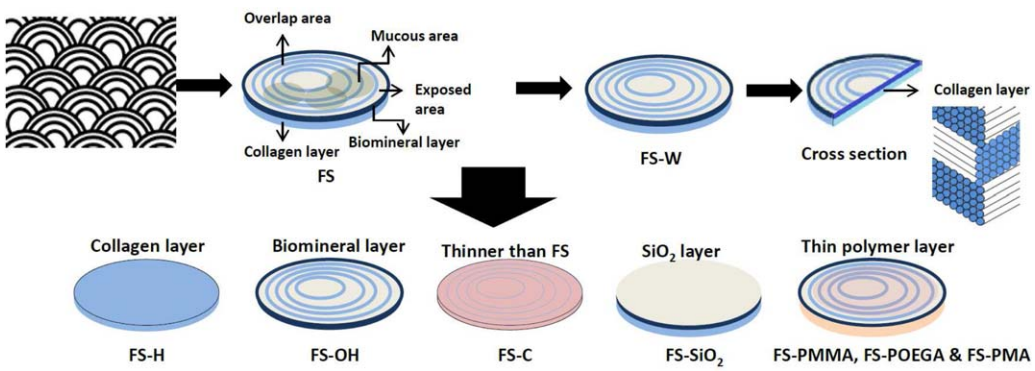

Figure 1. The diagram illustrates different scale structures of unmodified scales (top row) and chemically modified scales (bottom row). From left: an assembly of scales showing their overlap (top left); a representation of an individual scale before and after washing, and the alternating collagen rod layers. The second row represents the expected changes in structure resulting from chemical modifications. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table I. Summary of Designations

Scale designation	Description of scale processing
FS	As-received scales. Hand washed in water.
FS-W	FS-W scales with additional washing in water in an ultrasonic bath (40 kHz) 1.5 h.
FS-H	FS-W scales 3.3% (wt %) HCl(aq) solution in an ultrasonic bath (40 kHz) for 1.5 h.
FS-OH	FS-W scales 3.3% (wt %) NaOH(aq) solution in an ultrasonic bath (40 kHz) for 1.5 h.
FS-C	FS-W scales washed 2.5 min in Clorox® solution of 2 tsp/gal water.
FS-SiO_2-C	FS-W scales immersed in TEOS/HCl (Si/H^+ 8:1) for 0.5 h, then heat treatment (70°C/4 h).
FS-SiO_2	FS-W scales immersed in TEOS/HCl (Si/H^+ 8:1) for 1 h, followed by heat treatment (70°C/4 h).
FS-PMMA	FS-W scales immersed for 1 h in monomer containing 1 wt % AIBN initiator, followed by heat treatment (70°C/4 h).
FS-POEGA	FS-W scales immersed for 1 h in monomer containing 1 wt % AIBN initiator, followed by heat treatment (70°C/4 h).
FS-PMA	FS-W scales immersed for 1 h in monomer containing 1 wt % AIBN initiator, followed by heat treatment (70°C/4 h).

in Table I, along with an outline of the procedure used to prepare that sample.

Cleaning Fish Scale Surfaces (FS and FS-W). As-received fish scales (FS) had been previously hand-washed in water. These scales were immersed in D.I. water (ca 2 g fish scale in 20 mL D.I. water), and placed in an ultrasonic bath for 90 min at 42 kHz. These fish scales were decanted and rinsed three times with D.I. water, then air dried at room temperature, and used as unmodified control specimens. These scales are differentiated from the as-received FS scales by the designation FS-W.

Fish Scales Treated by Acid or Base (FS-H and FS-OH). Scales (2 g) were immersed in 20 mL of a 3.3 wt % HCl(aq) solution, and then placed in an ultrasonic bath for 90 min at 42 kHz. The fish scales were decanted and rinsed three times with D.I. water. The fish scales were air dried at room temperature, and are designated as FS-H. The same procedure was used for scales that were immersed in a 3.3 wt % NaOH(aq) solution. These scales are designated FS-OH.

Fish Scales Treated by Clorox® (FS-C). Scales (FS) were immersed in a Clorox® solution (~10 mL per gallon of water, active agent NaOCl) with stirring for 2.5 min. The scales were decanted, rinsed three times with water, and then air-dried. These scales are designated FS-C.

Fish Scales Modified with SiO_2 (FS-SiO_2 and FS-SiO_2-C). Fish scales were immersed in TEOS and acidified water (H^+/Si = 8:1 mol:mol). The mixture was stirred at room temperature for 60 min. The scales were decanted, and the surface rinsed with D.I. water. The scales were then placed in a convection oven at 70°C and heated for 4 h. These scales are designated as FS-SiO_2. For comparison, the second specimen was prepared in the same manner, except it was soaked in the same TEOS/HCl solution for only 30 min. It was also placed in a convection oven at 70°C and heated for 4 h. These scales are designated as FS-SiO_2-C.

Polymer-Modified Fish Scales (FS-PMMA, FS-POEGA, and FS-PMA). Fish scales (FS or FS-W) were immersed in the selected monomer (MMA, OEGA, or MA) into which AIBN (1 wt %) had already been dissolved. The fish scales were stirred in the solution for 60 min at room temperature. Excess monomer was decanted and the scales were heated at 70°C for 4 h in a convection oven. The resulting scales are designated as FS-PMMA, FS-POEGA, or FS-PMA.

Preparation of Modified Alginate Hydrogel

Fish Scales Modified with Sodium Citrate (FS-NaC). Fish scales were cut into small pieces (1–2 mm). The cut scales (2 g) were added into a solution of sodium citrate (1 g in 50 mL of D.I. water). Then the solution was adjusted to pH 10 using NaOH(aq). The mixture was stirred overnight at room temperature, filtered to collect the modified scale pieces (FS-NaC), and further washed with D.I. water until no NaOH residue remained. The FS-NaC pieces were dried in air.

