Living organisms have evolved a multitude of specialized structured surfaces that are useful for interacting with their environment. These are a rich source of inspiration for biomimetic applications, from gecko-inspired sticky eyes to the smooth skin of chameleons. Other areas of interest are the patterning of surfaces to enhance their fluid mechanical properties, a field in which sharks have garnered attention. Shark skin harbors microscopic denticles whose shape and size vary not only between different species of shark but also within different body locations on the same shark. The denticles themselves are rigid but their tilt angle is variable and dependent on both the swimming motion and speed of the shark. All of these elements make it extremely challenging to model the effect of a combination of natural shark-like motion and denticle structure on fluid mechanics during swimming. While most studies report a reduction in drag, others have reported the opposite effect, and it is currently thought that the size and distribution of the denticles as well as cruising speed are all essential to obtain the desired effect. However, computational simulations remain extremely difficult due to the complexity and diversity of the denticle’s shape. Therefore, the development of a low-cost and easy-to-implement manufacturing procedure is a necessary step toward deepening our understanding of the fluid dynamics of complex structures such as shark skin through experimental fluid mechanics.

Commercial products such as Speedoo’s Fastskin line of swimsuits, which were highly publicized as being “shark-like”, do not represent the structure of true shark skin denticles, and their effectiveness is controversial. A straightforward approach to the production of truly biomimetic shark skin is to produce silicone-replica of natural shark skin, as demonstrated by a study on the copper shark Squalus suckleyi at x10 scale with general equipment that may be purchased and operated fairly easily by any laboratory. The dilemma of 3D printing is that the manufacturing costs both in terms of time and budget are inversely related to feature size, so that the large-scale production of custom micro-patterned surfaces remains a challenge. Here, we describe a relatively low-cost method to generate enlarged biomimetic surfaces for the experimental measurement of their fluid mechanics properties in large towing tanks, and we illustrate this method by producing a repetitive pattern of denticles from the shark Squalus suckleyi at x10 scale with general equipment that may be purchased and operated fairly easily by any laboratory.

**Methods**

**DNA extraction and analysis of biological samples.**—DNA was extracted from the flesh located immediately underneath the shark dermis using TRIzol Reagent (ThermoFisher Scientific, Inc.) according to the manufacturer’s instructions. The quantity and quality of the extracted DNA was assessed by Nanodrop ND-1000 spectrophotometer. The 18S rRNA gene was amplified by PCR using HF Phusion polymerase (New England Biolabs, Inc.) in a thermocycler and sequenced by capillary sequencing on a 3130xl Genetic Analyzer (Applied Biosystems, Inc.) using the BigDye Terminator v3.1 cycle sequencing protocol. DNA alignments were performed using nBLAST 2.8.1+. The DNA ladder used in Figure 2 was the 100 bp DNA Ladder, Version 2 (#DM420) (DynalMarker, Inc.).

**Equipment and software for design and manufacturing.**—The microscope used for imaging shark skin was the variable-pressure scanning electron microscope (SEM) FlexSEM 1000 (Hitachi High-Technologies, Inc.) equipped with the 3D-SEM option. Molds were designed using the academic license for Netfabb additive manufacturing software provided by Autodesk. The 3D printing settings and slicing were conducted in Ultimaker Cura version 2.7 and printed on an Ultimaker 3 (Ultimaker, Inc.) using orange PLA 2.85 mm filament. The silicone materials were purchased from Shin-Etsu, and standard MDA-015 vacuum pump (ULVAC KIKO, Inc.) and chamber was used to eliminate air bubbles.

**Additional hardware requirements.**—During mold design, the same 3D pattern may be repeated thousands of times. Furthermore, the method described in this paper requires processing of the data via a graphical user interface or GUI, leading to prohibitive memory requirements. We recommend using a computer equipped with at least 20 GB RAM and a hardware-accelerated graphics card.
Figure 1. Workflow for the production of biomimetic silicone rubber films.

