# Comparison of Replica Leaf Surface Materials for Phyllosphere Microbiology

Rebecca Soffe,[1*] Nicola Altenhuber,[1] Michal Bernach,[1,2] Mitja Remus-Emsermann,[2] Volker Nock[1*]

[1] Department of Electrical and Computer Engineering, University of Canterbury, Christchurch, New Zealand

[2] School of Biological Sciences, University of Canterbury, Christchurch, New Zealand

Email: rebecca.soffe@canterbury.ac.nz; volker.nock@canterbury.ac.nz

Phone: +64 3 369 3999

## Keywords

biomimetic surface, surface characterization, artificial surface, microorganism, bacteria

# Abstract

Artificial surfaces are routinely used instead of leaves to enable a reductionist approach in phyllosphere microbiology, the study of microorganisms residing on plant leaf surfaces. For instance, flat surfaces such as nutrient agar, enable the influence of nutrient supply on microorganisms to be investigated. In contrast microstructured surfaces, such as isolated leaf cuticles or reconstituted leaf waxes enable the influence of physicochemical properties to be investigated. However, interest in replica leaf surfaces as an artificial surface is growing. As replica surfaces offer an improved representation of the complex topography of leaf surfaces. The use of replica leaf surfaces has to date primarily been focused on replicating the superhydrophobic surfaces of leaves. Whereas in this paper, we investigate potential replica surface materials for phyllosphere microbiology studies. Using a test pattern, we investigated the resolution, the degradation characteristics in environmental conditions, surface energy, and bacterial survival characteristics for each potential replica material. Our results indicate that PDMS is the most suitable material for producing replica leaf surfaces. Due to the high resolution achieved through replica molding, extended stability, hydrophobic properties, and bacterial survival characteristics comparable to isolated leaf cuticles. Our experiments highlight the importance of considering the inherent material properties, when selecting a replica leaf surface for phyllosphere microbiology studies. A replica leaf produced in PDMS offers a control surface that can be used for investigating microbe-microbe and microbe-plant interactions in the phyllosphere. Thus, in turn enabling mitigation strategies against pathogens to either the plant host or humans to be developed.

# 1 Introduction

Many microorganisms thrive on plants. They reside either permanently or temporarily in the plant environment. Furthermore, microorganisms contribute to the health of the plant host. These microorganisms live in three interconnected compartments: the spermosphere, rhizosphere, and phyllosphere.[1-6] To date research has primarily been focused on the rhizosphere. The rhizosphere is the microbial habitat in the vicinity of the plant roots, which is influenced by the products secreted by the roots.[6, 7] However, recently phyllosphere microbiology, the study of microorganisms which reside on plant leaf surfaces, has gained increasing interest. This increase is attributed to growing interest in the role that microorganisms in the phyllosphere, have on the health of the plant host. Microorganisms in the phyllosphere are in direct contact with the plant cuticle. Where the plant cuticle, a protective waxy film that coats the leaves of plants and prevents pathogenic attacks against the plant host.[8] In addition, the cuticle prevents water, ion, and nutrient loss.[9]

Leafy greens are exposed to a range of potential contamination sources during growth on a farm, resulting in unwanted microorganisms in the phyllosphere. This is of importance for produce grown for human consumption, such as lettuce, rhubarb, and parsley.[4-7, 10] Potential contamination sources include: irrigation water, soil, fertiliser, farm workers, and equipment used around a farm.[11, 12] Unwanted contamination can result in diseases detrimental to plant health or produce unwanted pathogens that are harmful to humans. Furthermore, leafy greens are often consumed raw or with minimal processing. Which does not remove or kill unwanted contamination.[4, 10-14] In some cases, contamination can lead to outbreaks that can cause severe illnesses.[15-17] Unwanted leaf contamination is of an increased concern with increasing produce demand, large-scale production, and distribution. Consequently, further studies to understand phyllosphere microbiology are imperative for developing mitigation strategies. For instance, microbe-microbe and microbe-plant interactions need to be studied in greater detail to determine said mitigation strategies. Potential mitigation strategies may include: introducing other microorganisms to prevent against pathogens detrimental to either the plant host or humans; or developing different cleaning protocols of leafy greens.[17, 18]

The use of artificial surfaces is common practice in phyllosphere microbiology. Such surfaces are often utilised to provide an insight into microorganism communities in the phyllosphere of leafy greens. Artificial surfaces are used instead of a living leaf to enable a reductionist approach. Such an approach, allows one to identify the impact of individual factors on microorganism functioning and viability.[13] For instance, an artificial surface provides a controlled environment to study contamination of leafy greens.[19, 20] Artificial surfaces can be classified as either flat or microstructured, due to their lateral heterogeneity.

(1) Flat surfaces. A flat surface is defined as a surface that is laterally homogenous. In general, a flat surface will have no lateral chemical or biological heterogeneity at the time of inoculation with microorganisms. Commonly used flat surfaces include nutrient agar or inert surfaces (for example, metal, plastic, and glass). Nutrient agar is commonly used to investigate the influence of different nutrient compositions on microorganism interactions and colonisations. For example, Jacobs *et al.* investigated the role of pigmentation, ultraviolet radiation tolerance, and leaf colonisation strategies in epiphytic survival using nutrient agar.[21] Inert flat surfaces on the other hand, are commonly utilised for the investigation of attachment processes of microorganisms. For example, Rivas *et al.* observed variation in surface attachment amongst strains of Shiga toxigenic *Escherichia coli* (*E. coli*) on stainless steel surfaces.[22]

(2) Microstructured surfaces. Microstructured surfaces are defined as surfaces which are laterally heterogeneous. Furthermore, microstructured surfaces generally have no lateral chemical or biological heterogeneity at the time of inoculation. A microstructured surface is more representative of the surface of the leaf, as the cuticle of a leaf is physically (and chemically) laterally heterogeneous.[23] Commonly used microstructured surfaces include reconstituted leaf wax, leaf peels, isolated leaf cuticles, and microfabricated surfaces.[24-26] For instance, Remus-Emsermann *et al.* examined the permeability of fructose through isolated poplar (*Populus × canescens*) cuticles, to assist in explaining microorganism growth patterns in the phyllosphere.[27]

Although microstructured surfaces are suitable for their respective applications, they do not entirely represent the complex nature of the topography of plant leaves.[28, 29] Recent studies have utilised double-casting protocols to overcome this limitation. As such replica leaf surfaces have been produced in agarose, dental wax, or polydimethylsiloxane (PDMS).[28-31] To date leaf surfaces have been predominately reproduced for their self-cleaning properties.[32-34] In phyllosphere microbiology, studies are beginning to appear using microfabricated replica leaf surfaces. In one example, Zhang *et al.* produced replica spinach leaf surfaces in agarose. They investigated the interaction of *E. coli* on flat agarose and agarose replica leaf surfaces.[31] However, studies to date, including the aforementioned did not compare the potential influence of the replica leaf material have on microorganism viability and behavior.

In general, materials used as replica leaf surfaces to date have not been fully investigated for their use in phyllosphere microbiology studies. As the inherent properties of a material can influence microorganism behaviour, it is imperative that the suitability of a material as a replica leaf surface is characterized comprehensively. Investigations should include: (1) the resolution and degradation characteristics of the replica leaf surfaces. To determine if the replica can suitably mimic the complex topography of a leaf surface and maintain the topography throughout an experiment. (2) The

hydrophobicity of a replica leaf material and how representative this is of a leaf surface. As hydrophobicity influences the attachment processes of microorganisms.

We selected three commonly-used biocompatible materials to investigate their suitability for replica leaf surface materials, for phyllosphere microbiology studies. (1) Agarose. Agarose has been used as a replica leaf surface, has well established microfabrication protocols, and is routinely used in phyllosphere microbiology studies.[13, 31, 35] (2) PDMS. PDMS is routinely used in biological applications, such as the foundation of lab-on-a-chip devices and in bioimprinting.[36-42] (3) Gelatin. Gelatin, was initially used as a gelling agent for microbiology growth media, until it was succeeded by agar.[43] In addition, well established microfabrication protocols exist for gelatin.[44] In total, three concentrations of each material were used in our comparison.

For our investigation, we used a test pattern comprised of regular circular pillars. The pillars were designed to have a height of 5 µm and a diameter of 15 µm to mimic microfeatures found on leaves – trichomes, stomata, and grooves.[20] For each material we measured: (1) Optical surface profiles. (2) The degradation of the materials over three days. (3) The hydrophobic properties against two generic isolated leaf cuticles (*Citrus × aurantium*, and *Populus × canescens*). In addition, to investigate the biological suitability of each material we used a model leaf colonising bacterium *Pantoea agglomerans* 299R. We compared the bacteria survival results of the materials, to bacterium on isolated *Citrus × aurantium* cuticles.

## 2 Materials and Methods

An overview of the fabrication protocols to produce the patterned materials is provided in **Fig. 1**, with accompanying procedures explained in the following sub-sections. In brief, a negative-tone photoresist mold master was fabricated using standard soft-lithography processes. The mold master comprised of an array of circular pillars, with a height of 5 µm and a diameter of 15 µm. From the mold master the polydimethylsiloxane (PDMS) templates were produced. In turn, the PDMS template was used to fabricate patterned agarose, PDMS, and gelatin substrates. The patterned materials were then used in our investigation towards finding a suitable leaf surface replica material. In this process the mold master and PDMS template are representative of a living leaf surface and leaf imprint, respectively. Whereas, the patterned materials are representative of a replica leaf [28-31]. We selected PDMS as our template material as PDMS is well-established in microfabrication. In addition, PDMS can exhibit nanoscale pattern resolution.[41, 42]

### 2.1  Mold Master Fabrication

For the mold master substrate a 4" prime grade silicon wafer was used. The wafer was dehydrated at 185 °C overnight in an oven to improve resist adhesion. The wafer was then removed from the oven and left to cool to room temperature. The wafer was subsequently cleaned in oxygen plasma for 10 minutes. ADEX05 (DJ MicroLaminates), an epoxy dry film photoresist, was then processed to produce a mold master.[39] Prior to casting the PDMS template, the mold master was treated with Trichloro(1H,1H,2H,2H-perfluorooctyl)silane (448931, Sigma-Aldrich) for two hours. This treatment was undertaken to facilitate the removal of the PDMS template.

