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Polyhydroxyalkanoates and their advances for biomedical applications

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Abstract

Polyhydroxyalkanoates (PHAs) are sustainable, versatile, biocompatible and bioresorbable **polymers**, suitable for biomedical applications. Produced via bacterial fermentation, under nutrient limiting conditions, they are unravelling a new horizon for devices in biomedical applications. A wide range of cell types including bone, cartilage, nerve, cardiac, and pancreas readily attach, grow and are functional on PHAs. The tuneable physical properties and resorption rates of PHAs allows them to be a toolbox for biomedical engineers in developing devices for hard / soft tissue engineering applications and drug delivery. The versatility of PHAs and the vast range of different PHA-based prototypes are discussed. Current ***in vitro***, ***ex vivo*** and ***in vivo*** development work are described, and their regulatory approval reviewed.

Polyhydroxyalkanoates - Bacterially derived polymers In the pursuit of sustainable biocompatible and bioresorbable materials for biomedical applications, Polyhydroxyalkanoates (PHAs) have been gaining an enhanced interest, which has been shown in the recent surge of publications in this area [1-3]. In contrast to synthetic polymers, often obtained from fossil fuel sources, PHAs are extracted from bacterial species such as *Pseudomonas putida, Cuprivadus necator, Alcaligenes latus, Pseudomonas mendocina* and *Bacillus subtilis*, under nutrient-limiting conditions [4], (**see Figure 1)**. PHAs degrade under physiological conditions via surface erosion into natural metabolites such as 3-hydroxybutyrate and hydroxyacyl-CoAs. This is a key factor as to why PHAs are non-immunogenic, biocompatible and exhibit excellent bioresorbability, allowing them to be easily incorporated into biological systems [4,5]. PHAs are classified into two main groups, short-chain length (SCL) and medium chain length (MCL). SCL-PHAs, such as Poly(3-hydroxybutyrate) (P(3HB)) are generally more brittle, stiff, with high melting temperatures, lower **elongation at break** and high **crystallinity**, ideal biomaterial properties for hard tissue engineering applications. MCL-PHAs, in contrast are characterized as highly **elastomeric**, with lower melting temperatures [4], making these ideal scaffolding materials for most soft tissue engineering applications, (**see Figure 1**) [6,7]. The mechanical and thermal properties of PHAs can be tuned by forming blends or composites of both SCL and MCL PHAs, or creating covalently linked copolymers, [5] the latter which can be achieved by means of altering substrate sources during the production stage [8].

In this context we discuss the current state of the art applications PHAs have been applied to in the areas of soft tissue engineering, hard tissue engineering, drug delivery as well as the current status in biomedical prototype developments based on PHAs. We consider both the current *in vitro* and *in vivo* status. Furthermore, the current state of clinical trials considered, and regulatory approval of PHA-based products are explained.

PHA-based Biomedical Prototype Development:

The vast array of biomedical applications based on PHAs can be categorised into four main subgroups: soft tissue, hard tissue, drug delivery and medical device related applications,**(Figure 2).** For some of

these applications PHAs have been used as coatings for decellularised matrices to prevent immune responses observed from the implantation of an **allograft** or **xenograft** made using native tissue such as in a heart valve [9,10]. A further development of using PHAs as coating is their application in recently developed Gyropinning technology where core sheath fibres can be sum with PHAs as the sheath and another polymer such a PLA as the core, giving benefits of the biocompatibility of PHAs for a variety of applications [11,12]. In addition to using PHAs as a structural device they have also been used as a mechanism to deliver cells to a target area during implantation, where cells are seeded onto the PHA scaffolds prior to use [13]. In the following sections the large variety of biomedical devices will be discussed based on the specific application. Highlights relevant to clinicians are described in the **Clinician's corner**.

Soft tissue engineering

Due to their elastic properties MCL-PHAs (e.g. P(3HO) and P(3HO-*co*-3HD)) are preferentially used in soft tissue applications. These include cardiac patches [14], vascular grafts [15], heart valves [16], auricular reconstructions [17], sutures and wound dressings [18], nerve conduits [19-21], and cartilage tissue [22-25]. The tuneability of PHA mechanical properties via the production of blends or copolymers e.g. P(3HB)/P(3HB-*co*-3HHx) and P(3HB)/P(3HO), making the materials slightly stiffer for applications such as cartilage tissue, have shown promising results [26].

Cardiac patches.