64GB DDR4 RAM and to allocate a swap memory size of 256GB on a dedicated solid state hard drive.

Experimental

Overview.—This method starts with shark sampling and acquisition of microscopy data of shark skin denticles using either scanning electron microscopy or X-ray microtomography. A mold is then designed using 3D software by extracting a pattern unit which is then repeated and 3D printed in polylactic acid (PLA) at 54 micron layer height. This PLA mold is then used to cast a silicone rubber film, which can then be attached to an airfoil or other relevant surface for water flow measurement in a towing tank (Figure 1).

Shark sampling and species identification.—Fishermen from the Shonai area, Yamagata Prefecture, Japan, donated sharks that were accidentally captured during fishing activities in the Sea of Japan (Figure 2A). A total of 10 fresh shark specimens of body length of 0.7–0.9 meters were caught between February and April 2017, including males and females of 4 species encompassing 3 different orders of shark (Table I). The described adult length was 1.0–1.6 meters for all species represented in our collection, meaning that most of our specimens were at least about half of their adult size. For this study, we chose *Squalus suckleyi* for three reasons: (a) it is classified as “least concern” according to the IUCN Red List of Threatened Species, and abundant enough in our geographical area to make it a relatively common bycatch; (b) they inhabit a wide depth range, from shallow waters (mostly 50–149 m) down to 1,236 m, meaning that they are comfortable swimming at various depths; (c) the closely related *Squalus acanthias*, or Atlantic spiny dogfish, is one of the most well-studied Squaliformes in terms of molecular biology, with the availability of multi-tissue transcriptomics data and embryo-derived cell lines.

To confirm the species, DNA was extracted from the flesh located underneath the shark dermis (Figure 2B, Experimental). The 18S ribosomal RNA (rRNA) gene is commonly used as a molecular marker for species identification. Therefore, DNA primers were designed to bind to conserved regions of the 18S rRNA gene, and 1,800 base pairs within this gene were amplified by polymerase chain reaction (PCR) (Figure 2C, Experimental). The PCR product was then sequenced and aligned against the NCBI database, confirming that the specimen we selected belonged to the *S. suckleyi* species.

3D microscopy of shark skin denticles.—To acquire the morphology of shark skin denticles of *S. suckleyi*, a 2 × 2 millimeter area was cut out from right below the rear dorsal fin (Figure 2B). Using a scanning electron microscope (SEM), the same specimen was captured from 4 different angles at x100 magnification for surface reconstruction (Figure 3A, Experimental). This method reminiscent of photogrammetry is able to capture the morphology of the sample surface but is unable to register elements that are completely hidden or shadowed, such as the bottom part of the denticles. To capture the full 3D morphology of the denticles, X-ray microtomography (μCT)
was carried out using a customized machine at the University of Tohoku Museum at a resolution of 2 × 2 × 2 μm (Figure 3B). Because μCT is able to discriminate between different material densities, single denticles are easily distinguished from the surrounding tissue. In both cases, the data is output as an STL file.

**Mold design.**—The following process is written with the SEM data in mind, but may be easily adapted to the μCT data as well. When handling STL files for 3D printing, one should keep in mind that not all STL files are compatible with 3D printing. The STL format describes an object’s surface in terms of the coordinates and orientation of a series of triangles fitting that surface. Sometimes, the orientation of some triangles does not match the curvature of the surface, in which case subsequent slicing into layers and 3D printing may fail. Furthermore, 3D printers are not able to handle 2D surfaces with zero thickness, and STL files may sometimes contain such elements. The Netfabb software (Autodesk) specializes in editing, troubleshooting, and optimizing STL files specifically for additive manufacturing, and is free of charge for academics.

