ABSTRACT: The eradication of biofilms remains an unresolved challenge across disciplines. Furthermore, in biomedicine, the sampling of spatially heterogeneous biofilms is crucial for accurate pathogen detection and precise treatment of infection. However, current approaches are incapable of removing highly adhesive biostructures from topographically complex surfaces. To meet these needs, we demonstrate magnetic field-directed assembly of nanoparticles into surface topography-adaptive robotic superstructures (STARS) for precision-guided biofilm removal and diagnostic sampling. These structures extend or retract at multilength scales (micro-to-centimeter) to operate on opposing surfaces and rapidly adjust their shape, length, and stiffness to adapt and apply high-shear stress. STARS conform to complex surface topographies by entering angled grooves or extending into narrow crevices and “scrub” adherent biofilm with multiaxis motion while producing antibacterial reagents on-site. Furthermore, as the superstructure disrupts the biofilm, it captures bacterial, fungal, viral, and matrix components, allowing sample retrieval for multiplexed diagnostic analysis. We apply STARS using automated motion patterns to target complex three-dimensional geometries of ex vivo human teeth to retrieve biofilm samples with microscale precision, while providing “toothbrushing-like” and “flossing-like” action with antibacterial activity in real-time to achieve mechanochemical removal and multiverse pathogen detection. This approach could lead to autonomous, multifunctional antibiofilm platforms to advance current oral care modalities and other fields contending with harmful biofilms on hard-to-reach surfaces.

KEYWORDS: reconfigurable, multiscale, complex topography, shear force, antimicrobial, diagnostic sampling, antibiofilm

Biofilms are composed of microbial cells enmeshed in an extracellular matrix that are firmly attached on a variety of surfaces. A major challenge is targeting biofilms formed on surfaces with arbitrary orientations and complex topographical features like crevices. Such complex, biofilm-covered surfaces pervade health care and industry causing chronic infections and costly contaminations. Furthermore, the ability to sample biofilm contents could inform more effective treatment and ultimately enable precision medicine to increase successful clinical outcomes. However, while methods to molecularly identify pathogens have advanced dramatically, sampling methods, the first step in a diagnostics process, have not advanced at the same pace. Sampling is particularly challenging due to the heterogeneous distribution of diverse pathogens, requiring biofilm disruption and collection, often from within crevices or grooves. To address these needs, we develop a microrobotic system to access and remove adhesive biofilms and perform sampling for pathogen detection using iron oxide nanoparticles (IONP), a versatile class of materials with catalytic and magnetic properties.

IONPs have been used for biofilm treatment since they display peroxidase-like activity that efficiently catalyzes hydrogen peroxide for antimicrobial effects, while applied magnetic field enables control of positioning. However, current approaches cannot be applied on complex topographies and lack the ability to retrieve biofilm samples and their distinct

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components from difficult-to-reach locations for diagnostic purposes. The treatment and retrieval of biofilms on surfaces with irregular features and positioning, such as human teeth, requires a different conceptual approach. Rather than simply pulling and aggregating nanoparticles on a horizontal surface, techniques are needed to generate assemblies that extend toward the biofilm-contaminated surface and that conformally adapt while applying sufficient stresses to remove highly adhesive biofilms.

To meet these challenges, we design a magnetic control platform with robotic principles to assemble, reconfigure, and actuate IONP superstructures to target adherent biofilms on vertically oriented surfaces (Figure 1). We discover the formation of bristle-like structures that physically extend and conform to surfaces while adjusting their shape and cohesive strength through the spatial and temporal coordination of magnetic fields. Notably, the catalytic property of these structures is preserved throughout reconfiguration, providing mechanochemical treatment in real-time. We find that biofilm removal depends on type of motion, interparticle cohesion, and force at the interface as determined by the size, arrangement, and dynamically-controlled spatial location of the bristle superstructure, allowing multiaxis targeting with microscale precision. We then develop automated motion patterns adapted to remove biofilms from the complex surface contours of ex vivo human teeth and retrieve biofilm samples for analysis. Our key advancements are two-fold. We assemble a dynamic telescoping, reconfigurable superstructure that conforms to targeted surface topography with controlled stiffness capable of applying high surface forces. We then exploit this adaptability of shape and strength as a form of physical intelligence with in situ catalysis to provide a unifying approach to collect site-specific microbes and eradicate biofilms from complex surfaces. By introducing surface topography-adaptive robotic superstructures (STARS), we enable chemical treatment, mechanical removal of adhesive biofilms, and multikingdom pathogen detection, while creating automated motion dynamics for a precise multitasking system to target surfaces in arbitrary arrangements.

RESULTS AND DISCUSSION

Development of Reconfigurable STARS. We design an automated electromagnetic platform to treat biofilms on vertically oriented surfaces, which require self-supporting functional elements that withstand gravitational forces. As illustrated in Figure 1, we develop a field-directed technique to dynamically assemble and actuate magnetic bristles from IONPs in solution (Figure S1). These bristle-shaped superstructures can extend horizontally from a vertical base to interact with an opposing vertical surface (Figure 1A,B). The forward electromagnet core guides the bristles across the target surface with topography-adaptive property. (C) (top-view) IONPs (1 mg mL$^{-1}$) are initially collected in a low aspect ratio mound and then extended into a high aspect ratio bristle-like formation as they are swept laterally. (D) IONPs are multifunctional with peroxidase-like activity, generating free radicals at the site of mechanical cleaning providing both antimicrobial treatment and physical biofilm removal. (E) Bristle motion is controlled to disrupt biofilms through mechanochemical action and retrieve biofilm contents (microbes, extracellular polysaccharides, biomolecules) for diagnostic sampling. (F) Programmable motions enabled target treatment and optimized cleaning via automated routines. (G) Multifunctional and multitasking capabilities integrated into STARS.
emagnets positioned on either side of the vessel controls the formation and multimodal action of the magnetic bristles. The magnetic field generated from both electromagnets is coordinated via a programmable microcontroller. Furthermore, the position of the forward iron core can also be controlled, while the rear ferrite core is fixed. This design enables flexible positioning and control of bristle-like superstructures that form on a vessel wall and can span the vessel width while moving in multiple directions, as directed by the local field. For example, in one modality, the magnetic field is oriented to drive the superstructure assembly with its base on the vessel wall near the mobile forward electromagnet core in the $xz$-plane (Figure 1A), extending in the $y$-direction (orthogonally) toward the rear electromagnet. By cyclically changing the position of the forward magnetic core, the superstructure position can be controlled. The resulting bristles enable biofilm removal from a vertically oriented surface placed in the vessel, such as the human tooth (Figure 1B). When viewed from above, the bristles extend during the first 1–2 s of the cycle oriented toward the rear electromagnet and translate across the vertical vessel wall (Figure 1C); the superstructure self-supports as the length increases during the lateral motion (Movie S1; red arrow). Furthermore, we exploit IONPs (Figure S2, see Experimental Section for details) which are catalytic with peroxidase-like activity and can activate hydrogen peroxide ($\text{H}_2\text{O}_2$) to produce free radicals on site for antimicrobial activity (Figure 1D).

