Received: 12 May 2022 | Revised: 20 June 2022 | Accepted: 21 June 2022

DOI: 10.1002/pat.5792

SHORT COMMUNICATION

polymers for advanced technologies WILEY

# Measuring contact angles on hydrophilic porous scaffolds by implementing a novel raised platform approach: A technical note

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**Funding information**
N.C. Paxton acknowledging funding from Advance Queensland (AQIRF2020), CSIRO, 3D Systems and Edale Capital, as well as from Queensland University of Technology (Early Career Research Scheme Grant), and QUT Centre for Biomedical Technologies (Industry Engagement Grant).

## Abstract

Contact angle (CA) analysis is a widely employed technique to assess the surface properties of solid samples, including in tissue engineering research where scaffolds are typically designed to be both porous and hydrophilic to enable cell and tissue infiltration. Paradoxically, the types of scaffolds that possess the most optimal hydrophilic surface properties for cell attachment are the most challenging surfaces to attain accurate CA measurements. Here, we propose the use of a small 3D printed platform to elevate samples above the CA measurement substrate and demonstrate reproducible and accurate CA measurements on a range of popular polymer scaffolds that had undergone 5 min plasma treatment to instigate hydrophilicity. Using four polycaprolactone or high-density polyethylene scaffolds with porosity ranging from 35.8%–93.1%, 0° CAs were reproducibly observed by measuring the CA while the scaffolds were elevated on the 3D printed platform, compared to the highly variable false-positive results when measuring the scaffolds while directly sitting on measurement substrates of various materials. This versatile, low-cost modification to CA hardware overcomes the challenges associated with measuring the surface properties of porous, hydrophilic scaffolds and provides a simple tool for tissue engineering researchers to perform CA measurements for any biomaterial scaffolds to ascertain hydrophilicity which is used to infer the suitability of scaffold surfaces for cell attachment.

**KEYWORDS**
biomaterials, contact angles, scaffolds, wetting

## 1 | INTRODUCTION

Contact angle (CA) analysis, a technique established over 2 centuries ago, remains an integral component of biomaterial research and materials analysis.[1] CA is a robust technique for measuring the interaction between three phases, often used to measure the wettability of solid materials by measuring the angle at which a liquid-vapor interface meets the solid in air. Hydrophobic materials are typically characterized by large CAs often (>90°), whereas hydrophilicity is characterized by low CAs (<90°).[2] Routine water CA measurements using the sessile drop technique feature the deposition of droplets of water using a syringe and needle controlled by a syringe pump to deposit the water at a nominal flow rate or to produce a standardized volume. Once the water droplet falls onto the experimental material below, a back-lit

