

Water-Processable, Biodegradable and Coatable Aquaplastic from Engineered Microbial Biofilms

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Abstract

Plastics have become an integral part of humans' day-to-day activities and nearly 80% of the plastics produced globally have accumulated in landfills and bodies of water. Contamination from non-biodegradable plastics and microplastics (<5 mm fragmented particles) are causing potentially irreversible damage to our ecosystems. Herein, we report aquaplastic, a new class of microbial biofilm-based biodegradable bioplastic that is water-processable, robust, imprintable and coatable. *E. coli* was genetically engineered to produce protein-based hydrogels, which are cast and dried under ambient conditions to produce "aquaplastic" that can withstand strong acid/base and organic solvents. In addition, aquaplastic can be healed and welded to form three-dimensional architectures using water. The combination of straightforward microbial fabrication, water-processability, and biodegradability make aquaplastic a unique material worthy of further exploration as an alternative to conventional plastics in certain applications.

With over 8.3 billion tons having accumulated on Earth, plastics are one of the most abundant human-made materials to date, and their continuing manufacture accounts for what is currently a trillion-dollar industry¹. The broad utility and cheap manufacture of plastics have undoubtedly contributed to a range of modern lifestyles, spanning all geographies and socio-economic strata. However, their success as components of consumer goods, building materials, and as pillars of

our global material economy comes at the expense of their environmental and health impacts. More recently, the accumulation of microplastics has been increasingly cited as the cause of death for marine life and it is likely that many, if not most, humans have some level of microplastic accumulation in their bodies due to ingestion²⁻⁵. Although the proliferation of policy measures designed to curb plastic accumulation, like “reduce and recycle” initiatives, and bans on single-use plastics, should be regarded as positive signs in terms of the prevalence of political and social will to solve these problems, there is a need for more drastic changes if the negative impacts of plastic pollution are to be avoided^{6,7}.

One potential solution to further plastic accumulation is to substitute bioplastics, especially those that are biodegradable and produced from biological foundries (i.e. bio-based), for petrochemical plastics⁸. Although biological production of polyethylene, polyethylene terephthalate and other polymers and precursors from engineered microbes alleviates fossil fuel demand, it leads to the same end-of-life consequences as traditional plastics. At present, bioplastics account for ~0.6% (2 million tons) of global plastic production annually⁹. Bioplastics composed of biodegradable polymers such as polylactide, polyhydroxyalkanoate, and starch blends are slowly becoming more prevalent, but still share only ~0.3% of the global plastics market. Because these polymers are usually derived from crops, the current market also requires about 0.8 million hectares of arable land, prohibiting its significant expansion without drastic increases in land commitments or further technological innovations.

In order to address the challenges outlined above, a growing subset of plastics are designed to dissolve or degrade in water over various time scales. Polyvinyl alcohol (PVA) is the most prevalent water-soluble plastic, and used in a wide range of applications related to packaging and coatings¹⁰. Although bulk materials composed of PVA can dissolve in water, the polymer is synthesized from petrochemically derived ethylene by free-radical polymerization. Moreover, PVA has been consistently found to have limited biodegradation (several months to years) in natural-solid matrices such as soil and compost, as well as in water-bodies that lack PVA-degrading microbes¹⁰. Here we demonstrate a new water soluble bioplastic that is fabricated directly from bacterial culture with minimal purification and easily be processed from a hydrogel state to form bulk materials (Fig. 1A-C). Plastics that can be produced from biological components in water offer greener fabrication methodologies than those obtained from synthetic and non-biodegradable components in non-aqueous media¹¹. Since this new bioplastic is microbially-derived, water-processable, and biodegradable, it could offer a more holistically sustainable alternative to conventional plastics.