### 2.2  PDMS Template Fabrication

The PDMS template was produced using standard replica moulding techniques **(Fig. 1a)**.[45] The PDMS (Sylgard 184, Dow Corning) was prepared at a ratio of 10:1 w/w (base to curing agent). The base and curing agent were thoroughly mixed together, and then degassed in a vacuum desiccator (Z119016, Sigma-Aldrich) until no bubbles remained. Following this, the PDMS was poured onto the photoresist mold master and degassed again. Once no bubbles remained, the PDMS and mold master were placed on a hot plate for two hours at 80 °C to allow the PDMS to set. Once set, the PDMS template was carefully removed from the mold master. The PDMS template was then placed on a hotplate for a further two hours at 80 °C - to improve the durability of the template.

Several templates were fabricated to produce enough patterned samples for all experiments. All patterned substrates were fabricated to an overall height of 3 mm. This was achieved by using rings with an internal diameter of 66 mm and filling the ring to the 3 mm line. From the patterned substrates

smaller samples were taken using a cork borer (Usbeck, Germany), with an internal diameter of either 7.8 or 11.5 mm.

## 2.3  Patterned Agarose Fabrication

In preparation of casting the patterned agarose, the PDMS templates were placed under vacuum for two hours. The agarose (Agarose Low EEO, A0576, Sigma-Aldrich) was added to phosphate buffered saline (PBS, P4417, Sigma-Aldrich) to produce a concentration of 2.5, 5, or 7.5 % w/v. The agarose solution was then placed on a hot plate at 200 °C, until the agarose was fully dissolved – approximately 15 minutes. Once the powdered agarose was fully dissolved, the solution was then poured onto the degassed PDMS template. This stack was then placed in a vacuum desiccator (Z119016, Sigma-Aldrich) for ten minutes with an open outlet valve **(Fig. 1b(*i*))**. Following this, the stack was left at room temperature to allow for the agarose to set for an hour. Once set, the patterned agarose was carefully peeled off the PDMS template.[35, 46]

## 2.4  Patterned PDMS Fabrication

In preparation for casting the patterned PDMS, the PDMS template was treated with 0.1% w/v hydroxypropylmethylcellulose (H8384, Sigma-Aldrich) in a phosphate buffer saline (PBS, P4417, Sigma-Aldrich) for 10 minutes. The patterned PDMS was prepared at a ratio of 5:1, 10:1, or 20:1 w/w (base to curing agent). The base and curing agent were thoroughly mixed together, and the mixture was then degassed until no bubbles remained **(Fig. 1b(*ii*))**. The PDMS was then poured onto the PDMS template and degassed again. Once no bubbles remained, this stack was then placed on a hot plate for two hours at 80 °C to allow the patterned PDMS to set. Once set, the patterned PDMS was carefully removed from the PDMS template.[47, 48]

## 2.5  Patterned Gelatin Fabrication

Gelatin from porcine skin (gel strength 300, G2500, Sigma-Aldrich), was added to PBS (P4417, Sigma-Aldrich) to produce a concentration of 10, 12.5, or 17.5 % w/v. The gelatin solution was then placed on a hot plate at 50 °C until the gelatin was fully dissolved – approximately 30 minutes **(Fig. 1b(*iii*))**. The microbial transglutaminase (mTG, Ajinomoto Co., Inc., activity of approximately 1000 U g$^{-1}$) solution was prepared in 1 ml of PBS. The mTG solution was prepared to a final concentration of 10 U of mTG per one gram of gelatin. The mTG solution was thoroughly mixed to ensure the mTG was fully dissolved. Once dissolved, the mTG solution was added to the gelatin solution and quickly mixed together. Once thoroughly mixed, the gelatin-mTG solution was immediately poured over the PDMS template. After pouring, the gelatin on the PDMS template was left at room temperature for five minutes to stabilise. The stack was then placed into an oven at 37 °C for four hours to set the

gelatin. To enable the gelatin to be readily peeled off the PDMS template, the stack was removed from the oven and placed in a fridge at 4 °C for 30 minutes. The patterned gelatin was then carefully peeled off the PDMS template.[44]

## 2.6   Optical Profiles

All optical profiles for the patterned substrates were obtained within 30 minutes after peeling from the PDMS template. This was done to minimise potential effects from degradation due to the ambient conditions in the laboratory. All 3D optical profiles were obtained using a Profilm3D optical profilometer (Filmetrics Inc., USA), equipped with a 20 × objective (CF Plan 20×/0.40 DI, Nikon). For agarose and gelatin, the high sidewalls of the pillars made imaging difficult due to light scattering. To correct for this, the data was processed using the inbuilt *remove outliers* function in the Profilm3D software 2018 (ver. 3.2.7.2, Filmetrics Inc., USA). For the *remove outliers* function, the *invalid pixels filled in* method was used, and with *maximum slope* set to 10. During post processing, all images were also filtered using a 3-point level function in Profilm3D software to compensate for non-level placement of the substrates.

## 2.7   Atomic Force Microscopy Scans

Atomic force microscopy (AFM) scans were obtained for the mold master, PDMS template, and patterned PDMS. Due to the patterned agarose and gelatin degrading during the required imagining period, the AFM tip would lose contact. As a result, AFM images were not possible for agarose and gelatin. All images were taken using a Digital Instruments Dimension 3100 (Vecco, USA) equipped with TAP300-G tips (BudgetSensors, USA) operating in tapping mode. All AFM scans were analysed using Gwyddion (Version 2.49).

## 2.8   Degradation Measurement Method

Two conditions were examined to test the degradation of the patterned materials: (1) 30 °C at a relative humidity of 25%, and (2) 30 °C at a relative humidity of 75%. A temperature of 30 °C was selected as it sustains bacterial life. Whereas, the humidity levels were selected to: (1) mimic dry conditions, similar to a climate-controlled laboratory; and (2) a higher humidity that would slow the degradation of the patterned samples (prepared as detailed in the following sub-section). Five samples per the three different concentrations for each material were used. Each sample had an initial diameter of 11.8 mm. Weight measurements were taken at: 0, 2, 4, 6, 12, 24, 48, and 72 h. A weightless percentage measure was then determined for each of the time points. Results are presented as mean ± SEM (standard error mean).

## 2.9  Humidity

The degradation of agarose and gelatin is undesirable for investigating microorganisms in the phyllosphere. Consequently, an environment was sought to minimise the degradation of the materials. In addition, the environment must be favourable for microorganisms, and minimise the potential swelling of the materials. To achieve this a saturated salt solution was prepared by dissolving 72 g of sodium chloride (71382, Sigma-Aldrich) in 200 ml of deionised water. This solution was then kept in an open top container alongside samples for degradation and bacteria recovery experiments, inside an airtight container. The airtight container was placed in an oven at 30 °C, which resulted in a constant relative humidity of 75 % (see **Supplementary Fig. S1** for humidity data).[49-51]

## 2.10 Contact Angle Measurement with Water Method

All contact angle measurements were undertaken with a CAM200 (KSV Instruments Ltd, Finland), integrated with KSV CAM Optical Contact Angle and Pendant Drop Surface Tension Software (ver. 4.01, KSV Instruments Ltd, Finland). Flat samples were compared against patterned samples for each material at the three selected concentrations. Five samples were measured for each concentration. All samples had a diameter of 11.8 mm. This diameter was selected to minimise the potential occurrence of edge effects on the water droplets. For agarose, which is considerably hydrophilic, all results presented are for droplets with a volume of less than 40 µl. Whereas for PDMS and gelatin, water droplets with a volume less than 60 µl were analysed. Prior to the contact angle measurements all samples were dried with dry nitrogen gas. This was done to minimise any potential effects from surface moisture. In addition, experiments were conducted within an hour of peeling the material away from the PDMS template. Deionised water was used to determine the surface energy of the materials.

Results are presented as mean ± SEM (standard error mean). For statistical analysis, Student's t-test or ANOVA was performed using GraphPad Prism 7 (GraphPad Software, USA). P values less than 0.05 were considered significant (*$P<0.05$, **$P<0.01$, ***$P<0.001$, and ****$P<0.0001$).

## 2.11 Bacteria Culture Protocol

*Pantoea agglomerans* 299R, a model leaf colonising bacterium isolated from a healthy leaf of a pear tree, was grown overnight on nutrient agar plates (13 $gL^{-1}$ Lysogency broth and 15 $gL^{-1}$ bacteriological Agar, Oxoid) at 30 ˚C.[52] The *P. agglomerans* was then harvested using a sterile inoculation loo and resuspended in 5 ml of sterile phosphate buffer (8 $gL^{-1}$ NaCl (LabServ), 0.2 $gL^{-1}$ KCl (LabServ), 1.44 $gL^{-1}$ $Na_2HPO_4$-GPR (AnalaR), 0.24 $gL^{-1}$ $KH_2PO_4$ (AnalaR), pH 7.4). Following this, bacteria were washed by centrifugation at 1150 RCF for five minutes at 10 °C. The supernatant was discarded, and the bacteria was suspended in fresh phosphate buffer to an $OD_{600\ nm}$ of 0.2, corresponding to approximately $2 \times 10^8$ bacteria per mL.

## 2.12 Bacteria Viability Protocol

For bacteria viability experiments samples with a diameter of 7.8 mm were used. Five samples were taken per time point. All samples were sterilised for 15 minutes with ultra-violet sterilisation. Then 100 µL of bacterial solution ( ~2 x $10^7$ bacteria) was inoculated to the patterned samples, using an air brush (KKmoon T-180 Airbrush, China) at $1\times10^5$ Pa.[53] Patterned samples coated with bacteria were then incubated at 30 ˚C at a relative humidity of 75%.