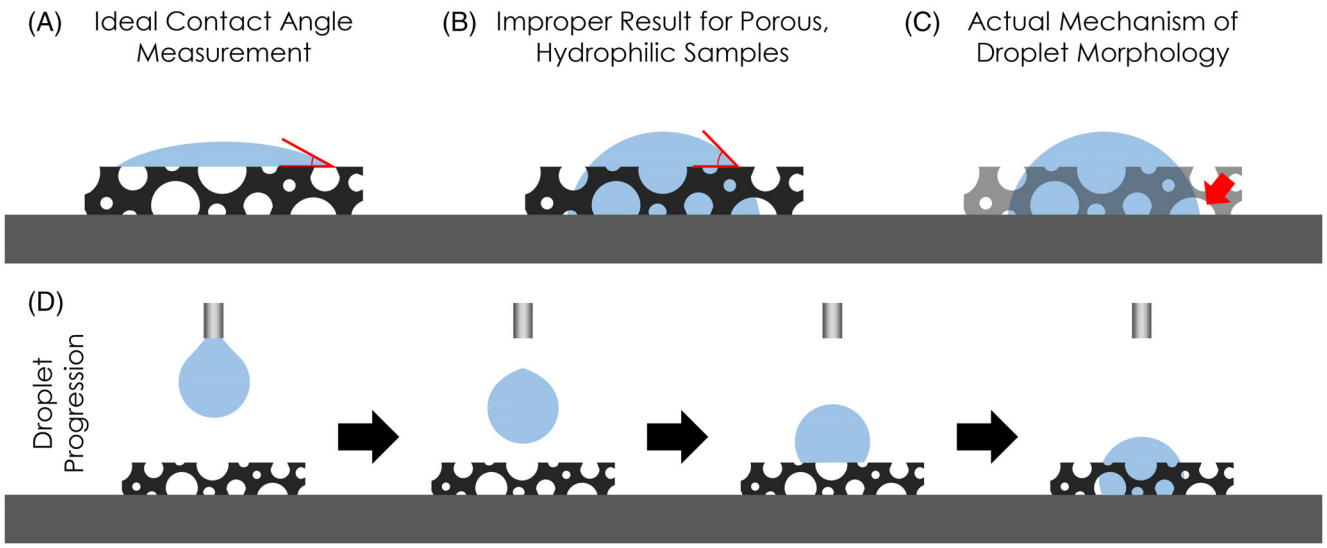



**FIGURE 1** Schematic diagram of contact angle measurements of a water droplet (blue) on porous scaffolds (black) placed on a substrate (gray). (A) The ideal measurement for a hydrophilic sample, (B) improper measurement of a droplet that has been absorbed into the scaffold and (C) and transparent view indicating that the droplet may be interacting with the substrate below the sample. (D) The progression of a contact angle measurement on hydrophilic samples

image of the droplet on the surface in profile is captured by a high-resolution camera. The static CA, defined as the angle subtended by the surface and water-air interface, can be measured using digital image analysis software. More complex experimental configurations are available for the measurement of CA interactions under dynamic conditions, with the material at varying or inverted angles, and material morphologies, described elsewhere.[1,3] CA is calculated via the Young equation which fundamentally presumes a flat, smooth solid surface from which the angle is calculated. For rough, porous, or chemically heterogeneous surfaces, CA measurements can be highly variable and no longer represent the true material property as the result is also a function of the varying surface geometry.[4,5] This presents a challenge in biomaterials research.

In biomaterials literature, a wide variety of materials have been developed, including implantable materials for permanent surgical implantation and regenerative tissue engineering. Many of these materials are designed to have rough surfaces which are known to improve cell attachment in some contexts, as well as porosity, which plays a vital role in enabling tissue ingrowth for implanted materials.[6] Furthermore, hydrophilicity is often highly desired, particularly for polymer biomaterials, to improve the ability to seed and attach cells to biomaterial surfaces.[7] Many physical and chemical treatment strategies have been proposed to induce hydrophilicity and the effects of such surface property modifications have been well-studied.[8] Most notably, a strong relationship between hydrophilicity and cell attachment has been identified; not only because hydrophobic scaffolds impede the penetration of aqueous cell suspensions and thereby limit cell seeding efficacy,[9] but also functional groups known to drastically enhance cell adhesion on scaffold surfaces are readily introduced onto scaffold surfaces through wetting techniques such as plasma treatment.[10] However, the porous and ideally hydrophilic nature of biomaterials can lead to the inconsistent interaction between deposited droplets and the material surface, and therefore inaccurate measurement of true biomaterial properties and compromised validity of CA quantification.

Fundamentally, CA on hydrophilic porous scaffolds cannot be accurately measured. Porous scaffolds with entirely wettable surfaces absorb the deposited water droplets, which may then pass through into the porous structure and interact with a fourth phase, the substrate below the sample, yielding an improper measurement of the CA on the top of the scaffold surface (Figure 1). While several models exist for approximating CA on rough surfaces such as the Wenzel or Cassie–Baxter models (Figure S1), porous, hydrophilic scaffolds cannot be approximated as rough surfaces since fundamentally, roughness is not limited to the surface of the sample with the porosity permeating the entire sample. This introduces interaction between the air, water, sample, and a fourth surface, the measurement substrate, which these models do not consider (Figure S1).[11]

Therefore, this study proposes a simple and low-cost modification to CA measurement apparatus to enable the accurate measurement of CA on porous and hydrophilic scaffolds to ensure valid measurements are obtained for samples with near-zero CA. This study proposes the use of a 3D printed platform to elevate a sample above the measurement stage to observe complete wetting and full absorption of water droplets through highly porous and hydrophilic samples, a first in the literature.