Preparation of Alginate Hydrogel Samples. The unmodified alginate hydrogel was prepared by sodium alginate, $CaCO_3$, and GDL.[22] First, sodium alginate (1.2 g) was dissolved in deionized water (30 mL) to get 4.0 wt % sodium alginate solution. $CaCO_3$ (0.111 g in D.I. water, 5 mL suspension) was added to the sodium alginate solution under stirring, giving a molar ratio of calcium ion to carboxyl of 0.2 (Ca^{2+}:COO^- = 1:5). Then, a fresh GDL solution (0.395 g GDL in 5 mL D.I. water) was added. The molar ratio of $CaCO_3$ to GDL was 1:2 to maintain the hydrogel at a neutral pH. The mixed suspension (40 mL) was transferred into a mold and stored in a high humidity chamber at room temperature for 24 h. The resulting alginate hydrogel (3.0 wt %) was cut using a 15 mm punch tool to obtain samples for rheological test. For hydrogels with modified or unmodified fish scale, the scale pieces were added to the sodium alginate solution (1 g of the desired fish scale pieces) giving 3.3 wt % scales with respect to the alginate solution and 2.5 wt % with respect to the final solution) to get the modified alginate hydrogels.

Preparation of Scale Cross-Sections

Scales were embedded in an epoxy resin (EpoxiCure; Buehler, Lake Bluff, IL, USA) inside a cylindrical mold. The embedding resin was allowed to harden (24 h, room temperature), and then removed from the mold. Each sample was polished using silicon-carbide paper under a continuous water jet to wash away abraded material. The specimens were polished until a cross-section near the center of the scale was reached.

Derjaguin–Muller–Toropov (DMT) Modulus Using Atomic Force Microscopy (AFM)

All AFM experiments were carried out with a Dimension ICON AFM system (Bruker, USA). Peakforce Tapping mode was

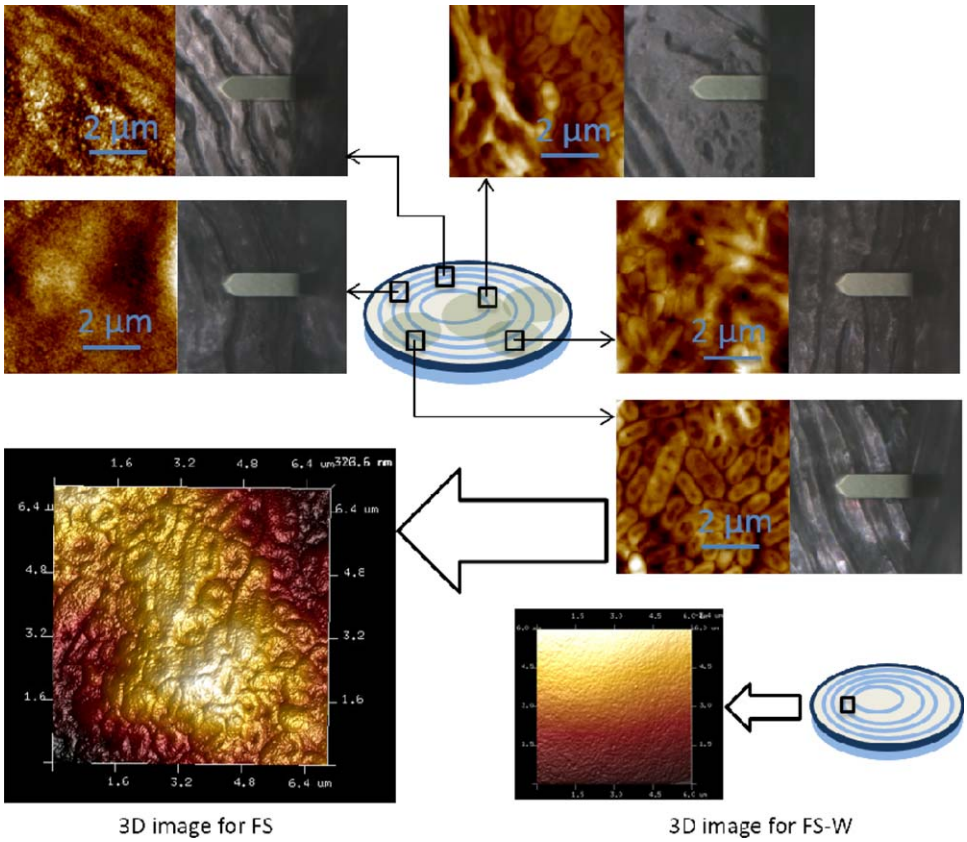

Figure 2. The biomineral surface of FS control scales imaged by optical microscope and AFM. The probe tip in the optical images has a 40 μm diameter. AFM images show a 6 × 6 μm area. At bottom, a topographical AFM image maps the roughness of the biomineral layer. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

applied for all the nanomechanical measurements and topography imaging.[23] RTESPA silicon cantilevers (Bruker, USA) were used. The spring constant was calibrated using Sader's methods[24] before each experiment, and the calibrated values were in the range from 32 to 49 N/m. The measurements were made using Peakforce QNM mode[25] to characterize the nanomechanical properties of fish scale. At each imaging pixel, the AFM tip was brought to contact with the sample surface with specified contact force, e.g. 500 ~ 600 nN in our experiments. The AFM system recorded the curve of tip–sample separation vs interaction force, and fitted the curve with the DMT model. In this way, the elastic modulus at each imaging pixel could be calculated, and the modulus mapping was achieved. The mechanical properties that were provided by Peakforce Tapping mode were along the out-of-plane direction, e.g., normal to the measured surface in our experiment.

Other Characterization Methods

FTIR spectra were recorded on a Perkin–Elmer Spectrum One FTIR Spectrometer from 4000 to 500 cm^{-1} using a 4.0 cm^{-1} resolution. Equilibrium moisture content (EMC) was obtained by placing samples of precisely known mass (±0.0001 g) in an EMC chamber at 25°C with 80% relative humidity. The measurements were repeated three times. The rheological properties of alginate hydrogels were determined by using a rheometer (HR-2, TA Instruments, New Castle, DE). The samples were punched to 15 mm in diameter and 2 mm thick. The hydrogels were subjected to 10% strain at 25°C and a frequency sweep of 0.1–100 Hz was carried out while maintaining a constant gap of 2200 μm.

