

Bioinspired microhooked patches for targeted delivery of molecules in plant leaves

Isabella Fiorello
Bioinspired Soft Robotics Laboratory
Istituto Italiano di Tecnologia
Pontedera, Italy
isabella.fiorello@iit.it

Barbara Mazzolai
Bioinspired Soft Robotics Laboratory
Istituto Italiano di Tecnologia
Pontedera, Italy
barbara.mazzolai@iit.it

Abstract — In the present work, we mimic the strong shear-dependent leaf attachment of the hook-climber *Galium aparine* to propose new patches with micropatterned hooks for targeted delivery of molecules in leaf tissues. We first built biodegradable and dissolvable isomalt-made micro-hooked patches using high-resolution micromanufacturing techniques including two-photon lithography. Secondly, we characterize the mechanical properties of plant leaves and the attachment forces of the micro-hooked patches to leaf surfaces. Lastly, we tested the micro-hooked patches for *in situ* release of fluorescein molecules in plant vascular tissues, producing biodegradable environmental-friendly machines. This research highlights the potential to use plant-like sustainable miniaturized machines to cure plants, reducing the use of pesticides and preserving natural ecosystems.

Keywords— biomimetics, plant-inspired machines, 3D micromanufacturing, drug delivery, sustainability.

I. INTRODUCTION

Life on Earth is dominated by plants which play a key role in maintaining the ecological balance[1]. The development of new sustainable and smart tools capable to preserve the plant's health and reduce the consumption of natural resources is becoming more and more important. For this reason, there is an increasing interest in miniaturized technologies capable of precisely operating over plant organs for *in situ* monitoring applications[2], molecular delivery to plant vascular tissues[2, 3] or energy harvesting[4]. Natural organisms can inspire scientists in the prototyping of disruptive bioinspired soft materials or robotic systems capable to work in complex unstructured real-world environments[5]. Among natural organisms, the hook-climber *Galium aparine* uses a unique parasitic ratchet-like attachment mechanism via microscopic hooks to strongly anchor its body over the surrounding host vegetation, especially to host leaves[6] (Fig. 1a, b). Here, we mimic the strong shear-dependent leaf attachment of the hook-climber *Galium aparine* to propose new patches with micropatterned hooks for targeted delivery of molecules to leaf tissues (Fig. 1c)[2]. Firstly, we built artificial microhook-based patches using high-resolution micromanufacturing techniques in combination with molding and casting of biodegradable and dissolvable materials. Then, we characterized the biomechanics of plant leaves as well as the attachment behavior of the microprinted prototypes to plant surfaces. Finally, we tested the prototypes for *in situ* delivery of fluorescein molecules to plant vascular tissues.

II. BIOINSPIRED MICROHOOKED PATCHES

The artificial microhooks were designed and modelled by

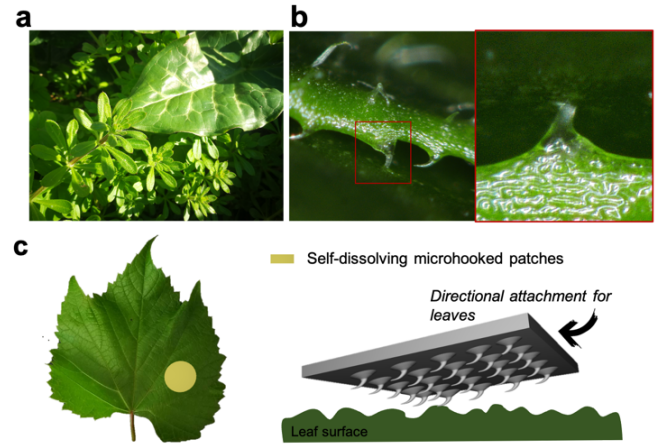


Fig. 1. a) The hook climber *Galium aparine* in natural environments. b) Microscope images of *G. aparine* leaf on a host plant (left) and focus on natural abaxial microhook interlocking with the leaf surface (right). c) Schematic of direction-based microhooks attachment system to leaves. Adapted from[2].

