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POLYHYDROXYALKANOATES (PHAs) FOR TISSUE ENGINEERING APPLICATIONS: BIOTRANSFORMATION OF PALM OIL MILL EFFLUENT (POME) TO VALUE-ADDED POLYMERS

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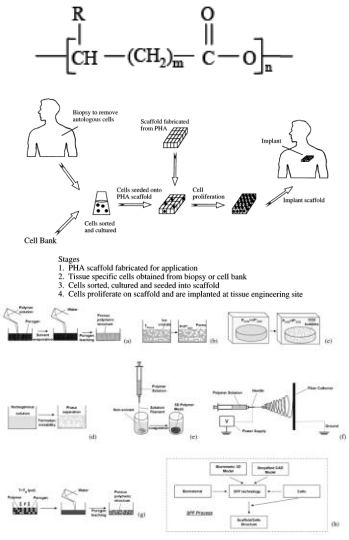
Graphical abstract



Abstract

The study of cancer cell has been hindered by the lack of appropriate *ex vivo* models, which can mimic this microenvironment. It is hypothesized that the fabrication of porous 3-D scaffolds for the biomimetics growth of cancer cells *ex vivo* could facilitate the study of the disease in its native 3-D niche. For that reason, biomaterials are used for fabrication of 3-D scaffold, in general, may be natural polymers such as proteins, collagens and gelatin, or synthetic biopolymers. Among the various available biodegradable polymers, polyhydroxyalkanoates (PHAs) have gained significant interest as one of the value-added materials which can be synthesized from abundantly available source of palm oil mill effluent (POME). Down the group of the PHA, poly-3-hydroxybutyrate (PHB) and copolymerizing this PHB that produced PHBVs; these two polymers have the most prevalent polymer used for scaffolds fabrication. A physico-chemical and biological modification has developed to improve wetting, adhesion, and printing of polymer surfaces, generally by introducing a variety of polar groups. These techniques must be tailored to introduce a specific functional group when the surface modification is a precursor to attach a bioactive compound. There are a few methods in order to fabricate porous 3-D scaffolds such as solvent casting, particulate leaching, thermally induced phase separation, gas forming, fiber bonding, electrospinning and also solid free form method. A review of the polyhydroxyalkanoates (PHAs) for tissue engineering applications is presented, beginning with the basic naturally derived polymerization of PHAs, biotransformation of palm oil mill effluent (POME) to the value-added polymers, novel methods of scaffold fabrication capabilities and its physico-chemical and biological surface modifications to increase cell-biomaterial affinity.

Keywords: Biomaterials, polyhydroxyalkanoates (PHAs), palm oil mill effluent (POME), 3-D scaffold, surface modifications, cell-biomaterial affinity

Abstrak

Kekurangan model ex *vivo* yang boleh meniru persekitaran sebenar sel telah menghadkan kajian keatas sel kanser. Fabrikasi struktur perancah berliang 3-D untuk pertumbuhan sel kanser biomimetik secara ex *vivo* boleh memudahkan kajian keatas penyakit yang khusus secara 3-D. Oleh yang demikian, biobahan yang digunakan untuk fabrikasi struktur perancah 3-D, secara umum, mungkin daripada jenis polimer semula jadi seperti protein, kolagen dan gelatin, atau biopolimer sintetik. Antara pelbagai polimer terbiodegradasi yang tersedia ada, polimer daripada keluarga *polyhydroxyalkanoates* (PHAs) telah menarik perhatian sebagai salah satu bahan tambah nilai yang boleh disentisiskan daripada sumber yang didapati dengan banyaknya iaitu effluen kilang kelapa sawit (POME). Dalam kumpulan polimer PHA, *poly-3-hydroxybutyrate* (PHB) dan pengkopolimeran PHB yang dihasilkan PHBVs; kedua-dua polimer ini mempunyai potensi sebagai bahan utama dalam fabrikasi struktur perancah. Pengubahsuaian fiziko-kimia dan biologi telah dibangunkan untuk meningkatkan sifat pembasahan, lekatan, dan percetakan permukaan polimer, secara amnya dengan memperkenalkan pelbagai kumpulan kutub. Terdapat beberapa kaedah untuk fabrikasi struktur perancah berliang 3-D seperti acuan pelarut dan larut lesap zarah, pemisahan fasa haba teraruh, pembentukan gas, ikatan fiber, electrospinning dan juga kaedah pepejal bebas. Kajian semula terhadap *polyhydroxyalkanoates* (PHAs) bagi aplikasi kejuruteraan tisu dibentangkan, bermula dengan pempolimeran asas semula jadi yang diperolehi daripada PHAs, biotransformasi effluen kilang kelapa sawit (POME) kepada nilai tambah polimer, kaedah fabrikasi struktur perancah dan seterusnya pengubahsuaian fiziko-kimia dan biologi permukaan bagi meningkatkan affiniti sel terhadap biobahan.

Kata kunci: Biobahan, *polyhydroxyalkanoates* (PHAs), effluen kilang kelapa sawit (POME), struktur perancah 3-D, modifikasi permukaan, affiniti sel-biobahan

1.0 INTRODUCTION

Tissue engineering has emerged as a multi-disciplinary field combining biology, materials science and surgical reconstruction in order to provide living tissue products that restore, maintain or improve tissue functionality [1, 2]. Due to out of a lack of donor organs and tissues, the need for this approach has been a primary option recently. To be more specific, biomaterials (any matter, surface or construct that interacts with biological systems) have gained significant importance in the field of tissue engineering. Often these biomaterials (derived either from nature or chemically synthesized) are used for tissue regeneration when the surrounding defective tissue exhibits the natural potential for tissue regeneration. However, in situations where the tissue lacks this ability of regeneration, relevant cells as well as growth factors have been used to accelerate tissue regeneration. In addition, these biomaterials have also been combined with drugs and used as drug delivery systems, thereby reducing the microbial infections while maximizing tissue regeneration [3]. In general, there are three distinct approaches currently being used in the tissue engineering and regenerative medicine applications.

These are (1) infusion of isolated autologous cells or cell substitutes, (2) implantation of tissue-inducing acellular (no containing cells) scaffold materials *in vivo* (e.g., natural polymers and inorganic materials) by allowing the patient's cells to repair the tissue guided by the scaffold and (3) implantation of expanded *in vitro* autologous cells seeded in scaffolds [2].

1.1 Biomaterials for Tissue Engineering Applications

A biomaterial is a "material intended to interface with biological systems to evaluate, treat, augment or replace any tissue, organ or function of the body" [4]. Over the past fifty years, biocompatible materials (biomaterials) have been used widespread in biomedical and tissue engineering applications and can now be considered "third-generation biomaterials" [5]. Initially, biomaterials were chosen because of their biological inertness, the goal was to minimize the body's immune response to the foreign material (i.e., metallic implants were used for skeletal injuries) [6]. Though this goal is still valid today, scientists have come to understand that complete biological inertness is a synonym to non-recognition by the body. This lack of biological recognition is often accompanied by fibrous tissue encapsulation and chronic inflammation which in turn compromise the mechanical performance and long-termbiocompatibility of the prosthesis. Thus, second-generation biomaterials were developed seeking to tailor or enhance biological recognition in an attempt to improve the biomaterial-body interface.