Results

Fabrication of aquaplastic. Aquaplastic is composed of recombinantly produced biofilm matrix proteins. Biofilms are communities of microbial cells embedded in an extracellular matrix that provides them adhesion capabilities, and protection from the surrounding environment¹². We leverage the curli system of *E. coli*, which is the primary proteinaceous component of its biofilm matrix, formed by the secretion and extracellular self-assembly of CsgA protein monomers into a fibrous mesh¹³. In previous work, we have demonstrated that a refactored curli expression system can serve as a platform for the biological fabrication of numerous functional materials¹⁴⁻¹⁸. In one specific example, fusions of the curli fiber monomer, CsgA, to a human cytokine, trefoil factor 2 (TFF2), enabled us to harvest hydrogels simply by filtering bacterial cultures, using sodium dodecyl sulfate (SDS) as a gelator (Fig. S1, S2)^{19,20}. In this work, we denote these hydrogels as either *biofilm aquagels*, which contain viable bacterial cells, or *curli aquagels*,

which are inert gels from which the cells have been removed or inactivated. Here we show that both types of gels can be cast onto surfaces or molds to create plastic films, which we call *biofilm aquaplastic* and *curli aquaplastic*, respectively.

Unlike traditional thermoplastics and thermoset plastics, which are melted at high temperatures to form semi-solids that are then molded into desired shapes, aquaplastics are made by casting the aquagels and drying them under ambient conditions to mold their form. When the *biofilm/curli aquagels* are cast on a flexible or soft flat surface, they form plastic films that can be easily peeled off to form free-standing structures (Fig. 1D, Fig. S2). For the *biofilm aquaplastics*, these films were flexible enough to be twisted repeatedly into helical shapes without wrinkling or cracking. Because of the remarkable robustness of the constituent curli fibers, the *biofilm aquaplastics* can also be molded into three-dimensional architectures, like cones, bowls, tubes, and spheres by casting them on a sacrificial polystyrene foam mold, followed by selective dissolution of the mold (Fig. 1E, Fig. S3).

Physical and chemical properties of aquaplastic. Thermogravimetric analysis (TGA) showed that both the *biofilm aquaplastic* and *curli aquaplastic* start to degrade above 200 °C (Fig 2A). Differential scanning calorimetric (DSC) analysis of aquaplastics revealed glass transitions for *biofilm aquaplastic* and *curli aquaplastic* at 150 °C and 145 °C, respectively, during the cooling cycles (Fig. S4). Wide-angle X-ray scattering analysis (WAXS) on both *biofilm aquaplastic* and *curli aquaplastic* revealed the characteristic signatures of cross- β secondary structure of CsgA with d -spacing values of 0.98 nm and 0.46 nm (Fig. 2B), corresponding to the inter- and intra- β -sheet distances, respectively, and indicating that the curli amyloid structure is maintained through the aquaplastic processing steps²¹. The mechanical properties of the aquaplastics were investigated by nanoindentation, which showed Young's modulus values, E , of 2.2 ± 0.7 GPa (*biofilm aquaplastic*) and 4.3 ± 0.9 GPa (*curli aquaplastic*), respectively²². The corresponding hardness, H , values at maximum load were found to be 167 ± 109 MPa and 252 ± 140 MPa, while the yield strength, σ_y , values (estimated using the relation $\sigma_y = H/3$) were 55 ± 36 MPa and 84 ± 47 MPa, respectively (Fig. 2C-F)²³. Thus, the mechanical properties of aquaplastics are as good as any other commercially available conventional plastics and bioplastics, which typically exhibit $E \sim 0.5$ -4 GPa.

The biodegradability of the aquaplastics was investigated under aerobic conditions by incubating them for 45 days in the presence of a mixed culture of microorganisms obtained from the primary effluent of a wastewater treatment plant²⁴. Microorganisms in the mixed culture media metabolize samples into biogas and water-soluble species (*i.e.*, ions or simple protein/sugars) resulting in mass loss for each sample. The aquaplastics were compared to nanocellulose, which is known to undergo rapid and complete biodegradation. Both *biofilm aquaplastic* and *curli aquaplastic* were highly biodegradable under these conditions, losing 93% and 89% of their initial masses, respectively, over the course of 45 days, while the nanocellulose control lost 85% mass over the same time span (Fig. 2G).