The STL file containing the 3D data is imported into Netfabb, and the freecut function is used to cut a representative pattern out from the 2D surface (Figure 4, upper left). At this point, the single denticle contained 271,226 triangles, for a file size of 50 MB. Since we were planning to fit 1,152 denticles on an 18 × 18 cm mold, this was likely to have produced an extremely large file of more than 50 GB, which is more than most computers can handle in a GUI interface. This block is then rotated 45 degrees and duplicated as many times as necessary (Figure 4, upper right). The ensemble of block units is then trimmed so that half of the blocks located on the edges are cut exactly in half. All block units are then merged to generate a single mesh. Basically, the mold was designed so that the 3D printed layers are oriented parallel to the denticles, to make sure the subtle lines generated by layer deposition were oriented in the same direction as the water flow.

**3D printing.**—The prints were tested in many orientations and we found that printing the mold vertically instead of horizontally drastically improved the quality of the print. Printing horizontally is unrealistic because the XY resolution of FDM printers is limited by the size of the nozzle. The nozzle we used has an inner diameter of 0.25 mm (250 μm), which is only 1/8th of the width of a single denticle. Furthermore, filament retraction becomes an issue as the printer should retract the filament just enough to prevent unwanted deposition of filament while moving through empty spaces. All of these problems were solved by printing vertically because the Z-axis resolution of this printer is 60 μm, and the nozzle does not need to cover any empty space. We were successful in printing 0.5 × 18 × 18 cm molds vertically without using any brim, skirt, or raft to secure the polyactic acid (PLA) print to the printer bed. However, proper calibration was essential to get the first layer to stick uniformly enough to support the print throughout. Unsurprisingly, the taller the print, the more wobbly it got toward the end of the print. Past the height of 9 cm, wobbling significantly altered print quality, so we chose to print 0.5 × 18 × 9 cm molds. Instead of generating new STL files, the height may be simply arranged by changing the position of the mold along the Z-axis in the 3D printer software (here, Cura version 2.7) so that it looks like the

<table>
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<tr>
<th>Order</th>
<th>Latin name</th>
<th>Common name</th>
<th>Depth range (m)</th>
<th>Number</th>
<th>IUCN status</th>
</tr>
</thead>
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<td>Squaliformes</td>
<td>Squalus suckleyi</td>
<td>North pacific spiny dogfish</td>
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<td>3</td>
<td>Least concern</td>
</tr>
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<td>Squaliformes</td>
<td>Dalatias licha</td>
<td>Kitefin shark</td>
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<td>Vulnerable</td>
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<td>Heterodontiformes</td>
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<td>japanese bullhead shark</td>
<td>6–37</td>
<td>4</td>
<td>Least concern</td>
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<tr>
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<td>Mustelus griseus</td>
<td>Spotless smooth-hound</td>
<td>5–51</td>
<td>2</td>
<td>Data deficient</td>
</tr>
</tbody>
</table>

**Table I. List of shark specimens collected during this study.**

![Figure 3](image_url). Data acquisition. (A) Reconstruction of the 2D surface of shark skin using an SEM by taking four pictures at different angles; (B) Acquisition of the full 3D structure of individual shark skin denticles by X-ray computed microtomography. Note: the data shown here was from *Heterodontus japonicus* (Table I).
Figure 4. Mold design and 3D printing. [Top] Isolation of pattern unit from the SEM data by cutting out a single denticle (left), creating a “block unit” carrying the complementary surface (middle), and duplicating this block as many times as necessary (right); [Bottom] The STL file of the mold is converted to gcode according to optimized printer-specific settings (Table II) (left); a representative example of a complete simultaneous print of four 0.5 × 18 × 9 cm molds. The initial yellow PLA spool was replaced by a blue one in the middle of the print.

mold goes right through the print bed (Figure 4, bottom left). In Cura, the slicer will interpret this as printing only the part that is above the print bed, as shown by a picture a finalized print (Figure 4, bottom right). It should be noted that the color of the PLA is irrelevant and may be changed halfway through the print if it looks like the printer is going to run out of filament. After a series of tests, the Ultimaker AA 0.25 mm nozzle and the combination of a number of parameters such as layer height, line width, infill line width, travel speed, etc. were optimized and presented in Table II.