In more recent research polyhydroxyalkanoates (PHAs), in particular, poly(3-hydroxyoctanoate), P(3HO), has been investigated as a left ventricular cardiac patch for **myocardial infarct** repair. The mechanical properties of P(3HO) were analysed at body temperature (37°C) and measured a Young's modulus of 1.5 ± 0.4 MPa. This value decreased to 0.41 ± 0.03 MPa with an increased porosity of the patch, placing it in the range of that of the adult human **myocardium** at 0.02 ± 0.05 MP. This polymer is also highly elastomeric at body temperature, with an elongation at break of 447 ± 5% for the porous patch, enabling it to cope with the continuous contraction and relaxation of the heart muscle [14]. These results show how some PHAs have ideal material properties for cardiac applications and have the ability to cope with the pressures of the heart muscle, which give PHA-based patches great advantage in the field.

Stents and vessels.

A key issue with the gold-standard metallic stents that are used in a clinical setting is the risk of restenosis, whereby the artery becomes narrowed again around an implanted stent. To prevent this,

the production of a biodegradable stent which carries out its purpose of opening a blocked artery and then degrades before restenosis can occur, is a necessary progression in this field. In recent years there has been advancement in this area, for example biodegradable stents of PLLA/P(4HB) were implanted in a porcine model and exhibited promising results, i.e. lesser degree of stenosis when used alongside an oral atorvastatin drug compared to the same conditions with permanent 316L stents [27]. This suggests opportunity for an improved gold-standard stent design, with PHAs being a very promising material for this application.

The elastomeric MCL-PHAs are a promising option for blood vessels owing to their flexible nature, thereby allowing for expansion due to blood flow pressures. A recent study found that P(3HO) modified with bacterial cellulose nanofibres could be successfully melt processed for tube extrusion; these improved thermal and mechanical properties suggest a high feasibility for tissue engineered blood vessels *in vivo* [28].

Sutures.

The skin constantly regenerates, and under normal conditions, minor skin injuries often heal by themselves [29]. However, depending on factors, such as depth and severity of the defect, location of the wound, amount of microbial invasion and health of the patient, material intervention may be required in the form of sutures, wound dressings, and in the case of large defects, skin grafts and tissue engineered skin [30]. Sutures can be absorbable and non-absorbable however, the material used as the suture must be biocompatible, antibacterial, have high tensile properties, easily sterilisable, and be tied easily with a good grip [31]. Polyhydroxyalkanoates are advantageous materials for use as sutures of which P(3HB), P(4HB), P(3HB-*co*-3HV) and P(3HB-*co*-3HHX) have all been investigated for their potential [32]. To highlight their suitability for this in 2007, the FDA approved P(4HB) for use as the suture material SCL-PHA (commercial name TephaFLEX®) P(4HB) is an attractive material for absorbable sutures, as it's degradation product is less acidic than PGA and PLLA, and it degrades faster than PLLA, PCL and other PHAs such as P(3HB) [33]. Since 2007, two further PHA based suture materials have been FDA approved; Phantom Fiber™ (Tornier Co.) and MonoMax® (Braun Surgical Co.), which are both manufactured using P(4HB) [31]. Current suture research, involving PHAs, focuses on further modifying P(3HB-*co*-3HV) and P(3HB-*co*-3HHx) for use as suture materials [32]. Blending of the two low molecular weight polymers P(3HB-*co*-3HHx) and PLLA (ratio 20:80) in a film improved mechanical properties, increased toughening and increased degradation rate, in which the blend was confirmed as an excellent choice of material to manufacture 'resorbable medical sutures' [34]. This research has shown that PHAs perform better than other commonly used polymers for wound healing, promoting important aspects such as cell proliferation and ultimately

improving wound closure and the addition of the recent blended PHA materials can further improve PHAs for use in bioresorbable suture materials.

Wound dressings.