Five samples were randomly selected per measurement time point – 0, 2, 4, 6, 12, 24, 48, and 72 h. Each individual sample was suspended in 1 mL of fresh phosphate buffer in a 1.5 mL Eppendorf tube. Individual samples were then placed in a Bead Ruptor 24 (Omni International Inc., USA) at 2.6 ms$^{-1}$ for two sets of five minutes, with a five minute interval. Following this, the samples were placed in an ultrasonic bath (E Easy Elmasonic, Elma Schmidbauer GmbH, Germany) for five minutes. For each sample 100 µL of bacterial solution was recovered.[54, 55] The recovered solution was then plated onto nutrient agar by undertaking serial dilutions using PBS. At each dilution step 10 µL aliquots were taken. With a final dilution of $10^{-6}$ being plated. Colony-forming units (CFU) were expressed as bacterial cell density per unit area for each sample (7.8 mm diameter). The cell density was normalised to the initial number of cells after inoculation. This was undertaken to enable a comparison between the survival curves of the bacterial colonies on the different materials. Results are presented as mean ± SEM (standard error mean).
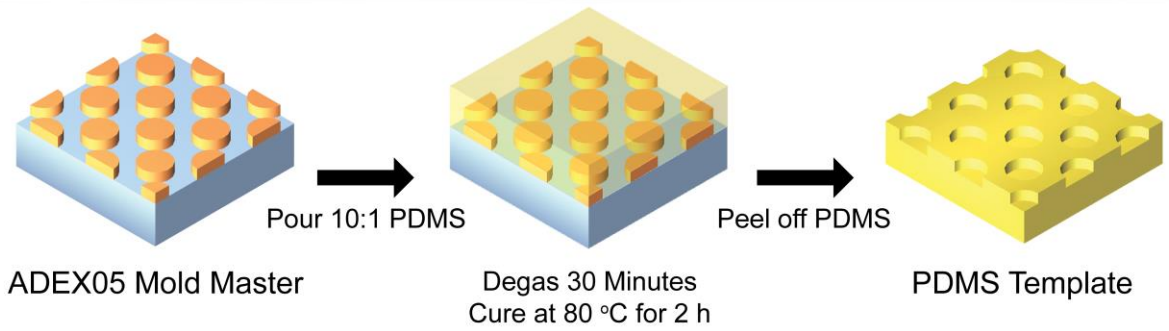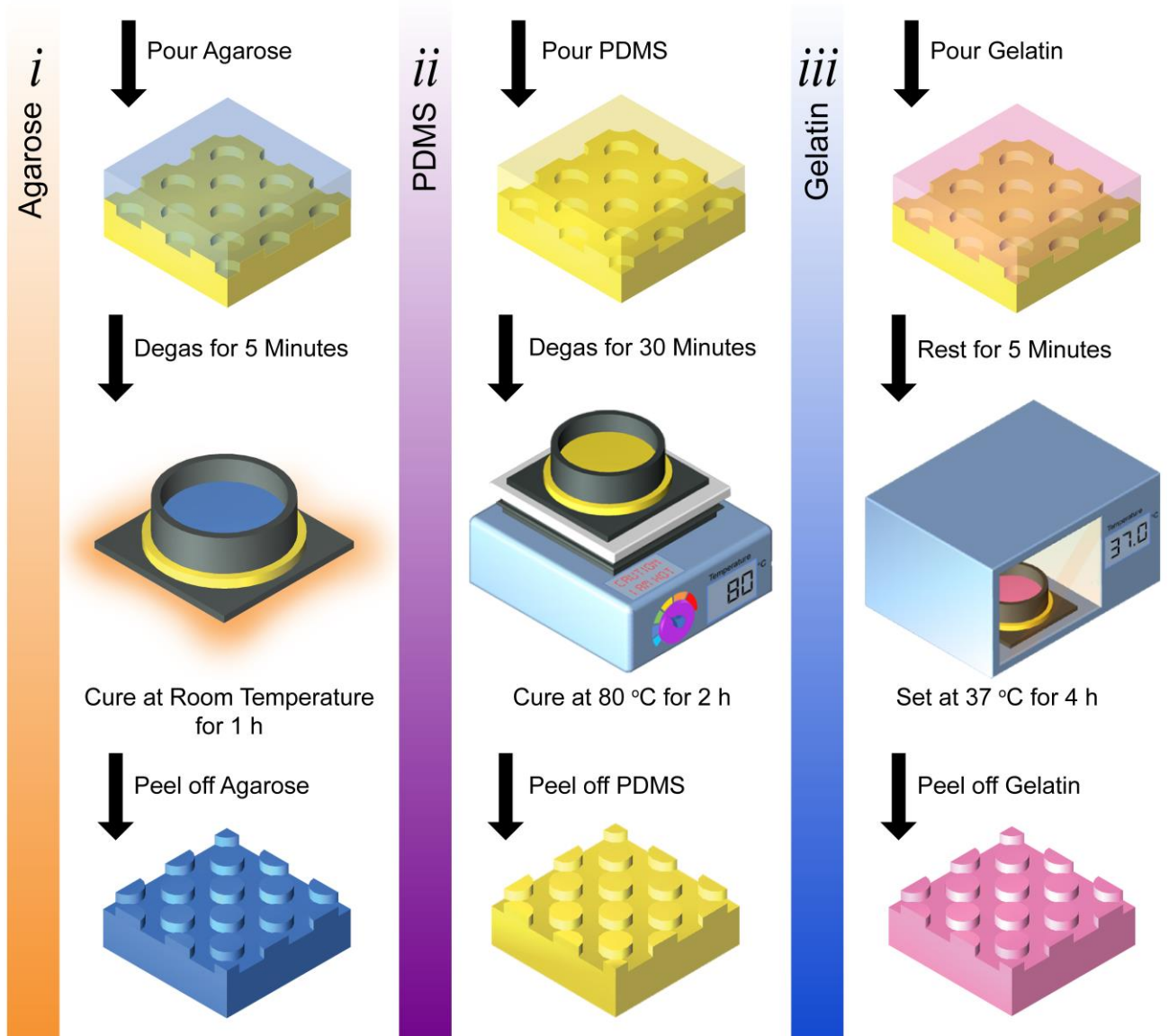
**Fig. 1**. Schematic overview of the fabrication protocols. (a) Protocol used to produce the PDMS templates. (b) Protocols used to produce the patterned: (*i*) agarose, (*ii*) PDMS, and (*iii*) gelatin.

# 3 Results and Discussions

## 3.1 Pattern Resolution

The microstructures found on leaves are dependent on the plant species and can range in complexity. Microstructures found on leaves include groves to stomata (pores) to the more complex structure of trichomes (leaf hairs). The ability to sufficiently replicate the surface topography of a living leaf into a replica, is important for producing replica leaf surface that can be used as a control surface in phyllosphere microbiology. In addition, inherent damage due to the replication process could produce inaccuracies of the replica leaf surface. This is important as the topography of the leaf surface has the ability to influence colonisation and attachment behaviours of microorganisms.[4, 20]

To investigate this with our selected materials we used a regular test pattern. The test pattern consisted of circular pillars with a height of 5 µm and a diameter of 15 µm. Such dimensions are comparable to the features found on leaf surfaces – trichome, stomata, and grooves. For example, *Pelargonium zontal* stomata have a length and width of 16 µm by 9 µm.[56] In contrast, trichomes on a *tomato leaf* have a width of approximately 17 µm at their base.[20] Comparisons of the patterned materials were undertaken using 3D optical profiles (**Fig. 2**). Optical profiles were selected over more commonly used techniques to compare microstructure resolution, such as atomic force microscope (AFM) and scanning electron microscopy (SEM). This was due to the inherent properties of agarose and gelatin influencing the ability to obtain high-resolution images. The ability to use AFM imaging was hindered by the softness and quick degradation of both agarose and gelatin. As such, when investigating the suitability of AFM imaging, we regularly observed the AFM tip losing contact during imagining. On the other hand, for SEM imaging a conductive coating would be required. As both agarose and gelatin are non-conductive. To produce a conductive coating the samples would have to be placed under vacuum. A short three minute vacuum was tested which resulted in a noticeable structural degradation of both materials (data not shown). Obtaining an optical profile instead requires no modification to be undertaken on the materials, and scans can be completed in approximately three minutes - mitigating any affects from degradation (**see section 2.6 Optical Profiles for more information**).

To prove the validity of optical profilometry for our materials we compared our results to AFM scans undertaken on the mold master, PDMS template, and the patterned PDMS (**see Supplementary Information S2** and **Table S1**). The measurements obtained using the AFM and optical profilometer are in agreeance (**Table 1** and **Table S1**). For example, the height of the mold master was measured to be $4.50 \pm 0.03$ µm using the optical profilometer - which lies in the range measured by the AFM of $4.44 \pm 0.16$ µm. Furthermore, the measured widths were $14.64 \pm 0.47$ µm and $14.94 \pm 0.21$ µm for

images taken by the AFM and optical profilometer, respectively. In consequence, this indicates no significant difference between 3D profiles taken by either AFM or an optical profilometer.

A summary of optical profilometry measurements of the height and width of the pillars is presented in **Table 1** – or in the case of the PDMS template, the depth and width of the well. Twenty pillars were measured for each dimension measurement.

In the case of agarose, the pillars were smaller by 1.54 ± 0.57 µm (in height) and 4.93 ± 0.25 µm (in width) in comparison to the mold master **(Table 1)**. In addition, a larger variation in measured heights for agarose was observed. We conjectured this variation was most likely a result of non-uniform shrinkage occurring while the agarose sets during fabrication.[57]

In addition, pillars produced in gelatin were also smaller. With the height and width of the pillars in gelatin 0.61 ± 0.03 µm and 1.14 ± 0.27 µm smaller than the mold master, respectively **(Table 1)**. We conjectured this was due to the gelatin degrading while setting, during fabrication. In addition, we observed that pillars regularly broke when peeling the gelatin off the PDMS template. We minimised the occurrence of the pillars breaking by following the recommendations made by Paguirigan and Beebe. In which the gelatin and PDMS template were placed in the fridge prior to peeling off the gelatin.[44] Trichomes (leaf hairs) would more than likely not withstand being peeled off the PDMS template. This problem is compounded by trichomes being quite intricate and considerably fragile.[20]

In summary, patterned PDMS was more representative of the patterned pillar microstructures used as mold master. Dimensions of the pillars produced in PDMS measured within 10% of the pillars of the mold master. Whereas, pillars produced in agarose and gelatin differed from the mold master by more than 10%. This indicates that from a pure pattern replication perspective, a replica leaf made from PDMS would have more representative topography of a living leaf. When comparing the replica fidelity of PDMS to gelatin or agarose.