Silicone rubber casting.—The silicone rubber KE-106 RTV (Shin-Etsu, Inc.) was selected for the following properties: compatibility with PLA molds, the lack of a requirement for mold coating, relatively long working time at room temperature (2 hours at 23°C), and the possibility of letting it cure also at room temperature as PLA is susceptible to deformation in the presence of heat. The expected hardness on the Durometer A scale is 56A, and tensile strength 8.0 MPa.

As described in Figure 5, the silicone solution is mixed with the curative agent CAT-RG (Shin-Etsu, Inc.) at a 100:10 ratio and black toner was added until transparency was sufficiently low. This was tested by spreading the silicone solution onto a piece of paper with characters printed in black; we stopped adding toner when it became difficult to distinguish the letters. This coloring was done to avoid the laser reflecting through a transparent silicone rubber shark skin during subsequent measurements. The mixing of silicone, curative agent, and toner was done manually but there are machines to automate this. To remove bubbles, we subjected the solution to vacuum treatment. The vacuum was regularly paused to prevent the solution from overflowing, and this process was iterated until bubbles no longer appeared and the solution settled down. Then, using a 10 mL syringe, the silicone rubber was cast onto the 3D printed molds by carefully and slowly applying it to each denticle shape by following a rectilinear path. Then, a 2 mm-thick acrylic board was pressed against the mold to make sure the silicone film is both as thin as possible and perfectly smooth on one side. At this point, the whole setup was left at room temperature (23°C) for at least two days before unmolding. Finally, the silicone rubber was removed from the mold, leaving a 3D replica of the shark skin.

Table II. List of Cura Print Settings (bold: changed from default).

<table>
<thead>
<tr>
<th>Setting</th>
<th>Value</th>
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<tr>
<td>Layer Height</td>
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<tr>
<td>Line Width</td>
<td>0.219 mm</td>
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<tr>
<td>Infill Line Width</td>
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<td>Wall Thickness</td>
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<td>Outer Wall Wipe Distance</td>
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<td>Top/Bottom Thickness</td>
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<td>Wall Speed</td>
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<td>Travel Acceleration</td>
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<tr>
<td>Print Jerk</td>
<td>25 mm/s</td>
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<td>Travel Jerk</td>
<td>30 mm/s</td>
</tr>
<tr>
<td>Enable Print Cooling</td>
<td>Yes</td>
</tr>
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</table>
Figure 5. Silicone rubber casting. [Top] From left to right: RTV silicone rubber (KE-106) is mixed with curative agent (CAT-RG) at a 10:1 ratio and black toner was added until the mixture was opaque. Vacuum treatment ensured removal of air bubbles. The silicone rubber was then applied to the mold; [Bottom] An acrylic board was pressed onto the silicone rubber. Two days later, the silicone rubber film was unmolded, manually trimmed, and assembled into a larger surface.

rubber films were manually trimmed and assembled to the desired size (Figure 5, bottom right).

Results

Assembly on airfoil with 20 cm chord.—The panels covered in denticles may be easily placed by rotating in increments of 90° to produce the desired denticle orientation relative to the flow of water (Figure 6). The airfoil we chose to test had a chord of 20 cm. To cover the entire surface, several panels were assembled as shown in Figure 6 to obtain a $23 \times 37$ cm area covered in 3,309 denticles. Water-resistant double-faced tape was used to apply the film to the stainless steel airfoil, and the remaining few centimeters until the trailing edge were completed by adding an appropriate length of additional denticle-covered silicone rubber film. The direction of the denticles is indicated in red and blue and the direction of the water flow by blue arrows (Figure 6). The denticles were oriented so that the leading edge (toward which the water flow arrows are pointing) had denticles in the same direction as the upper surface (blue) whereas the lower surface, which cannot be seen in this picture, had denticles facing the opposite direction (red).