RESULTS AND DISCUSSION

Visualization and Analysis of Unmodified Scales: FS and FS-W

Cycloid scales, like the more studied ctenoid scales, have two types of layers: the outer surface biomineral (largely hydropxyapatite) layer and the inner layer consisting of alternating layers of collagen rods. As Figure 2 shows, the biomineral surface is rough with regular ridge features of ~30–40 μm diameter that alternate with depressions having a similar or slightly smaller diameter. These ridges form, like growth rings on a tree, as the scales grow so scales have variable thicknesses with the newer edges being thinner than those at the scale center. These are the dominant features of the scales, but other small variations in features depend on the scale's placement on the fish with respect to other scales. The upper scale surface that is exposed to the environment is generally rougher than the portion of the scale that is overlapped by other scales (Figure 1). Most of these features are similar to those that have been described for other types of scales by others.[16,26] However, we also observed that the portion of the scale that is covered by other scales shows unusual features that appear cell-like but seem to be too small

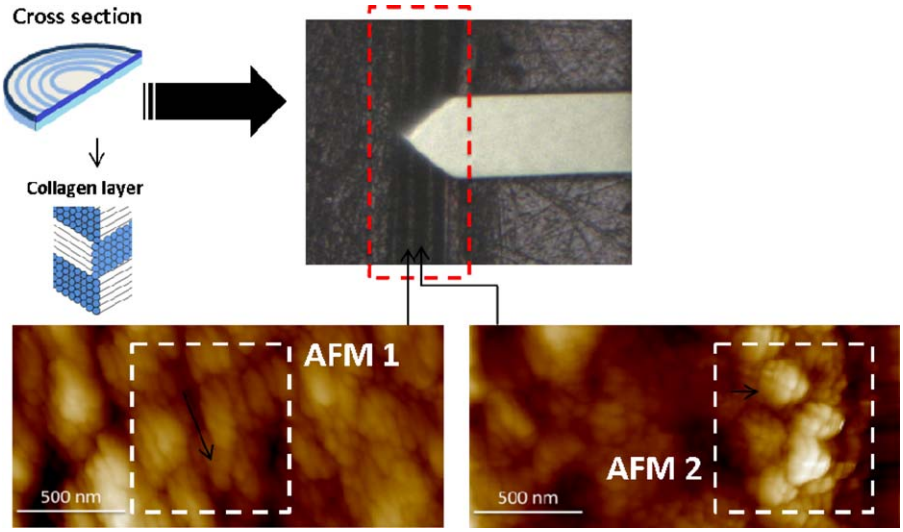

Figure 3. Cross-section of FS-W showing the bundles of collagen rods. Image at left shows the side view of the collagen bundles within a collagen layer; image at right shows the end-on view. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

for cells ($\leq$1 μm). The topographical AFM image at the bottom of Figure 2 shows that these features are oval to circular. Interestingly, these features are only observed in the FS scales. Since they were not found even in FS-W scales, these features were removed by low-energy ultrasonic washing; it seems likely that the features might be the dried remains of the scales' mucous layers, which possess the mucous/goblet cells.[27] Because each side of an individual scale is complex with varied features, the degree of chemical modification and resulting nanomechanical properties can also vary significantly depending on the site of testing. For that reason, the nanomechanical testing site is noted both for the surface tested and the location on that surface, or when a cross-sectional interface was tested then where within that cross-section.

Figure 3 shows the cross-section of an FS-W scale. The optical image shows multiple layers of collagen bundles with different orientations as evidenced by the light and dark grey striations. The AFM images confirm these collagen layers and the projecting collagen bundles made up of individual collagen rods are clearly seen at two different angles in the AFM images. These alternate in a similar manner to those reported for the ctenoid scales of arapaima gigas.[16] AFM1 (left) shows the collagen bundles in a collagen layer from an side direction (perpendicular to the direction of the bundle) while AFM2 (right) shows the collagen bundles in a collagen layer end on (in the direction of the bundles of collagen rods), so the tops of the collagen rods are observed. These images show that the hierarchical structure, and especially the collagen rod structure, is retained after the ultrasonic washing, which is important because the alternating layers with bundles of collagen rods are what impart toughness to the scales.

Partial Removal of a Scale Component: FS-H, FS-OH, and FS-C

FTIR Analysis of Changes in Composition and Structure. The baseline composition of the biomineral and collagen surfaces of FS and FS-W scales was assessed (Figure 4) by FTIR to identify changes in each layer after modification. Key bands in the collagen layer are the N—H stretching band (3288 cm^{-1}), the C═O stretch band (1633 cm^{-1}), and the N—H bending band (1540 cm^{-1}). The only differences observed in the

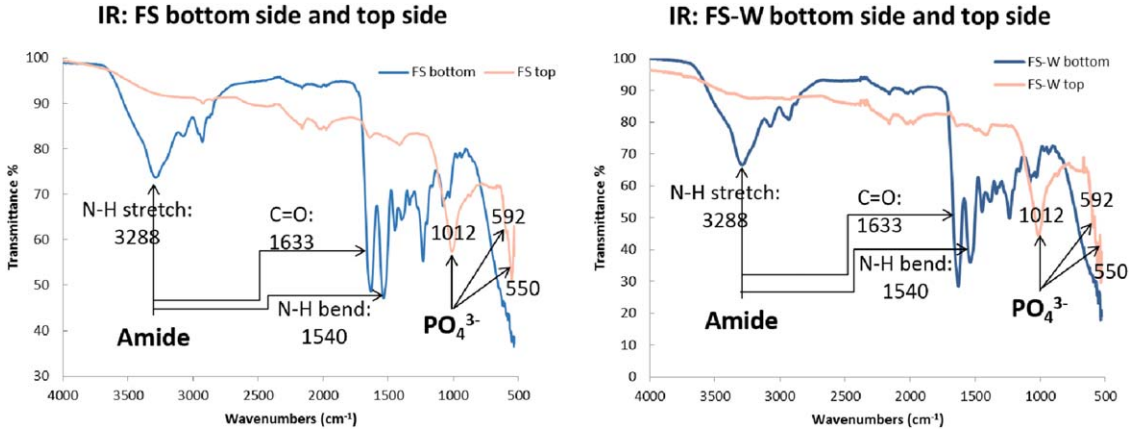

Figure 4. FTIR of (a) FS and (b) FS-W. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FS-W compared to FS was the relative size of the amide N—H bending band (1540 cm^{-1}) compared to the amide C═O stretching band (1633 cm^{-1}). A small reduction in one of the alkyl C—H bands, near 3000 cm^{-1}, may have occurred on the bottom (collagen) surface. The removal of residual mucous layer components from FS by ultrasonic washing can account for this.