Such magneto-catalytic properties combined with magnetic field modulation allow automated, reconfigurable bristles to be formed with multiple programmable functionalities, including extension and retraction, topographical adaptability, and tunable stiffness gradients. These STARS chemically treat and mechanically remove biofilms (Figure 1D and Figure S3) and can penetrate biofilms to retrieve samples for diagnostic analysis (Figure 1E). Automation of STARS bristles movement enables varying motion dynamics with precise spatial (multi-axis) control that can be evaluated for the most efficient biofilm removal and localized diagnostic sampling on the tooth surface (Figure 1F).

**Physical Characterization of STARS.** The magnetic field generated by the electromagnets enables control over bristle formation, position, and properties. We develop a three-dimensional (3D) finite element model to better understand the balance of magnetic fields and forces that control these properties and compare prediction to the experimental outcome. As shown in Figure 2A and Figure S4, STARS bristles align along the direction of the magnetic field, extending from the position of the positionable forward
Electromagnet core toward the fixed rear electromagnet. The values for magnetic flux density in the center of the vessel, where the vertical biofilm-covered surface will be situated, vary from 35 to 70 mT, depending on the instantaneous position of the forward core (Figure 2A). Electromagnets are cycled on and off during the cleaning cycle, enabling three important aspects of control. First, IONPs can be flexibly reconfigured and released at will, allowing brushing motions (Figure 2A). Second, as long as the magnetic forces are focused in a small region, and bristles closely track the movement. The rear ferrite core is larger in diameter (10 mm) and generates weaker forces that serve primarily to define the direction of bristles’ extension. As the forward core translates, the bristle closely follows the highest magnetic field strength. As the forward core translates, the bristle closely follows the highest magnetic field, as shown in the finite element model (Figure 2A). As long as the magnetic fields are generated, STARS bristles remain assembled and self-supported. Thus, to collect IONPs at the end of a cleaning sequence, both coils are de-energized to allow the bristles to dismantle and settle to the bottom of the vessel, which occurs within 1–2 s (Movie S2). After the bristles settle, the forward coil is re-energized, and the cycle is repeated. To summarize, bristle assembly and the motion dynamics involve three sequential and interdependent processes. In the initial process, IONPs are collected by energizing the forward electromagnet. Thereafter, the rear electromagnet is energized, which guides bristles formation and extension toward the position of the vertical biofilm-contaminated surface. Finally, the lateral motion creates changes in bristle shape and length guided by the magnetic field.

Bristle length depends on the IONP suspension concentration and can range from 1 mm in length at 0.5 mg mL$^{-1}$ to as long as 7 mm at 2 mg mL$^{-1}$. The bristle shape is influenced by the movement of the electromagnet core, extending during the first few seconds (Figure 1C and Movie S1). The effective length of the bristles can be visualized by creating a composite of accumulating sequential images of the sweeping process.

The core of the forward electromagnet is then actuated to move bristles in a lateral motion repeatedly across a vertically oriented surface in a sweeping motion (see Experimental Section for details) with linear velocities between 6 and 48 mm s$^{-1}$. Since the forward core is small in diameter (3 mm), the magnetic forces are focused in a small region, and bristles closely track the movement. The rear ferrite core is larger in diameter (10 mm) and generates weaker forces that serve primarily to define the direction of bristles’ extension. As the forward core translates, the bristle closely follows the highest gradient of the magnetic field, as shown in the finite element model (Figure 2A). As long as the magnetic fields are generated, STARS bristles remain assembled and self-supported. Thus, to collect IONPs at the end of a cleaning sequence, both coils are de-energized to allow the bristles to dismantle and settle to the bottom of the vessel, which occurs within 1–2 s (Movie S2). After the bristles settle, the forward coil is re-energized, and the cycle is repeated. To summarize, bristle assembly and the motion dynamics involve three sequential and interdependent processes. In the initial process, IONPs are collected by energizing the forward electromagnet. Thereafter, the rear electromagnet is energized, which guides bristles formation and extension toward the position of the vertical biofilm-contaminated surface. Finally, the lateral motion creates changes in bristle shape and length guided by the magnetic field.

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(Figure 2B). Bristle length also depends on their lateral velocities; at higher velocities, fluid drag forces disrupt the cohesion among IONPs at the distal end of bristles resulting in shorter lengths (Figure 2C and Figure S5). For example, 2 mg mL$^{-1}$ of IONP can generate up to 7 mm bristle in 8 mm width vessel, and by further optimizing the magnetic field and IONP amounts (4 mg mL$^{-1}$), it can reach 1 cm in length in 12 mm vessel (Figure S6). Bristle length also shows nonmonotonic dependence on magnetic field strength, first extending with magnetic field strength, but then retracting at higher field strengths as IONPs pack tightly (Figure 2D). A bristle length of 2.2 mm is sufficient to reach the planar vertical surfaces placed in the vessel between the two electromagnets that are used for experimental characterizations, where IONP concentrations at or above 1 mg mL$^{-1}$ increase the surface coverage (Figure 2E).

Given the extensibility of the bristles, we investigate bristle interaction with topographically complex surfaces. Using lithographical methods, we generate surfaces with repeated circular, square, triangular patterns (Figure 2F–H, Movie S2 and S3) and find that the bristles can readily reach and adapt to enter the surface recesses, including corners in the square patterns and cusp-like features at the intersection between the two circular shapes. This shape-adjusting, topography-adaptive functionality is a form of physical intelligence that could enhance the ability to remove biofilms formed in difficult-to-reach surfaces.