Quality assessment of biomimetic shark skin film.—To assess the quality of the final product, a small portion consisting of just 40 denticles was cut out and observed by scanning electron microscopy (SEM) in backscattered electron topographic mode (BSE-TOPO mode) at low magnification ($x37$). The scales harbored fine lines corresponding to the 3D printed layers on the mold. The average layer height was estimated at 54 $\mu$m, which is just below the 60 $\mu$m layer height that we had selected for the settings (Figure 7). The shape of the denticles were according to planned, with 3 clearly visible keels (ridges) and corresponding valleys. The average size of the denticles was $4 \times 5 \times 1.1$ mm, and the thickness of the silicone rubber film itself was about 0.3 mm.

Discussion

Challenges in manufacturing scale and cost.—The resolution of most consumer 3D printers – either fused deposition modeling (FDM) or stereolithography (SLA) – is usually around 50 $\mu$m per layer, and rarely goes below the 20 $\mu$m mark. For instance, the XY resolution is often worse than the Z-axis resolution due to a combination of the limitations of stepping motors and the properties of the materials used. To our knowledge, the maximum resolution currently available for an SLA printer is the Pico2 (Asiga, Inc.), advertised for jewelry and...
dentistry with a minimum voxel size of $39 \times 39 \times 1 \mu m$ and a consequently small build volume of $5.12 \times 3.2 \times 7.5 \text{ cm}^3$ (122.9 cm$^3$). In a completely different league both in terms of price and precision, the Photonic Professional GT2 (Nanoscribe, Inc.) applies 2-photon laser technology to reach submicrometer resolution, bringing the minimum lateral feature size down to 0.16 $\mu m$. However, the build size is limited to just about 0.8 cm$^3$, with print time increasing as a function of resolution. All of these factors combined result in an inability of current 3D printing technology to mass produce large surfaces of shark denticles at biological scale (150–500 $\mu m$ per denticle).

The first completely artificial biomimetic shark skin was published by the Lauder lab at Harvard in 2014. They were able to produce 17.7 $\times$ 8.7 cm biomimetic films based on a $\mu$CT-scanned model of a Mako shark’s 150 $\mu m$ denticle at x12.4 scale (1.86 mm per 3D printed denticle) using the Objet Connex500, a multi-color, multi-material 3D printer with a 50 $\times$ 40 $\times$ 20 cm build volume and a minimum layer height of 16 microns, which is based on inkjet (PolyJet) technology. In a subsequent study, they 3D printed airfoils with shark skin denticles directly attached to them. However, even a second-hand purchase of this printer would require an initial investment of $50,000–$100,000, and the version currently on the market (Objet500 Connex3) has a public price tag of $350,000. In fact, few 3D printers are more expensive than this.

Strategic choices – mold versus direct printing, denticle 2D surface versus 3D shape.—Our current method describes the manufacturing of a shark skin-like silicone rubber film that mimics the simplified 2D surface of a shark’s skin. As shown in Figure 3, while SEM is only able to capture surface topology, $\mu$CT shows that denticles have an intricate 3D shape with a base that is hidden in the SEM data. 3D printing of the full 3D shape remains challenging for several reasons: supporters are required for 3D printing overhangs of $\leq 30^\circ$ relative to the horizontal plane, and these supporters would need to be removed during post-processing. This may be possible with dual extrusion, using PLA for the mold and a water-soluble polyvinyl acid (PVA) filament for the supporters, however, in this case the unmolding of a very thin silicone rubber film without tearing or ripping would be difficult. In this case, direct 3D printing of the shark skin shape should be possible if the angle and/or base of the denticle is trimmed or tilted just enough to circumvent the overhang problem; this strategy seems to have been adopted by the Lauder group. In contrast with direct 3D printing, the mold strategy we adopted here allows for re-use of the molds to increase the manufacturing output. Therefore, in order to develop and optimize 3D printed molds for silicone rubber casting, we opted for the more simple 2D surface version as our first prototype.