The biomineral surface shows the expected hydroxyapatite PO_4^{3-} bands at 1012, 592, and 550 cm^{-1}. A small loss in the minor band near 3000 cm^{-1} is likely from the organic components, predominantly proteins and glycoproteins, from residual mucous layer, but this makes little distinctive contribution to the FT-IR spectra.

In our initial study, we adapted published procedures designed to completely remove one or the other layer, to preferentially but only partially degrade on of the layers. Acidic solutions degrade or destroy the biomineral layer, having little effect on the collagen layer,[28] so here a dilute HCl solution was used to give FS-H scales (see Supporting Information Figure S1a). Basic solutions decompose collagen, leaving the biomineral layer behind,[7] so here dilute NaOH was used to give FS-OH (see Supporting Information Figure S1b).

The absorptions from PO_4^{3-}, which are strong in FS and FS-W, are significantly reduced in FS-H scales, with the top surface showing only a very small band at 1012 cm^{-1}. Also both the top and bottom surfaces of the FS-H include obvious bands from amide bonds of collagen. These bands are barely evident in the top surface of FS and FS-W. Therefore, even the mild acid wash removed a significant portion of the biomineral layer, but appears to have left the collagen layer intact. A shortened soak cycle would be needed to retain more of the biomineral layer if that were desired.

Similarly, the use of NaOH is the established technique to remove the collagen layer to recover hydroxyapatite[7] since it decomposes collagen faster than the biomineral layer. After soaking the scales in NaOH(aq) under mild conditions the FTIR spectra of the top and bottom surfaces of FS-OH retained the key absorption bands observed in FS and FS-W, so a mild base treatment allowed each layer to retain significant character of the original surfaces. But a comparison of the relative areas of key amide bands from collagen shows that it was reduced.

FTIR of the top and bottom surfaces of a dilute solution of Clorox®-soaked scales (FS-C, Supporting Information, Figure S1c) were similar to the FS and FS-W (Figure 4), showing some character from both layers was retained, however the hydroxyapatite peaks (PO_4^{3-}) had strengthened relative to collagen peaks (3288, 1633, and 1540 cm^{-1}). However, the fish scales were visibly thinner and more brittle than FS and FS-W scales, so mild Clorox® oxidation is destructive to both layers, but the collagen layer is affected more.

Changes in EMC. The EMC of the scales was significantly increased by all the modifications. Even the FS-W caused the EMC to nearly double (6.2 ± 0.5 to 12.0 ± 0.2 wt %) as seen in Figure 5. The washing step did not induce chemical changes but did likely remove any residual mucous layer, which indicates

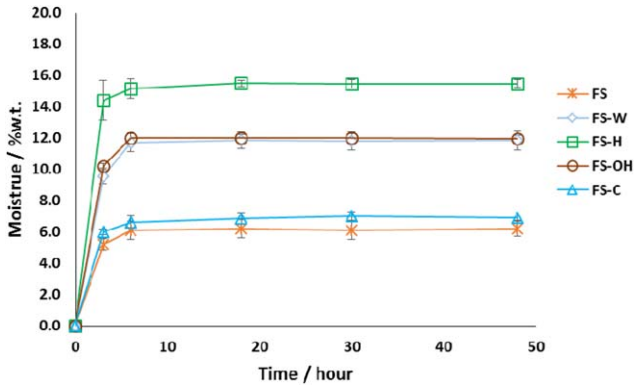

Figure 5. EMC of FS, FS-W, and modified scales FS-H, FS-OH, and FS-C. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

that this component plays a significant role in reducing moisture uptake by the scales. The ultrasonic washing may also have disrupted the mechanical integration of the scale components, but we had no way to test for this. Interestingly though, the EMC of the FS-C scales is quite close to that of FS (6.2 ± 0.5 to 6.9 ± 0.2 wt %, respectively). The Clorox® soak affected both layers, but here we attribute the reduced EMC to oxidation of the hydrophilic amines and amides of collagen. The FTIR (Supporting Information, Figure S1c) supports this, as it shows a clear decrease in N—H stretching and bending bands.

Polymer-Modified Scales

Changes in Composition by FTIR. Four different modifications of the scales were performed to chemically change the scale surfaces by adding a polymer component to one or both scale layers. FS-W treated with an acidic solution of TEOS followed by heating proved to be a nonselective modification, as evidenced by the clear addition of the Si—O—Si band at 1055 cm^{-1} in the FTIR spectra of both the biomineral and collagen surfaces of the FS-SiO_2 scales (Supporting Information, Figure S2a). But the modification was more extensive at the biomineral surface because the PO_4^{3-} bands of the biomineral surface were completely obscured while the amide bands of the collagen layer remained significant.

In contrast to this, FS-W scales modified via radical polymerization of the hydrophilic OEGA monomer showed a significant preference for modification of the collagen layer. The FTIR of both top and bottom surfaces (Supporting Information, Figure S2b) show an increase in the C—H stretch at 2873 cm^{-1}, but it is much more significant in the collagen layer than the biomineral layer. The C═O stretching band at 1725 cm^{-1} and the C—O stretching band at 1103 cm^{-1} were also strong in the collagen surface's spectrum but negligible in the biomineral surface's spectrum. Because the OEGA is hydrophilic, it likely adsorbs to both surfaces but may be sufficiently compatible with the collagen to also diffuse into this layer. If this is the case, similar results can be expected with other hydrophilic organic monomers.