taking inspiration from natural abaxial hooks of *G. aparine* as detailed in Ref. [7]. The microhook-based patches were microfabricated by strategically combining two-photon lithography with PDMS micromolding and casting of isomalt-made materials (Fig. 2a). Isomalt is a low-cost and recyclable sugar alcohol with unique mechanical properties, which enables the microfabrication of high-resolution and water-dissolvable patches with microhooks capable of anchoring and penetrating leaves in shear direction (Fig. 2b-e).

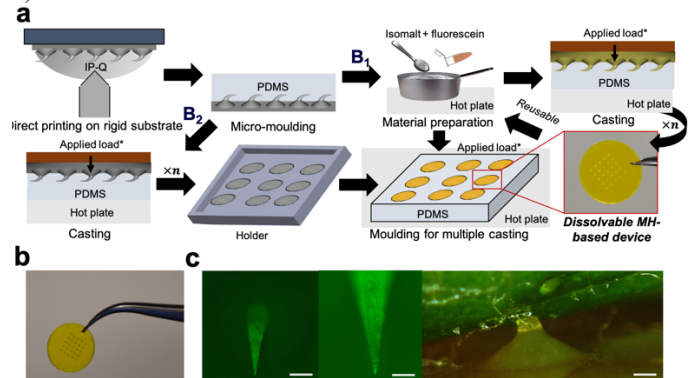


Fig. 2. a) Schematic of the microfabrication process which includes the direct printing of microhook arrays, PDMS molding and casting of isomalt materials. b) Picture of a microfabricated patch. c) Artificial isomalt-made hook and its anchoring to a leaf surface. Scale bars, 200, 50 and 200 μm , respectively. Adapted from[2].

III. MECHANICAL CHARACTERIZATIONS

We investigated the mechanical properties of the leaf surfaces and the attachment behavior of the microfabricated patches (Fig. 3). For instance, we characterized the leaves of the grapevine *Vitis lambrusca* by using scanning electron

microscopy, optical profilometer and nanoindentation techniques (**Fig. 3a, b**). The plant cuticle of *V. lambrusca* shows a rough surface with convex cells (average roughness of about 15 μm) (**Fig. 3a**) and an average hardness (E) of about 13 MPa (**Fig. 3b**). The microhook-based patches were capable to strongly anchor the leaves of *V. lambrusca*, showing pull-off forces of $0.5 \pm 0.2 \text{ N/cm}^2$ (**Fig. 3c**) and shear forces of $3.4 \pm 0.6 \text{ N/cm}^2$ (**Fig. 3d**).

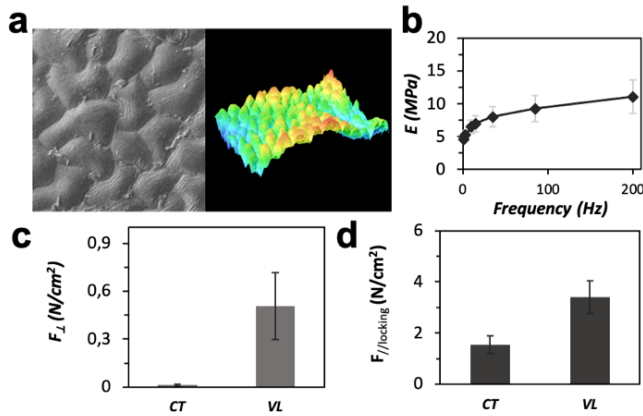


Fig. 3. a) Scanning electron microscope (left) and optical profilometer (right) views of the adaxial side of *V. lambrusca*'s leaf. b) Nanoindentation results at different frequencies. c, d) Maximum pull-off forces (c) and maximal shear-locking forces (d) generated by microhooked patches in the interlocking direction over the adaxial side of *V. lambrusca* leaf. As a control, a device without hooks was tested (CT). Adapted from [2].