Although, the aquaplastics are highly biodegradable, their constituent curli fibers are known to be resistant to disassembly in the presence of solvents, harsh pH values and detergents²⁵. We found that both aquaplastics were stable in non-polar solvents like *n*-hexane, even after 24 hours of incubation (Fig. 3A, Fig. S5). Aquaplastics were also stable in chloroform, which is known to dissolve coatings, rubber and many plastics such as polystyrene and polyvinyl chloride (Fig. 3A, Fig. S5). Surprisingly, both aquaplastics remained intact and unchanged after 24 h in saturated

sodium hydroxide. On the other hand, upon incubation in concentrated sulfuric acid (98%), both aquaplastics formed gelatinous films with double the original film dimensions within a few minutes (Fig. 3A, Fig. S6A). Similar exposures to concentrated nitric acid (70%), led to a slight yellow coloration and degradation after 6 hours for both aquaplastics (Fig. S6B and S7). The aquaplastics maintained a constant dry mass during incubation in the organic solvents (Fig. S8). Only the *curli aquaplastic* retained its mass in sulfuric acid, while the *biofilm aquaplastic* lost ~55% of its mass. Mass retention experiments could not be determined accurately after exposure to NaOH because of precipitation of salts during the drying process.

Coatable and imprintable aquaplastic. The chemical resistance of the aquaplastics and the known adhesive properties of curli fibers further inspired us to fabricate protective coatings, which are currently made with non-biodegradable plastics²⁶. Unlike traditional plastic powder-based coating strategies²⁷, aquaplastic coatings were formed by casting aquagels onto various surfaces followed by air-drying under ambient conditions. Aquaplastics could be applied easily to create strongly adhered coatings on convoluted surfaces, like leather or plywood, or on flat surfaces, such as mobile phone touch screens, aluminum automobile exterior body parts, and copper wire (Fig. 3B, Fig. S9). The results demonstrate the versatility of aquaplastics in their ability to adhere to materials with a range of roughnesses and compositions (e.g. biomaterials, electronics, metals).

Another feature of aquaplastic fabrication is the ability to imprint patterns onto its surface during the drying process. Aquaplastics of either type, after being cast on nylon meshes composed of 30-35 μm width fibers, were imprinted with the mesh pattern with high fidelity (Fig. 4 and S10). More irregular patterns could also be imprinted onto aquaplastics this way. Leaves of *Colocasia esculenta* (i.e. taro), are known to be hydrophobic on their dorsal side due in part to their surface nanotopography. After casting aquaplastics on the leaf and peeling them off to form self-standing films, we observed pockets formed on the film surface by the 10-15 μm mushroom-like structures on the leaf surface by FESEM (Fig. 4), along with other features down to $\sim 1 \mu\text{m}$. We tested the size resolution limits of this imprinting by casting aquaplastics on the ventral surface of a *Nelumbo nucifera* (i.e. lotus) leaf. In addition to the imprinting of the characteristic papillae structures (pillars with 5-10 μm diameters) from the leaf surface, the aquaplastics successfully captured leaf surface features as small as $\sim 30 \text{ nm}$, originating from waxy crystalline aggregates (Fig. 4 and S10-11). This imprinting capability could also be leveraged to alter surface wettability, with aquaplastics cast on the ventral surface of the hydrophobic taro leaf being imparted with hydrophobic characteristics (contact angle of $117^\circ \pm 4.3$), which result from the leaf nanotopography, compared to non-imprinted surfaces (contact angle of $58.6^\circ \pm 1.7$) (Fig. 4)²⁸.

Water-processable aquaplastic. Although the ability of aquaplastics to rehydrate after fabrication hinders their direct comparison to petrochemical plastics for certain applications^{11,29}, it does lead to unusual and possibly beneficial material performance characteristics. Incubating a square sheet of *biofilm aquaplastic* in milliQ water caused it to swell by 80% along its lateral dimensions, whereas a corresponding sheet of *curli aquaplastic* under the same conditions led to swelling only by 50% (Fig. S12). The rehydrated aquaplastic films retained their swollen lateral dimensions upon re-drying. We exploited the water-responsiveness of the aquaplastics to demonstrate their ability to self-heal. Scratches made on the surface of a *biofilm aquaplastic* could be removed completely by spraying a few microliters of water onto the scratch and allowing it to air dry for 2 minutes (Fig. 5A). Full-thickness cuts in the *biofilm aquaplastic* could

be healed in a similar manner. *Curli aquaplastic* could also heal but slightly less efficiently, showing “scars” after the aqua-healing process when analyzed by FESEM (Fig. S13).