We characterize bristle mechanical properties using two complementary methods, both of which are based on force estimates derived using Euler’s beam deflection approximation:

$$F = \frac{\delta_b L^3}{3EI}$$

where $\delta_b$ is deflection, $L$ is beam length, $E$ is Young’s modulus, and $I$ is area moment of inertia (see Experimental Section for details). First, we measure the cohesion force of STARS bristles using a PDMS microcantilever to determine the physical strength of the bristle itself (Figure 3A). The deflection of the cantilever tip by the lateral sweeping motion of the bristles is recorded. Using known values of the length, Young’s modulus, and moment of inertia of the microcantilever, the force exerted by the bristles on the microcantilever, and therefore the reaction force exerted by the PDMS microcantilever on the bristles, is determined. The ability of the bristles to withstand this reaction force can be used to characterize STARS bristle mechanics. The force was increased until the bristles broke, allowing the cohesive strength to be determined. We first measure maximum applied force at a fixed position while varying field strength. There is a linear relationship between the lateral force applied by the bristles and magnetic field strength (Figure 3B). This demonstrates the feasibility to dynamically tune bristle stiffness during operation. By placing the microcantilever at various positions along the long axis of the magnetic bristles at a constant field strength of 69 mT, we estimate cohesion forces ranging from 8.2 $\mu$N at the distal end to 180.5 $\mu$N near the base of the bristles (1.75 mm measuring height, Figure 3C). Furthermore, using the value for applied shear force, we approximate the applied shear at the surface ($\tau = F/A$, $A$ is contact area) by estimating the applied cross-sectional area of the bristles at the interface. Using the force measurements obtained using the PDMS microcantilevers, we estimate shear stresses ranging from 10.4 to 229.8 N m$^{-2}$.

At the interface of the STARS bristles with the treatment surface, the dominant stresses are the lateral shear stress applied by the bristles, which is opposed by a combination of fluid drag and adhesion between the biofilm and the substrate. Unlike typical monolithic bristles, the IONPs near the bristles ends continuously reconfigure and change the bristle structure under high shear near the vertical surface. To verify that these dynamic structures can produce significant stresses, we fabricate a micropillar composed of PDMS with 50 $\mu$m diameter and 240 $\mu$m height (aspect ratio = 4.8, Figure S7). This single micropillar interacts with the small-scale features at the ends of the bristles, allowing characterization of the local force generated by these structures (Figure 3D and Movie S4).

The micropillar is vertically placed at the position of the target surface and imaged during bristle contact to measure deflection. Based on its deflection, we approximate the shear stress applied at the 2.2 mm distance to be as high as 83.3 N m$^{-2}$ depending on the magnetic field strength (Figure 3E,F). The shear stresses measured by both the cantilever and pillar measurement systems are comparable in magnitude and demonstrate that the shear stresses produced at the vertical target surface or tooth surface are significantly greater than the minimum values necessary for biofilm removal even in narrow, difficult-to-access spaces. In addition, the shear stress can be adjusted to remove biofilms at different distances from the surface by adjusting magnetic field strength.

STARS bristle movement is opposed by viscous drag, which increases with actuation speed. We approximate this contact force as drag on a cylindrical body at constant velocity. The Reynolds number, $Re = \frac{\rho u L}{\mu}$, is on the order of 10 during movement, where $\rho$ and $\mu$ are the density and dynamic viscosity of the fluid, $u$ is the flow speed, and $L$ is the characteristic linear dimension. The viscous drag force on the translating bristle can be estimated as $f_D = 1/2 C_D \rho u^2 A$, where the drag coefficient $C_D$ is ~5, $u_b$ is the velocity of bristle, and $A$ is the area of the bristles facing the fluid. This yields an estimated maximum drag force on the bristle of 0.8 $\mu$N at 12 mm s$^{-1}$, which is an order of magnitude below the applied shear force at the vertical target surface/bristle interface.

These complementary analyses reveal four essential properties of STARS bristles: (i) The support structure varies along the length of the superstructures, with a highly cohesive base that supports the extended bristles with decreasing, yet strong cohesion along the length of the structure. (ii) The cohesion and associated capability of the bristle to mechanically remove strongly adhesive biofilms are retained at the distal end, where stresses exceed the values necessary for biofilm removal. (iii) Even as the STARS bristles reconfigure and adapt to fine-scale surface topography, applied shear stress remains sufficient to remove biofilm, as evidenced by shear stress measurements of the smallest bristles. (iv) This shear stress can be dynamically adjusted by modulating the magnitude of the magnetic field, enabling fine-tuning of the maximum shear for controlled biofilm removal. Hence, the “stiffness gradient” provides strong cohesion at the base of the bristle to withstand gravity and extend horizontally, while flexibility at the tip provides enough shear force to dislodge biofilms while continuously adapting to the topography.

**Antibiofilm Functionality of STARS.** The process of STARS bristle assembly and their physical properties establish...
the fundamentals for targeted magneto-catalytic action for biofilm treatment and sampling. We use an oral pathogen (Streptococcus mutans) to form one of the stickiest biofilms with matrix-enmeshed bacteria which is exceptionally difficult to remove.31,32 Next, we assess the removal of biofilms on vertically oriented surfaces. To mimic the anatomical positioning of teeth in the upper arch, slabs are 3D-printed and placed in a vertical position similar to upper incisors (Figure 4A). Biofilms of S. mutans are formed on the slabs (see Experimental Section for details). The slab material’s surface characteristics have been verified to have similar biofilm adhesion properties as human enamel, that is, requiring 0.184 N m\(^{-2}\) for biofilm removal.29

To characterize biofilm scrubbing on the surface, we devise an automated, standardized process to ensure consistency and repeatability. During forward surface biofilm removal efficacy studies, we fix the geometry and hold the magnetic field strength constant at 69 mT and focus on two experimental parameters, specifically, IONP concentration and scrubbing velocity. The distance between the vessel wall and the biofilm specimen during all experiments is fixed at 2.2 mm. IONP suspensions at concentrations ranging from 0.5 to 2.0 mg mL\(^{-1}\) are added to the vessel. A uniform 10 mm lateral sweeping...
motion is used to quantify biofilm removal at all IONP concentrations and sweeping velocities. This focused motion is intended to provide a quantifiable metric for efficacy rather than to remove the biofilm from the entire surface. As such, we measure biofilm removal in terms of pre- and post-treatment based on binarized before/after images using a threshold value (Figure 4B).