Second generation biomaterials used bioactive components that were specifically designed for use within the human body and promoted specific responses by the surrounding tissues, such as synthetic hydroxyapatite and Bioglass®. Both were used as porous scaffolds, coatings or powders, and by the mid-1980s these new bioactive materials had attained clinical use for various dental and orthopaedic applications. The biomaterial-body interface problem was also addressed by exploiting resorbable materials and thus could eliminate such problem. For that reason, another class of second-generation biomaterials was introduced which is resorbable polymers. Resorbable polymers are the main example of these resorbable materials, namely polylactic, polyglycolic acid and polyhydroxyalkanoates which decompose hydrolytically into biological accepted molecules, H_2O and CO_2. They are used as sutures, screws in orthopaedics and in controlled-release drug-delivery systems. In these devices, cytostatic agents, cytotoxic agents, antithrombotic agents and/or anti-inflammatory agents are incorporated within resorbable polymeric matrices [6]. Therefore, the creation of third-generation biomaterials is to promote or inhibit specific cell activities.

Currently, biomaterial research efforts involve the development of materials that promote an 'appropriate host response for a given application' [7]. For example, artificial tissues being fabricated by placing cells within scaffold materials which help guide cell proliferation and differentiation. The polymers used in biomedical and tissue engineering scaffold fabrication can be divided into broad categories of synthetic, biodegradable synthetic and naturally derived polymers (biopolymers) [8], with a middle ground of semi-synthetic materials rapidly emerging [9]. Most of the biocompatible materials commonly in use in tissue engineering are adapted from other surgical implantation/uses, such as sutures, haemostatic agents and wound dressings [10].

Meanwhile, naturally derived polymers (biopolymers) can be of both plant and animal origin. Lignocellulosic (biofibres), sodium alginate or natural rubber are those example of polymer derived from plant, whereas collagen, chitosan or gelatine are those derived from animal. Natural polymers offer the advantage of biological recognition, which reduces problems such as antigenicity, cytotoxicity and indiscriminate protein adsorption. The disadvantages of using the natural polymers are they often require chemical or physical pre-treatment to enhance their material properties so that their resistance to enzymatic or chemical degradation can be increased. These treatments however may have the toxic effects and affect cell growth (i.e., cross-linking withglutaraldehyde). Moreover, natural polymers may also include pathogenic impurities, variations in batch [11] and in general offer low reproducibility.

Biodegradable synthetic polymers, on the other hand, offer high reproducibility and the possibility of large-scale production, as well as controlled mechanical and biodegradability properties [8]. They lack, however, biological activity (cell-biomaterials recognition) and may be very hydrophobic. Despite of that, this type of polymer do offer an advantage over naturally derived polymers in that they can be tailored to give a wide range of properties and which are more predictable [12]. In particular, many investigations have concentrated on synthetic biodegradable polymers that are already approved by the food and drug administration (FDA). The most common biodegradable synthetic polymers being used or studied include polyhydroxyalkanoates (PHAs), polylactic acid (PLA), polyglycolic acid (PGA), polyanhydrides, polyfumarates (PF), polyorthoesters, polycaprolactones (PCL) and polycarbonates (PC) [13].

2.0 POLYMER OF MICROBIAL ORIGIN POLYHYDROXYALKANOATES (PHAS)

Polyhydroxyalkanoates (PHAs) are a class of natural polyesters that are produced by numerous organisms as an internal carbon and energy storage, as part of their survival mechanism [14]. Poly(β-hydroxybutyrate) (PHB), synthesized from *Bacillus megaterium*, was first mentioned in the scientific literature in 1925 by the French scientist Lemoigne [15]. He reported this bacterium can literally accumulate an intracellular homopolymer that consisted of 3-hydroxybutyric acids which were linked through ester bonds between the 3-hydroxyl group and the carboxylic group of the next monomer. Bacterially synthesized polyhydroxyalkanoates (PHAs) have attracted much attention because they can be produced from a variety of renewable resources, truly biodegradable and highly biocompatible thermoplastic materials [15]. Although a great variety of materials of this family can be produced, the use of PHAs in tissue engineering has been mainly restricted to PHB and poly(hydroxybutyrate-co-valerate) (PHBV) [1, 16]. To date, only several PHA (Figure 1) including poly(3-hydroxybutyrate) (PHB), copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV), poly(4-hydroxybutyrate) (P4HB), copolymers of 3-hydroxybutyrate and 3-hydroxyhexanoate (PHBHHx) and poly(3-hydroxyoctanoate) (PHO) are available in sufficient quantity for application research [17].

Since the discovery of PHB more than 90 genera of archae (extremophiles: halophiles (salt lovers), thermophiles (heat lovers), and acidophiles (acid lovers)) and eubacteria ($gram^+$ and $gram^-$) have been detected in aerobic and anaerobic habitats, they are able to produce PHAs in wide range of molecular weight [18]. Today, PHAs are separated into three classes: short chain length PHA (scl-PHA, C_3 to C_5), medium chain length PHA (mcl-PHA, C_6 to C_{14}), and long chain length PHA (lcl-PHA, > C_{14}) [19].

Research over the past 20 years focused on the substrate specificity of PHA polymerases. It was found that the supply of cells with a particular fatty

acid is frequently reflected in the monomeric composition of the PHA. To date, more than 100 different monomers have been reported as PHA constituents [20, 21] but only few of these PHAs were produced in large quantities [22]. As a consequence little is known about the chemical and mechanical properties of the polymers. To date, PHA monomers with straight, branched, saturated, unsaturated, and also aromatic monomers were found. Of special interest are functionalized groups in the side chain that allow further chemical modification, e.g., halogens, carboxyl, hydroxyl, epoxy, phenoxy, cyanophenoxy, nitrophenoxy, thiophenoxy and methylester groups [21, 23]. The length of the side chain (R) and its functional group considerably influence the properties of the bioplastic, e.g., melting point, glass transition temperature and crystallinity (stiffness/flexibility). Also the average molecular weight and the molecular weight distribution are dependent on the carbon source [21].

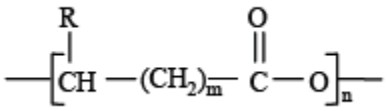


Figure 1 General molecular structures of polyhydroxyalkanoates. m = 1, 2, 3, yet m = 1 is most common; n can range from 100 to several thousands. R is variable (side chain). When m = 1, R = CH_3, the monomer structure is 3-hydroxybutyrate, while m = 1 and R = C_3H_7, it is a 3-hydroxyhexanoate monomer [16]

2.1 Sustainable Production of Polyhydroxylalknoates (PHAs)

Malaysia is one of the world leaders in the production and export of crude palm oil. In Malaysia, the oil palm industry has contributed vastly towards the country's economic well-being. During the economic crisis in the late 1990s, the industry helped to cushion the impact of the economic downturn through its export-oriented activities which provided the much needed foreign exchange for the country. Crude palm oil (CPO) production has increased from only 1.3 million tonnes in 1975, to 4.1 million tonnes in 1985 and 7.8 million tonnes in 1995 to 17.56 million tonnes in 2009 [24]

The Malaysian palm oil industry has created various products as a consequence of the cultivation of oil palm and the production of the main product (palm oil) and secondary products (palm kernel oil and cake). The oil palm products are employed in numerous food and non-food applications [25]. In 2009, there were 418 crude palm oil mills, 59 refineries, 57 downstream industries and 18 oleochemical plants. The Malaysian Palm Oil Board's long-term programme is to establish a biodiesel plant that will produce methyl ester (biodiesel) which can be used to replace petroleum diesel. There were 28 biodiesel plants with a production of 2.7 million tonnes per year methyl ester, respectively [24]. Another potential revenue generator is to convert the large quantity of biomass (13.2 million tonnes dry weight) into added value products [26]. However, this important economic activity generates an enormous amount of liquid effluent from the milling processes [27]. Palm oil mills with the wet milling (grinding) process accounted for the major production of waste [28]. Hence, the increase in number of mills will generate more environmental problems.