## 2 | EXPERIMENTAL

### 2.1 | Scaffold preparation

Several porous polymer scaffolds manufactured via various molding and 3D printing methods were used as representative samples

of typical polymeric porous biomaterials. High porosity melt electrowritten scaffolds were fabricated using a custom-built MEW device, described previously.[12,13] Polycaprolactone (PCL) PC12 (Purac, Corbion) was loaded into a 3 mL syringe (Nordson EFD) and fitted with a 21 G blunt-tip needle (Nordson EFD). The syringe was then loaded in a grounded custom heater jacket with a heating supply and regulated air pressure. After heating for 10 min at 90°C, the material was then extruded onto a motorized collector plate system controlled using Repetiteur Host software. Gcode was programmed to instruct the fabrication of 30 layer high cross hatch scaffolds, 10 × 10 × 1.5 mm in size, with 1 mm and 0.5 mm spacing between parallel fibers, according to previously published methods.[12] Additionally, two commercial porous high-density polyethylene (pHDPE) scaffolds, MEDPOR® and StarPore® were purchased from Stryker and supplied by Anatomics respectively. Scaffold sheets were trimmed into 10 × 10 × 1.5 mm scaffold sections.

## 2.2 | Micro-computed tomography

Micro-computed tomography (μCT) was used to 3D image the polymer scaffolds at high resolution to calculate porosity. μCT scanning was performed using a μCT50 (Scanco Medical AG, Brüttisellen, Switzerland) on all scaffold types, with an isotropic voxel size of 5 μm, 45 kVp energy, 200 μA current, 9 W intensity, and 1246 ms integration time. The scan data was analyzed using ImageJ with the BoneJ and Scanco μCT plugins.[14,15] Thresholding was applied to distinguish polymer material from background and BoneJ was used to calculate porosity.

## 2.3 | Plasma surface treatment

Plasma surface treatment of scaffolds was achieved using vacuum plasma cleaner (PDC-002-HP Harrick Plasma, United States). Scaffolds were placed in the vacuum chamber which was then evacuated and flushed with 50% $Ar_2$ and 50% $O_2$. Plasma treatment was performed for 5 min on "medium" setting (38 W)[13] before being removed and stored in a petri dish for CA analysis 2 h later.

## 2.4 | 3D Printed platform design and manufacturing

A small platform was designed in Autodesk Fusion 360, with a footprint of 6 × 9 mm and two walls 6 mm in height and 2 mm in thickness, therefore separated by 5 mm. 3D models were exported as STL files and loaded in an open-source slicing software (Ideamaker version 4.1.1, Raise 3D Technologies, Inc., Irvine, California, United States) for preparation for 3D printing using a Raise3D Pro 2 Plus (Raise 3D Technologies, Inc.) with poly-l-lactic acid (PLA) 1.75 mm diameter filament (Spectrum filaments, Piece, Poland).

## 2.5 | CA measurement

Water CA was performed on Drop Shape Analyzer (Biolin ThetaFlex, Västra Frölunda, Sweden). Distilled $H_2O$ was dispensed to create a droplet of 50 μL onto dry samples placed in various configurations on a motorized z-stage. CA measurements were performed on non-treated scaffolds on the measurement stage substrate (anodised aluminum), followed by measurement of plasma treated samples (with known hydrophilicity[7,13]) on the measurement substrate and raised on the 3D printed platform. Next, the CA of various measurement substrates were measured, including the native measurement stage in the Drop Shape Analyzer (anodised aluminum), a coated glass microscope slide (ÜberFrost® Printer Slides, InstrumeC), a metal plate (stainless steel) and a petri dish (polystyrene). The CA of plasma treated scaffolds was then measured on each of the substrates. Videos and images were acquired and the angle between the sample surface and edge of the water droplet was measured automatically using OneAttension Software and reported as average ± SD ($n = 10$).