Given that IONPs display peroxidase-like activity, we also interrogate whether STARS bristles would provide an on-site source of free radicals for catalytic reaction-generated antimicrobial effects. We use an established method based on a colorimetric assay using 3,3′,5,5′-tetramethylbenzidine (TMB) to demonstrate the generation of reactive oxygen species (ROS) from H$_2$O$_2$ by the catalytically active STARS bristles. The hydroxyl radicals produced from H$_2$O$_2$ oxidize colorless TMB to blue-colored reaction products which can be visualized and assayed by measuring the absorbance at 652 nm.$^{15,15}$ Catalytic activity of the STARS bristles is determined in the same conditions as the biofilm removal assay. The assay indicates rapid generation of ROS during the first 2 min of cleaning (Figure 4C–E). The catalytic activity can be readily visualized in the close-up image showing free radical reaction with TMB (in blue) immediately surrounding the bristles in real time, which accumulated over time (time-lapse panel). The free radicals generated from catalytic activity can chemically kill bacteria embedded in the biofilms. Further, we compare the catalytic activity of the STARS bristles with freely dispersed IONPs. As expected, free nanoparticles have higher catalytic activity due to a significantly larger surface-to-volume ratio compared to the STARS bristle for the peroxidase-like reaction (Figure S8). However, ROS has a limited lifespan$^{33,34}$ and does not diffuse over long distances. This can limit the targeting capacity of ROS generated from freely dispersed IONPs. In contrast, STARS can be precisely steered to the desired treatment area whereby the bristles provide direct contact and physically interact with the biofilm while generating ROS in close proximity to its surface for in situ bacterial killing.

To demonstrate the bacterial killing effect, we determine the cell viability (viable counts) of the removed biofilms post-treatment. The data show complete bacterial killing with nondetectable viable cells following treatment with the STARS bristles in the presence of H$_2$O$_2$, on two-dimensional slabs, indicating localized ROS generation during scrubbing motion for effective bacterial killing (Figure 4F). However, the removed biofilm from the control group harbored more than 10$^6$ colony-forming units (CFU) mL$^{-1}$ of viable cells after bristle treatment without H$_2$O$_2$, demonstrating efficiency of the magneto-catalytic bristles for biofilm removal and bacterial killing. Given that H$_2$O$_2$ is a known disinfectant, we evaluated cell viability of biofilms treated with H$_2$O$_2$ without STARS and found that the hydrogen peroxide alone is incapable of eradicating biofilms which harbored significant amounts of viable cells (>10$^6$ CFU mL$^{-1}$; Figure S9). We have previously shown that IONP (the building blocks of STARS) activates H$_2$O$_2$ and enhances its antibacterial effects against S. mutans biofilms.$^{36}$ H$_2$O$_2$ is neither capable of degrading the biofilm EPS matrix nor performing mechanical removal of biofilms from surfaces on its own. Conversely, STARS treatment (Figure 4F) shows complete biofilm eradication. A key factor is that STARS bristles not only have catalytic property that potentiates H$_2$O$_2$ but can also perform mechanical scrubbing, providing both physical removal of biofilm and ROS generation in situ for bacterial killing. Hence, STARS mechanochemical treatment is substantially more effective than the use of H$_2$O$_2$ alone for biofilm eradication.

Biofilm removal efficacy is first evaluated as a function of magnetic field strength (Figure 4G). Biofilm removal is quantified by capturing images using fluorescent labeling, binarizing the targeted region, and calculating the portion of biofilm removed from the targeted region (Figure 4B and Figure S10, see Experimental Section for details). Removal efficacy increases as bristle stiffness increases, until the field strength becomes great enough to compact and slightly retract the bristle (Figure 2D). Optimized parameters for further biofilm removal experiments were based on this result. As IONP concentration increases, the availability of IONPs in suspension for bristle formation increases, which corresponds to greater length after bristle assembly. At the lowest concentration (0.5 mg mL$^{-1}$), bristles formed with lengths between 1 and 2 mm and were shown to have limited efficacy at scrubbing the surface. The efficacy at 0.5 mg mL$^{-1}$ is further reduced as scrubbing velocity is increased to the level where viscous forces interfere with the integrity of the distal ends of the bristle, as shown at 0.5 mg mL$^{-1}$ and 48 mm s$^{-1}$ (Figure 4H). As the IONP concentration is increased, scrubbing efficacy increases due to the lengthening of the bristle. Biofilm removal is significantly enhanced as IONP concentration increases to 1–2 mg mL$^{-1}$, achieving above 90% biofilm removal in the targeted area (Figure 4H and Figure S11). When STARS scrub adherent biofilm, some portion of STARS can be detached from main bristle assembly by mechanical friction between target biofilm surface and contacted STARS, but STARS continuously reattach to reach and mechanically scrub the target surface, leading to efficient biofilm removal. In addition, any detached portion of STARS is readily recollected by controlled magnetic fields and immediately reassembled to regenerate STARS.

Furthermore, we assess the reusability of STARS for biofilm elimination. We perform three consecutive biofilm treatments with the same IONPs. The results show that STARS has robust reusability over repeated treatment cycles (Figure S12). During consecutive treatments, even though a slight reduction in catalytic activity is observed (Figure S12A), the physical removal of biofilm (Figure S12B) and biofilm killing efficacy (Figure S12C) are stably maintained. Interestingly, we found that biofilm components (microbial cells and EPS matrix) were entrenched into the extended STARS bristle during the biofilm removal (Figure 4I) and can be retrieved together with the collected bristle as a structured biohybrid complex (Figure 4JK).

Altogether, STARS bristles operate through multiple complementary mechanisms that are capable of catalysis, physical intelligence (surface conforming, shape-adapting reconfiguration, and adjustable shear strength), and generation of tunable lateral forces and antimicrobial reagents in situ. Such functionality enables efficacious biofilm removal at multiple spatial and length scales, while capturing biofilm components. This provides multimodal features for designing autonomous motion routines for precise biofilm treatment and sampling by combining controlled mechanical and chemical activation occurring simultaneously with topography-adaptive functionality in real-time.