Using a similar protocol, independently fabricated aquaplastic films could be “aqua-welded” together using only water to trigger their adhesive properties. Rectangular aquaplastic films could be attached to one another in perpendicular and parallel (overlapping) orientations just by spraying water at the interface and allowing for 2 min of drying (Fig. 5B and S14). This strategy could be leveraged to create self-standing three-dimensional structures, such as a miniature house with a 4 cm x 4 cm footprint, composed of ten aqua-welded panels (Fig. 5C). Notably, the aqua-welded house was strong enough to support its own weight for at least 18 months after its original construction.

Discussion

None of the properties described for the aquaplastics above apply to the wild-type CsgA protein, despite its ability to self-assemble into fibrous structures. We found that both the fused TFF (or other protein) domains and the presence of SDS in the washes was necessary to arrive at a hydrogel, which, when dried, exhibited moldable properties. Our preliminary data support the conclusion that both of these factors contribute to the formation of a hydrated network of curli fiber polymers that adopts a plastic state upon drying, whereas in the absence of either component, the protocol leads to brittle films that crack upon drying (Fig S15). These observations are also consistent with SDS’s known role as a gelator, though this is the first report of its role as a plasticizer.

Although there are many examples of solvent-cast biopolymer films in the literature, these frequently form amorphous (soft) or crystalline (rigid) structures upon drying, hindering their utility as plastics that can hold a three-dimensional shape³⁰⁻³⁴. In contrast, our system combines the features of moldability into arbitrary 3D shapes, complete water processability, ambient fabrication, microbial production, and genetic programmability. These properties result in a biomaterial fabrication platform with considerably more versatility in material properties compared to other microbially-derived bioplastics, like polylactides and polyhydroxyalkanoates. Ongoing work will focus on increasing the yield and production scale of the curli fiber-based bioplastics (currently at 100 mg of aquaplastic per liter of bacterial culture) and exploring the use of engineered curli fibers with various fused domains in conjunction with other materials to form composite materials with an even wider range of properties relevant for plastics. These efforts, in addition to advances in biomanufacturing technology and synthetic biology will provide opportunities for the production of biodegradable plastics from sustainably-derived molecular components and energy sources.

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Supplementary Materials:

Materials and Methods

Figures S1-S15

References (1-34)