In general, the palm oil milling process can be categorized into a dry and a wet (standard) process. The wet process of palm oil milling is the most common and typical way of extracting palm oil, especially in Malaysia. According to the industrial standard, the milling process produces wastewater in the range 0.44 - 1.18 m^3/tonne fresh fruit bunches (FFB) with the average figure of 0.87 m^3/tonne FFB. It is estimated that for each tonne of CPO that is produced, 5 - 7.5 tonnes of water are required, and more than 50% of this water ends up as palm oil mill effluent (POME) [29]. It has been reported that for every tonne of the CPO produced, about 3.5 m^3 of POME is generated, which indicates that with some 500 palm oil mills, more than 17.5 million tonnes of CPO is produced annually. It is estimated that about 61.25 million m^3 of POME is generated from the palm oil industry annually. POME is an oily wastewater generated by palm oil processing mills and consists of various suspended components. On average, for each tonne of FFB processed, a standard palm oil mill generated about 1 tonne of liquid waste with biochemical oxygen demand (BOD) 27 kg, chemical oxygen demand (COD) 62 kg, suspended solids (SS) 35 kg and oil and grease (O&G) 6 kg [30]. The composition and characteristic of the raw POME obtained from a local palm oil mill factory is summarized in Table 1.

2.2 The Science of Biopolymers

The usage of conventional plastics has resulted in environmental degradation [31]. The production of biodegradable polymers is seen to be a viable alternative with the increasing environmental pressure to replace conventional plastics. The problem that is faced by industry is the high production costs of biopolymers. Biodegradable polymers derived from polyhydroxyalkanoates (PHAs) are considered to be good candidates for biodegradable plastics due to their large range application and capability of being produced from renewable resources [32, 33]. These materials have attracted interest because of their potential use as biodegradable alternatives to petroleum-based synthetic plastics such as polypropylene (PP) and polyethylene (PE). Polyhydroxyalkanoates (PHAs) are mainly produced by microbial fermentation processes, and a major challenge is to reduce their production costs [32]. A feasibility study using fermentative volatile fatty acids (VFAs) as carbon source to synthesise PHA by activated sludge (treating sewage and industrial waste waters using

air and microorganisms to reduce the organic content of the sewage) was carried out to simultaneously reduce the production cost of PHAs and disposal amount of organic wastes [34].

Several efforts have been investigated to produce PHAs by microbial fermentation on organic waste (palm oil mill effluent (POME), olive oil and kitchen waste). The production of biodegradable polymers from oil palm industry can be seen as beneficial to the environment as well as contributing to sustainable development [35, 36]. Until recently, the remaining 90% discharged (empty fruit bunches, fibres, fronds, trunks, kernels, POME) was considered as waste and either burned in the open air or left to settle in waste ponds [34, 37]. By utilizing the POME and empty palm oil fibre bunch (EPFB) as carbon source and support matrix, the disposal of POME that needed further treatment could be reduced.

Although POME consists of high organic acids and is suitable to be used as a carbon source, POME is usually present in a complex form that cannot directly be utilised by PHA producing bacterial species for PHA synthesis. Typically, raw POME is difficult to degrade because it contains significant amounts of oil (tryacylglycerols) and degradative products such as diacylglycerols and monoacylglycerols and fatty acids [35, 38]. The fatty acids composition (C_{12} - C_{20}) of each of this fraction are different from one another and contribute to a high value of pollution load in POME. Therefore, anaerobic treatment has been proposed to reduce the POME characteristics. It is one of the naturally occurring processes involving decomposition and decay, in which complex organic matters are broken down into their chemical constituents. Hydrolysis and acidogenesis are the first step to convert the wastes to short-chain VFAs (i.e., acetic, butyric and propionic acids) as shown in Table 2. After that, the VFAs will be utilised by PHA-producers for PHA production [34, 39].

2.3 Biopolymer Production using POME

The price of PHAs is mainly dependent on substrate costs, accounting for about 40% of the total production cost [40]. In the last decade, a variety of low cost carbon substrates (e.g., starch, tapioca hydrolysate, whey and molasses) have been tested for PHA production to reduce the production cost. POME can be considered as an alternative with no cost of using it for PHA production. The production of VFA and PHA using POME as substrates are listed in Table 3. According to Ali *et al.* [41], with a content of 50% PHA in the dried cells and 2% dissolved in the chloroform, the calculated minimum cost for obtaining PHA from POME is below USD2/kg. By increasing the PHA content in the cell from 50% to 80%, the unit cost of PHA could be slightly reduced; whereas an increase in the amount of PHA dissolved in chloroform from 2% to 5% would result in a remarkable reduction of the PHA cost to less than USD1/kg. The above mentioned price was in line with the latest market price which is quoted at about €1.50/kg (~USD2/kg) produced by one of the largest volume manufacturers (Mirel™) [32]. This price was much higher than that of starch polymers and other bio-based polyesters due to high raw material costs (30% to 40% of total cost), small production volumes and high processing costs (particularly for purification) [32].

Table 1 Composition and chemical properties of raw POME

Composition [42]	**% content**
Water	94.2 to 95.3
O&G (oil and grease)	0.7 to 0.8
Total suspended solids	4 to 5

Parameters [30]	**Units**	**Results**
pH	-	4.8 ± 0.21
Total suspended solids	mg/L	35,000 ± 200
Turbidity (cloudiness/haziness)$^{\Psi}$	NTU	21,000 ± 300
COD	mg/L	65,000 ± 800
BOD	mg/L	27,000 ± 800
O&G (oil and grease)	mg/L	8,000 ± 300
TOC (total organic carbon)	mg/L	12,300 ± 570
Phosphorus	mg/L	142 ± 19
Ammonia	mg/L	62 ± 10

*Note: values represent means of triplicate determination.
$^{\Psi}$A nephelometer, measures how much light is scattered/reflected by suspended particles in the water. The greater the scattering, the higher the turbidity.