IV. DEMONSTRATION OF *IN SITU* DELIVERY OF MOLECULES

The microhook-based patches were able to both anchor plant leaves and directionally penetrate the leaf tissue and fast dissolve inside it to inject a small amount of molecules (e.g., fluorescein) (**Fig. 4**) [2]. This technology has a high potential to be used as a novel plant-like tool for targeted release of fungicides/bactericides, micro/nanoparticles, genetic materials and/or other molecules to leaf tissues through the hook array (**Fig. 4a, c**).

The isomalt material is cheap and sustainable and seems to be particularly suitable for 1) loading the payload, 2) building microhooks of sufficient strength to penetrate the tissue in a shear direction and 3) dissolving inside the plant sap to release the payload.

The microhooked patches were loaded with fluorescein molecule's and directionally injected in *V. lambrusca* leaves (**Fig. 4a, c**). After the injection, the fluorescein molecule's delivery and mobility through the vascular tissue were recorded by using a fluorescence microscope (**Fig. 4d, f**). The fluorescence intensity decreased during the time at the penetration site of the hooks (**Fig. 4d**). Vice versa, the fluorescence intensity increases at the point of the vascular tissue "far" from the injection site (**Fig. e, f**), suggesting that the fluorescein-loaded isomalt is absorbed and transported in the plant vascular tissues (e.g., phloem).

Finally, we tried the fast dissolution of the microhooked patches in water at room temperature, obtaining a complete dissolution in less than 10 min (**Fig. 4g**). Moreover, we investigated the effect of environmental humidity and direct water exposure in a single hook (**Fig. 4h**).

The tip of the hook dissolved in about 10 seconds when directly exposed to water, suggesting a fast molecular delivery in the plant sap (**Fig. 4h**, lower side). In addition, the hook tip partially dissolved in about 150 minutes as response

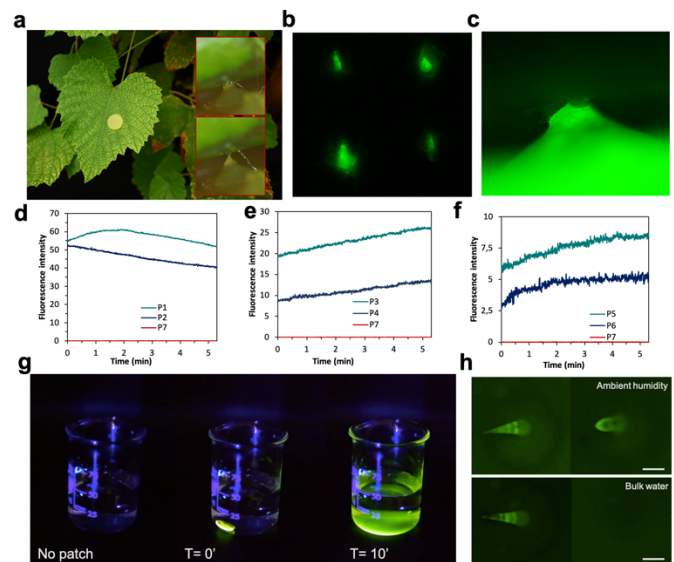


Fig. 4. A) Isomalt-made microhooked patch attached to a leaf of *V. lambrusca*. b, c) Fluorescence view of the injection site of microhooks. d-f) Fluorescence intensity variation over time in the vascular plant tissue. g) Dissolution of patches into water over time. h) Dissolution at single hook level. Scale bar, 100 μm .

to environmental humidity, showing high potential for fully biodegradable and environmental-friendly machines (**Fig. 4h**, upper side).

V. CONCLUSION AND FUTURE PERSPECTIVES

These results suggest that our technology could be used as a novel sustainable plant-inspired tool for *in situ* molecular delivery to plant leaf tissues, opening several scenarios of applications in precision forestry and agriculture fields.

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