**Table 1:** Pattern Resolution Measurements. Height and width measurements were taken from 20 pillars (or wells, as was the case from the PDMS template). All data is represented as mean ± standard deviation. Standard deviation has been used to show the scattering in measured values. See **Supplementary Information S2** for AFM and optical profilometer image comparison for the PDMS patterned replication process.

| | AFM | Optical Profilometer | | | | |
|---|---|---|---|---|---|---|
| | ADEX05 Mold Master | ADEX05 Mold Master | PDMS Template | Patterned Agarose | Patterned PDMS | Patterned Gelatin |
| Height (µm) | 4.44 ± 0.16 | 4.50 ± 0.03 | 4.18 ± 0.01 | 2.96 ± 0.60 | 4.71 ± 0.01 | 3.89 ± 0.06 |
| Width (µm) | 14.64 ± 0.47 | 14.94 ± 0.21 | 15.11 ± 0.52 | 10.01 ± 0.46 | 14.00 ± 0.38 | 13.50 ± 0.48 |
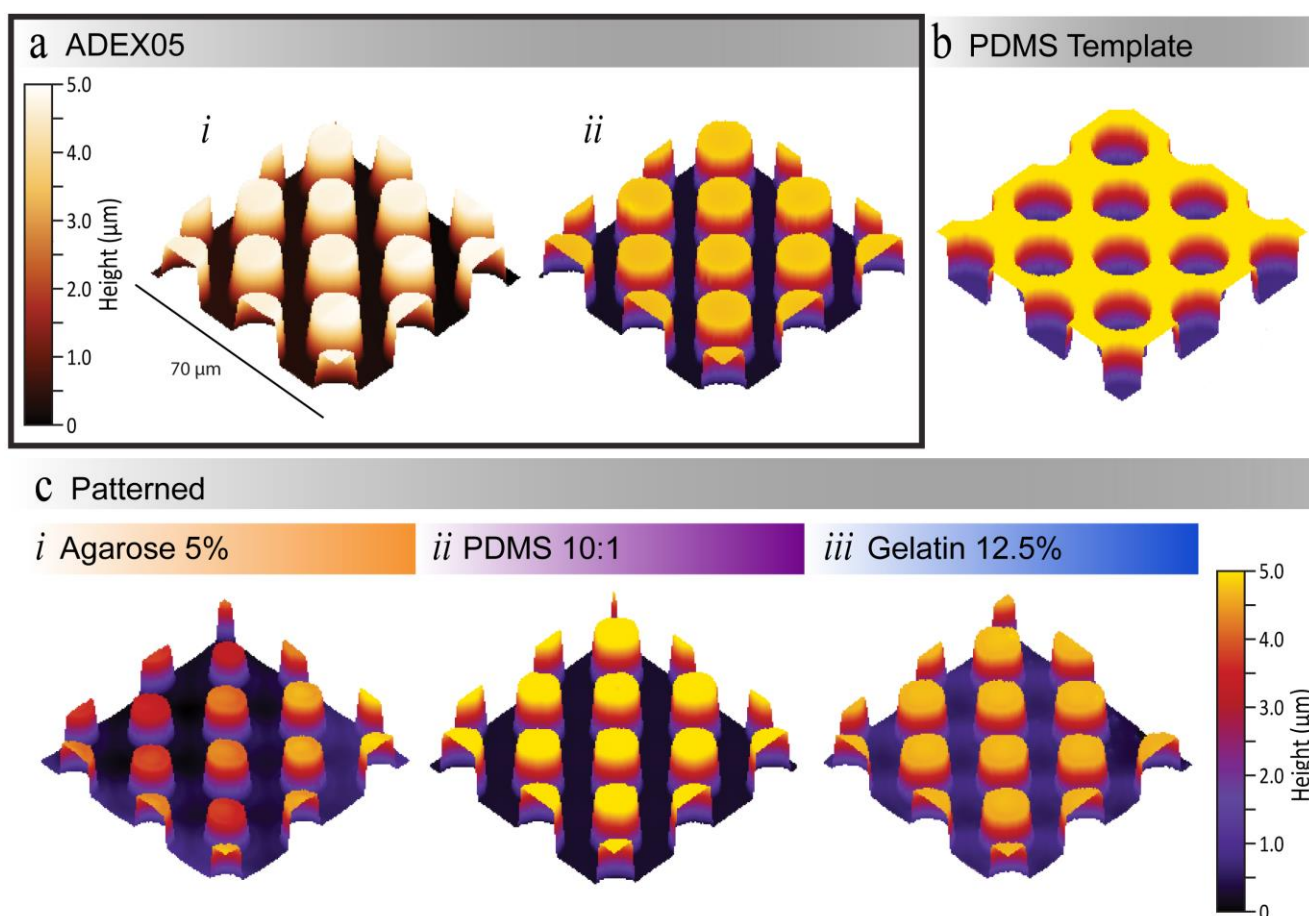


**Fig. 2**. Resolution images**.** (a) Comparison of the photoresist mold master (ADEX05) undertaken by (*i*) AFM and an (*ii*) optical profilometer. (b) PDMS 10:1 template 3D optical profile. (c) Optical profiles for (*i*) agarose 5 % w/v, (*ii*) PDMS 10:1 w/w, and (*iii*) gelatin 12.5 % w/v. All optical profilometer images were filtered using a 3-point level function in Profilm3D Software (Filmetrics). See **Supplementary Information S2** for AFM and optical profilometer image comparison for the patterned PDMS replication process.

### 3.2 Degradation Measurements.

Microbiology experiments can take anywhere from a few minutes or hours to days and even weeks. Consequently, a replica material should not degrade during the duration of the experiment as this will result in a moisture and topography changes, which influences microorganism behaviour.[6, 13] Thus, topography changes may prevent certain experiments from being undertaken. For example studies investigating the influence of chemicals or air quality on microorganism behaviour.[4]

To study material stability, we examined the degradation of the pattern materials over three days under two conditions at 30 °C (**Fig. 3**). A temperature of 30 °C was selected as it is a common temperature to cultivate environmental bacteria. The two humidity conditions are as follows: (1) 25% relative humidity. This humidity was selected to mimic the humidity often found in dry conditions. (2) 75% relative humidity. This humidity was selected to slow the degradation of the patterned samples. These two relative humidities can be found in cities around the world.[58]

For patterned agarose a relative humidity of 75% effectively slowed the degradation to the equilibrium point by 36 hours (**Fig. 3a).** The equilibrium point is defined as the time in which the material reaches an equilibrium moisture exchange with the surrounding environment. Regardless of the concentration of agarose, the equilibrium point (88 ± 1 % weight loss) was reached within 24 h and 48 h for 25% and 75% relative humidity, respectively. For a humidity of 75% no significant difference in the degradation characteristics were observed. Thus, indicating that there was no advantage in increasing or decreasing the concentration of agarose from 2.5 to 5 % w/v – the concentration of agarose routinely used in microfabrication.[31, 35]

In contrast, for gelatin a relative humidity of 75% lowered the equilibrium point (**Fig. 3c**). Furthermore, regardless of the concentration of gelatin the equilibrium point (80 ± 1 % weight loss) was reached within 24 h in both humidity conditions. Similar to agarose, changing the concentration of gelatin in PBS provided no advantage in degradation characteristics.

For patterned PDMS no degradation was observed over the 72 h experimental duration in either humidity conditions. In addition, no concentration dependence was observed (**Fig. 3b)**.

In summary, the degradation results indicate that a replica leaf produced from PDMS, would be stable in environmental humidities that plants occupy around the world. Whereas, agarose and gelatin degrade within 48 h of fabrication in environmental humidity ranges. In literature, agarose and gelatin has been reportedly stored in water or in a fridge to minimise degradation, respectively.[44, 46] However, these conditions are not compatible with phyllosphere microbiology experiments, and affect the resolution of microstructures. For instance, storing agarose in water results in swelling and hence changes the microstructure topography. Furthermore, the majority of microorganisms that reside on plants generally do now grow in low temperatures, such as those experienced in a fridge – 0 to 4 °C. PDMS additionally offers the ability to easily send samples to laboratories on the other side of the

world, as the PDMS replica would not degrade due to the environmental conditions during transportation. Whereas, agarose and gelatin would quickly end up in a liquefied state, unless stored in the aforementioned unfavourable conditions.
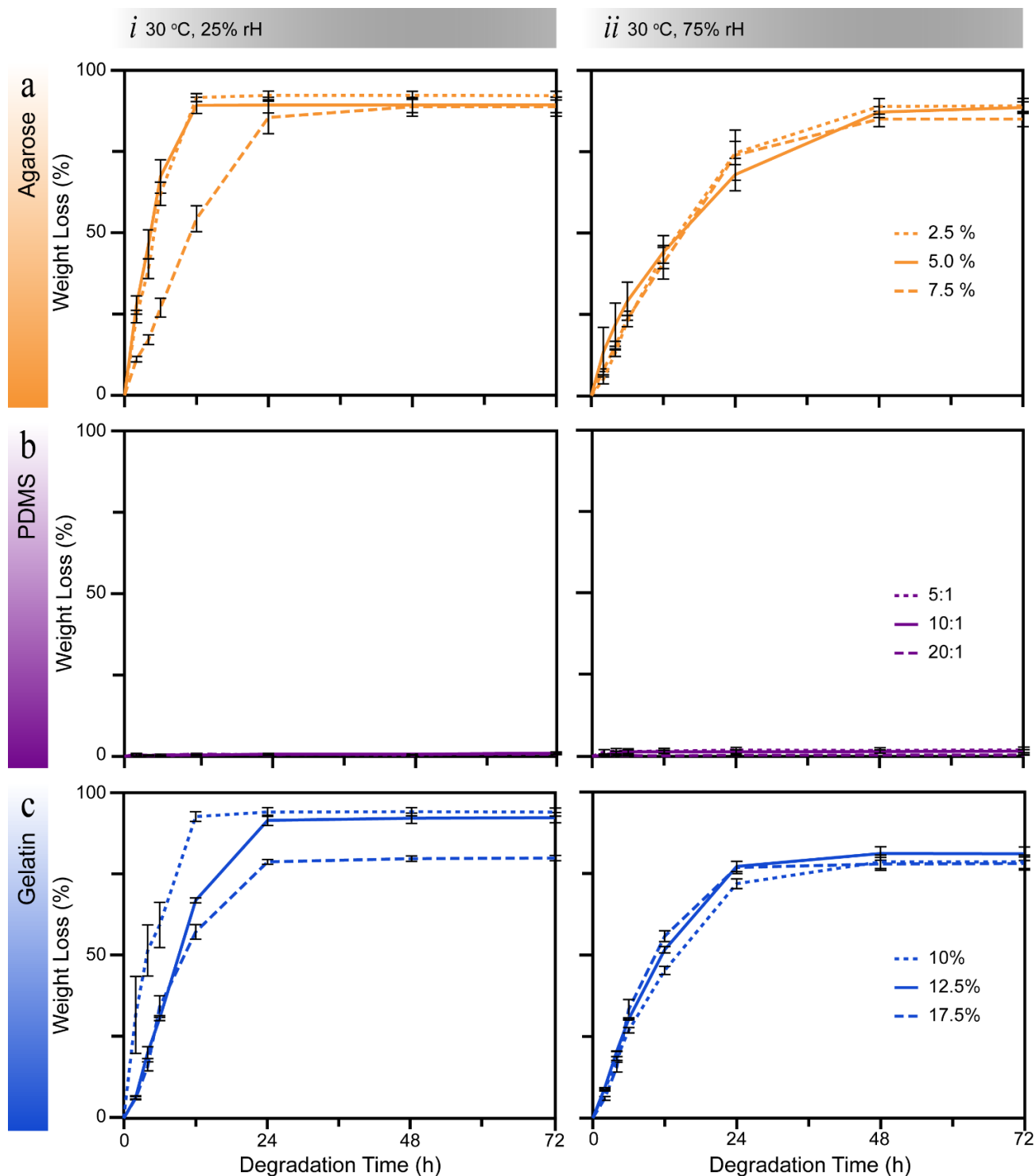


**Fig. 3. Degradation measurements for the three selected materials.** The weight loss over 72 h for (a) agarose, (b) PDMS, and (c) gelatin was investigated at relative humidities (rH) of (*i*) 25% and (*ii*) 75% at 30 °C. Five samples were measured for each material at each concentration.