So far, no one has tested an artificial shark skin configuration with just the top 2D surface of the denticles, so it is not known for sure which design is the best and in which context. However, the simpler design is much more likely to be applicable to industrial-scale production on a wider variety of instrumentation, for the reasons described above. Previous research also tested a single row of a ‘continuous shark-inspired profile’, which is basically a continuous 2D surface with denticle-like peaks and valleys, and they obtained promising results albeit in a specific context. This suggests that the 2D surface structure alone is sufficient to enhance the fluid dynamic properties. Therefore, our current design is likely to address some of the scalability issues of the full 3D model in terms of manufacturing, while still retaining the potential for drag-reducing effect in certain configurations which remain to be determined. Our technique also has the advantage of enabling the rapid and reproducible production of relatively wide surfaces of flexible shark skin-like films.

Low-cost manufacturing of reasonably large biomimetic surfaces.—In this study, we used a commercially available FDM-based 3D printer, the Ultimaker 3 (UM3), the retail price of which is comparable to that of an expensive laptop ($3,500). The UM3 has the advantage of being compatible with open source software (CuraEngine) and comes with dual interchangeable nozzles. The resolution and print time may be optimized by choosing an adequate nozzle size. According to the maker’s website, the lowest possible Z-layer height (20 $\mu m$) is supposed to be attainable with the 0.4 mm nozzle. However, in our hands, the best resolution we could achieve was with the 0.25 mm nozzle at 54 $\mu m$ average layer height, which is slightly better than the shortest layer height for that nozzle (60 $\mu m$). This means that shark denticles of approximately 400 $\mu m$ width, printed at x10 scale, comprised 74 layers, which was sufficient to reliably reproduce the shape of the denticles. The resolution claimed by the Harvard study is still approximately 3 times better, but our solution is significantly cheaper, to the point that it may be reproduced and built on by any laboratory. Our current working size for the mold is limited by the requirement to print it vertically, but we were reproducibly successful with 0.5 $\times$ 9 $\times$ 18 cm molds, on par with previous studies. The 0.5 $\times$ 18 $\times$ 18 cm vertical prints may be improved by designing support bars to be printed along with the molds to prevent the significant wobbling encountered past 9 cm of height. The printing of one 0.5 $\times$ 9 $\times$ 18 cm mold took approximately 30 hours, and two UM3’s working in parallel were able to produce 8 reusable molds in...
4.5 days. The casting of silicone rubber required an additional 2 days, meaning that the production time for a 23 × 37 cm surface (Figure 6) was about 1 week.

Things to consider when measuring fluid mechanics.—Another aspect that is important to consider prior to manufacturing is the ability to measure the flow of water on the shark skin surface. This is done by attaching the artificial shark skin to a given surface, such as an airfoil, and towing the model through a water tank filled with particles of a given size. The movement of these particles is captured by a laser and high speed camera. If the denticles are to-scale, then a higher resolution camera is required to visualize the flow. If the denticles are 10 times bigger, then the airfoil and the test facility should also be 10 times bigger. On the other hand, the airfoil should travel at 1/10 of the shark’s swim speed to keep the same Reynolds number. This enlarged setup is therefore advantageous in terms of the required spatial and temporal resolution of the high speed cameras, but requires the production of larger surface areas as presented here. Therefore, the present method’s purpose was to decrease the production cost for enlarged models of the shark skin denticles while also increasing the working surface sufficiently to enable experimenting in large towing tanks. We expect this method to be useful for research teams investigating the fluid mechanic properties of various types of micropatterned surfaces.

Conclusions

Our method enables the production of relatively large surfaces of orientable micropatterned repetitive structures at a very reasonable cost-performance. This method will benefit researchers looking to test a wide range of biomimetic shapes for experimental fluid dynamics measurements in large towing tanks.

Acknowledgments

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