Table 2 VFAs composition (%) of fermented POME in anaerobic reactor [30]

Systematic name	**Generic/trivial name**	**Compound**	**% constituent**
Ethanoic Acid	Acetic acid	CH_3COOH	57
Butanoic Acid	Butyric acid	C_3H_7COOH	33
Propanoic Acid	Propionic acid	C_2H_5COOH	6
Pentanoic Acid	Valeric acid	C_4H_9COOH	4

Nevertheless, POME is usually presented in complicated forms that cannot be directly reused by PHA-producing species such as *Ralstonia eutropha*, a representative bacterium for PHA synthesis [35]. It was proposed that an anaerobic treatment of POME could be coupled with PHA production using heterotrophic bacteria (bacteria that use organic (carbon-containing) compounds as a source of energy and carbon) to reduce PHA production costs [30]. According to Ali *et al.* [43], it was critical to maintain the pH at 7 in the anaerobic treatment of POME sludge in the first stage of the process, in order for only acetic and propionic acid to be produced and not formic acid and biogas. With increasing concentrations of formic acid (for a pH maintained below 4), the PHA yield and content in *Rhodobacter sphaeroides* IFO 12203 dropped from 0.50 g/g and 67% to 0.21 g/g and 18%, respectively. Hassan *et al.* [44] later found that the presence of sludge in the anaerobically

treated POME inhibited PHA accumulation by *R. sphaeroides* IFO 12203. Based on the studies, it seems that the PHAs are being produced in a POME without sludge as opposed to a treated POME with sludge. Moreover, a low concentration of ammonium would accelerate the PHA production in a synthetic waste with an organic acid profile which was observed during POME treatment. However, Hassan *et al.* [44] found that addition of ammonium and phosphate to anaerobically treated POME was required to maintain the cell activity and production of PHA since neither ammonium nor phosphate was present in the anaerobically treated POME. In total, the organic acid concentrations obtained from anaerobically treated POME were too low [34, 43] for it to be reused as raw material in the production of PHA on an industrial scale. The underlying reason was that this would require a production reactor with a much larger size than that of a reactor for normal bioplastic production (lab scale anaerobic reactor (organic acid production: 19 L; lab scale aerobic reactor (PHA production): 6 L) [30].

The organic acids in the anaerobically digested POME could be concentrated by evaporation for use as substrates in the fed-batch non-sterile PHA fermentation system using *R. eutropha* ATCC 17699. Although the proposed overall zero emission system appeared to be practical, major drawbacks were found, including the rather low yield and productivity of PHA by *R. eutropha* when the concentrated organic acids from POME were used as compared to synthetic organic acids. This could be due to the high presence of ammonium (1.5 g/L) or other compounds in the anaerobically digested POME concentrate [45].

Md. Din *et al.* [46] proposed the suitability of using mixed cultures (mixed with different bacteria strains) to produce PHA in POME since most prokaryotes are capable of PHA production. The study noted that by using mixed cultures and POME, different types of PHA-constituents could be obtained. The harvesting of these PHA-constituents was more reliable for use as biodegradable plastics material as opposed to a single PHA-constituent.

A type of mixed culture was maintained in a SBR and a high concentration of POME proposed for this system in order to generate autotrophic (an organism that produces complex organic compounds (such as carbohydrates, fats and proteins) from simple substances present in its surroundings (light, inorganic (CO_2) and organic matters)) rather than heterotrophic bacteria (depending only on organic matters as an energy source) in the production of PHA. However, the average PHA production by using POME could only reach 44% of the CDW, indicating that an optimisation of the PHA sludge content must be carried out by varying the oxygen rate, feeding regime or transient conditions (passing with time before changing to other condition).

With all the rising issues, POME could still be the main choice of substrate in synthesizing a promising yield of biopolymer. The usage of POME could also be promoted in producing PHAs via microbial fermentation process as added value materials especially for tissue engineering applications. The added value here is a rough estimation based on the above mentioned information and current market price. The rough estimation of PHAs revenue per annum that could be procured is shown in Table 4.

3.0 POLYMERS FOR TISSUE ENGINEERING APPLICATIONS:POLYHYDROXYALKANOATES (PHAS)

With all the advantages and disadvantages comprising from both naturally derived and synthetic biodegradable polymers as described in the earlier section, one of the synthetic biodegradable polymers that could compromise with all of the major requirements on fabricating three-dimensional (3-D) porous scaffold (i.e., porosity, mechanical strength, surface properties, biocompatible, etc.) is the naturally synthesized polyhydroxyalkanoates (PHAs). PHAs are used for tissue engineering of 3-D cell culture system due to its acceptable biodegradability [47], biocompatibility [48] and resemble as polypropylene (PP) in which offering a wide range of mechanical properties [1]. PHAs have already been studied to some extent for tissue engineering applications mainly for scaffold materials in combination with ceramic materials [49] as a vehicle for drug delivery [50] and also as a material for cardiac tissue engineering [16]. The potential use of PHAs in tissue engineering is illustrated in Figure 2.

To be useful as tissue engineering scaffolds, PHAs must possess the five key properties. They must be (1) biocompatible, (2) support cell growth, (3) guide and organize the cells, (4) allow tissue ingrowth and (5) ultimately, be able to degrade to non-toxic products [1]. If these key properties aremet by the PHAs, then the additional benefits of using PHAs in tissue engineering are huge. For example, unlike many other degradable polymers, the properties of PHAs can be tailored with a wide range of building blocks, various treatments can be used to attach bioactive factors, alter surface and mechanical properties and a number of methods can be used to provide a range of degradation rates [1]. For instance, the brittleness of PHB was improved through copolymerization process of β-hydroxybutyrate with β-hydroxyvalerate [15] which was first commercialized in 1990. The copolymerization produces less crystalline, more flexible and more readily processable materials than pure PHB. Depending on the requirements of different applications, PHA can be either blended, surface modified or combined with other polymers, enzymes or even inorganic materials such as hydroxylapatite to further adjust their mechanical properties or biocompatibility [16]. In addition, tissue engineers have determined that the surface structure is also an important factor. Porous surfaces can be produced by the leaching technique, which is done by blending of PHA with a

salt that can be washed out with water. The surface of PHA materials can be rendered more hydrophilic as was shown by the treatment of P(HB-*co*-HV) with allyl alcohol gas plasma that led to an increase of wettability [51].

Apart from tailoring its physico-chemical properties, the biocompatibility of two PHA polymers, namely, PHB and PHBV have been studied by a number of different research groups. For instance, the polymers have been reported not to induce any prolonged acute inflammatory responses when implanted *in vivo* (subcutaneously into the neck folds of mice) for up to 5 weeks in the form of tablet [52]. Separately, it has also been reported that when a highly porous well-interconnected PHBV structure was seeded with fibroblasts, it sustained a cell proliferation rate similar to that observed in collagen sponges for 35 days, with a maximum cell density being observed on day 28 [53]. An interesting aspect of PHA scaffolds is the fact that the tissue-engineered cells can be implanted together with the supporting scaffold [1]. This approach was exemplified by Sodian and coworkers [54] who used scl-PHA for the fabrication of a tri-leaflet heart valve scaffold. A porous surface was achieved with the salt leaching technique resulting in pore sizes between 80 and 200 µm. The scaffold was seeded with vascular cells from ovine carotid artery and subsequently tested in a pulsatile flow bioreactor. The cells formed a confluent layer on the leaflets. The authors pointed out that the whole scaffold was molded completely and that a simple melting process was used to fix the leaflets to a model conduit.