Scales modified via radical polymerization of hydrophobic monomers—MMA or MA—yielded FTIR spectra (Supporting

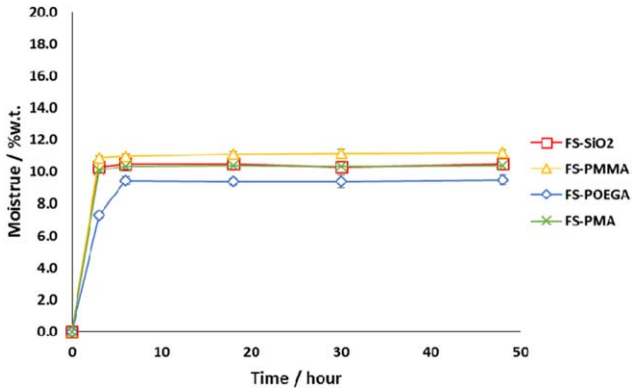

Figure 6. EMC of polymer-modified fish scales. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Information, Figure S3a,b) that showed no obvious differences compared to FS and FS-W. This indicates that the hydrophobic MMA and MA monomers failed to effectively wet or penetrate into the collagen. However, EMC does indicate an effect from these monomers even though changes in the FTIR spectra were not evident. Possibly a small amount of hydrophobic monomer penetrated into the fish scale without significantly altering the FT-IR spectra. It should be noted that these scales were soaked in monomer at room temperature. If a more extensive hydrophobic modification is desired, then it seems likely that the scales may need to be immersed in heated monomer, and/or a small amount of a suitable solvent added to aid penetration. However, here we sought to minimize altering the hierarchical structure of the scale, so solvents and high heat were both avoided.

Based on the FTIR spectra of modifications done under these conditions, modification by metal alkoxides and hydrophilic monomers is facile while hydrophobic modification of either scale surface appears negligible.

Changes in EMC of Polymer-Modified Fish Scale. As we expected, one application for organic-polymer-modified fish scales to be as an additive in some packaging materials, we looked at the EMC of these scales. In comparison to the EMC of the FS-W scales (11.8 ± 0.3 wt %), all the polymer-modified fish scales gave a small, but statistically significant reduction in EMC. Interestingly, the greatest reduction came from the most hydrophilic modification, FS-POEGA at 9.5 ± 0.3% (Figure 6). One of the hydrophobic polymer modifications (FS-PMMA) yielded the smallest reduction in EMC at 11.2 ± 0.2 wt %. The FS-PMA scales had an EMC of 10.4 ± 0.2 wt %, which is slightly lower than FS-PMMA, and statistically the same as FS-SiO_2 at 10.5 ± 0.2 wt % EMC. The fact that one of the hydrophobic modifications resulted in the smallest reduction in EMC is best explained by it being ineffective at modifying the FS-W scales. The effect of the FS-POEGA may have been due to it being marginally less hydrophilic than the scale layers coupled with its ability to add to both layers. The weak effect of PMA is attributed to little modification of either surface, but the reason why FS-SiO_2 and FS-PMA have equivalent EMC is not understood, because the SiO_2 is more hydrophilic than PMA and the data show far more significant modification of both scale sides by SiO_2 than by PMA. More studies are required to effectively correlate structural changes to EMC outcomes.

Nanomechanical Properties

Another likely application of fish scales is as composite reinforcement. We tested the effects of our modifications on the nanomechanical properties of the modified, but otherwise intact, scales. We tested the surface of the biomineral layer, and the collagen layer in cross-section, both from the middle of the layer and closer to the edge of the biomineral layer (Figure 1).

DMT Modulus of Biomineral Surfaces. Table II shows the DMT moduli obtained from the peak-force tapping mode in the AFM[29] for the biomineral surfaces of all the scales studied here. The modulus of the as-received FS was 4.0 GPa, but rose slightly to 4.2 GPa for the FS-W. This further supports the evidence that washing the scales removed some residual mucous layer which is soft.

The modulus of biomineral surface of the FS-H scales was 57% less than FS-W, at only 1.8 GPa. This is explained by the partial degradation the biomineral layer. However, the modulus of the FS-OH scales also gave a significant reduction in modulus of the FS-OH scales to 2.2 GPa. The base is intended to degrade the collagen but also weakened the biomineral layer. So the mechanical properties of both layers were compromised. Again, these data are consistent with the data gained from FTIR, which showed that the dilute acid and base treatments gave preferential but not completely selective degradation of the respective target layer.

The most interesting result from the non-polymer modifications of scales is from the FS-C. The modulus of the FS-C scales was 4.7 GPa, which is greater than that of the FS or FS-W scales. The FTIR of the oxidized biomineral surface showed decreases in peaks at 1540 and 1633 cm^{-1} (protein) relative to the peak at 1012 cm^{-1} (PO_4^{3-}). When compared to the FS spectra, changes in the relative ratio of the peak areas occurred in the IR spectrum of both sides of the scale, so the rise in modulus is most probably due to loss of softer proteins in the biomineral layer allowing the modulus to be dominated by the HAP, but the exact changes are unknown.

The most substantial change that resulted from the supplemental polymerization modifications came from the in situ hydrothermal conversion of TEOS to SiO_2 nanoparticles. Figure 7 shows the optical and AFM images of the biomineral surface of

Table II. Averaged Modulus (GPa)[a] of the Biomineral Surface of Modified Fish Scales

Modified fish scale		Polymer-modified fish scale	
FS	4.0	FS-SiO_2-C	8.6
FS-W	4.2	FS-SiO_2	22.8
FS-H	1.8	FS-PMMA	4.9
FS-OH	2.2	FS-POEGA	1.2
FS-C	4.7	FS-PMA	3.8

[a] Modulus data in Table II was the averaged modulus measured at 5 different positions on each sample.