### 3.3 Contact Angle Measurements

Surface energy and hydrophobicity in particular, constitute an important physical property of the cuticle of a leaf. A surface is classified as either hydrophilic, hydrophobic, or superhydrophobic, when the contact angle of water is < 90°, > 90°, and >150°, respectively. We selected enzymatically isolated leaf cuticles from *Citrus × aurantium* (bitter orange) and *Populus × canescens* (poplar) plant species to compare our materials against (**Fig. 4**).[23] For each of the three replica materials we compared both flat and patterned surfaces (see **Supplementary Fig. S3**, for contact angles at different material concentrations).

The hydrophobicity of a surface is important, as the presence of water on a surface impacts resource availability and colonisation patterns of microorganisms in the phyllosphere. Furthermore, the hydrophobicity of the surfaces also influences the microorganism attachment processes. Microorganisms can achieve attachment by (1) adapting to enable attachment, or (2) by forming biofilms [13, 59].

Our results indicate that patterned PDMS (95.5 ± 0.6°, N = 5) is hydrophobic. Whilst, agarose (15.9 ± 0.9°, N = 5) and gelatin (78.3 ± 1.0°, N = 5) are hydrophilic. Furthermore, our results are in agreeance with literature.[41, 60] Patterning the different materials with our test pattern did not influence the hydrophobic properties of the materials (**Fig. 4**). This is attributed to the size of the pillars, as there is no change in pinning of the water droplet observed on this scale.[61] In addition, changing the concentration also provided no significant change in the hydrophobicity of each of the materials (**Supplementary Fig. S3**).

Both bitter orange and poplar leaf cuticles were determined to be hydrophobic, with contact angles measured to be 97.9 ± 2.7° (N = 5) and 103.8 ± 0.7° (N = 5), respectively. In general, contact angles of plant leaves can vary considerably from hydrophilic to superhydrophobic.[32, 62, 63] No significant difference was measured between bitter orange and PDMS. However, a slight difference was measured between Poplar and PDMS ($P < 0.05$, N = 5). This is in contrast to the significant difference observed between the leaf cuticles and both agarose and gelatin (**Fig. 4**).

In summary, the hydrophobicity of PDMS is comparable to bitter orange and poplar. Whereas, agarose and gelatin are considerably more hydrophilic. Conversely, PDMS as a replica surface would be a suitable artificial surface for attachment studies of hydrophobic leaves. In addition, the degree of hydrophobicity of PDMS can be modified temporarily through oxygen plasma or extended in duration with polyvinylpyrrolidone (PVP) treatment. Both modifications are not harmful to microorganisms.[64] The use of PVP treatment would enable more extensive attachment studies to be undertaken using a PDMS replica surface. An area that has been highlighted as requiring more extensive studies to be carried out.[13]
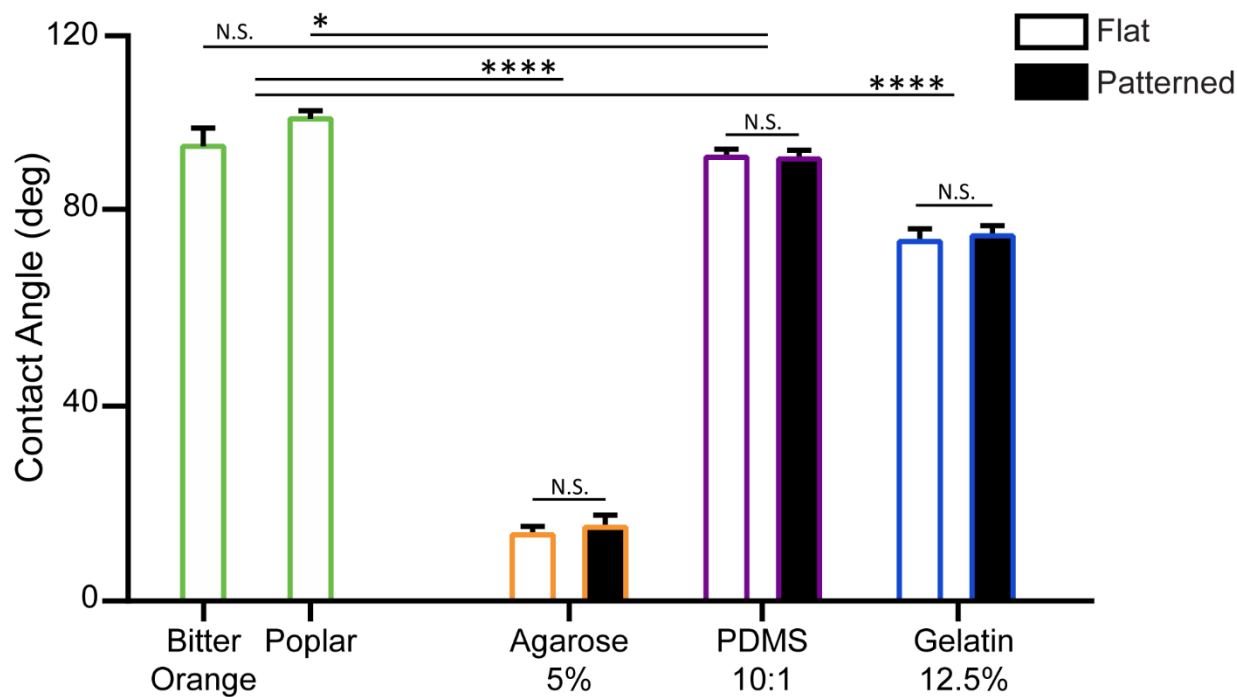
**Fig. 4**. **Contact angle comparison of leaf cuticles with the three selected replica materials.** Contact angles of bitter orange and poplar leaf cuticles, are compared with contact angles of agarose 5 % w/v, PDMS 10:1 w/w and gelatin 12.5 % w/v. See **Supplementary Fig. S3** for contact angle measurements for the three concentrations of each material. Data is presented as the mean ± SEM, *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001. N.S. indicates no significant difference between measurements.

### 3.4 Bacterial Survival.

To assess the suitability of the materials to support microorganism life we used bacterial survival experiments, with *Pantoea agglomerans* 299R as our model microorganism (**Fig. 5**). The bacterium *P. agglomerans* 299 was isolated from a Bartlett pear tree leaf. Strain *P. agglomerans* 299R is a spontaneous rifampicin resistant mutant of *P. agglomerans* 299.[52] We selected *P. agglomerans* 299R as our model microorganism as it is: (1) a model microorganism for leaf colonisation, (2) well characterised and fully sequenced, and (3) it is genetically amendable (able to produce mutants and bioreporters).[27, 52] We compared the bacterial survival characteristics from the replica materials against isolated *Citrus × aurantium* (bitter orange) leaf cuticles. This was done to determine which material resembled bacterial survival characteristics observed on the surface of leaves – in the absence of the nutrients from the leaf. This is of importance, as we are looking for a suitable material to form a replica leaf platform for phyllosphere microbiology studies.

In the case of agarose, the population of *P. agglomerans* 299R stabilised at a normalised cell density of $1.06 \pm 0.03$ (N = 5) after 24 h (**Fig. 5)** - regardless of whether the material was patterned or flat (**Supplementary Fig. S4**). We conjectured that the population stabilised due to the presence of moisture on the surface of the agarose. As bacterial life can be sustained when moisture is present in the environment. In this case the moisture on the surface of the agarose is a result of the agarose degrading. Furthermore, agarose is derived from red seaweed which is comprised of polysaccharides. Hence, agarose contains a potential nutrient source of carbon for bacteria. However, due to the bacteria not having the necessary enzymes to hydrolyse the α-$(1 \rightarrow 3)$ and β-$(1 \rightarrow 4)$ glyosidic bonds between the material monomers, this does not provide a nutrient source for bacteria multiplication.[65] As a result, no increase in the population of *P. agglomerans* 299R was observed.

Conversely, on gelatin an increase in the population of *P. agglomerans* 299R was observed in the first 12 h after inoculation (**Fig. 5**). This growth was attributed to gelatin providing a nutrient source, in the form of peptides and proteins. As the bacterial enzymes were able to hydrolyse the peptide bonds. Thus, producing a suitable nutrient source of carbon and nitrogen. After 12 h the population stabilised at a normalised cell density of $1.69 \pm 0.03$ (N = 5). Indicating that this is the largest population density gelatin can sustain. It is important to note that enzymatic breakdown of gelatin as a nutrient source by the bacteria would also influence the degradation characteristics of the material.[66] As this breakdown often results in gelatin becoming liquefied.[43]

In contrast, *P. agglomerans* 299R populations were not sustained on either isolated bitter orange cuticles or PDMS. Once a cuticle is isolated from a leaf, there is no nutrient support from the leaf and the cuticle itself does not provide any nutrients for the *P. agglomerans* 299R. Hence, no increase in the population of *P. agglomerans* 299R was observed. Furthermore, with the absence of moisture on the surface of the bitter orange cuticles, the *P. agglomerans* 299R died within 6 h (**Fig. 5**). In the case

of PDMS, bacteria do not have the enzymes necessary to degrade the PDMS to form a sustainable nutrient source. PDMS also did not degrade in the experimental conditions (**Fig. 3**), indicating that an exchange of moisture with the environment did not occur. As a result PDMS does not provide the necessary moisture to sustain bacterial life. The lack of nutrients and moisture resulted in the population of *P. agglomerans* 299R dying within 6 h of inoculation to the patterned PDMS surface. Which is comparable to the behaviour observed on the isolated leaf cuticles.

