

THE EFFECT OF SURFACE HETEROGENEITY ON WETTABILITY OF POROUS THREE DIMENSIONAL (3-D) SCAFFOLDS OF POLY(3-HYDROXYBUTYRIC ACID) (PHB) AND POLY(3-HYDROXYBUTYRIC-CO-3-HYDROXYVALERIC ACID) (PHBV)

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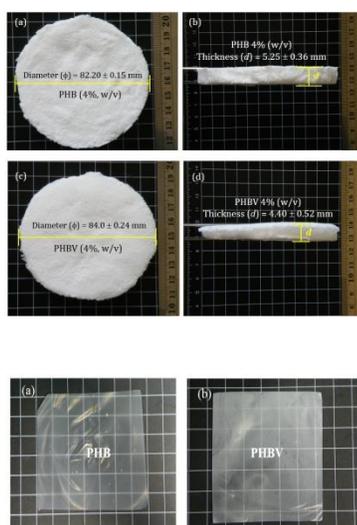
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Graphical abstract



Abstract

Biopolymers such as poly(3-hydroxybutyric acid) (PHB) and poly(3-hydroxybutyric-co-3-hydroxyvaleric acid) (PHBV) are preferred ingredients for the manufacture of materials especially in tissue engineering and regenerative medicine application because they are biocompatible, biodegradable and do not possess any environmental problems associated with their production, fabrication, use and disposal. For that reason, studies have been initiated to produce a 3-D biomimetic scaffolds with an improved thickness greater than 1 mm as an alternative material to synthetic polymers in tissue engineering application. The purpose of this study is to evaluate the physico-chemical surface properties of these polymeric 3-D structures and its corresponding thin films prior to be used as biomimetic materials. To measure the surface free energy (wettability), a Cassie-Baxter contact angle correction for heterogeneous wetting of two liquids (water and methylene iodide) were utilized. To verify its wettability, surface free energies were used to estimate the interfacial energy, work of adhesion and spreading coefficient. The results indicate that the calculated Cassie-Baxter contact angle correction method have proven to exhibit one dominant factor that cause a huge deviation from the true contact angles of its corresponding thin films which is surface heterogeneity. This material characteristic would possibly give a side effect (e.g., low cell attachment and proliferation) against cell-biomaterial affinity as highly hydrophobic material are most likely to be unfavorable for the absorption of essential extracellular matrix proteins (compounds that give a signal to cell to attach on the solid surface).

Keywords: PHB, PHBV, 3-D scaffolds, contact angle, surface free energy, hydrophobicity, hydrophilicity

Abstrak

Biopolimer seperti *poly(3-hydroxybutyric acid)* (PHB) dan *poly(3-hydroxybutyric-co-3-hydroxyvaleric acid)* (PHBV) adalah bahan-bahan yang lebih menjadi pilihan dalam pengeluaran material khasnya dalam bidang kejuruteraan tisu dan aplikasi perubatan regeneratif kerana ia bersifat bioserasi, biodegradasi dan tidak memberikan sebarang masalah terhadap alam sekitar dalam pengeluaran, fabrikasi, penggunaan dan pembuangannya. Atas alasan ini, kajian-kajian telah dijalankan untuk menghasilkan perancah/rangka biometrik 3-D dengan penambahbaikan daripada segi ketebalannya yang melebihi 1 mm sebagai suatu material alternatif kepada polimer sintetik dalam aplikasi kejuruteraan tisu. Objektif kajian ini adalah untuk menilai ciri-ciri fiziko-kimia permukaan struktur polimerik 3-D dan filem nipisnya sebelum ianya dapat digunakan sebagai suatu material biometrik. Bagi mengukur tenaga bebas permukaan (tahap kebolehasahan), pembetulan sudut kontak Cassie-Baxter untuk pembasahan secara heterogen kedua-dua cecair (air dan iodin metilena) telah digunakan. Bagi mengesahkan tahap kebolehasahannya, tenaga-tenaga bebas permukaan telah digunakan untuk mengagak tenaga antara fasial, tindakan pelekatan dan penyebaran koefisien. Hasil kajian menunjukkan bahawa kaedah pembetulan sudut kontak Cassie-Baxter yang dikira telah terbukti merupakan satu faktor dominan yang telah menyebabkan lencongan yang besar daripada sudut-sudut pertemuan sebenar daripada filem-filem nipisnya iaitu permukaan heterogen. Ciri material ini berkemungkinan akan memberikan kesan sampingan (contohnya, kekurangan pelekatan dan proliferasi sel) terhadap afiniti biomaterial-sel di mana material yang berhidrofobik tinggi adalah lebih berkemungkinan untuk tidak digemari bagi penyerapan protein-protein ekstraselular matrik penting (bahan-bahan yang memandu sel untuk melekat pada permukaan keras).