# 3 | RESULTS AND DISCUSSION

## 3.1 | Sample platform preparation

A 3D printed platform was designed in computer-aided design (CAD) software (Fusion 360, Autodesk) and 3D printed using a low-cost FDM printer, using approximately 220 mg filament, equal to 0.5 cents (AUD) in value (Figure 2A). When installed on the CA Analyzer, the platform raised a sample placed on top by 6 mm (Figure 2B), enabling the sample to be viewed through the camera without the substrate immediately below the sample (Figure 2C). This simplistic design and ability to manufacture through low-cost methods enables versatility in design to suit various sample geometries and measurement configurations (Data S1).

## 3.2 | Sample geometry and porosity

Several scaffolds representative of popular scaffold systems reported in the literature, including commercial samples, have been used in this investigation to validate this technique using samples with widely varying porosity. MEW scaffolds are emerging in popularity for a wide range of tissue engineering applications,[16] and are routinely plasma treated or etched using sodium hydroxide (NaOH) to achieve improved hydrophilicity and enable cell attachment.[12] Meanwhile, porous high-density polyethylene (pHDPE) scaffolds are the gold standard in commercial surgical implant biomaterials, particularly due to their porous structure enabling tissue ingrowth and ability to be sculpted and molded to conform to patient anatomy, or directly manufacturing into patient-specific implants.[7,17]

μCT analysis revealed the porosities of these scaffolds, ranging from 93.1% for the MEW PCL scaffold with 1 mm spacing, down to 35.8% for the MEDPOR® pHDPE scaffold samples (Table 1). These

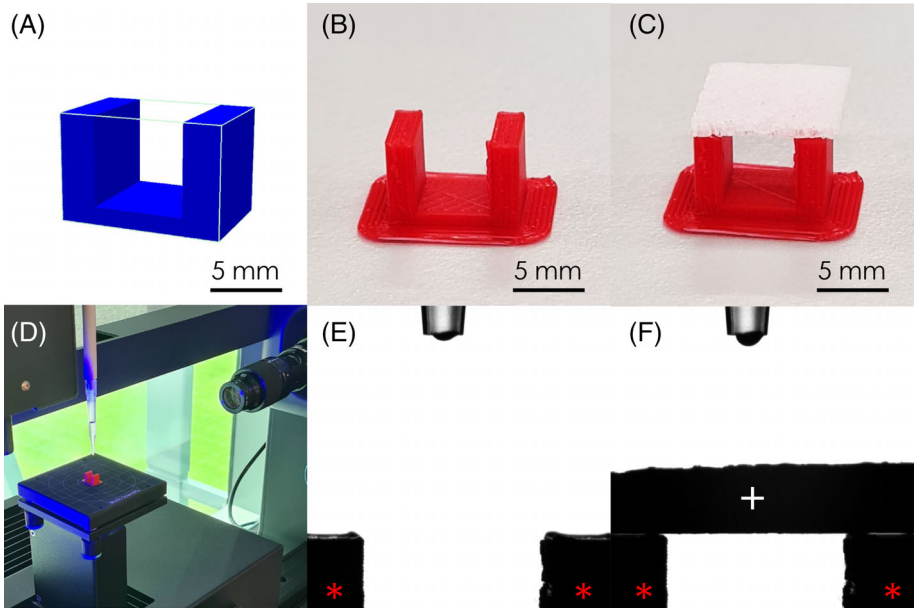
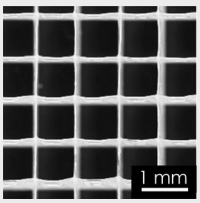
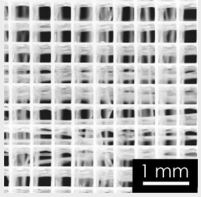
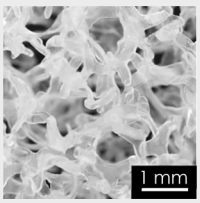
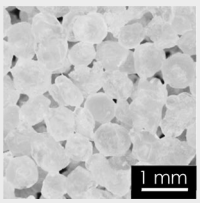