**Automated Biofilm Removal and Retrieval on Tooth Surface.** We employ 3D-printed tooth replicas and ex vivo human teeth to closely mimic the clinical conditions (Figure
Natural teeth have variations in physiological contours, geometries, and surface topography including varying degrees of surface curvature and angles. Interdental space between teeth is a confined hard-to-reach area where pathogenic biofilm can grow and flourish and requires manual flossing for removal. Our STARS bristles can conform to these variations through their adaptive nature. The bristle’s length can reconfigure to reach distant surfaces in confined spaces as it moves from flat to curved and through interdental spaces. We demonstrate this adaptability on a cross-sectional model of human teeth, which enables clear visualization (Figure 5B). As bristles are swept over the interdental space, they reconfigure and conform to the curvature of the surface, transforming from a “brush-like shape” to an extended “floss-like structure” (Figure 5C) that reaches the entire narrow gap (Movie S5 and Figure S14). These structures dynamically change their size with a tunable range over multiple length scales; the heights vary from submillimeter to >4.5 mm (Figure 5D) and widths range from 3 mm to submicrometer (Figure 5D,E and Figure S3), providing structural flexibility to adaptively conform to the interproximal region. To evaluate motion-controlled, 3D cleaning patterns, we created an experimental apparatus that enables combinations of different motions with fully automated cleaning routines (Figure S15).

Based on our results with vertical biofilm specimens (Figures S11 and S15), we created a set of fundamental STARS motion patterns, including circular, linear, and arced motions which mimic toothbrushing and test their cleaning efficacy on 3D-printed human tooth mimics (Figure 6A). Using a circular-linear dual motion, we demonstrate effective cleaning of the facial surface of anterior teeth (Figure 6A, top). However, this pattern does not effectively clean the curved interdental area in between two teeth. To address this, we add an arced motion following the contour of the interdental surface which targeted interdental cleaning (Figure 6A, bottom). Localized biofilm removal efficacy depends on the programmed motion as well as the targeted region. The circular motion is effective in broadly cleaning the facial tooth surfaces, while the arced motion directed along the space between teeth demonstrates high interproximal removal efficacy (Figure 6A, graph). We test the cleaning efficacy of the combined (multiaxis) motion, using an ex vivo human teeth model mimicking the natural tooth-gingival positioning and arrangement as well as its anatomical features. We demonstrate complete biofilm removal from the facial and interdental spaces on human teeth by the combined STARS motions (Figure 6B). In addition, we evaluate the killing efficacy following STARS treatment. The data show that the motion dynamics of STARS eradicated the biofilm (Figure S16A) with no viable cells detected (Figure S16B).
S16B), demonstrating mechanochemical properties for effective removal and killing of biofilms formed on human teeth.

An important aspect of STARS bristles is related to biosafety. The biocompatibility of IONP (the building blocks of STARS) has been thoroughly demonstrated in our previous study, showing neither cytotoxicity in vitro nor deleterious effects on oral mucosal tissues in vivo.

For further assessment of biocompatibility, we also investigated whether the mechanical action and scrubbing of STARS bristle can affect the integrity of the soft-tissue surface. We use an ex vivo pig jaw model whereby the gingival surface was directly exposed to STARS treatment and then collected for histological analysis. We found that standard (10 min) STARS treatment and scrubbing did not cause harmful effects on the gingival tissue surface (vs untreated control), showing intact keratinized layer (Figure S17).

As STARS continually reconfigure during biofilm cleaning at targeted areas, they incorporate biofilm contents into the bristle. This feature enables precise retrieval of biofilm contents with high spatial precision at the submillimeter level (Figure 6A, close-up). The heterogeneous distribution and non-uniform location of biofilms associated with human chronic infections make accurate sampling difficult, which would be particularly important for hard-to-reach biofilms such as those formed in the interdental space. A key challenge is to collect samples in targeted locations and in sufficient quantities that can be used to detect pathogens and biomolecules using currently available technologies.

To assess this capability, we use S. mutans and Candida albicans, which are bacterial and fungal pathogens found in virulent interkingdom biofilms on the tooth surface. Furthermore, the biofilm’s pathogenicity is associated with extracellular polymer (EPS)-producing exoenzyme termed glucosyltransferase (GTF), which can serve as virulence biomarker.

After biofilm sample collection, we analyze both the microbial content and enzyme activity (Figure 6C). We find bacteria, fungi, and EPS entrenched within the bristles (Figure 6D). Further analysis reveals that STARS can collect sufficient quantities of the biological materials, allowing detection of both bacterial and fungal pathogens by qRT-PCR at expected proportions as well as quantitative measurement of GTF activity (Figure 6D, graph). In addition, we also...
demonstrate that human coronavirus OC43 (HCoV-OC43) in biofilms can be also retrieved for viability detection (Figure S18), allowing interkingdom detection of bacteria, fungi, and viruses as well as their byproducts. The data indicate feasibility of applying STARS for diagnostic sampling of disease-causing biofilms, achieving localized sample retrieval for detection of infective agents and virulence-associated biomolecules, which would provide invaluable guidance for precise and personalized therapies. Together, STARS may lead to simultaneous therapeutic and diagnostic applications that combine autonomous, tether-free biofilm removal with concomitant data collection functionality.

CONCLUSION

In summary, we demonstrate the directed assembly of reconfigurable, cantilevered bristle-like superstructures composed of packed nanoparticles by spatially and temporally modulating magnetic fields. These surface topography-adaptive robotic superstructures (STARS) have tunable strengths, shapes, and reactivity that are effective for removing biofilms. We find a spatially ordered bristle superstructure with controllable stiffness that provides strong cohesion at the base, where support is required, and remains reconfigurable at the distal end, where surface conformality is desirable. This gradient in properties allows STARS to self-support, reach, and adapt to variations in surface topography in arbitrary orientations, while generating sufficient shear stress to dislodge biofilms. Furthermore, by introducing scalable and reversible architectures, the STARS length can be extended or retracted while conforming to crevices and other complex features, enabling treatment and retrieval of distinct microbes from within biofilm on hard-to-reach surfaces with microscale precision. Notably, the catalytic property is preserved throughout reconfiguration, providing mechanochemical function afforded by the assembled superstructures.