Kata Kunci: PHB, PHBV, perancah/rangka 3-D, sudut kontak, tenaga bebas permukaan, tahap hidrofobik, tahap hidrofilik

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1.0 INTRODUCTION

In tissue engineering, as in many other engineering fields, the design of the artificial structural may be as crucial as the material it is made of. Tissue engineering scaffold plays the fundamental role of promoting cell: adhesion, growth, proliferation, differentiation, and will help steer the course for tissue development [1]. The scaffolds, cells and signals are the main building blocks of tissue engineering. Moreover, scaffolds must provide the cells with the appropriate physical support (i.e., porosity, pores interconnectivity and pores size distribution), chemical signals (i.e., biosignaling components from ECM proteins) and mechanical strength to allow them to generate specific cells and tissues. Scaffold design and development is mainly an engineering challenge and is in fact one of the main goals of this study. In scaffold-based tissue engineering strategies, a key component of the scaffold that serves as a template for cells interactions and for the formation of the extracellular matrix (ECM) is providing structural support to the newly formed tissue. A temporary 3-D scaffold mimicking the physiological functions of the ECM is critical to preserve cells ability to differentiate into their native phenotypes and to constitute a structural template to fill the tissue lesion. Tissue engineering scaffolds are meant to be colonized by cells and should transmit the chemical and physical signal necessary to ensure adequate cells and tissues growth. An ideal tissue engineering scaffold should

fulfill a series of requirements [2, 3]. One of the important requirements is surface properties.

Scaffold surface properties such as morphology, hydrophilicity, surface energy and charge are factors controlling *in vitro* cell adhesion, migration, phenotype maintenance and intracellular signalling as well as *in vivo* cell recruitment and healing at the tissue-scaffold interface [4-6]. Cell response to the biomaterial is not mediated by a direct contact but rather through an interfacial layer formed on material surface once it is in contact with a physiological environment. Such a layer is created as result of non-specific adsorption of ECM proteins. Polymer-surface engineering is a useful tool to improve scaffold multifunctionality and to design biomimetic materials able to interact with the surrounding environment by biomolecular recognition [7]. Due to the rough nature of any fabricated polymeric 3-D scaffolds topography characteristics, studies of its physico-chemical are essential to determine wetting capability as most of the mammalian cells are responsive to high surface free energy calculated by means of surface properties analysis. For that reason, the aim of this study was to determine its hydrophobicity/hydrophilicity based on the surface free energy and spreading coefficient of both polymeric 3-D scaffolds with an improved thickness greater than 1 mm. In addition, validation was made to verify the hydrophobicity/hydrophilicity of both polymeric 3-D scaffolds and the corresponding solvent-cast thin films.

Real solid surfaces are usually rough and chemically heterogeneous. On such surfaces, the actual contact angle may vary from point to point. Several studies have proved that liquid fills up the roughness grooves (homogeneous wetting regime) and air bubbles are entrapped inside the grooves (heterogeneous wetting regime), underneath the liquid [8-10]. A rough hydrophobic surface was used as a basis for studying equilibrium wetting established many years ago by Wenzel and Cassie-Baxter (CB) [8]. The homogeneous wetting regime as mentioned earlier relates with Wenzel theory. It yields the Wenzel apparent contact angle, θ_w , Young contact angle, θ_Y and the roughness ratio, r can be expressed as follows:

$$\cos \theta_w = r \cos \theta \quad (1)$$

The roughness ratio is defined as the ratio of the true area of the solid surface to its nominal area. This ratio is determined using BET surface area analysis to produce true surface area and the nominal area is related to its geometrical shape area. Moreover, this equation shows that when the surface is hydrophobic ($\theta_Y > 90^\circ$), roughness does increase the contact angle to some extent. Meanwhile, the extensions of the Young's equation for rough substrates, known as the Wenzel and Cassie-Baxter equations, predict the so-called apparent contact angles. This CB equation describes the heterogeneous wetting regime and gives θ_{CB} , the CB apparent contact angle, as:

$$\cos \theta_{CB} = r_f f \cos \theta_Y + f - 1 \quad (2)$$

In this equation, f is the fraction of the projected area of the solid surface that is wet by the liquid, and r_f is the roughness ratio of the wet area. When $f = 1$, $r_f = r$ and the CB equation turns into the Wenzel equation (Equation 1) [8].

2.0 EXPERIMENTAL

2.1 Materials

Poly(3-hydroxybutyric acid) (PHB; $M_w = 300,000 \text{ g mol}^{-1}$; CAS No. 29435-48-1), poly(3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV; $M_w = 680,000 \text{ g mol}^{-1}$ with 12% (w/w) polyhydroxyvalerate (PHV) content; CAS No. 80181-31-3) and Alirazin Red S (Sigma-Aldrich™) were purchased from Sigma-Aldrich® (Dorset, United Kingdom). Chloroform 99.9% in purity (AnalaR®) was obtained from VWR International (Leicestershire, United Kingdom).

2.2 Preparation of the Porous 3-d Scaffolds

Solvent-casting particulate-leaching (SCPL) process was employed to produce porous 3-D scaffolds with an improved thickness greater than 1 mm. Polymer concentrations of 4% (w/v) for PHB and PHBV were prepared in 60 ml of boiled reflux chloroform (99.9%

purity) at 60°C. Once cooled, the polymer solutions were added evenly over an aluminum foil-coated glass Petri-dish. Sodium chloride crystals were then poured evenly and mixed into the polymer solutions with continuous stirring until the polymer-solvent solution became pasty, thick and packed. Subsequently, the packed-in pasty were put immediately inside a lyophilization flask (to avoid the etching effect) and air-dried for 2 days for complete solvent evaporation [11]. The dried-casting polymers were peeled carefully from the aluminum foil and then dialyzed with 10 liters of cold deionized water (DIW) at 21°C for each polymer for 2 days to remove all sodium chloride crystals. Later, they were frozen at -70°C for 3 hrs and then transferred into a cryostat bath containing ethylene glycol (-15°C) to lyophilize the remaining deionized water and chloroform via the sublimation process. Scaffolds were then cut into cuboids with an approximate size of 10 mm x 10 mm x 5 mm prior to the surface roughness, BET surface area, water contact angle and surface free energy analysis.

2.3 Fabrication of solvent-cast thin film

PHB and PHBV thin films were prepared and used to compare its wetting behaviour with porous 3-D scaffolds. A solvent-cast film was made by pouring a polymer solution of 4% (w/v) into a casting block on a clean polypropylene (PP) sheet with the size of 30 cm x 30 cm. Then, the casting block was immediately pushed upwards towards the end of the sheet so that the polymer solution can be smeared evenly. The sheet coated polymeric thin film was left overnight in a fume cupboard at $20 \pm 1^\circ\text{C}$ for complete solvent evaporation prior to the wetting analysis.

2.4 Surface Roughness

The surface roughness of 3-D scaffolds was calculated based on Wenzel's theory [12]. The surface roughness factor (r) is defined as the ratio of the area of the actual surface (measured using BET surface area) to that of a smooth surface having the same geometric shape and dimensions. The equations of roughness factor (r) and its geometric surface area of porous 3-D scaffold (cylindrical shape: Figure 1) are expressed as follows:

$$\text{Roughness factor } (r) = \frac{\text{True surface area of porous 3-D scaffolds}}{\text{Geometric surface area of porous 3-D scaffolds}} \quad (3)$$

$$\text{The geometric surface area of porous 3-D scaffold (cylindrical shape)} = 2(\pi)(r)^2 + 2(\pi)(r)(d) \quad (4)$$

where r is the radius of the 3-D scaffolds, d is the diameter and π is the constant pi (3.14). True surface area of the porous 3-D scaffolds was determined using BET surface area analyzer. Assumptions were made prior to the evaluations which are as follows: 2) the efficiency of salt-leaching process was 100% and 3)

there was no loss of polymer materials throughout the fabrication and salt-leaching process (2.4 g = 4% (w/v) PHB and PHBV).