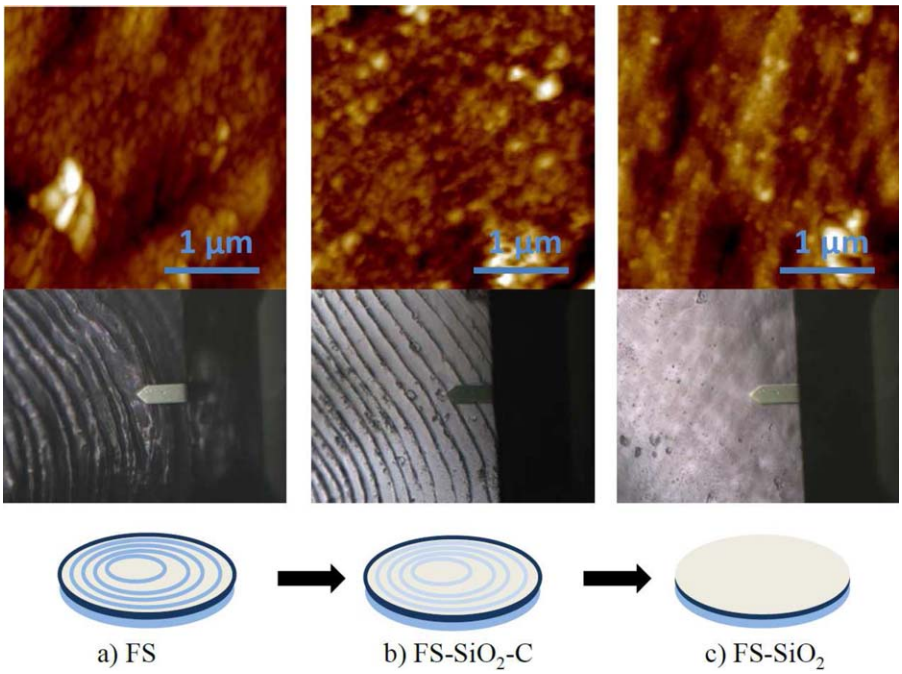

Figure 7. Optical and AFM images showing changes in surface features of (a) FS, (b) FS-SIO_2-C, and (c) FS-SiO_2. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

FS, FS-SiO_2-C, and FS-SiO_2. As seen from Table II, the modulus of the as-received FS was only 4.0 GPa, but more than doubled to 8.6 GPa for FS-SiO_2-C, and rose to 22.8 GPa for FS-SiO_2. The structure of the biomineral surface was also visibly changed from FS [Figure 7(a)], where ridge features were clearly visible. With a 30 min immersion in acidified TEOS followed by thermal treatment, the depressions begin to fill in with SiO_2 nanoparticles [Figure 7(b)], and the ridge features start to become obscured. After a 60 min immersion followed by the same thermal treatment, the surface is nearly featureless [Figure 7(c)].

The scales modified by hydrophobic polymers—PMMA and PMA—gave small or negligible effects on the modulus (4.9 and 3.8 GPa, respectively). This further reinforces the FTIR data that

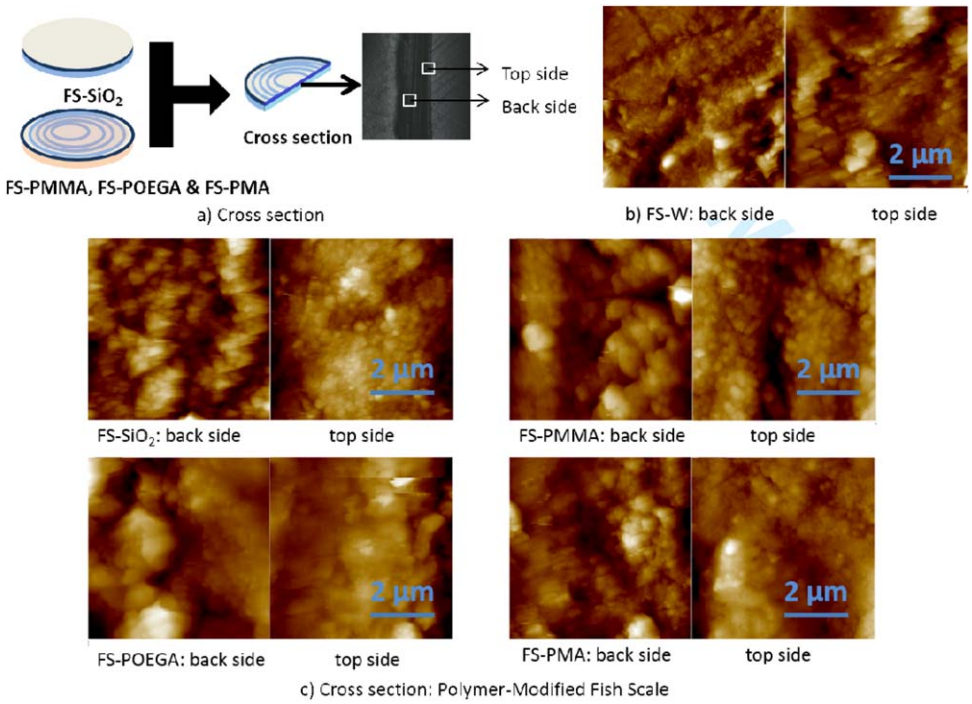

Figure 8. Cross-section AFM test (6 × 6 μm). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Table III. Modulus of Cross-Sectional Layers of Scales (GPa)

Modulus	FS-W	FS-SiO_2	FS-PMMA	FS-PMA	FS-POEGA
Near top edge	5.2	12.7	4.3	3.3	6.9
Near back edge	2.2	10.2	3.6	2.2	2.1

showed negligible modification of the scales, and was attributed to the hydrophobic monomers failing to adsorb to and penetrate the hydrophilic scale surfaces. However, the hydrophilic OEGA monomer gave POEGA-modified scales that showed a significant reduction in modulus (1.2 GPa), which shows that the OEGA adsorbed to, and polymerized onto, the biomineral surface. This is consistent with the FTIR of the biomineral surface showing the presence of the POEGA, and accounts for the reduction of surface modulus because of the soft POEGA membrane on the surface. The modification of the biomineral surface is expected to be by adsorption of POEGA to the surface, and not grafting to the surface, but the adsorption was sufficiently strong that it was not removed by washing with water.

Cross-Section of the Samples. To determine if the monomer reactants penetrated into the collagen layer, we tested the mechanical properties of scale cross-sections. The modulus of the collagen was measured near the biomineral interface (labeled as "top side") and nearer the center of the collagen layer (labeled as "back side") as illustrated in Figure 8(a).