**FIGURE 2** (A) Computer-aided design (CAD) model of the 3D printed platform and (B, C) photographs of the 3D printed platform (B) without and (C) with a scaffold placed on top for measurement. (D) Photograph of the 3D printed platform installed on the contact angle (CA) device to elevate samples above the measurement stage substrate. (E, F) The view through the camera showing (E) the platform (*) in position beneath the pipette tip with (F) a scaffold (+) elevated by the 3D printed platform in preparation for CA measurement

**TABLE 1** Summary of the scaffolds used in this study, including a description of their geometry, porosity measured using μCT and a representative image of their structure

| | MEW PCL (1 mm spacing) | MEW PCL (0.5 mm spacing) | HDPE StarPore® | HDPE MEDPOR® |
|---|---|---|---|---|
| Geometry | Crosshatch microfiber | Crosshatch microfiber | Sintered star-shaped particles | Sintered ellipsoid-shaped particles |
| Porosity | 93.1% | 84.7% | 67.6% | 35.8% |
| Reference | 12,13 | 12 | 7 | 7 |
| Image |   |   |   |   |

sample also varied with surface roughness, characterized by 1 or 0.5 mm voids between fibers of the MEW scaffolds compared to ~1 mm diameter particles fused together for the pHDPE scaffolds (Table 1).

### 3.3 | CA of non-treated and plasma treated scaffolds

CA measurements were performed in replicate on each of the scaffold types, both prior to and following plasma treatment which has been previously reported to increase the hydrophilicity of the polymer surfaces suitable for successful cell attachment (Figure 3A). Quantification of the CA on non-treated scaffolds confirms that the PCL and HDPE scaffolds initially exhibited hydrophobic properties, with CAs measured between ~160° for the PCL scaffolds and ~110° for the HDPE scaffolds (Figure 3B). Following plasma treatment, the CA reduced to near-zero, but measurements were highly variable due to the sporadic absorption of the droplet into the porous scaffolds largely dependent on the placement on the droplet with respect to the scaffold struts or fibers. On several occasions where a small droplet, with CA typically below 20°, was observed above the scaffold surface (Figure 3A), an improper measurement was recorded, resulting in a false CA measurement due to the droplet being absorbed into the sample and clearly interacting with the hydrophobic substrate below (Figures 3C and S2). Where measurement of the droplet volume is achievable using the Drop Analyzer Software, the droplet size remaining on the surface of the scaffold was measured to be smaller than the target 50 μL droplet, indicating absorption into the scaffold.

By comparison, 0° CA was measured for all plasma treated scaffolds elevated on the stands as the droplets were observed to be completely absorbed, or pass through, the porous scaffolds without interacting with the hydrophobic substrate below (Figure 3A). Independent of scaffold porosity or architecture, the measurements performed with the samples elevated more accurately depict the hydrophilic behavior of these porous scaffolds and limits the likelihood of experimental error obscuring accurate CA measurements with