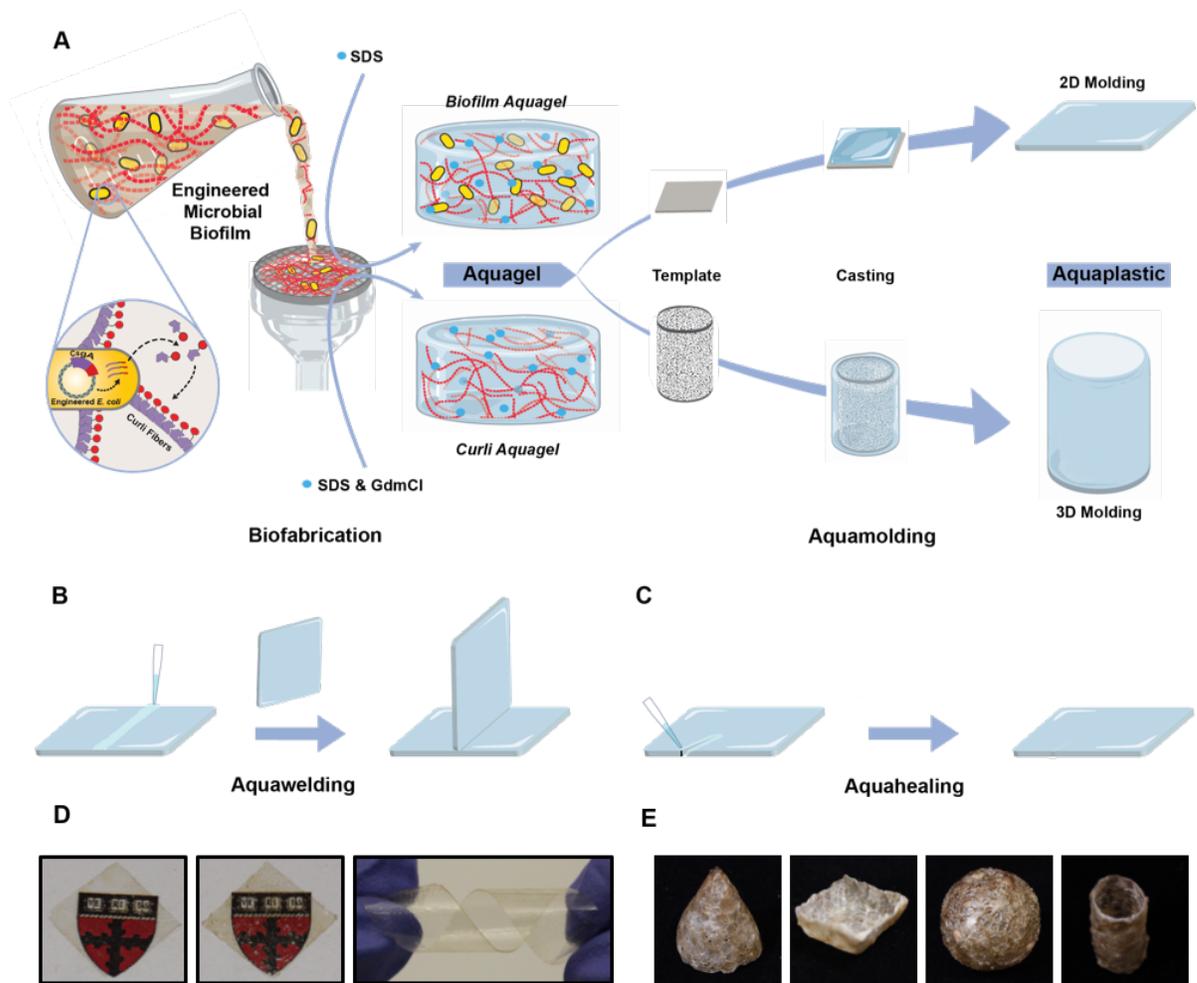


Figure 1. Fabrication of aquaplastic directly from engineered microbial biofilms. Schematics of aquaplastic fabrication from genetically engineered bacteria programmed to produce a functional curli fiber-based aquagel that can be molded into 2D and 3D architectures (**A**), and aqua-welding (**B**) and aqua-healing (**C**) of aquaplastics. SDS: sodium dodecyl sulfate; GdmCl: guanidinium chloride. **D**. Images of *biofilm aquaplastic* (left), *curli aquaplastic* (middle), and the flexibility of *biofilm aquaplastic* (right); **E**. Images of 3D-molded aquaplastic – cone, bowl, sphere and cylinder.