The modulus of the back and top edges of the FS control was measured at 2.2 and 5.2 GPa, respectively (Table III). The AFM images [Figure 8(b) left and right] clearly show the collagen rods. The higher modulus from the top-side collagen rods is from the reinforcing effect of the intact biomineral layer.

When the scales were modified by TEOS, the AFM image [Figure 8(c), top left] shows the SiO_2 nanoparticles, though the size of individual SiO_2 nanoparticles is not clear. In the top-side image, these nanoparticles completely obscure the appearance of the bundles of collagen rods, but in the backside images, the bundles are still visible indicating less extensive modification, The presence of these nanoparticles within the collagen layer confirms the FTIR results that showed SiO_2 in both the biomineral and collagen layers. The reinforcing effect of the SiO_2 on the collagen phase is confirmed by the significant rise in modulus both near the biomineral surface and deep within the collagen layer too (12.7 and 10.2 GPa, respectively).

The hydrophobic modifications of the scales showed that the modulus near the biomineral layer of FS-PMMA and FS-PMA was reduced, compared to FS-W. Interestingly, the modulus of FS-PMMA within the collagen layer was increased relative to FS-W, while that of FS-PMA was unaffected. The FTIR did not show evidence that either surface of the scales was modified by MMA or MA, but the change in the modulus suggests that there might have been some degree of modification. However, these changes are small, so the reasons for them need to be better understood.

When the scales were modified with OEGA, the modulus within the collagen layer showed only no significant change (2.1 GPa compared to 2.2 GPa for FS-W), but the modulus near the biomineral surface rose to 6.9 GPa compared to the FS-W control at 5.2 GPa. The FTIR confirmed the chemical modification of both surfaces by the hydrophilic OEGA monomer but the higher modulus for the collagen edge near the biomineral layer is less clear. Because the bundles of collagen rods are less distinct than in the other images, there is a possibility that the POEGA disrupts the collagen arrays. However, all these modifications need to be studied further.

Effect of Surface-Modified Scales on Alginate Hydrogel

A simple surface modification of fish scales was performed to test how much the modified scales enhanced the properties of an alginate hydrogel, and if the improvement exceeded that of unmodified scales. Because alginates gel via ionic bonding, the scales were modified using sodium citrate (giving FS-NaC scales). The expected modification of the biomineral surface, along with the FTIR (Figure 9) verifying the modification, is confirmed by the significant increase in the carbonyl bands at 1633 cm^{-1} and a significant reduction in the PO_4^{3-} bands at 1012 cm^{-1}.

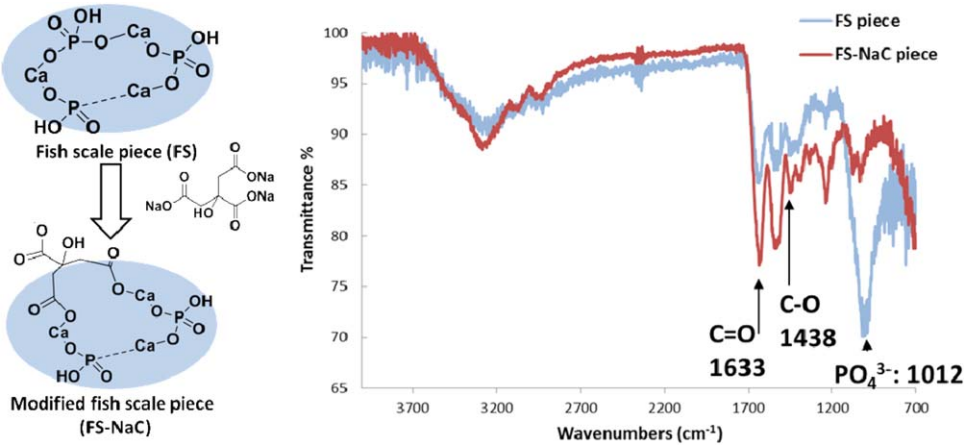

Figure 9. Expected surface modification resulting from treatment of FS-W scales with sodium citrate, and the FTIR spectra proofs. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

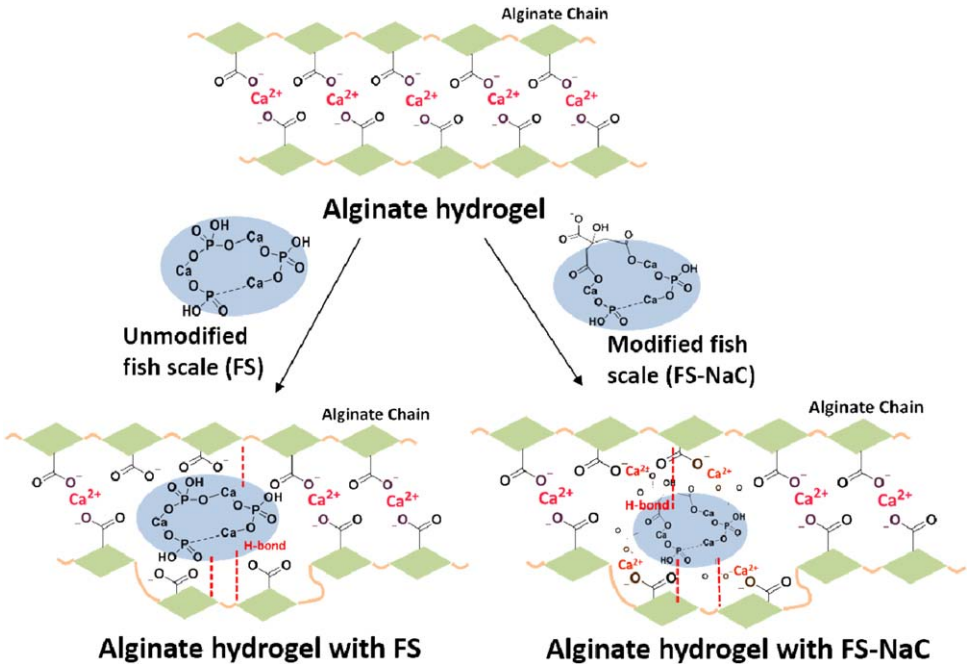

Figure 10. Interactions between the alginate chain and the fish scales. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 10 illustrates the expected interactions of FS-W and FS-NaC scales with the alginate. When unmodified FS-W scales bond with the alginate chains, the interactions are through H-bonds. However, when the FS-NaC scales bond with the alginate chains, the interactions are both H-bonds and ionic bonds, and this results in the increased ability of these scales to reinforce the hydrogel.