Table 3 Various products in bioprocesses using POME as substrates

Product	Microorganism, fermentation substrate and fermentation conditions	Maximum production	References
VFA	Mixed cultures, POME + palm oil sludge. 30 °C, pH was controlled at 7, SBR (sequenced batch reactor), 24 h	7.8 g/L	Ali *et al.* [43]
VFA	Mixed cultures, POME + palm oil sludge with a ratio of 1:1, 300 rpm, 30 °C, pH was controlled at 7, stirred tank bioreactor fermentation, 84 h	10 - 14 g/L	Yee *et al.* [55]
VFA	Mixed cultures, POME + palm oil sludge, pH was controlled at 7, SBR, 96 h	10.27 g/L	Cheong *et al.* [56]
Single constituent PHA	*Rhodobacter sphaeroides* IFO 12203, Synthetic waste based on organic acid profiles obtained during POME treatment. 30 °C, pH was controlled at 7, photobioreactor fermentation, ≈ 200 h	≈ 4.0 g/L	Ali *et al.* [43]
Single constituent PHA	*Rhodobacter sphaeroides* IFO 12203, anaerobically digested POME, 30 °C, pH was controlled at 7, photobioreactor fermentation	> 2.0 g/L	Ali *et al.* [41]
Single constituent PHA	*Alcaligenes eutrophus* H16(ATCC 17699), standard medium with feeding of acetic acid obtained from anaerobically digested POME (single VFA), 400 rpm with aeration rate of 0.75 L/min, 30 °C, pH was controlled at 7, stirred tank bioreactor fermentation, 17 h	1.8 g/L	Hassan *et al.* [44]
Single constituent PHA	*Ralstonia eutropha* ATCC 17699, concentrated organic acid from anaerobically digested POME (100 g/L of total acids with acetic:propionic = 3:1), 400 rpm with aeration rate of 0.75 L/min, 30 °C, pH was controlled at 7, bioreactor fermentation, ≈ 65 h	≈ 6.25 g/L	Hassan *et al.* [45]
Multiple constituents PHA	Mixed cultures (mixed with different strains), high concentration of POME with 490 COD/N ratio (g COD/g N) and 160 COD/P ratio (g COD/g P), 1000 rpm with aeration rate of 1.5 L/min, 30 °C. pH was controlled at 7, SBR	24.24 g/L	Md. Din *et al.* [46]

Table 4 Rough estimation of PHAs revenue per annum on utilizing POME

Raw POME generated annually [29]	61.25×10^6 m^3 = 612,50,000,000 L
Concentrated POME (90% H_2O removal)	0.1 × 612,50,000,000 L = 6,125,000,000 L
Maximum PHA production (24.24 g/L) [46]	24.24 g/L × 6.13×10^9 L = 1,500 million kg/year
Current market price (Mirel™)	USD1.20/kg
Value added of PHAs production (revenue)	USD1.20/kg × 1,500 million kg/year = ∴USD1,800 million/year

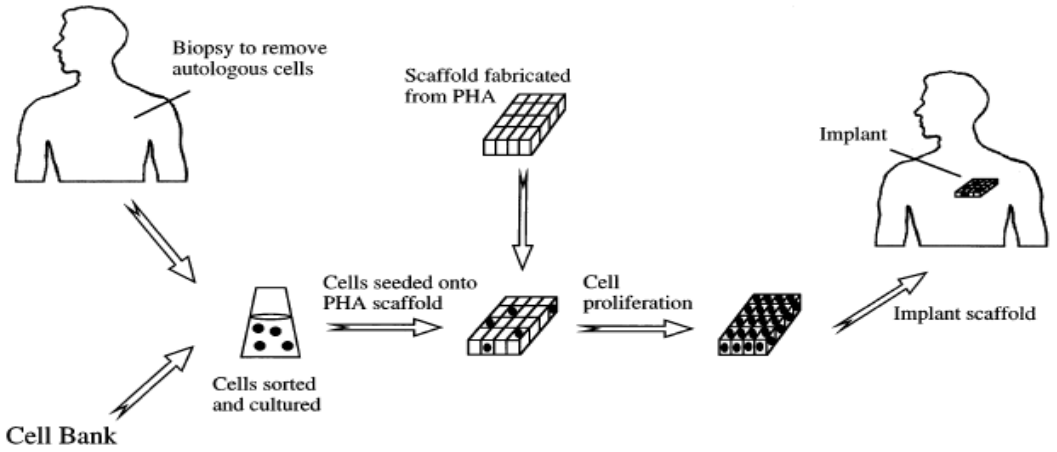


Figure 2 Role of PHAs in Tissue Engineering [1]

4.0 SCAFFOLDS REQUIREMENT FOR TISSUE ENGINEERING APPLICATIONS

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. As was mentioned earlier, 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 TE 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 which are as follows [1, 57]:

1. The scaffold should be non-immunogenic, non-toxic, biocompatible and easily manufactured.
2. The scaffold material has the ability to biodegrade. Its degradation products should not be toxic and should be easily eliminated from the implantation site by the body (i.e., secretion via urine or respiratory system; H_2O and CO_2).
3. Macrostructure: Macro and micro-structural properties affect not only cells survival, signaling, growth, propagation and reorganization but play also a major role in modelling cell shape and gene expressions, both related to cell growth and preservation of native phenotypes [58, 59].
4. Porosity and pore interconnectivity: Scaffolds should possess interconnected open pore geometry and spread porosity (usually exceeding 90%) with a highly porous surface and microstructure. This would allow *in vitro* cell adhesion, ingrowth and reorganization and would provide the necessary space for neovascularization *in vivo* [60]. Pore interconnectivity directly influences the diffusion of physiological nutrients and gases to cells as well as the removal of metabolic waste and by-products from cells [58]. The diverse nature of tissue architecture requires different microenvironments for regeneration, including the employment of scaffolds with optimal pore sizes. In addition, since the mechanical strength decreases by increasing the porosity, the void

volume in scaffolds for load-bearing tissues must be tuned to allow both the accommodation of the large number of cells and the maintenance of the structural strength required [61].

5. Pore size and surface area: Moreover, a good distribution of the large number of cells requires high internal surface area to volume ratios [58]. Since pore size and internal surface area are related linearly, a compromise between both properties has to be determined depending on the scaffold's application. When the pore diameter is too small, cells may provoke pore occlusion (blockage of pore) and prevent cellular penetration within the scaffold.
6. 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 signaling as well as *in vivo* cell recruitment and healing at the tissue-scaffold interface [62-65]. 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 [66]. Moreover, surface modification is critical to elicit specific cellular responses and direct new tissue formation. Bioactive ligands, such as peptides and polysaccharides, may either be physically adsorbed (non-covalent interactions) or covalently grafted onto the surface (chemically immobilized) or included in the bulk (during the fabrication process) to promote specific cell adhesion. Usually, the bio-molecular recognition of the material by the cells is achieved by incorporating cell-binding peptides in the form of native long chain of ECM proteins such as fibronectin, laminin and vitronectin [67] or more frequently, in the form of short peptide sequences derived from ECM proteins, such as arginine-glycine-aspartic acid (RGD) peptides [68]. The employment of the long chain proteins during adsorption onto the biomaterial surface is the most advantageous thanks to its stability, straightforward synthesis and absence of random folding [68].
7. Mechanical properties: The scaffold should have sufficient mechanical strength during *in vitro* culturing to maintain the spaces required for cell ingrowth and matrix formation. Moreover, it must provide sufficient temporary mechanical support, matching the mechanical properties of the host tissue as closely as possible, to bear *in vivo* stresses and loading. Therefore, the materials must be selected and/or designed with degradation and resorption rate and mechanical properties, such that sufficient structural integrity of the scaffold is retained until the newly grown tissue is able to support loads and stresses and can assure its structural role [69].