The findings on the interdependence of superstructure conformability, topography adaptation, physical force adjustment, and spatiotemporal magnetic field control may lead to further investigations at the intersection of reconfigurable soft-matter, functional nanomaterials, and microrobots. In addition, these dynamic structures can be actuated remotely and precisely with translational and circular motions using automated, programmable control algorithms. We demonstrate an application to potentially advance current oral health care modalities which have remained unchanged for decades. We create an automated, tetherless multitasking platform that integrates “toothbrushing-like” and “flossing-like” action simultaneously with antimicrobial activity in real-time and sample retrieval for compositional analysis and pathogen detection. We contemplate STARS as an operator-independent process that can be useful, for example, in oral health care, by persons with disabilities who have difficulty brushing their own teeth. Future studies will compare the STARS-based device with current manual handling biofilm removal technologies, including ultrasound-based devices and electric toothbrushes. Lastly, we envision development of feedback-guided STARS for on-demand motion patterns, control, and functional assembly that will lead to highly configurable structures able to adjust to different 3D surface geometries and adhesion strengths to achieve autonomous biofilm removal and diagnostics.

EXPERIMENTAL SECTION

Synthesis and Characterization of Iron Oxide Nanoparticles. The iron oxide nanoparticles (IONPs) used in this study were synthesized via a facile solvothermal method and characterized as detailed previously in ref 13. In a typical procedure, 0.82 g of iron(III) chloride was dissolved into 40 mL of ethylene glycol. Then, 3.6 g of sodium acetate was added to the solution under vigorous stirring at room temperature for 30 min. Subsequently, the mixture was transferred to a custom-built 50 mL Teflon-lined stainless-steel autoclave and heated for 12 h. After the autoclave was cooled to room temperature naturally, the IONP precipitate was collected, rinsed several times with ethanol, and then dried at 60 °C for 3 h. The synthesized IONPs were characterized using scanning electron microscopy (SEM, FEI Quanta 600, FEI, Portland, OR, USA). For IONPs size distribution, the region of IONPs was automatically detected and characterized by the size invariant circle detection method implemented in MATLAB built-in function “imfindcircles” (Figure S1) as described in ref 45.

Magnetic Field Control Device. The magnetic fields are controlled with a device combining programmable electromagnets and servo motors. The magnetic field for the reconfigurable STARS bristle was formed by two coaxially arranged electromagnets at a distance of 8 mm. Between the two electromagnets, an actuation vessel with dimension of 15 × 6.7 × 13 mm³ (width × depth × height, about 1.3 mL) was designed to accommodate IONPs suspension, and a holder was constructed to maintain an 8 mm gap between the two electromagnets. Both the electromagnets and servo motors were mounted around the actuation vessel and remained fixed in the same position for all experiments. A ferrite core with a diameter of 10 mm was situated in the center of the rear electromagnet. An iron core with a diameter of 2 mm was positioned in the center of the forward electromagnet. The position of the forward iron core is moved in varying trajectories using servo motors.

For side-to-side motion, a microservo motor (SG90) was programmed to move 50° (between 65° and 115°) with an arm of 14 mm length. A symmetrical arc-shaped movement of approximately 12 mm was implemented with various linear velocities from 6 to 48 mm s⁻¹. For circular motion, a continuous rotation servo motor (FS90R) was used and programmed to allow unrestricted movement at angles using a relatively short arm of 2 mm length. A continuous circular movement was generated centered with the axis of electromagnets with a linear velocity 12 mm s⁻¹ in clockwise or counterclockwise directions.

To create multimodal motions with a high degree of repeatability in position and timing, we created a sliding vessel. The body of the device consisted of two different parts: a holder for the electromagnets and servos and a sliding vessel. One servo drives a pinion gear attached to the rack of the sliding vessel for left-to-right motion, and the other servo drives the forward iron core for circular or arc-shaped motions. The sliding vessel holds the tooth replicas and natural teeth and was actuated by a programmable micro servo motor. The motion of the sliding vessel was coupled with the programmable motion of the electromagnet iron core, which allowed combination of motions to mimic brushing patterns.

The high-gradient magnetic field was directed by the repositioning of the iron core, and the various positions were mainly implemented by the movement of the servo controlling the iron core within the forward electromagnet (side to side and circular by regular and continuous micro servo), during which the magnetic field remained constant. Actuation in all experiments was performed via the application of a magnetic field and servo programmed to achieve the desired motion pattern. The movement of the microservo arm and the magnetic fields of electromagnets were controlled using a microcontroller (Arduino Nano, Arduino, Somerville, MA, USA). Each electromagnet was energized independently using a programmable DC power supply (Sorenson XTR60-14, AMETEK Programmable Power, San Diego, USA) and Arduino integrated development environment software for system coding.
The geometries of the actuation vessel and the body of the device were developed and designed with Onshape computer-aided design (CAD) software (Onshape Inc., Cambridge, USA). The actuation vessel and the body of the device were fabricated using a low-force stereolithography (SLA) 3D printer (Form 3B, Formlabs Inc., MA, USA) with 25 × 25 μm² lateral resolution and 50 μm layer thickness with a biocompatible photopolymer resin (Dental SG V1 resin, Formlabs Inc., MA, USA). For 3D printed parts, rinsing was performed for 20 min in fresh 99% isopropanol (IPA) and air drying for 30 and 60 min postprint ultraviolet light curing (405 nm light at 60 °C) (FormCure, Formlabs Inc., MA, USA).

Bristle-like STARS Formation. The bristle-like superstructures were assembled using a magnetic field control system as described above. The dispersed IONPs were collected by magnetic fields from the forward electromagnet for 5 s forming a rounded dome shape, and the strength of the magnetic field used for the standard biofilm removal experiments was measured to be 90 and 25 mT (MF100; Extech Instruments, Boston, MA, USA) at the forward and rear regions, respectively. The STARS were then designed to orthogonally extend from the forward vessel wall to form elongated bristle-like structures for 25 s by energizing the rear electromagnet. When moving the bristles from the front to the rear, the strength of the magnetic field was measured to be 35 and 70 mT at the forward and rear regions, respectively. At the beginning of the next cycle, IONPs were recollected at the forward region. A cycle of 30 s was repeated up to 20 times during dental biofilm removal experiments. The appearance and physical/mechanical properties of STARS bristles can be precisely controlled by the concentration of IONPs (0.5–2.0 mg mL⁻¹), the sweep velocity of the servo (6–48 mm s⁻¹), and the strength of magnetic fields (13.3–96.4 mT) at the center of the actuation vessel.