Rheology was used to measure loss and storage moduli (G'' and G') to show the relative improvements in properties of the weak alginate gels. Figure 11(a) plots both G' and G'' versus frequency while Figure 11(b) shows the tan δ versus frequency. The frequency sweep from 0.1 to 100 Hz was performed at a constant strain of 10% and using a parallel-plate geometry. In the lower range of frequencies, the loss modulus (G'') of the hydrogels was higher than its storage modulus (G'), showing that the gel had viscous liquid-like characteristics in this range of frequencies [Figure 11(a)]. Beyond 45 Hz, however, the G' was greater than G'', indicating that there was a transition from the liquid to the solid phase. To further understand this phenomenon, the loss tangent (δ) was plotted against frequency [Figure 11(b)]. The point at which tan $\delta = 1$ is around 46 Hz, and this frequency translates into the gel point. This is corroborative evidence for the G' and G'' values and the related change of phase.

When the effects of the unmodified FS-W and surface-modified FS-NaC scales on the G' and G'' are compared with the control alginate gel, it is seen that the G' of the FS-NaC-modified alginate is an order of magnitude greater than that of the control alginate and significantly greater than that of the FS-W-modified alginate. The difference in the G' of the two modified alginates proves the improved interfacial adhesion leading to improved properties from the modified scales. In fact, while the FS-W-modified alginate also has an initial higher G' than the

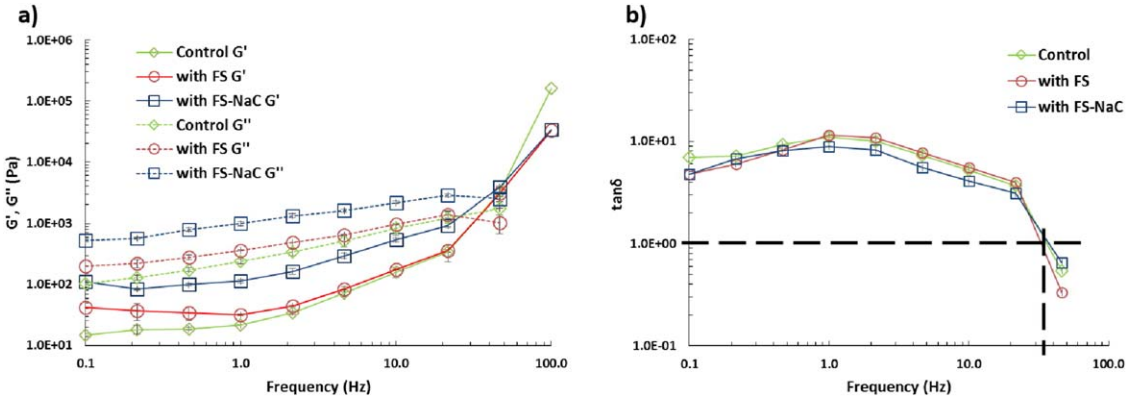

Figure 11. The properties of unmodified alginate and FS-W- and FS-NaC-modified alginate hydrogels plotted as (a) G′ and G″ and (b) tan δ versus frequency. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

alginate control, this enhancement is almost gone by 1.0 Hz while the FS-NaC-modified alginate maintains its higher G' until the gel point at ~46 Hz. This is due to the additional ionic bonds with the Ca^{2+} ions and the new active sites in the form of highly electronegative O^- atoms of the modified fish scales, compared to only H-bonds between the alginate and the unmodified FS-W for strengthening the hydrogel. Because the G'' trends are the same as the G' trends, these ionic bonds to the FS-NaC scales are more effective at allowing dissipation of the viscous energy of the system than the hydrogen bonds to the FS-W scales.

CONCLUSIONS

The inherent design of fish scales, with a biomineral upper surface and alternating arrays of collagen layers as their lower surface, imparts both high modulus and high toughness, yet this abundant and growing waste resource is little used, and most of the uses are based on harvesting the individual scale components. This work sought to explore the possibility of expanding the use of fish scales as a waste resource, by exploring the potential to modify one or the other surface, measured by FTIR, and their effect on nanomechanical properties and EMC. It is the first research to investigate chemical modifications of fish scales, and their effects on functionality and nanomechanical properties of modified fish scales, and their relative effects on the biomineral and the collagen layer of the scale. The data showed that modifications can be targeted to the biomineral or the collagen layer, but while some modifications were "preferential", none were truly selective, so all the modifications had some effect on both layers. The types of chemical modifications ranged from a targeted partial removal of one or the other layer, and "supplementation" of one or the other layer by inorganic and hydrophilic or hydrophobic organic polymers. The range of surface modification possible was explored because the most likely use for "intact" fish scales seems likely to be as an additive for biodegradable packaging or composite materials, and these potential applications benefit from good interfacial adhesion. The study concluded with a brief exploration of a sodium-citrate-modified scale as an additive to a weak alginate hydrogel and showed that the modified scale significantly improved both loss and storage moduli of the weak gel at lower frequencies compared to both the pure alginate hydrogel and the hydrogel containing unmodified fish scales, proving the significant benefit from even simple surface modifications of scales as a reinforcement. The overall significance of these results is that a wide range of modifications is possible to adjust the modulus and polarity of a scale interface for a wide range of polymer matrices. However, before "intact" scales, be the modified or unmodified, can find wide use, a suitable mechanical cutting tool must be devised, as grinding the scales to a powder sacrifices the hierarchical design, and hand cutting is both unrealistic and leaves the scale pieces are too large for truly effective distribution within a matrix. If the modified scale pieces were more effectively distributed within a matrix, possibly even greater mechanical benefit would be achieved.

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