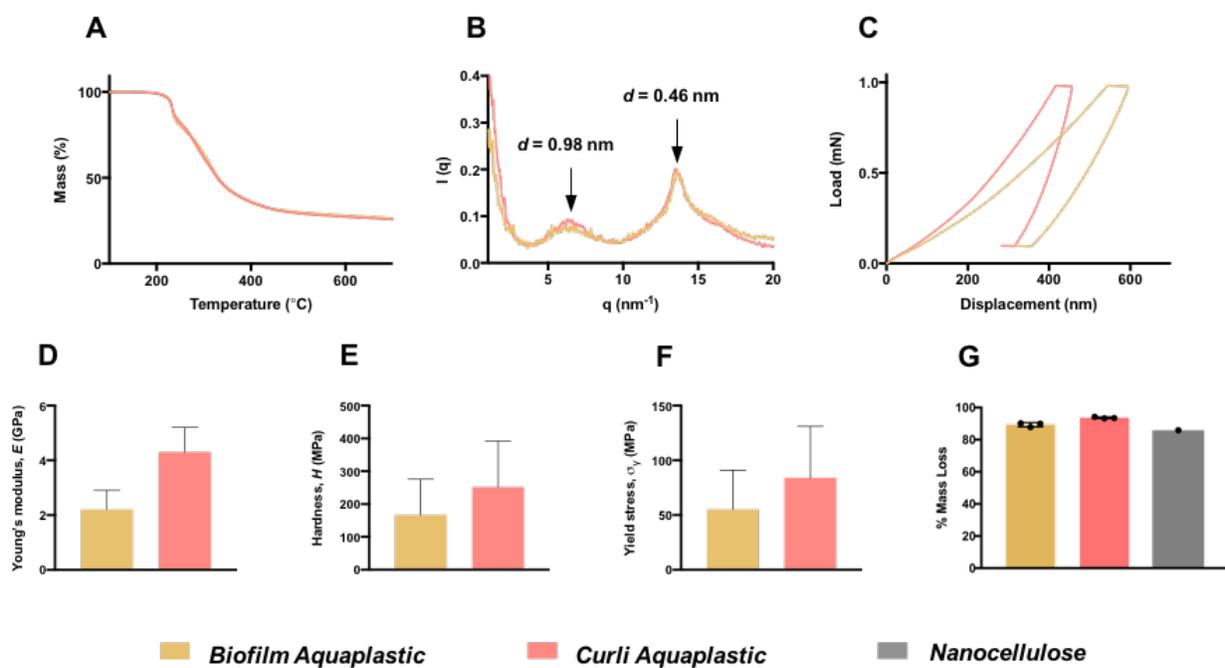


Figure 2. Physical and chemical properties of aquaplastics. **A.** Thermogravimetric Analysis (TGA) thermogram; **B.** Wide-Angle X-ray Scattering intensity profile; **C.** Load–displacement curves under a maximum indentation load of 1 mN in a single indentation cycle; **D.** Young’s modulus; **E.** Hardness; **F.** Yield stress for *biofilm aquaplastic* and *curli aquaplastic*. **C-F** n=128 for *Biofilm aquaplastic* and n=82 for *curli aquaplastic*. **G.** Biodegradation analysis of *biofilm aquaplastic* and *curli aquaplastic*, compared to nanocellulose, based on mass loss after exposure to a wastewater-derived microbial consortium. The bar graphs represent mean values and the error bars are standard deviation.