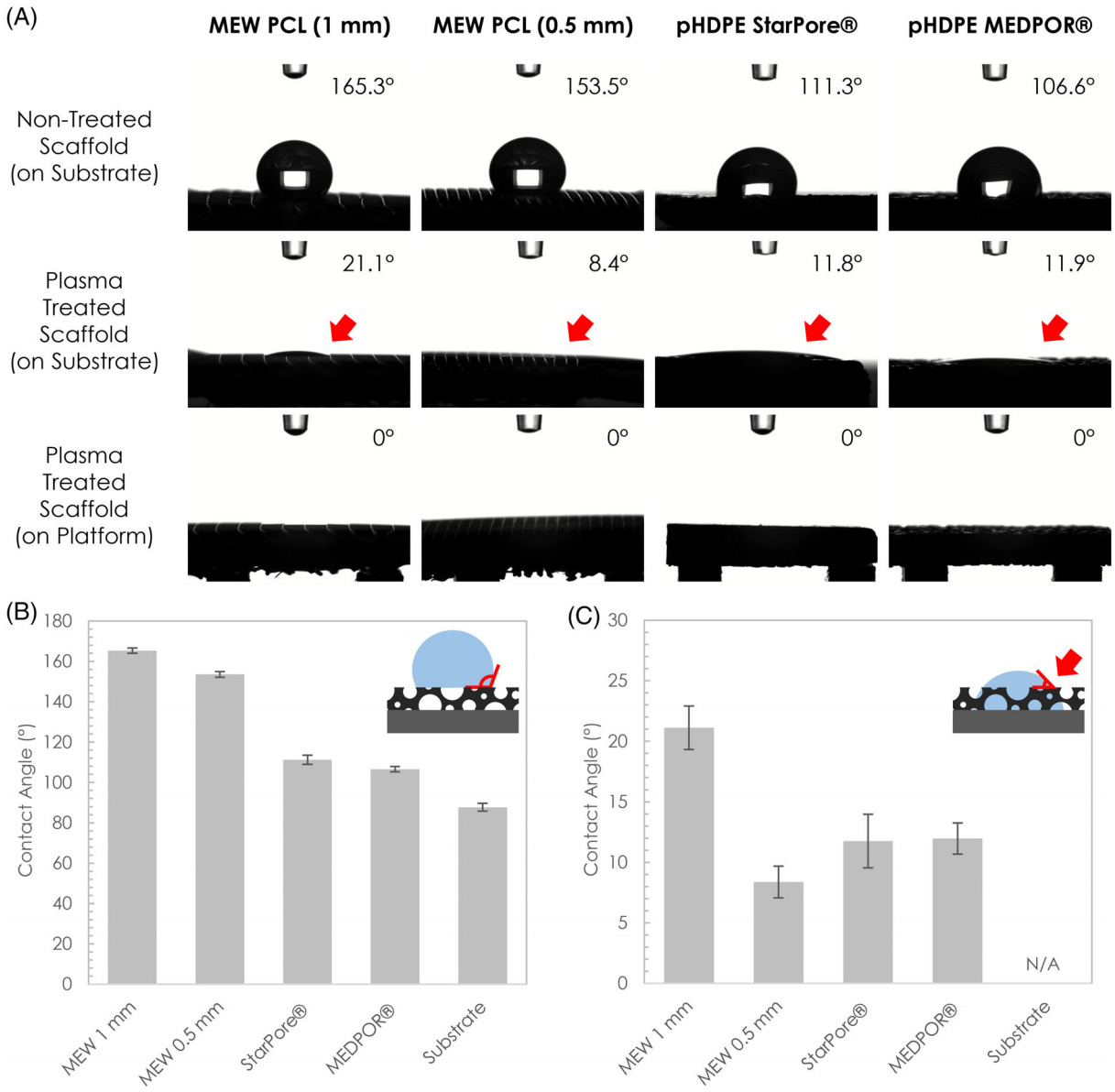



**FIGURE 3** (A) Still images from contact angle (CA) measurements of non-treated or plasma treated scaffolds measured while directly sitting on the substrate or elevated on the 3D printed platform. Artifact water droplets are indicated with red arrows. Contact angle measurements for (B) non-treated scaffolds as well as the measurement substrate, and (C) plasma treated scaffolds measured on the substrate. Values reported as average ± SD ($n = 10$)

unnecessary interplay between the droplets and substrate below the sample, due to the porous nature of the scaffolds (see Video S1).