5.0 TECHNIQUES FOR POLYMERIC SCAFFOLDS PREPARATION

5.1 Solvent-Casting and Particulate-Leaching (SCPL)

The solvent casting and particulate leaching method was developed by Mikos *et al.* [70] amongst others for polylactic acid (PLA) and polyglycolic acid (PGA) polymers, and several authors have used the method to manufacture composite scaffolds [71, 72]. It consists of dissolving a polymer in a solvent and then adding particles of a leachable porogen (i.e., salt particles, glucose, paraffin spheres, etc.). The mixture forms a thick paste which is left to dry in air or under vacuum until the solvent has evaporated completely. The porogen is then leached out and leaves behind a network of interconnected pores. In the case of composites, the second phase is added with the porogen and remains within the structure after the porogen is leached out [73, 74]. Additionally, a thermal treatment can be used to modulate the crystallinity of the polymer by melting the polymer and controlling the cooling rate. The advantages of the solvent casting method are that it is a simple and fairly reproducible method which does not require sophisticated apparatus. The disadvantages include thickness limitations intrinsic to the particle leaching process, limited mechanical properties and some authors question the homogeneity and interconnection of the pores in the scaffolds, as well as the presence of residual porogen and solvent [75].

5.2 Thermally Induced Phase Separation (TIPS)

Thermally induced phase separation was first applied to PLA scaffolds by Schugens *et al.* [76] and several authors have applied this technique to composite scaffolds [77, 78]. It consists of inducing a solid-liquid or liquid-liquid of polymer-solvent phase separation. This is done by dissolving the polymer in a solvent and quenching the solution at a certain temperature. The quenching induces a phase separation into a polymer-rich phase and a polymer-poor phase. As the solvent crystallized, the polymer follows accordingly to the crystallized solvent microstructural foam. The solvent must then be removed from the phase separated solutions either by freeze-drying (sublimation process) or by solvent extraction [79]. The solvent leaves behind a microstructural foam of polymer as the solvent is sublimed during the freeze drying process. The main advantage of the phase separation method is that pore morphology and orientation can be tailored by altering the thermodynamic (quenching temperature) and kinetic parameters of the processing (time of phase separation). Its disadvantages include the use of potentially toxic

solvents and a high degree of anisotropy (not isotropic; not in the same direction) of the porosity.

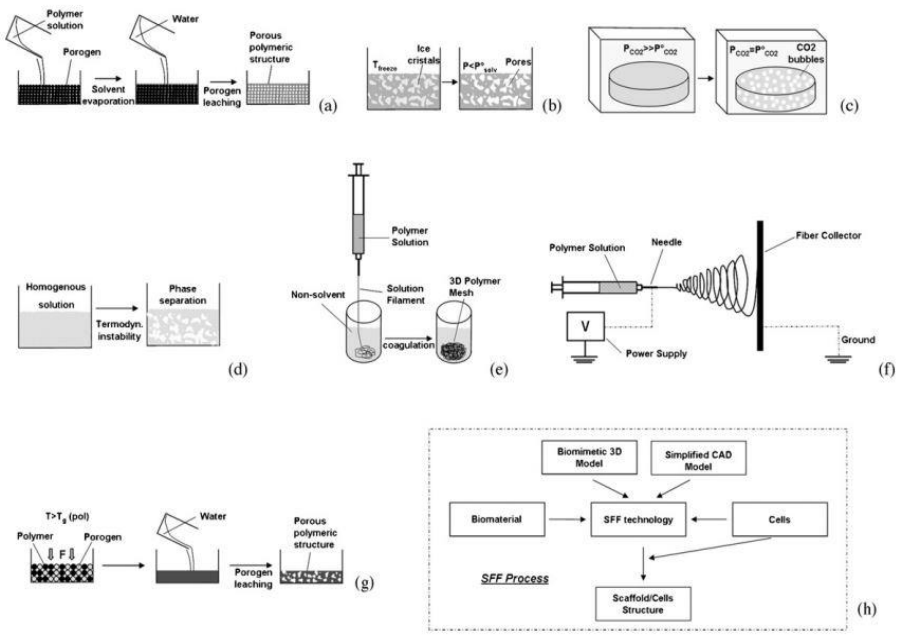


Figure 3 Schematic of commonly used techniques for scaffold production: (a) Solvent casting-particle leaching process. (b) Freeze drying process (TIPS - below freezing point < 0°). (c) Supercritical fluid technology. (d) Phase separation technology (TIPS - above freezing point > 0°). (e) Wet spinning process for the production of 3-D polymer scaffold. (f) Electrospinning process. (g) Melt molding technique. A mould filled with polymer powder and porogen component is heated above the polymer glass-transition temperature (Tg) and a pressure (F) is applied to the mixture. Then the porogen is leached out leaving a porous structure. (h) Layout of Solid Free Form (SFF) process. A 3-D computer model, directly designed in CAD software (simplified CAD model) or obtained from a medical imaging technique (bio-mimetic model) is expressed as a series of cross-sectional layers. From the data, the SFF machine fabricates the physical model (Adapted from [60])

5.3 Gas-Foaming (Super Critical Fluid Technology)

The gas foaming process is used to fabricate highly porous foams without the use of organic solvents [80]. Organic solvents may leave residues behind which can have toxic effects *in vitro* and may cause inflammation *in vivo*. This high pressure CO_2 gas technique can avoid the use of toxic solvents in scaffold fabrication. The process consists of saturating the polymer mix with gas at high temperatures and pressures. Then, a thermodynamic instability is created by quickly decreasing the temperature and pressure which stimulates the nucleation and growth of pores of gas within the polymer. Gas-foaming yields high porosities (up to 93%) and varying the temperature, pressure and rates of parameter reductions can modulate the pore sizes. The main disadvantage is the resultant scaffolds contain a nonporous surface film with mixed open and closed cell structures (poor interconnectivity) which is not suitable for application as tissue engineering scaffolds [12].

5.4 Fibre Bonding

The fibre bonding method was first developed by Cima *et al.* [81] who produced scaffolds made of polyglycolic acid (PGA) polymer. They took advantage of the fact that PGA was available as sutures and thus in the shape of long fibres. In fibre bonding, two fibre materials can be attached to each other via 'heat fuse' or 'embed' methods and then one material is dissolved in a selective solvent to obtain the fibre scaffold [82]. Mikos *et al.* [83] improved the structural stability of the constructs developing a fibre bonding technique in which the PGA fibres are joined at their cross-linking (interconnected) points by 'sintering' (bonding via heating) above their melting point temperature. The main advantage of the fibre bonding technique is the very high surface area-to-volume (m^{-1}) ratio which makes them ideal for tissue engineering applications. Although the fibre bonding technique can produced highly porous scaffolds with interconnected pores that are suitable for tissue regeneration, this method involves the use of solvents that could be toxic to cells if not completely removed [84] which could reduce the ability of cells to form new tissue *in vivo*.