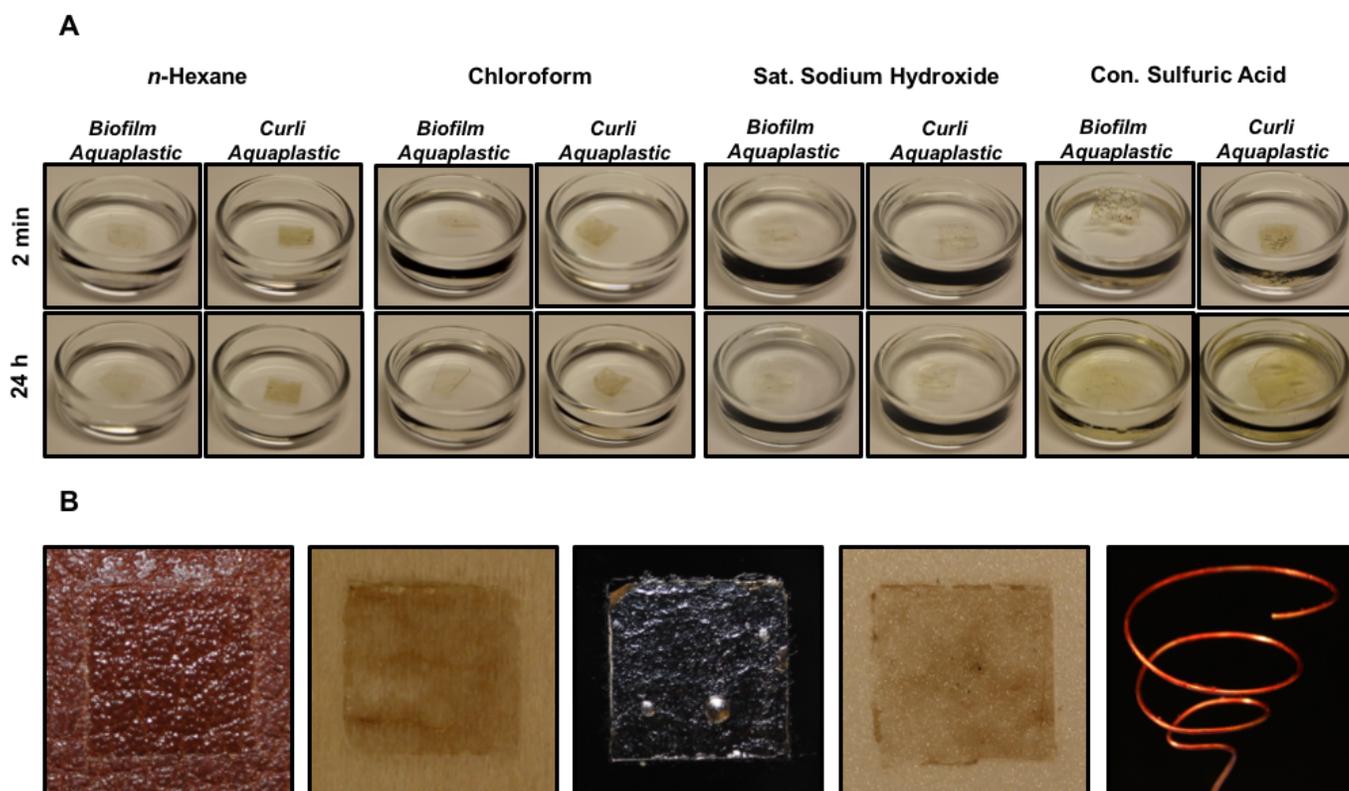


Figure 3. Coatable and chemically resistant aquaplastic. A. Images showing the stability of a 1 cm² sample of aquaplastic in organic solvents (*n*-hexane, chloroform), strong acid (concentrated sulfuric acid) and strong base (saturated sodium hydroxide) after 2 minutes or 24 hours of exposure; **B.** *Curli aquaplastic* coatings on biomaterials - cow leather (1st from left), plywood (2nd), mobile phone touch screen (3rd), aluminum automobile exterior body part (4th) and copper wire (5th).

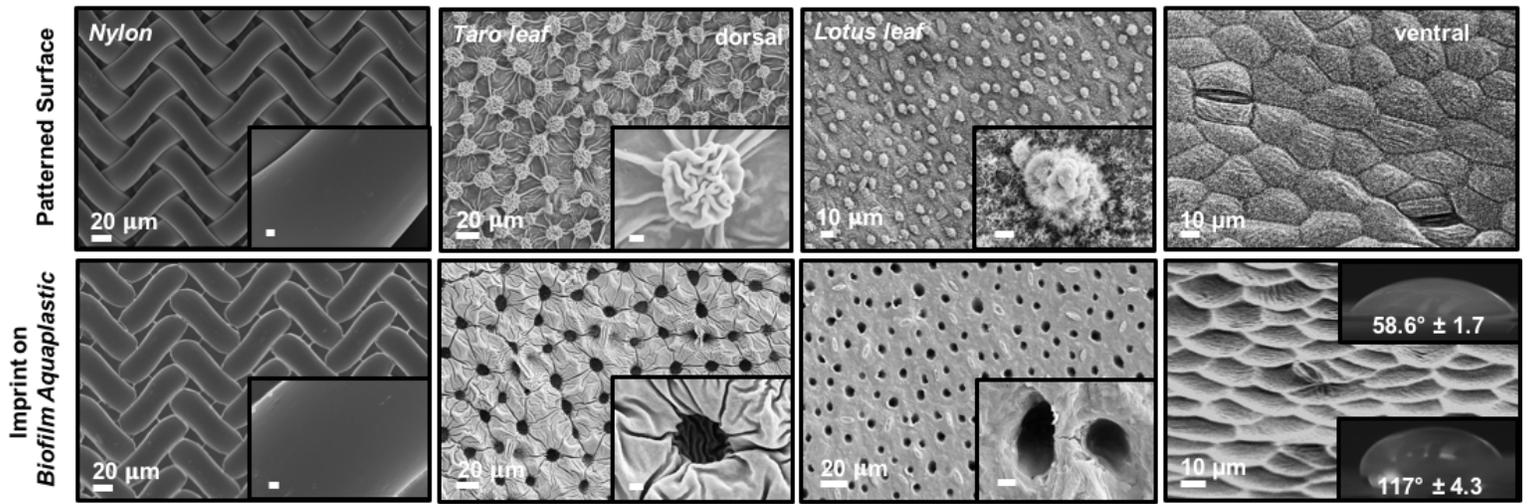


Figure 4. Imprinting on aquaplastic. FESEM images showing imprinting (*bottom*) of various patterned surfaces (*top*) on *biofilm aquaplastic* by ambient drying of *biofilm aquagel*. Insets in the first three columns show higher magnification of surface features (scale bar = 2 μm). Insets in the last column are water contact angles for non-imprinted (*top*) and imprinted aquaplastic surfaces (*bottom*), showing increased hydrophobicity.