Different measurement substrates materials also impacted the observation of false-positive CA measurements. Hydrophobic materials, including a polystyrene petri dish and anodised aluminum CA measurement stage substrate resulted in observed false-positive measurements when measuring entirely hydrophilic plasma treated MEW PCL (1 mm) scaffolds on these surfaces (Figure 4), compared to the 0° CA consistently measured when the sample was elevated above the substrate using the 3D printed platform (Figure 3). Comparatively, hydrophilic materials such as the coated glass slide exhibited a very small intrinsic CA and therefore did not elicit a perceivable false-positive CA measurement for the plasma treated scaffolds. While this may offer a promising alternative to the 3D printed sample holder for more accurately measuring CA of porous, hydrophilic samples, the temporal nature of glass slide hydrophilicity, which may diminish with age, wear and use of cleaning agents,[18] presents a challenge for CA apparatus upkeep, measurement reproducibility and cost. When not interacting with a fourth phase by being elevated above any measurement substrate material, truly hydrophilic, porous scaffolds can be accurately characterized using CA.

As CA remains one of the most popular and accessible method for characterizing hydrophilicity of biomaterials, accurate measurements are vital to providing insight into the interactions between

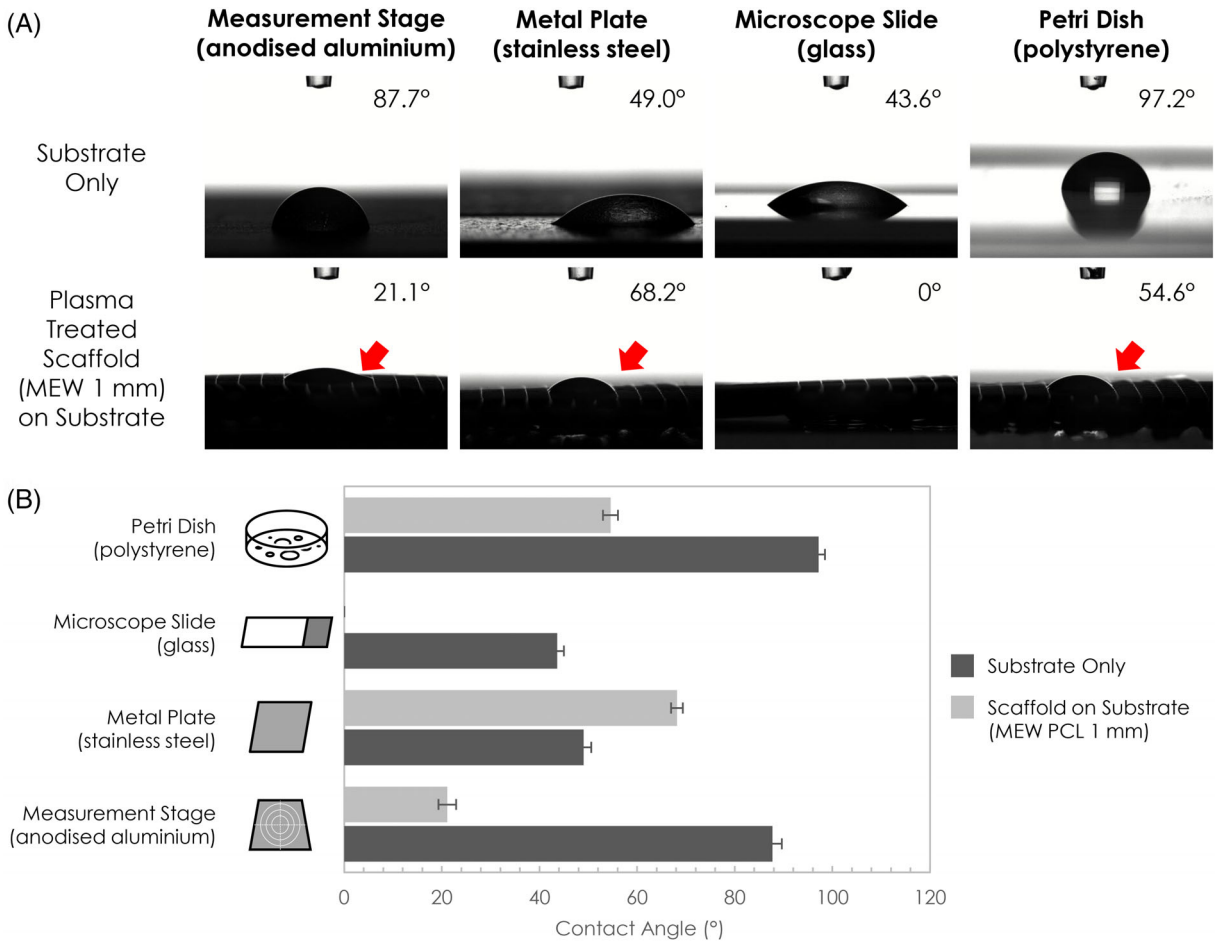



**FIGURE 4** (A) Still images from contact angle (CA) measurements of various substrates and plasma treated MEW PCL (1 mm) scaffolds measured while directly sitting on the substrate. Artifact water droplets are indicated with red arrows. (B) Contact angle measurements for each substrate alone as well as plasma treated scaffolds measured on the substrate. Values reported as average ± SD ($n = 10$)

scaffolds and other media, including for assessing suitability for successful cell seeding and cell attachment.[19–21] False-positive CA measurements may lead to over-application of surface treatments, such as NaOH etching time or concentration,[22] or plasma exposure time or energy, which are known to contribute to accelerated aging, degradation or other uncontrolled damage to the scaffolds and may limit their performance.[13]