The STARS bristle formation and movement videos in the actuation vessel were captured using a Zeiss Axio Zoom.V16 fluorescence upright stereo zoom microscope system (Carl Zeiss Microscopy GmbH, Jena, Germany) with a 1× objective (numerical aperture, 0.25) at a video frame rate of about 100 fps. The videos were processed using Zeiss Zen Blue software to determine the position, velocity, and length of STARS bristle.

Surface Conforming Property of STARS Bristle. To evaluate reconfigurability and adaptation of STARS bristles, surfaces with various topographies including repeated circular, square, and triangular patterns were designed with Onshape. All patterned surfaces were formed on teeth extracted in the dental clinic (for surgical reasons) at the School of Dental Medicine were collected and repurposed for this study without any identifiers. The teeth were matched into pairs based on morphology and dimensions. Tooth pairs were scanned using a CERECA Omnicam Intraoral Scanner and integrated CEREC 5.0 software (Dentsply Sirona, USA) to implement a tooth mimic model. The scanned high-resolution 3D CAD images were used to generate 3D-printed teeth replicas and design a custom 3D-printed holder to mimic the gingival margins of the natural teeth using 3D CAD software (Blender, version 2.91.0; Blender Foundation, Amsterdam, Netherlands). 3D printing was done using Formlabs 3B printer with a biocompatible Dental SG V1 resin, and the 3D-printed teeth replicas were polished as described above.

The polished 3D-printed tooth pairs and the natural tooth pairs were sterilized in an autoclave for 20 min at 121 °C, placed in the custom-fit holder, and subsequently used for biofilm formation. The biofilms formed on teeth were placed in the actuation vessel and subjected to STARS bristle treatment.

Biofilms were formed on saliva-coated 3D printed slabs, 3D-printed tooth pairs, or ex vivo human tooth pairs. Streptococcus mutans UA159, a biofilm-forming model oral pathogen, was grown in ultrafiltered (10-kDa cutoff; Millipore, MA, USA) tryptone-yeast extract (UFFY) broth containing 1% (w/v) glucose at 37 °C and 5% CO₂ to midexponential phase following the protocol described in ref 15. Both saliva-coated 3D printed slab and tooth pairs were mounted vertically in 24-well plates and inoculated with ~2 × 10⁹ CFU of
actively growing *S. mutans* cells per milliliter in UFTYE containing 1% (w/v) sucrose at 37 °C with 5% CO₂ for 43 h. The culture medium was changed twice daily (at 19 and 29 h) until the end of the experimental period (43 h). In a separate experiment, *Candida albicans* SC5314 (a well-characterized opportunistic fungal pathogen) was used to generate mixed-species biofilms with *S. mutans*. For inoculum preparation, *C. albicans* (yeast form) and *S. mutans* cells were grown in ultrafiltered UFTYE broth (at pH 5.5 and pH 7.0 for *C. albicans* and *S. mutans*, respectively) containing 1% (w/v) glucose at 37 °C and 5% CO₂ to midexponential phase. Salisbury-coated 3D printed slabs were mounted vertically in 24-well plates and inoculated with \(2 \times 10^8\) CFU of actively growing *S. mutans* cells and \(2 \times 10^5\) CFU of *C. albicans* (yeast cells) per milliliter in UFTYE containing 1% (w/v) sucrose at 37 °C with 5% CO₂ for 43 h.

**Biofilm Disruption and Removal.** Biofilms were treated with STARS bristles assembled from IONPs (0.5, 1, and 2 mg mL\(^{-1}\) final concentrations) solution in the actuation vessel as described above. We used our previously optimized IONP formulation designed to enhance the catalytic bioactivity with EPS-degrading enzymes (dextranase/mutanase, 25/5 U) and 1% H₂O₂ (1% v/v) described in ref 19. Briefly, biofilms were placed in the actuation vessel containing IONP solution for 10 min followed by the addition of 1% H₂O₂ and subsequent modulation of the magnetic field, which forms STARS bristles. The velocity, trajectories, and motion frequencies were controlled as described in the previous sections. Biofilm removal in all experiments was performed via the application of an external magnetic field and servo movement preprogrammed to achieve the desired trajectories, velocities, and motion patterns.

Standard culturing method (number of viable cells by CFU determination) and stereotype-based fluorescence imaging were performed to assess the biofilm removal by the STARS bristles. For the culturing method, the total number of CFU per biofilm was determined after biofilm treatment. Briefly, the removed biofilm was collected and homogenized via water bath sonication followed by probe sonication (10 s pulse at an output of 7 W; Branson Sonifier 150; Branson Ultrasonics, CT, USA). Homogenized biofilm suspensions were serially diluted and subjected to microbiological analysis. The total number of viable cells was determined by CFU counting following the protocol in ref 15. For fluorescence imaging, SYTO9 (485/498 nm; Molecular Probes, Carlsbad, CA, USA) fluorescent probe was used for labeling bacterial cells, and Alexa Fluor 647 dextran conjugate (647/668 nm; Molecular Probes, Carlsbad, CA, USA) was used for labeling EPS. Before and after removal, images of the samples were taken with the Zeiss Axio Zoom.V16 fluorescence upright stereo zoom microscope system (Carl Zeiss Microscopy GmbH, Jena, Germany) with 1× objective (numerical aperture, 0.25).

To analyze the area of biofilms on the slab, open-source Fiji software was used. Images were processed for the green channel (SYTO9) due to significant cross-talk from the red channel (Alexa Fluor 647) from stereo microscopy. A standardized and constant rectangular-shaped region of interest (ROI; \(4000 \times 4000 \mu \text{m}^2\), width and height) was selected to calculate and compare only the biofilm area on the 4 × 2 mm² substrate. A median filter (radius = 2) was applied to eliminate noise and reduce false segmentation of background and out-of-focus signal. Otsu’s automatic global thresholding algorithm ref 46 featured in Fiji was used to classify and segment the colonized area of biofilms on the ROIs. The biofilm removal efficacy was calculated as \((a - b)/a\), where ‘\(a\)’ was the segmented area of biofilm before treatment and ‘\(b\)’ was the segmented area of biofilm after treatment for comparing removal efficacy of biofilms under the various actuating conditions as the normalized area of biofilm.