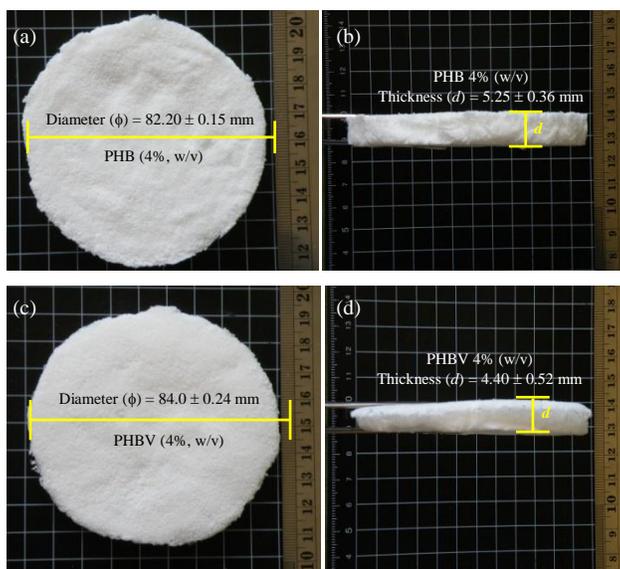


Figure 1 Morphology of the polymeric porous 3-D scaffolds in a cylindrical shape prepared using the solvent-casting particulate-leaching (SCPL) technique [11]. They were later cut into a rectangular shape with an approximate size of 5 mm × 10 mm × 10 mm: (a) Aerial view of the PHB 4% (w/v) porous 3-D scaffold; (b) Side view of the PHB 4% (w/v) porous 3-D scaffold with the thickness of 5.25 ± 0.36 mm; (c) Aerial view of the PHBV 4% (w/v) porous 3-D scaffold with the thickness of 4.40 ± 0.52 mm

2.5 BET Surface Area

Surface area analyzer gas absorption BET (ASAP® 2010; Micromeritics® Instrument Corporation, United States of America) was used to determine the surface area of the polymeric porous 3-D scaffolds using nitrogen adsorption isotherms at 77 K [13, 14]. Surface contaminants were removed prior to the gas sorption experiments by degassing gradually the whole chamber filled with polymeric porous 3-D scaffolds pieces. Under vacuum condition, the samples were dried inside the measuring cell at temperature below their glass-transition (T_g) temperature overnight. For the analysis part, nitrogen (the adsorbate) was admitted into the evacuated sample chamber. The theoretical and standard procedures for the measurements performed are reported and thoroughly explained by Li and Favis (2001) [15].

2.6 Water Contact Angle and Surface Free Energy

Static water contact angle (θ_{H_2O}) on the air surface of PHB and PHBV (4%, w/v) porous 3-D scaffolds and the corresponding solvent-cast thin films (Figure 2) were measured using a drop shape analyzer (DSA30E, KRÜSS GmbH, Germany) [16-18]. Re-distilled water of approximately 10 μ l was gently plated on the vertical cross section and on top of the surfaces

of the porous 3-D scaffold and thin film respectively ($n = 10$). All measurements were conducted at 20 ± 1 °C. To determine surface free energy (SFE) of the solvent-cast thin films (γ_s), Fowkes equation was chosen as the equation of state (EOS). The surface free energy was determined by contact angles of two types of reference liquids which are water (H_2O) and di-iodomethane (CH_2I_2) [19]. In addition, several calculations were made to verify the surface hydrophilicity/hydrophobicity of the thin films by means of the surface free energy (γ_{SL}), the work of adhesive (W_{SL}) and spreading coefficient at solid-liquid interfaces (Equation 8) [20]. All calculations were based on Young (Equation 6), Dupree (Equation 7) and Fowkes equations (Equation 5) and expressed as follows:

$$\gamma_L(1 + \cos \theta) = 2[(\gamma_L^d \gamma_s^d)^{1/2} + (\gamma_L^p \gamma_s^p)^{1/2}] \quad (5)$$

$$(\cos \theta)\gamma_L = \gamma_s - \gamma \quad (6)$$

$$W_{SL} = \Delta G = E_{adh} = \gamma_L + \gamma_s - \gamma_{LS} \quad (7)$$

$$(S_{H_2O/thin\ film}) = W_{SL} - W_{LL} \quad (W_{LL} = \Delta G = 2 \times \gamma_L = 72.8 \times 2 = 145.6\ mN/m) \quad (8)$$

where γ_L^d and γ_L^p are the dispersive component, and polar component, respectively; θ is the contact angles to water and to di-iodomethane (CH_2I_2),

respectively. Solid surface free energy (γ_s) is equal to solid-gas interface energy (γ_{SG}). The summarized adhesion parameters for the test liquids are shown in Table 1.

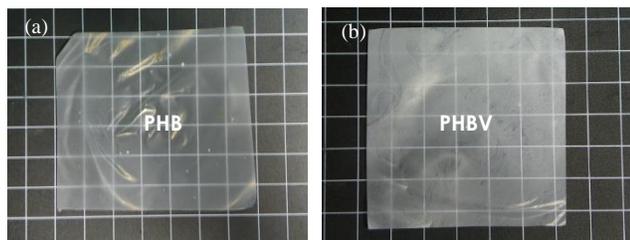


Figure 2 Morphology of solvent-cast thin polymeric films: (a) Aerial view of PHB (4%, w/v) and (b) Aerial view of PHBV (4%, w/v)

Table 1 Adhesion parameters for the test liquids [19]

Test liquid	γ_L (mN/m) = $\gamma^{dL} + \gamma^{pL}$	γ^{dL} (mN/m)	γ^{pL} (mN/m)
Water (H ₂ O)	72.8	21.8	51.0
Di-iodomethane (CH ₂ I ₂)	50.8	50.8	0

2.7 Statistical Analysis

Data was presented as means \pm standard deviation (SD) of mean values. Statistical comparisons were performed using Students t- t-test (PASW version 17.0 IBM Co.). A $p < 0.05$ was considered significant.

3.0 RESULTS AND DISCUSSION

Table 2 and Table 3 show surface free energy (SFE) of PHB and PHBV thin films in which correspond to its porous 3-D scaffolds. SFE of porous 3-D scaffolds were not determined as it produced inaccurate contact angle values due to severe hysteresis (effect of surface topography and roughness). For that reason, only thin films of PHB and PHBV were used to determine the surface free energy (SFE). Determination of surface free energy at the interfacial (SL) was based on the Young equation (Equation 6). The results show that PHBV produced fewer amount of surface energy as compared to PHB ($p < 0.05$) to indicate high in hydrophobic properties. To verify the results, a Dupree equation was used to determine the work of adhesive at interfacial (E_{adh}) and spreading coefficient of water over thin films ($S_{H_2O/thin\ film}$). The results in Table 3 show that PHBV produced fewer amount of (+) energy and (-) spreading coefficient as compared to PHB ($p < 0.05$).

These results clearly verified that PHB has a higher hydrophilic properties as compared to PHBV ($p < 0.05$). However, both polymers were considered as non-wetting materials as they produced (-) spreading coefficient during the analysis. In this verification measurement, the signage of (+) represent a non-

spontaneous process has occurred. This can be explained by the fact that an external energy is needed (e.g., surface treatment) to overcome the strong water intermolecular cohesive forces (non-wetting behavior) with the intention of wetting the polymer surface. Meanwhile, the (-) spreading coefficient means water will not spread over the thin film surface. If $W_{SL} > W_{LL}$, the intermolecular interactions at SL interface (e.g., hydrogen bonding (polar component), dipole-dipole (dispersive component) and Van der Waal (dispersive component)) are considered greater than cohesive forces of water. This will eventually promote spreading of water (wetting) on the solid surface. However, If $W_{SL} < W_{LL}$, no wetting will be occurred on the surface.