5.5 Electrospinning

The electrospinning process uses an electric field to control the formation and deposition of polymer fibres on a substrate. Sheets and cylindrical shapes can be fabricated with this technique. Electrospinning method is reported capable to fabricate polymer fibres range from a few nanometer to hundreds of microns [85]. The ability to co-spin polymers with various additives offers the possibility of functional fibres. PGA and PLA fibres are two commonly used biopolymers used with this technique [86]. They have been used either alone or combined with other biomaterials such as collagen to enhance their biocompatibility.

5.6 Solid Free Form (SFF)

Solid free form fabrication (SFF) is a developing technology that enables the fabrication of custom made devices directly from computer data such as computer aided design (CAD), computed tomography (CT) and magnetic resonance imaging (MRI) data [87]. The digital information is then converted to a machine specific cross-sectional format, expressing the model as a series of layers. The file is then implemented on the SFF machine, which builds customer designed 3-D objects by layered manufacturing strategy whereby 3-D objects are fabricated with layer-by-layer building via the processing of solid sheet, liquid or powder material stocks [60]. These techniques allow obtaining complex customized scaffolds design, minimal manpower requirement, highly accurate and consistent pore morphology, anisotropic structure, diverse range of processing conditions (solvent and/or porogen free, room temperature). Shortcomings include dependence on the processing technique, use of organic solvent, high temperature, lack of mechanical strength, limited material range, small pore size and pore occlusion (blockage) at boundary [58].

6.0 SURFACE ENGINEERING: SURFACE MODIFICATIONS OF POLYMERIC POROUS 3-D SCAFFOLDS

Several surface modifications techniques have been developed to improve wetting, adhesion, and printing of polymer surfaces by introducing a variety of polar groups (by introducing a random and non-specific groups surface rendering), with little attention to functional group specificity [88]. However, when surface modification is a precursor to attaching a bioactive compound (i.e., drug delivery application), these techniques must be tailored to introduce a specific functional group.

6.1 Physico-Chemical Modifications (Surface Treatments)

6.1.1 Wet Chemical Method

In wet chemical surface modification, a material is treated with liquid reagents to generate reactive functional groups on the surface. This classical approach to surface modification does not require specialized equipment and thus can be conducted in most laboratories. It is also more capable of penetrating porous three-dimensional substrates than plasma and other energy source surface modification techniques [89] and allows for *in situ* surface functionalization of microfluidic devices. For instance, concentrated sodium hydroxide and sulfuric acid have been used to generate carboxylic acid groups by base and acid hydrolysis of PMMA [90, 91]. Specifically, a 16 h treatment in 10M sodium hydroxide at 40 °C was reported to produce 0.66 nmol/cm^2 carboxylic acids on PMMA [90]. Unfortunately, these wet chemical methods are non-specific, producing a range of oxygen-containing functional groups. In addition, those which target modification of polymer side chains (as in PMMA ester modification) depend on the side chain surface orientation. The degree of surface functionalization may therefore not be repeatable between polymers of different molecular weight, crystallinity or tacticity [92]. These wet chemical methods also generate hazardous chemical waste and can lead to irregular surface etching [93]. Many of these techniques also require some extended treatment in concentrated corrosive solutions. For these reasons, while useful in the laboratory environment, these wet chemical processes may not be suitable for larger scale (e.g., industrial applications).

6.1.2 Ionized Gas Treatments

There are several types of ionized gas treatments (e.g., corona discharge, UV irradiation and flame treatment) but plasma is the most practical one. Plasma is a high energy state of matter, in which a gas is partially ionized into charged particles, electrons, and neutral molecules [92]. Plasma can provide modification of the top nanometer of a polymer surface without using solvents or generating chemical waste and with less degradation and roughening of the material than many wet chemical treatments [93, 94]. The type of functionalization imparted can be varied by selection of plasma gas (Ar, N_2, O_2, H_2O, CO_2, NH_3) and operating parameters (pressure, power, time, gas flow rate) [95]. Oxygen plasma is often used to impart oxygen containing functional groups to polymer surfaces such as PCL [96], PE [97] and PET [98]. In addition to oxygen, carbon dioxide plasma has been used to introduce carboxyl groups on PP and PE [99], PS [100] and air plasma has been used to oxidize PMMA [101]. Ammonia and nitrogen plasmas have been used to impart amine groups to the surface of PTFE [102] and PS [100], respectively. Inert gases (e.g., Argon) can be used to introduce radical sites on the polymer surface for subsequent graft copolymerization. However, with the exception of a recent development of an atmospheric plasma system condition [103], plasma generation requires a vacuum to empty the chamber of latent gases (e.g., atmospheric gases), which presents complications for continuous operation in a large scale industrial setting. Also, results are difficult to repeat between laboratories as there are many parameters involved to optimize conditions, including time, temperature, power, gas composition/flow/pressure, orientation of reactor and distance of substrate from plasma source [92]. It should be noted that in addition to the monomers and gases intentionally introduced to the plasma chamber, latent (hidden) chemicals from prior users may be present thus posing a risk of contamination. The plasma chamber should therefore be adequately cleaned, for example by

oxygen plasma, before introducing polymers for surface modification.

6.2 Biological Surface Modifications (Biological Surface Coating)

6.2.1 *Protein-Surface Interactions*

Protein adsorption on polymer surfaces has significant importance in biomedical applications both *in vitro* and *in vivo* [92]. The first event that usually occurs when a synthetic material in contact with a cell culture medium containing dissolved proteins is the adsorption of the protein to the surface. Other responses, such as the attachment of cells are secondary and depend on the nature of the adsorbed protein layer. Controlling adsorption from a single protein solution as well as more complex mixtures requires an understanding of (a) protein structure and property, in terms of stability, conformational dynamics and the tendency to aggregate; (b) the polymer surface that allows the introduction of functional groups at the surface to enhance biocompatibility with the biological surface; and (c) analytical techniques that can quantify the protein adsorption. The concept of biological surface modification is illustrated in Figure 4. Much published work on protein adsorption at the solid-liquid interfaces has mainly focused on the amount of protein adsorbed, with the little attention on the protein conformation, orientation and structural changes in the adsorbed layer. The major parameters that may influence protein-surface interactions include surface charge (electrostatic interactions) [104, 105], hydrophobicity/hydrophilicity of the surface [92] or possibly a combination of both parameters. Figure 5 illustrates some of the mechanisms of protein adhesion, including charge (electrostatic interactions) and polarity (hydrophobicity/hydrophilicity) [92].

Other factors include electrostatic forces between adsorbed molecules (e.g., electrostatic forces between other adjacent absorbed protein molecules), solvent-solute interactions and the morphology (e.g., surface roughness, pore size and porosity) and chemistry of the solid surfaces (e.g., surface free energy). The adsorption of proteins at the solid/liquid interface is a complex phenomenon that involves the following sequence of steps [106]:

1 Transport of proteins towards the interface.
2 Attachment at the interface.
3 Final molecular structural rearrangement in the adsorbed position.
4 Detachment from the interface.
5 Transport away from the interface. In protein-surface interaction, the governing factors are determined both by physical state of the material surface in contact with the protein solution, the molecular structure of the protein and the environment of the solution.