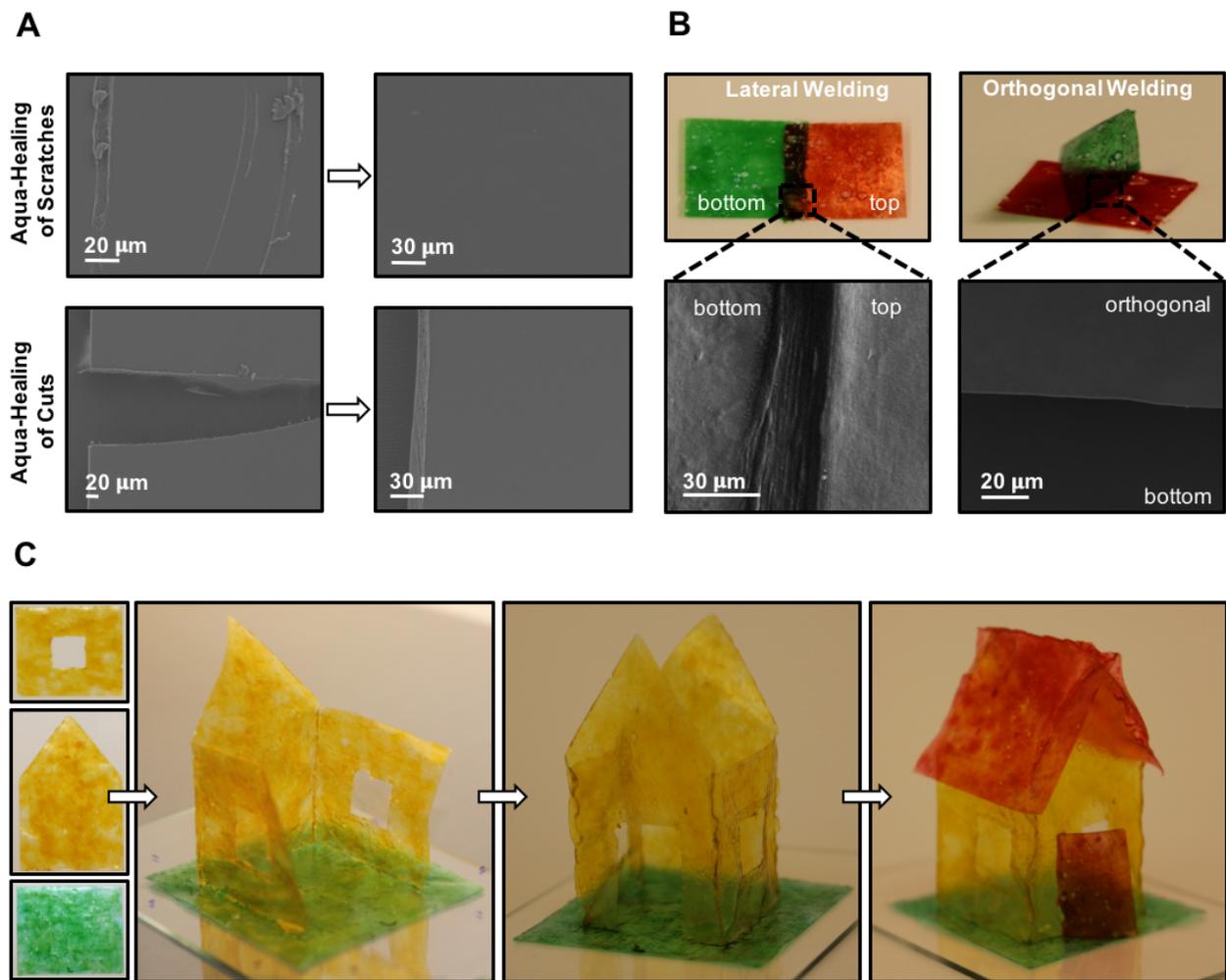


Figure 5. Aqua-healing and aqua-welding of aquaplastic. **A.** Scratches and cuts of *biofilm aquaplastic* were aquahealed by adding water at the site of abrasion; **B.** Aqua-welding of two *biofilm aquaplastic* panels in parallel and perpendicular orientations. **C.** Aquaplastic house formed by aquawelding 2D panels.

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