Table 2 Surface free energy (SFE) of PHB and PHBV

Sample	θ_{H_2O} (deg.)	$\theta_{CH_2I_2}$ (deg.)	Dispersive comp. (γ^{d_s}); mN/m	Polar comp. (γ^{p_s}); mN/m	Surface energy ($\gamma_s = \gamma^{p_s} + \gamma^{d_s}$); mN/m
Thin films (4% w/v)					
PHB	67.80 ± 1.2	42.80 ± 0.5	38.08 \pm 0.2	8.81 \pm 1.3	46.89 \pm 1.0
PHBV	75.10 $\pm 1.6^*$	45.80 ± 0.2	36.58 \pm 0.1*	6.02 \pm 0.8*	42.60 \pm 0.5*

*($p < 0.05$) - Results were considered statistically significant ($n = 4$) as compared with PHB. Example calculation for PHB: (1) Test liquid of CH₂I₂ = $(50.8)(1 + \cos 42.80) = 2[(50.8 \times \gamma^{d_s})^{1/2} + (0 \times \gamma^{p_s})^{1/2}]$, $(50.8)(1.73) = (14.24)(\gamma^{d_s})^{1/2} = 87.88/14.24 = (\gamma^{d_s})^{1/2}$, $\gamma^{d_s} = (6.17)^2 = 38.08$ mN/m. (2) Test liquid H₂O = $(72.8)(1 + \cos 67.80) = 2[(21.8 \times \gamma^{d_s})^{1/2} + (51.0 \times \gamma^{p_s})^{1/2}]$, $(72.8)(1.38) = 2[(21.8)(38.08)^{1/2} + 2[(51.0 \times \gamma^{p_s})^{1/2}]$, $100 = 57.62 + [(14.28)(\gamma^{p_s})^{1/2}]$, $100 - 57.62 = [(14.28)(\gamma^{p_s})^{1/2}]$, $42.38/14.28 = (\gamma^{p_s})^{1/2}$, $\gamma^{p_s} = (2.97)^2 = 8.81$ mN/m. (3) SFE of PHB ($\gamma_s = \gamma^{p_s} + \gamma^{d_s}$) = $8.81 + 38.08 = 46.89$ mN/m.

Table 3 Verification of hydrophobicity/hydrophilicity properties of polymeric thin films

Sample	θ_{H_2O} (deg.)	Interfacial (γ_{SL}); mN/m	Work of adhesive (W_{SL}); mN/m	($S_{H_2O/thin\ film}$) = $W_{SL} - W_{LL}$; mN/m
Thin films (4% w/v)				
PHB	67.80 ± 1.2	19.38 \pm 0.6	+100.31 \pm 1.1	-45.49 \pm 1.3
PHBV	75.10 $\pm 1.6^*$	23.88 $\pm 0.2^*$	+91.52 \pm 0.5*	-54.28 \pm 0.7*

*($p < 0.05$) - Results were considered statistically significant ($n = 4$) as compared with PHB.

The above mentioned condition was essential in tissue engineering application as favorability of any cells on biomaterials is highly depending on the interfacial surface energy between both different condition (surface material and cell growth media/blood plasma). Too high of interfacial energy signify fewer amount of energy available on the solid surface and vice versa. According to Blanco *et al.* (2009) some of the polymers (e.g., hydrophobic polymers with high molecular -C-C- chains) possess

high in interfacial free energy in aqueous solution that in fact tend to unfavorably influence cells and tissues during the initial stages of cell-biomaterial [21]. However, not all polymer materials have high interfacial free energy (γ_{SL}). As for PHB/PHBV, the surface free energy at the solid surface was considered much higher than at the interface ($\gamma_S > \gamma_{SL}$). For that reason, both polymeric 3-D scaffolds might give a good affinity between biomaterial and cell. For instance, the observed response of acute myeloid leukemia (AML) cell lines had a fairly good attachment and proliferation up to 14 days on the poly(L-lactic-co-glycolic acid) (PLGA) and polyurethane (PU) porous 3-D scaffolds [21].