This study does not address the challenge in measuring on rough or uneven surfaces, which are an intrinsic consequence of including porosity in a scaffold design. It was observed in this study that the alignment of the deposited droplet between or on top of scaffold microstructures, particularly repeating linear fibers prevalent in MEW scaffolds (Figure 4A), strongly influenced the likelihood of a droplet remaining on the top of the scaffold surface or being absorbed into the structure. Several mathematical approximations exist to correct for these geometric anomalies and their influence on CA measurements, which have been reported previously.[4,23] It is also recommended that where possible, the size of the water droplet is increased to a diameter significantly greater than the largest scaffold features (e.g., voids between fibers or polymer beads comprising the molded scaffolds) and a large number of repeats is required to achieve statistical power relevant to the investigation.

## 4 | CONCLUSIONS

This study has reported a novel, simple and low-cost modification to traditional sessile drop CA measurements to enable more widespread suitability for tissue engineering research. Using a 3D printed platform, several porous PCL and HDPE scaffolds having undergone plasma treatment to induce hydrophilicity were elevated above the measurement substrate to enable visualization of droplets interacting with the scaffolds, without being influenced by the substrate below. This technique was validated using both high (>90%) and low (<40%) porosity scaffolds to accurately characterize hydrophilic properties being induced on scaffold surfaces. Since complete wetting and hydrophilicity of porous scaffolds are so often required in tissue engineering research, this study provides a cheap, effective solution to enabling CA measurements to be performed accurately and reproducibly.

## AUTHOR CONTRIBUTIONS

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

## ACKNOWLEDGMENTS

The authors wish to acknowledge Mr Trent Brooks-Richards for his assistance 3D printing the sample platform. StarPore® scaffolds were kindly supplied by Mr Robert Thompson, Vice-President Product Innovation, Anatomics Pty Ltd, Melbourne, Australia. The data reported was obtained using the resources of the Central Analytical Research Facility within the Research Infrastructure Division, Queensland University of Technology, with funding from the Faculty of Engineering. Open access publishing facilitated by Queensland University of Technology, as part of the Wiley - Queensland University of Technology agreement via the Council of Australian University Librarians.

## DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the supplementary material of this article

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## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Paxton NC, Woodruff MA. Measuring contact angles on hydrophilic porous scaffolds by implementing a novel raised platform approach: A technical note. *Polym Adv Technol*. 2022;33(10):3759-3765. doi:10.1002/pat.5792