Work in literature has highlighted a number of biologically active coatings on engineered material surface either from serum-supplemented media [107], monocomponent ECM proteins in solution [92, 108] and the covalent attachment (grafting/bioconjugations) of the cell-adhesive peptide region of the ECM protein, Arg-Gly-Asp (RGD) [92, 109] where such modified surfaces have applications in bioartificial organs, medical devices and disposable apparatus [92].

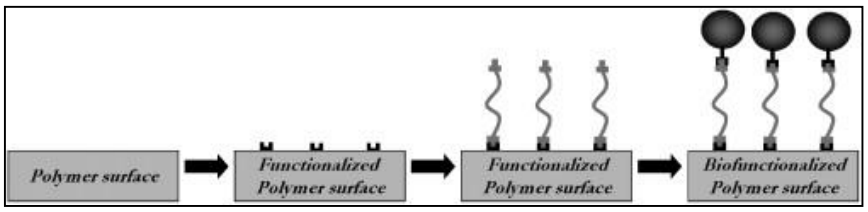


Figure 4 The concept of biological surface modification (Adapted from [92])

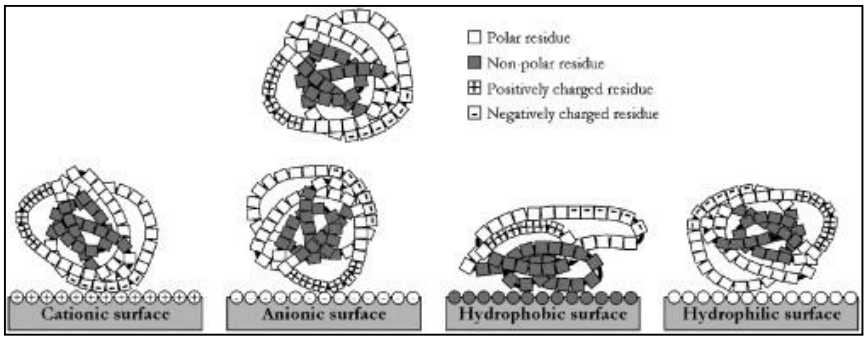


Figure 5 Mechanisms of protein adhesion (Adapted from [92])

The primary aim of coating solid surfaces with adhesion proteins and peptides mentioned above is to provide a biosignaling recognition template on the material surface for cell attachment through receptor-ligand interaction and to provide a strong mechanical contact between the cells and the surface [109, 110]. Coating surfaces with adhesive proteins derived from ECM such as fibronectin, vitronectin and collagen provides an 'adhesive interface' between the scaffold materials and the cells that resemble the native cellular milieu (environment), whose organization and production modulates and enhances cell adhesion through transmembrane integrin receptors [92]. The advantage of complete ECM proteins to peptide sequence is that they not only provide the cell-binding sequence for cell adhesion, but also provide secondary interactions with other ECM proteins and growth factors strengthening cell adhesion which results in enhanced cell growth and maturation. Furthermore, ECM proteins being the natural ligands found in *in vivo* do not cause any harmful side effects. Whilst, short-chain peptides (frequently found in numerous adhesive proteins) have lower binding to cells and selective activity for distinct integrin subtypes and are easily cleaved by enzymes [111]. However, short peptides in particular arginine-glycine-aspartic acid (RGD), do offer some advantages of enhanced stability during immobilization, increased likelihood of retaining proper orientation of binding domains after immobilization and the economic advantage of laboratory synthesis [92]. Whilst, native ECM protein tends to be randomly folded upon adsorption to the biomaterial surface such that the receptor binding domains are not always sterically (related to spatial arrangement of atoms in a molecule) available [68]. There are three major functions for the ECM:

1. They provide structural support to the tissue.
2. They provide substrate for cell adhesion and cell migration.
3. The ECM regulates cellular differentiation and metabolic function, for example by modulating the cell growth through the binding of growth factors.

Meanwhile, the *in vivo* ECM encompasses three major classes of biomolecules, which provide the complex structural entity surrounding and supporting the cells. These are:

1. Structural proteins (e.g., collagen and elastin).
2. Specialized proteins (e.g., fibronectin and laminin).
3. Proteoglycans (e.g., hydrophilic heteropolysaccharides, glycosaminoglycans (GAGs) -They form extremely complex high molecular weight components of the ECM.

The natural, ECM-derived polymers have biological properties (e.g., biosignaling mechanism) that synthetic polymers lack, whilst, synthetic polymers are cost-effective, exhibit less batch to batch variability and have physico-chemical properties which are readily modified to suit specific applications. Work in the literature on the 3-D ECM substrates for tissue engineering applications investigated a number of scaffolds that mimic the 3-D structures prepared from natural ECM-derived biopolymers, such as collagen, composite bioabsorbable (e.g., PLA and PGLA) with bioactive ceramic materials (e.g., hydroxyapatite, tri-calcium phosphate and wollastonite) or hybrid combination of natural and synthetic polymers [60]. For instance, the use of nanofibrous scaffolds (obtained by electrospinning) of combined poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) and type-I collagen for tissue-specific cells development in 3-D organization. The use of the above mentioned composite scaffolds resulted in acceleration of the adhesion and growth of NIH3T3 cells more effectively than the PHBV nanofibrous scaffold, thus making the former a good scaffold for tissue engineering [112]. Collagen is not the only ECM protein that has been extensively studied or applied in the hybrid biomaterials. Functional biomaterials have been designed utilizing fibronectin adsorption to poly(D,L-lactade) (PDLLA), poly(L-lactic-co-glycolic acid) PLGA, polystyrene (PS), poly(methyl-methacrylate) (PMMA), poly(caprolactone) (PCL) and polyurethane (PU) 3-D scaffolds to elicit cellular response and AML's leukaemic cell lines adhesion strength as well as assist in the development of the *ex vivo* 3-D leukaemia model [110].

7.0 CONCLUSION

Even though the studies of tissue engineering is set to contribute significantly to life sciences in the next millennium, this area faces various challenges such as the development of suitable scaffolds that temporarily provide mechanical support to cells at an early stage of implantation until the cells are able to produce their own extracellular matrix (ECM). The development of such scaffolds is based on the concept that cells seeded onto 3-D bioresorbable scaffolds, which can build native tissues under suitable *in vitro* and *in vivo* conditions. A number of novel scaffold materials have been developed and are under

investigation. Specifically, the PHB and PHBV are types of microbial polyester polymer which could gained a huge potential in term of its economic benefits as it can be synthesized from any known renewable carbon source waste (e.g. palm oil mill effluent (POME)). This two polymers gain much attention due to its biocompatible and biodegradable, as well as its mechanical properties, biocompatibility and biodegradability can be tailored. The use of just only polymers itself is not enough to have a good adhesion between scaffold and the environments. Thus, in order to improve adhesion as well as improve wetting, introduce a variety of polar groups with little attention to functional group specificity can be applied. From the view of materials science, the present challenge in tissue engineering is to design and fabricate reproducible bioactive and bioresorbable 3-D scaffolds of tailored porosity and pore structure, which are able to maintain their structure and integrity for predictable times, even under load-bearing conditions. Thus, a scaffold material should permit the application of suitable fabrications technology, so that a porous scaffold with any desired three-dimensional morphology as well as shape could be designed and fabricated, thus ease the study of complex human diseases, which then enable proper scientific investigation on its cure and medicine in future.

Acknowledgement

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