In general, hydrophilic surfaces has more than 100 mN/m surface free energy or at least more than the water surface tension 72.8 mN/m ($\gamma_S > \gamma_{SL}$: SFE at solid surface (s) is always higher than SFE at interfacial solid-liquid (SL)). As for the hydrophobic surface which is less than 100 mN/m, the total energy on the surface could be either be $\gamma_S > \gamma_{SL}$ or $\gamma_S < \gamma_{SL}$. In the case of $\gamma_S > \gamma_{SL}$ like PHB and PHBV, both polymeric surfaces are considered to have the ability to be wet by water owing to the SFE at solid surface is greater than SFE at the interfacial SL. In fact, this will affect the adsorption of the proteins whereby the proteins will rearrange its molecular structure to adsorb on top of the hydrophobic surface and later the cell attachment via ligand-receptor interactions [22]. However, if $\gamma_S < \gamma_{SL}$ (as observed on the other highly hydrophobic synthetic polymers (e.g., PS, PE, PMMA, PLA & etc.), less protein adsorption will occur during the initial contact of polymeric materials with the serum-supplemented solution. This is due to the less solid surface energy available to spread the liquid containing proteins on the pore surfaces. This will also affect liquid penetrability across a thick porous 3-D scaffold. This condition will trigger some of the cell to attach on the solid surface unfavorably as no bio-signaling compounds (extracellular matrix proteins) available on top of the solid surface [23]. Subsequently, the clustered cell will die due to starvation as cell growth media could not penetrate deep inside the porous 3-D scaffold.

3.1 Cassie-Baxter Contact Angle Corrections for Heterogeneous Wetting

A heterogeneous wetting condition of Cassie-Baxter regime was selected in describing the wetting condition of the porous material and predicting the corrected apparent contact angle. The results show that both polymeric porous 3-D scaffolds were observed to amplify the theoretical/corrected apparent water contact angle (θ_{apparent}) ranging from approximately 28° to 49° ($\theta_{\text{porous}} - \theta_{\text{apparent}}$) (Table 4). These theoretical/corrected apparent water contact angles (θ_{apparent}) were considered close enough to the solvent-cast thin films ($p > 0.05$: applicable only for PHB) to indicate that the Cassie-Baxter regime was quite relevant in explaining the highly observed surface hydrophobicity. Meanwhile,

the corrected apparent contact angles of PHBV 3-D scaffold produced lower value ($p < 0.05$) as compared to its corresponding thin film. This could possibly due to the unknown availability of surface roughness on PHBV thin film that has not been measured throughout the experimental works. However, both polymeric porous 3-D scaffolds and the corresponding thin films were considered as hydrophobic materials as they have a high degree of water contact angle of more than 65° [9].

Table 4 True contact angle of PHB and PHBV porous 3-D scaffolds

Surface physico-chemistry	Polymeric porous 3-D scaffolds		Control (Thin films)[a][b][c]	
	PHB (4%, w/v)	PHBV (4%, w/v)	PHB (4%, w/v)	PHBV (4%, w/v)
Surface roughness ratio, r	1.41 ± 0.02	1.62 ± 0.03*	N/A	N/A
Fraction of fluid contact area, f [f]	0.52 ± 0.04	0.34 ± 0.02*	N/A	N/A
Contact angle, θ_{apparent} (deg.)	65.79 ± 1.3	67.55 ± 1.1	67.80 ± 1.2	75.10 ± 1.6*
True contact angle, θ_{porous} (deg.)[d][e]	93.95 ± 0.8	117.20 ± 1.8*	N/A	N/A