In summary, agarose and gelatin will provide a moisture source to sustain bacterial life. In addition, gelatin is able to be degraded by the bacteria to produce a nutrient source that promotes an increase in population, until the maximum population sustainable by gelatin is reached. Thus, indicating that the inherent properties of the material influences bacterial viability. Which also indicates that the choice of replica material has the potential to influence phyllosphere microbiology studies. For instance, if one was to examine the influence of nutrient supply, the background supply due to the replica surface material will interfere with measurements. Thus, rendering any results obtained unreliable. Conversely, bacterial life was not sustained on either PDMS or bitter orange cuticles. Indicating that PDMS is more representative of a living leaf surface. In addition, modifying PDMS could allow a controlled nutrient or moisture supply to be introduced. For example, his could be achieved with the use of fillers, such as carbon nanotubes. Hence this would enable a nutrient supply more representative of the living leaf while retaining the physical advantages of PDMS.[67]
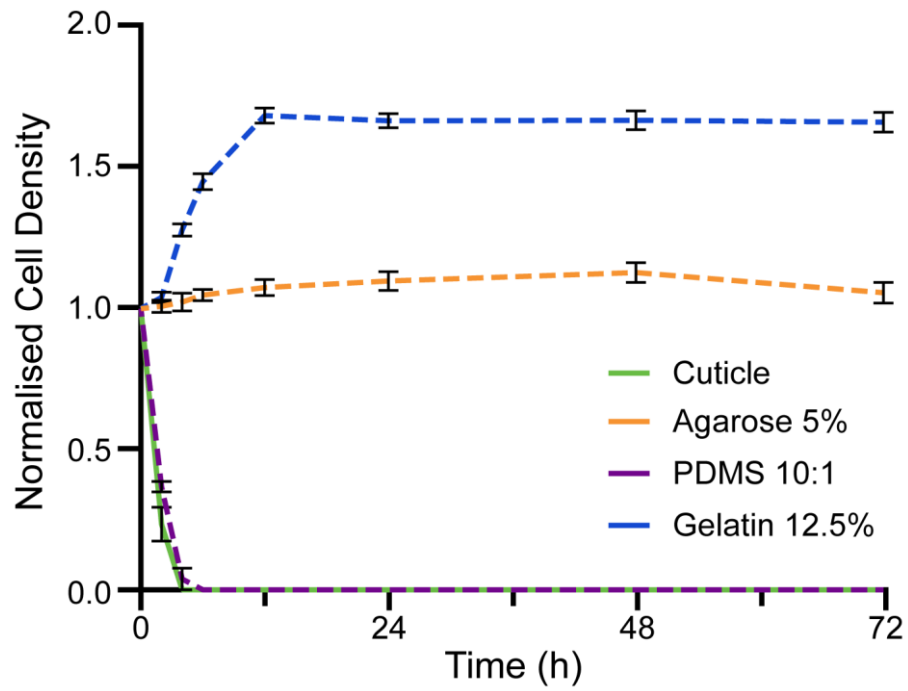
**Fig. 5**. **Normalised cell density of *Pantoea agglomerans* 299R.** The normalised cell density of *P. agglomerans* 299R was analysed over 72 h on: *Citrus* × *aurantium* (bitter orange) cuticles, agarose 5%, PDMS 10:1 w/w, and gelatin 12.5%. See **Supplementary Figure S4** for contact normalised cell density measurements for the three concentrations of each material. Data here is presented as the mean ± SEM.

## 4. Conclusions

Our work has demonstrated the potential of PDMS as a replica leaf material for phyllosphere microbiology. At the same time, our results highlighted the drawbacks of agarose and gelatin through comparing optical resolution, degradation characteristics, hydrophobic properties, and bacterial survival to PDMS.

Using optical profilometry, we demonstrated that agarose and gelatin replicas would not provide topography comparable to that of a living leaf. Dimensions of the test pillars reproduced in agarose and gelatin were found to differ from the mold master by more than 10%. Furthermore, agarose and gelatin degraded considerably within 72 h in both high and low humidity conditions at 30 °C. On the other hand, the height and width of pillars reproduced in PDMS were within 10% of the dimensions measured for the mold master. Furthermore, PDMS displayed no structural degradation within 72 h. These results indicate that in terms of topography, a replica leaf made from PDMS would be more representative of a leaf surface. In addition, PDMS would not degrade throughout the duration of an experiment. Therefore, will not influence microorganism behaviour. The suitability of PDMS was further supported by measured contact angles. The measured contact angles were comparable to those of isolated *Citrus × aurantium* and *Populus × canescens* leaf cuticles.

In addition, we examined the influence of the replica materials on bacterial survival in comparison with isolated *Citrus × aurantium* leaf cuticles. These results highlighted that PDMS is representative of a leaf cuticle, as both are unable to support bacterial life. Whereas, agarose and gelatin were able to sustain bacterial life. Thus, indicating that these materials would provide a nutrient source. As a result, rendering these materials unsuitable for nutrient supply studies.

In summary, the results presented here indicate that in contrast to agarose and gelatin, the combined properties of PDMS make for a suitable replica material for phyllosphere microbiology. Our experiments highlight the importance of considering the inherent material properties when selecting a material as a replica surface. In our current work, we are investigating the preparation of biomimetic leaf replicas made from PDMS with tailored nutrient permeability for plant-microbe interactions at a single-cell resolution.

# References

1. P. Lemanceau, M. Barret, S. Mazurier, S. Mondy, B. Pivato, T. Fort and C. Vacher, in *Advances in Botanical Research*, ed. G. Becard, Elsevier, 2017, vol. 82, ch. 5, pp. 101-133.
2. E. B. Nelson, *Annu. Rev. Phytopathol.*, 2004, **42**, 271-309.
3. R. L. Berendsen, C. M. Pieterse and P. A. Bakker, *Trends Plant Sci.*, 2012, **17**, 478-486.
4. C. Vacher, A. Hampe, A. J. Porté, U. Sauer, S. Compant and C. E. Morris, *Annu. Rev. Ecol. Evol. S.*, 2016, **47**, 1-24.
5. K. M. Meyer and J. H. Leveau, *Oecologia*, 2012, **168**, 621-629.
6. J. A. Vorholt, *Nat. Rev. Microbiol.*, 2012, **10**, 828-840.
7. S. Compant, M. G. Van Der Heijden and A. Sessitsch, *FEMS Microbiol. Ecol.*, 2010, **73**, 197-214.
8. L. Schreiber, *Trends Plant Sci.*, 2010, **15**, 546-553.
9. W. Aragón, J. J. Reina-Pinto and M. Serrano, *J. Exp. Bot.*, 2017, **68**, 5339-5350.
10. J. C. Heaton and K. Jones, *J. Appl. Microbiol.*, 2008, **104**, 613-626.
11. K. Herman, A. Hall and L. Gould, *Epidemiol. Infect.*, 2015, **143**, 3011-3021.
12. M. T. Gekenidis, D. Gossin, M. Schmelcher, U. Schöner, M. N. Remus-Emsermann and D. Drissner, *J. Appl. Microbiol.*, 2017, **123**, 916-932.
13. H. K. Doan and J. H. Leveau, *Phytopathology*, 2015, **105**, 1036-1042.
14. A. Newton, C. Gravouil and J. Fountaine, *Ann. Appl. Biol.*, 2010, **157**, 343-359.
15. J. A. Painter, R. M. Hoekstra, T. Ayers, R. V. Tauxe, C. R. Braden, F. J. Angulo and P. M. Griffin, *Emerg. Infect. Dis.*, 2013, **19**, 407-415.
16. M. Doyle and M. Erickson, *J. Appl. Microbiol.*, 2008, **105**, 317-330.
17. A. N. Olaimat and R. A. Holley, *Food Microbiol.*, 2012, **32**, 1-19.
18. D. Althaus, E. Hofer, S. Corti, A. Julmi and R. Stephan, *J. Food Protect.*, 2012, **75**, 1338-1341.
19. T. R. Scheublin, S. Deusch, S. K. Moreno-Forero, J. A. Müller, J. R. van der Meer and J. H. Leveau, *Environ. Microbiol.*, 2014, **16**, 2212-2225.
20. B. Sirinutsomboon, M. J. Delwiche and G. M. Young, *Biosyst. Eng.*, 2011, **108**, 244-252.
21. J. Jacobs, T. Carroll and G. Sundin, *Microb. Ecol.*, 2005, **49**, 104-113.
22. L. Rivas, N. Fegan and G. A. Dykes, *Int. J. Food Microbiol.*, 2007, **115**, 89-94.
23. L. Schreiber and J. Schonherr, *Water and Solute Permeability of Plant Cuticles*, Springer Berlin Heidelberg, 1st edn., 2009.
24. C. E. Jeffree, *Annual Plant Reviews: Biology of the Plant Cuticle*, 2006, **23**, 11-125.
25. M. Melotto, W. Underwood, J. Koczan, K. Nomura and S. Y. He, *Cell*, 2006, **126**, 969-980.
26. R. Rusconi, M. Garren and R. Stocker, *Annu. R. Biophys.*, 2014, **43**, 65-91.
27. M. N. Remus-Emsermann, S. de Oliveira, L. Schreiber and J. H. Leveau, *Front. Microbiol.*, 2011, **2**, 197(1-7).
28. K. Koch, A. Dommisse, W. Barthlott and S. N. Gorb, *Acta Biomater.*, 2007, **3**, 905-909.
29. K. Koch, A. J. Schulte, A. Fischer, S. N. Gorb and W. Barthlott, *Bioinspir. Biomim.*, 2008, **3**, 046002(1-10).
30. B. McDonald, P. Patel and B. Zhao, *Chem. Eng. Process Tech.*, 2013, **1**, 1012(1-8).
31. B. Zhang, Y. Luo, A. J. Pearlstein, J. Aplin, Y. Liu, G. R. Bauchan, G. F. Payne, Q. Wang, X. Nou and P. D. Millner, *ACS Appl. Mater. Inter.*, 2014, **6**, 12467-12478.
32. M. Sun, C. Luo, L. Xu, H. Ji, Q. Ouyang, D. Yu and Y. Chen, *Langmuir*, 2005, **21**, 8978-8981.
33. E. Lepore and N. Pugno, *BioNanoScience*, 2011, **1**, 136-143.
34. F. Wang, S. Li and L. Wang, *Colloid. Surface. A*, 2017, **513**, 389-395.
35. M. Mayer, J. Yang, I. Gitlin, D. H. Gracias and G. M. Whitesides, *Proteomics*, 2004, **4**, 2366-2376.
36. E. Berthier, E. W. K. Young and D. Beebe, *Lab Chip*, 2012, **12**, 1224-1237.
37. S. Nahavandi, S. Baratchi, R. Soffe, S.-Y. Tang, S. Nahavandi, A. Mitchell and K. Khoshmanesh, *Lab Chip*, 2014, **14**, 1496-1514.
38. D. Huh, H. J. Kim, J. P. Fraser, D. E. Shea, M. Khan, A. Bahinski, G. A. Hamilton and D. E. Ingber, *Nat. Protoc.*, 2013, **8**, 2135-2157.