**Biocompatibility of STARS Bristles.** To assess the biocompatibility of STARS, an ex vivo pig jaw model was used. Fresh pig mandibles (Sierra for Medical Sciences, CA, USA) were used to create buccal and lingual gingival block sections (~12 × 4 × 10 mm³) using a HP medium flexible coated double-sided diamond disc (Brasseler USA). The gingival blocks are composed of tooth structure, alveolar bone, and attached gingival tissues. Gingival block sections were placed in the treatment vessel with STARS bristles assembled from IONPs (2 mg mL\(^{-1}\)) solution against the gingival tissue surface. The velocity, trajectories, and motion frequencies were controlled as described in the previous sections. After the standard treatment time (10 min), the gingival tissue was dissected from the block with a 15c blade scalpel. STARS treated and untreated controls were processed for H&E for histological analysis to assess the STARS effect on the mechanical integrity of gingival soft tissues.

**Biofilm Entrainment by STARS and Visualization.** Super-resolution confocal microscopy was performed to visualize the biofilm components (bacteria, fungi, and EPS) that become entrained among the extended STARS bristles. The EPS glucan matrix was labeled with Alexa Fluor 647 dextran conjugate (Molecular Probes). *S. mutans* and *C. albicans* (if applicable) were stained with SYTO9 (Molecular Probes) and Concanavalin A-tetramethylrhodamine conjugate (Molecular Probes), respectively, as described in ref 44.

After biofilm removal, STARS bristles were collected and immobilized in 1% agarose for super-resolution imaging using a 40× water immersion objective (numerical aperture = 1.2) on an upright confocal microscope (Carl Zeiss LSM 800, Germany) with Airyscan. The STARS bristles were visualized using the reflection mode and a 405 nm laser. In a separate experiment, biofilm components entrained in STARS bristles were dehydrated through a graded ethanol series and examined by SEM (FEI Quanta 600, FEI, Portland, OR, USA).

**Analysis of the Biofilm Retrieval Components.** STARS bristles with entrained biofilm components were subject to molecular and enzymatic analyses after retrieval. The fungal and bacterial genomic DNA was extracted and purified from the sample using DNeasy PowerLyzer Microbial Kit (Qiagen, Germany), following the manufacturer’s instructions. The qPCR reaction was performed using a CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) and PowerTrack SYBR Green Master Mix (ThermoFisher, Waltham, MA, USA). Specific primers were used to detect each microbial species (*S. mutans*, forward: 5′-ACCAGAAAGGGAGGCTAAC-3′; reverse: 5′-TAGCCCTTTTACCTCAGTTTCTCGT-3′; *C. albicans*, forward: 5′-AGAAGGCTAATAAGGACGATGA-3′; reverse: 5′-AGTCATGTGATACTCATCTCA-3′).

Bacterial glucosyltransferase (Gtf) activity was analyzed using scintillation counting as described in ref 47. The Gtf activity in the sample was measured in terms of incorporation of [¹⁴C] glucose from radiolabeled sucrose substrate (New England Nuclear Research Products, Boston, MA) into the glucan product for 4 h at 37 °C. One unit (U) of Gtf enzyme was defined as the amount of enzyme needed to incorporate 1 μmol glucose into glucan over a 4 h reaction period.

For virus detection, *S. mutans* single species biofilms were incubated with human coronavirus OC43 (HCoV-OC43) at 5 × 10⁹ plaque-forming unit (PFU) mL⁻¹ at 4 °C for 4 h. The biofilms were washed 3 times with phosphate-buffered saline to remove unabsorbed virus and treated with STARS bristles as described above. The biofilms entrenched in STARS bristles were serially diluted and subjected to standard virus plaque assay to evaluate the amount of viable HCoV-OC43 using Vero E6 cells. HCoV-OC43 plaques were quantitated at 7 days after infection of Vero E6 cells.

**Statistical Analysis.** All statistical analyses were performed with GraphPad Prism 8.0 (GraphPad Software, Inc., La Jolla, CA). All data are represented as mean ± SD. Comparisons between multiple groups were performed using a two-sided one-way analysis of variance (ANOVA) with post hoc Tukey’s test, where \(P < 0.05\) was considered significant and \(P > 0.05\) was considered not significant. At least three independent experiments were performed unless otherwise stated.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available for free of charge at https://pubs.acs.org/doi/10.1021/acsnano.2c01950.
Supporting figures include size distribution of IONPs, magnetic actuation setup, SEM of IONP assembled superstructure, magnetic flux density profiles in the vessel, bristle-like superstructure using different concentrations and sweeping velocities, length of bristle-like superstructure under different conditions, PDMS micropillar for force measurement, TMB assay for peroxidase-like catalytic activity of STARS bristles and dispersed IONPs, viable cell counts (CFU) of H2O2 alone treatment without IONPs, localized biofilm removal quantification using computational imaging method, biofilm removal using different concentrations and scrubbing velocities, reusability of STARS, 3D-printed teeth model, time-series images (rewound frames) showing STARS bristle disassembly, automated motions for biofilm removal, biofilm removal efficacy in natural teeth model, histopathology of gingival tissue in pig jaws treated with STARS scrubbing, and cell viability of retrieved human coronavirus (HCoV-OC43) from biofilms using STARS bristles (PDF)

Movie S1: Top-view. The magnetic field is driving the superstructure assembly with its base on the vertical vessel wall extending orthogonally toward the rear electromagnet. By energizing the rear electromagnet and cyclically changing the position of the forward core, the bristle superstructure extends in length as it translates across the vertical vessel wall. Red arrows indicate the increase in length during the lateral motion while the superstructure self-supports orthogonally (MP4)

Movie S2: Top-view. STARS bristles can readily extend to enter the surface recess (corner in the square patterned surface). Then, forward-and-reverse video playback shows the disassembly process of STARS at the end of the cycle whereby the self-supporting structure dismantles and falls to the bottom of the vessel (MP4)

Movie S3: Top-view: STARS bristles extend and conform to the opposing vertical surfaces with different topographies (repeated circular, square, triangular patterns) demonstrating shape adaptation with varying lengths (MP4)

Movie S4: Top-view. Small-scale features at the ends of the STARS bristles interacting with the single PDMS micropillar allowing characterization of the local force generated to measure deflection (MP4)

Movie S5: Top-view. STARS bristles rearrange on a cross-sectional model of human teeth to reach, access, and conform into the narrow interdental space. As bristles are swept over the interdental space, they move inward and conform to the curvature of the surface, transforming from a “brush-like shape” to an extended “floss-like structure” (MP4)

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