* ($p < 0.05$) - Results were considered statistically significant ($n = 10$) as compared with PHB. [a] Fabricated by using the solvent casting. [b] Equilibrium contact angle of solvent-cast thin films on polypropylene sheet ($n = 10$). [c] Contact angle of polypropylene (PP) sheet without PHB and PHBV coating = $92.43 \pm 0.3^\circ$ [d] Cassie-Baxter regime for the heterogeneous wetting: $\cos\theta_{\text{porous}} = [r_f \cdot f \cdot \cos\theta_{\text{apparent}}] + f - 1$ (roughness ratio of the wet area, r_f was assumed to be as an average roughness ratio, r of polymeric porous 3-D scaffolds). [e] Initial true contact angle of redistilled water droplet resting on the porous 3-D scaffolds ($n = 10$). [f] Fraction of fluid area in contact with the material surface (f) - Random pores area were selected based on the SEM ($n = 3$) - All pores were assumed to be in a spherical form. ($f = 1 - f_{\text{air}}$). Example calculation: PHB: $\cos\theta_{\text{porous}} = r_f \cdot f \cdot \cos\theta_{\text{apparent}} + f - 1 = \cos 93.95 = [(1.41)(0.52)(\cos\theta)] + 0.52 - 1 - 0.07 = [0.733\cos\theta] - 0.48, -0.07 + 0.48 = [0.733\cos\theta], \cos\theta = 0.41, \theta = \cos^{-1}[0.41] = 65.79^\circ$; PHBV: $\cos\theta_{\text{porous}} = r_f \cdot f \cdot \cos\theta_{\text{apparent}} + f - 1 = \cos 117.20 = [(1.62)(0.34)(\cos\theta)] + 0.34 - 1 - 0.45 = [0.55\cos\theta] - 0.66, -0.45 + 0.66 = [0.55\cos\theta], \cos\theta = 0.38, \theta = \cos^{-1}[0.38] = 67.55^\circ$.

Thus, the highly observed surface hydrophobicity of the polymeric porous 3-D scaffolds in comparison with that of solvent-cast thin films (in accordance with the Cassie-Baxter regime) was probably due to the surface heterogeneity (e.g., availability of the surface roughness (Table 5) which could lead to hysteresis), air trapped inside the pore grooves (enhances the hydrophobicity because the drop is then partially sitting on air) and contaminants of salt on the rough surfaces (which could lead to a decrease of solid/vapor surface tension) [24].

Table 5 Surface roughness ratio (*r*) of polymeric porous 3-D scaffolds

Physical properties	Polymeric porous 3-D scaffolds	
	PHB (4%, w/v)	PHBV (4%, w/v)
Surface roughness ratio, <i>r</i>	1.41 ± 0.02	1.62 ± 0.03*
BET surface area, <i>A_s</i> , m ² /g	0.70 ± 0.02	0.82 ± 0.03*

*(*p*<0.05) - Results were considered statistically significant (*n* = 4) as compared with PHB

4.0 CONCLUSION

In this study, we produced an improved thickness greater than 1 mm 3-D porous scaffolds made from PHB and PHBV. To determine the corrected apparent contact angles of the porous 3-D scaffolds, the corresponding polymeric thin films were employed. A Cassie-Baxter contact angle correction for heterogeneous wetting procedure was used to characterize its surface properties (hydrophobicity/hydrophilicity). Moreover, the surface energies of these biodegradable polyesters in thin films were determined from the contact angles of water and methylene iodide. The results were then used to estimate the interfacial energy, work of adhesion and spreading coefficient. The results reported in this study demonstrated a highly surface hydrophobicity of PHBV in both thin film and 3-D porous structures as compared to PHB (*p*<0.05). The results were verified by the estimation of work of adhesion (+/-) and spreading coefficient (+/-) as both polymers were truly a hydrophobic material irrespective of its dimension. Furthermore, a Cassie-Baxter contact angle correction method was best to explain the dominant factors that might cause such deviation from the true contact angles of its corresponding thin films. As such, surface heterogeneity, air trapped inside the pore grooves and contaminants of salt on the rough surfaces during scaffold fabrication process (further verification is needed) were resorted here to explain such phenomenon. The negative effects (e.g., low cell attachment and proliferation) against cell-biomaterial affinity on this highly hydrophobic material are inevitable as most of the extracellular matrix proteins (compounds that give signal to cell to attach) would opt to absorb on moderate surface hydrophobic rather than on high surfaces.

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