39. A. Tayagui, Y. Sun, D. A. Collings, A. Garrill and V. Nock, *Lab Chip*, 2017, **17**, 3643-3653.
40. R. Soffe, S. Baratchi, M. Nasabi, S.-Y. Tang, A. Boes, P. McIntyre, A. Mitchell and K. Khoshmanesh, *Sensor. Actuat. A Chem.*, 2017, **251**, 963-975.
41. L. Murray, V. Nock, J. Evans and M. Alkaisi, *J. Biomed. Mater. Res. A*, 2016, **104**, 1638-1645.
42. I. Mutreja, T. B. F. Woodfield, S. Sperling, V. Nock, J. J. Evans and M. M. Alkaisi, *Biofabrication*, 2015, **7**, 025002(1-13).
43. J. Hollants, F. Leliaert, O. De Clerck and A. Willems, *FEMS Microbiol. Ecol.*, 2013, **83**, 1-16.
44. A. L. Paguirigan and D. J. Beebe, *Nat. Protoc.*, 2007, **2**, 1782-1788.
45. G. M. Whitesides, E. Ostuni, S. Takayama, X. Jiang and D. E. Ingber, *Annu. Rev. Biomed. Eng.*, 2001, **3**, 335-373.
46. J. He, M. Mao, D. Li, Y. Liu and Z. Jin, *J. Bionic Eng.*, 2014, **11**, 109-114.
47. L. Gitlin, P. Schulze and D. Belder, *Lab Chip*, 2009, **9**, 3000-3002.
48. L. Yang, X. Hao, C. Wang, B. Zhang and W. Wang, *Microsyst. Technol.*, 2014, **20**, 1933-1940.
49. F. Girard, M. Antoni, S. Faure and A. Steinchen, *Colloid. Surface. A*, 2008, **323**, 36-49.
50. M. G. Lawrence, *B. Am. Meteorol.Soc.*, 2005, **86**, 225-234.
51. L. B. Rockland, *Anal. Chem.*, 1960, **32**, 1375-1376.
52. M. N. Remus-Emsermann, E. B. Kim, M. L. Marco, R. Tecon and J. H. Leveau, *Genome Announc.*, 2013, **1**, e00036-00013.
53. M. N. Remus-Emsermann, R. Tecon, G. A. Kowalchuk and J. H. Leveau, *ISME J.*, 2012, **6**, 756-765.
54. C. Vogel, G. Innerebner, J. Zingg, J. Guder and J. A. Vorholt, *Appl. Environ. Microb.*, 2012, AEM. 00639-00612.
55. G. Innerebner, C. Knief and J. A. Vorholt, *Appl. Environ. Microb.*, 2011, **77**, 3202-3210.
56. S. H. Eckerson, *Bot. Gaz.*, 1908, **46**, 221-224.
57. T. Divoux, B. Mao and P. Snabre, *Soft Matter*, 2015, **11**, 3677-3685.
58. Intellicast, Relative Humidity, http://www.intellicast.com/Global/Humidity.aspx?region=World, (accessed 17/07/2018, 2018).
59. C. E. Morris and J.-M. Monier, *Annu. Rev. Phytopathol.*, 2003, **41**, 429-453.
60. T. Yasuda, T. Okuno and H. Yasuda, *Langmuir*, 1994, **10**, 2435-2439.
61. S. Robson and G. R. Willmott, *Soft Matter*, 2016, **12**, 4853-4865.
62. C. Brewer, W. Smith and T. Vogelmann, *Plant Cell Environ.*, 1991, **14**, 955-962.
63. H. Wang, H. Shi, Y. Li and Y. Wang, *Plos One*, 2014, **9**, e107062(1-10).
64. S. Hemmilä, J. V. Cauich-Rodríguez, J. Kreutzer and P. Kallio, *Appl. Surf. Sci.*, 2012, **258**, 9864-9875.
65. B. Hofsten and M. Malmqvist, *Microbiology*, 1975, **87**, 150-158.
66. H. L. Smith Jr and K. Goodner, *J. Bacteriol.*, 1958, **76**, 662-665.
67. C. Xue, G.-Q. Du, L.-J. Chen, J.-G. Ren, J.-X. Sun, F.-W. Bai and S.-T. Yang, *Sci. Rep.*, 2014, **4**, 5925(1-7).

# Supplementary Information:

# Comparison of Replica Leaf Surface Materials for Phyllosphere Microbiology

Rebecca Soffe,[1] Nicola Altenhuber,[1] Michal Bernach,[1,2] Mitja Remus-Emsermann,[2]
Volker Nock[1]


Department of Electrical and Computer Engineering, University of Canterbury,
Christchurch, New Zealand

[2] School of Biological Sciences, University of Canterbury, Christchurch, New Zealand

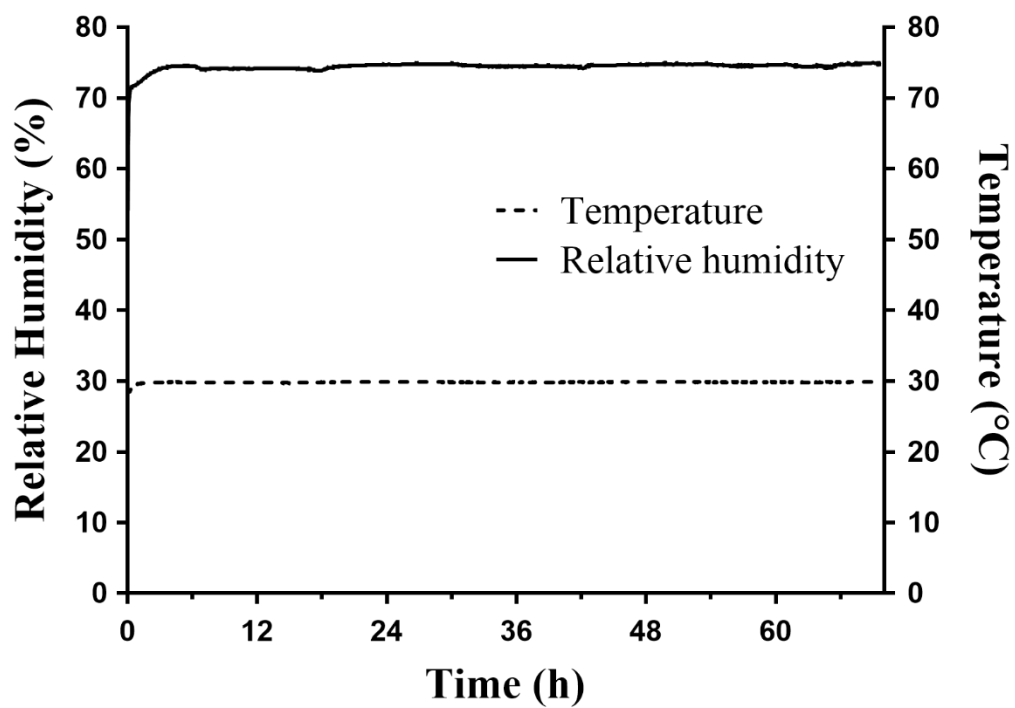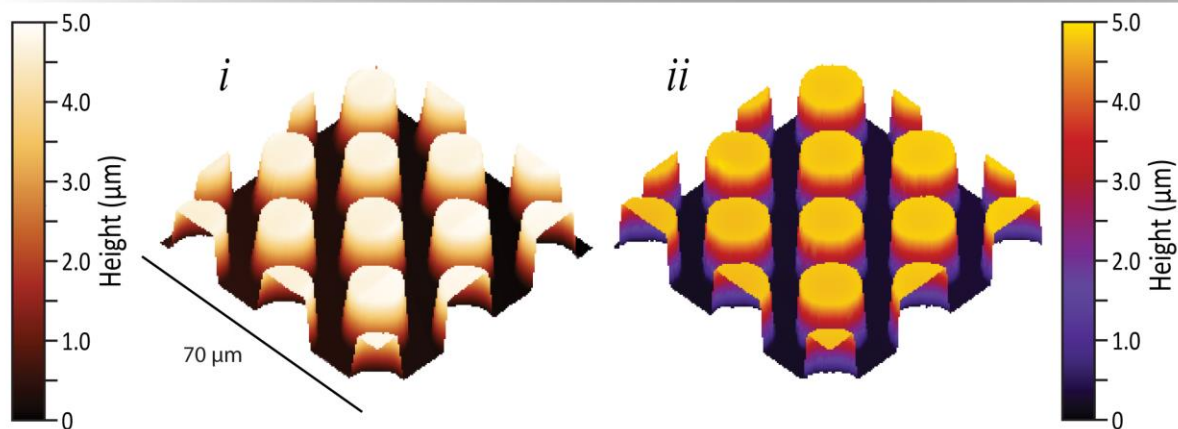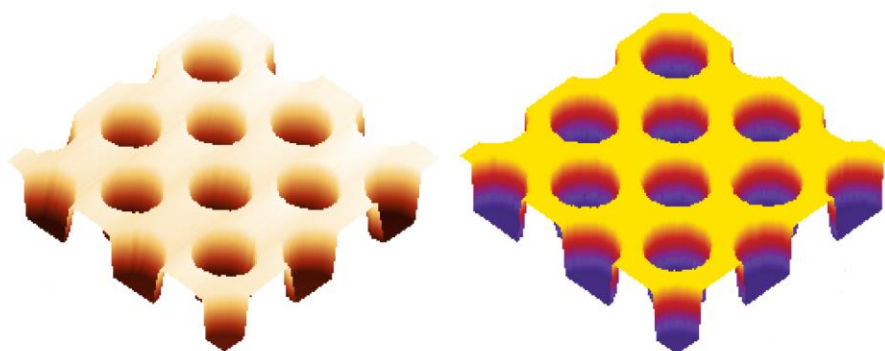**Supplementary Information S1: Humidity Control Experiment.**



**Figure S1. Humidity Experimental Control Experimental Results.** Experimental parameters detailed in section 2.8 Humidity.

## Supplementary Information S2: AFM and Optical Image Comparisons for Patterned PDMS Fabrication.
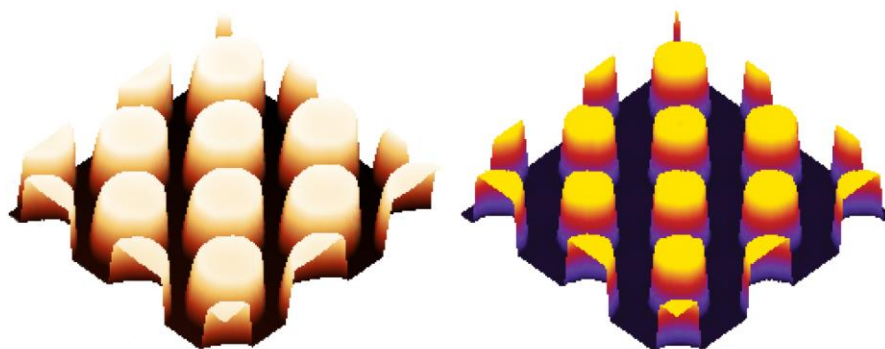


**Figure S2. AFM and Optical Image Comparison**. Comparison of the (a) photoresist mold master resolution; (b) PDMS 10:1 w/w template; and (c) patterned PDMS 10:1 w/w, undertaken by (*i*) AFM, and, an (*ii*) optical profilometer.

**Table S1: AFM and Optical Image Comparison Measurements.** Height and Width Measurements were taken from 20 pillars (or wells, as was the case for the PDMS template). All data is represented as mean ± standard deviation. Standard deviation has been used to show the scattering in measured values.

| | AFM | | | Optical Profilometer | | |
|---|---|---|---|---|---|---|
| | **ADEX05 Mold Master** | **PDMS Template** | **Patterned PDMS** | **ADEX05 Mold Master** | **PDMS Template** | **Patterned PDMS** |
| **Height (μm)** | 4.44 ± 0.16 | 4.20 ± 0.06 | 4.58 ± 0.11 | 4.50 ± 0.03 | 4.18 ± 0.01 | 4.71± 0.01 |
| **Width (μm)** | 14.64 ± 0.47 | 15.23± 0.57 | 14.93 ± 0.32 | 14.94 ± 0.21 | 15.11 ± 0.52 | 14.00 ± 0.38 |

**Supplementary Information S3: Contact Angle Measurements on Flat and Patterned Replica Materials.**
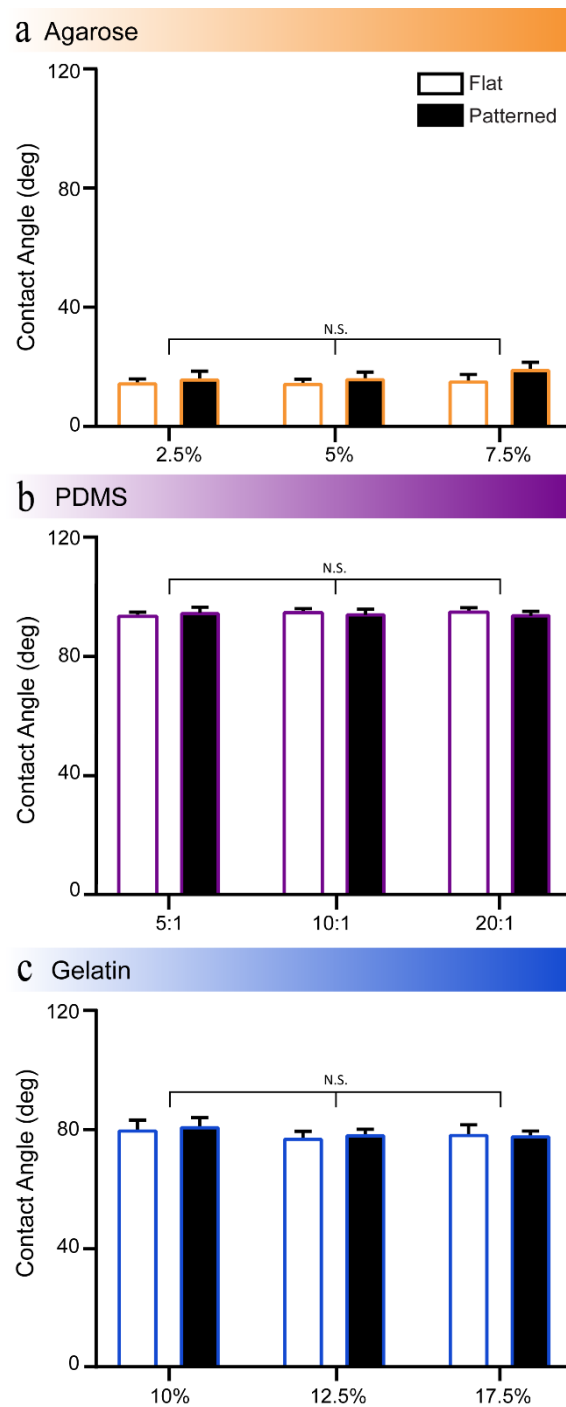


**Figure S3. Contact Angle Comparisons on Flat and Patterned Replica Materials at Three Different Concentrations.** Contact angles for (a) agarose, (b) PDMS, and (c) gelatin.

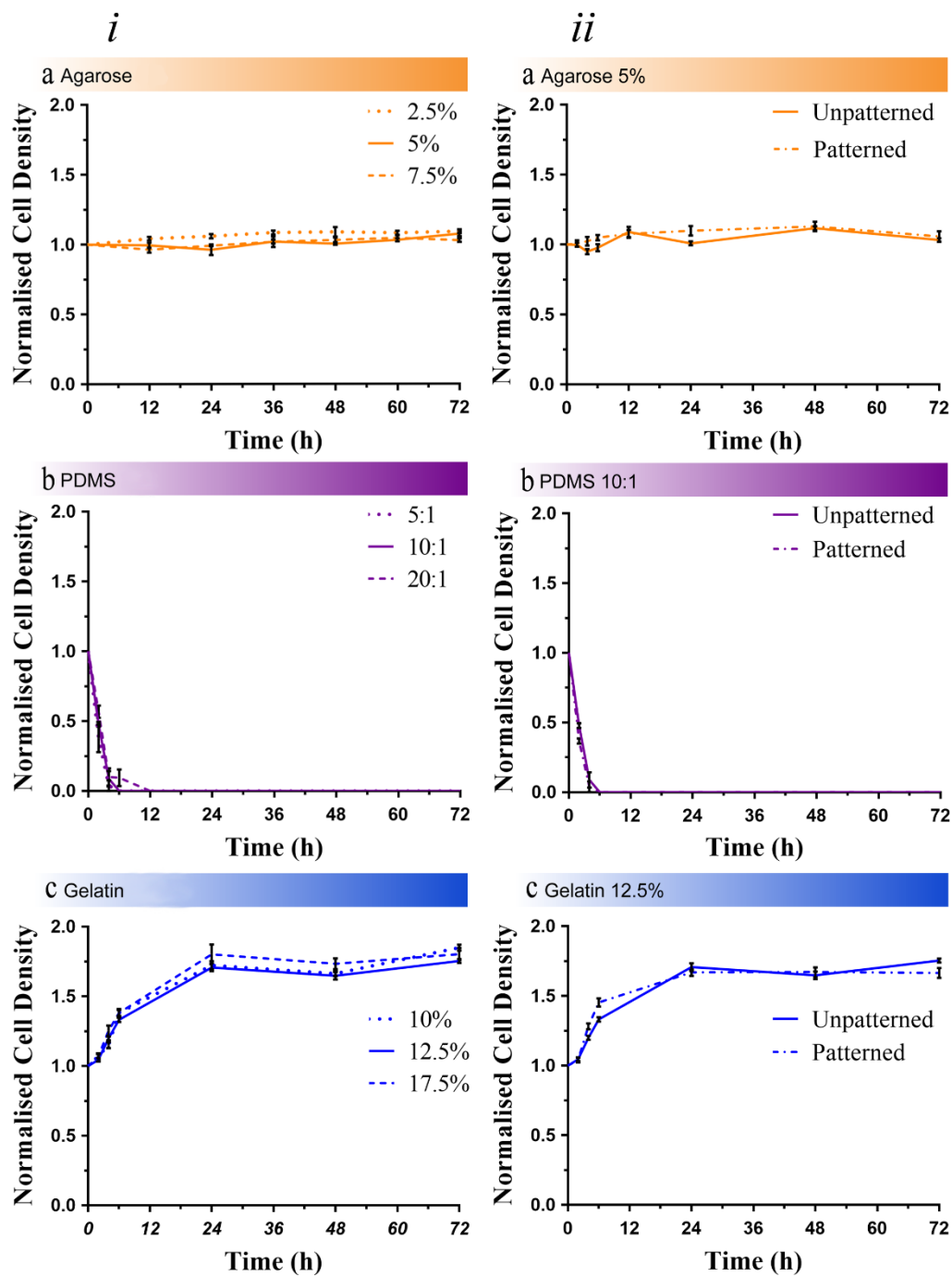**Supplementary Information S4: Bacterial Survival on Flat and Patterned Replica Materials.**



**Figure S4. Bacteria Viability of *Pantoea agglomerans* 299R.** Bacteria viability for bacteria at (*i*) different concentrations, for (a) agarose, (b) PDMS, and (c) gelatin. Comparison of (*ii*) bacteria viability on flat and patterned replica materials for (a) agarose 5 % w/v (b) PDMS 10:1 w/w, and (c) gelatin 12.5 % w/v.