Wound dressings are materials used to promote wound healing of acute and chronic wounds, whilst covering and protecting the wound [35]. The material chosen as the wound dressing, must be suitable for the wound type by being non-adherent, easy to use, sterile, non-toxic and prevent bacterial infection. It is chosen based on promoting angiogenesis, providing/maintaining a moist environment, allowing gas exchange, allowing keratinocyte, fibroblast and epidermal cell proliferation and migration . PHAs show much potential in their use as wound dressings, and in skin tissue engineering as previous studies have shown that keratinocytes and fibroblasts adhere and proliferate better on PHA based materials compared to other synthetic polymers, such as PLLA [36]. *In vitro* studies, using murine fibroblasts, showed P(3HB-*co*-4HB) nanofibers, containing collagen peptides, supported cell adhesion and proliferation, and *in vivo* studies, using a full thickness open excision-type skin wound rat model, showed that P(3HB-*co*-4HB)/collagen nanofibers was significantly better at promoting wound closure (98%) compared to the control treatment using gauze (63% wound closure) [37]. Blending P(3HB-*co*-3HV) with guar gum powder improved its mechanical properties for wound healing [38]. Samples were also loaded with curcumin, a drug known for its antibacterial and wound healing properties. Studies confirmed samples loaded with curcumin had significant bactericidal activity, compared to those samples without curcumin, and *in vitro* studies using NIH 3T3 fibroblasts, confirmed all samples were not **cytotoxic**. *In vivo* studies, using mice wound defects, also confirmed that P(3HB-*co*-3HV)/guar gum films loaded with curcumin promoted 90% of wound contraction, whereas the control (commercial gauze), only supported 55% wound contraction and scar tissue formation [38]. This research indicates that PHAs perform better than other commonly used polymers for wound healing applications, promoting important aspects such as cell proliferation and ultimately improving wound closure.

Nerve conduits.

Nerve guidance conduits (NGCs) are entubulation devices, used to protect and aid regenerating nerves after injury. Commonly, they are hollow tubes in design, used to bridge one end of the nerve to the other [39]. NGCs have had clinical success bridging nerve injury gaps of 10-30 mm in length, but for large critical gaps, an **autograft** is still the 'gold standard' treatment option [40]. This is because NGCs

lack associated extracellular matrix tissue, topographical cues, and cellular features that autografts possess [41]. However, a required second surgery, as well as donor site morbidity, is associated with autograft use and so research strategies focus on improving NGCs, such as the inclusion of cellular therapies, surface modifications, improved topography and physical guidance cues, for critical gap injury use [42]. MCL-PHAs, such as P(3HO), exhibit mechanical properties close to that of native nerve tissue, whereas SCL-PHAs exhibit excellent biocompatibility, processability and bioresorption profiles [43]. As a result, both SCL and MCL-PHAs have been investigated for use as outer tube materials as well as internal guidance scaffolds, on their own or in blends with other PHAs, as well as blends with other polymers and proteins. Solvent casted films from blends of P(3HO)/P(3HB) 25:75 and 50:50 significantly supported NG108-15 neuronal cells adhesion, proliferation and differentiation compared to P(3HO)/P(3HB) 75:25 blend and P(3HO) films[44]. Further work manufactured the P(3HO)/P(3HB) 25:75 blend into electrospun fibres, with varying diameters, for use as an intraluminal guidance scaffold. Small (2.4 ± 0.3 µm), medium (3.7 ± 0.3 µm) and large (13.5 ± 2.3 µm) fibres were manufactured and the large fibres significantly supported increased NG108-15 neuronal cell attachment and differentiation [45]. PHAs have also been blended with other materials for nerve tissue engineering applications, such as integrating graphene/gold (RGO/Au) into Poly(3 hydroxybutyrate-co-12 mol% hydroxyhexanoate), P(3HB-*co*-12 mol%-3HHx) fibres to investigate electrically conductive materials [46]. *In vitro* analysis, using Schwann cells, confirmed all scaffolds (PHAs, PHA/RGO/Au and PHA/RGO/Au plus electrical stimulation) supported Schwann cell attachment, proliferation and migration, and the study concluded the addition of the RGO/Au to the PHA scaffolds, and the use of electrical stimulation, further improved Schwann cell attachment, proliferation and migration [46]. Integrating 1% Bioactive glass® into PHA blends of 25:75 P(3HO)/P(3HB)significantly supported NG108-15 neuronal cell adhesion, differentiation and exhibited **tensile strength** (10.0 ± 0.6 MPa) similar to that of rabbit peroneal nerve [47]. PHA properties such as cell adhesion are vital in nerve conduits, enabling cells to bridge the gap where injury has occurred, and therefore they have been shown to be a very promising material for this particular application.

Cartilage implants.

The loss of cartilage, from trauma, age related degeneration, and developmental disorders, can lead to chronic pain and disability [48]. Cartilage tissue is **avascular** and lacks regeneration capabilities [49]. As a result, total joint replacement surgery is most often required to alleviate patient pain, distress, and disability. However, recent advances in cartilage tissue engineering, using materials such as PHAs, have shown success in treating early cartilage degeneration, offering alternative solutions to total joint

replacement. Previous studies have shown successful culturing of chondrocytes (human and rabbit) on PHA scaffolds on P(3HB-*co*-3HHX), P(3HB-*co*-3HV), and P(3HB) [25].

In a recent study nanofibres were fabricated of P(3HO) and P(3HB) blends using electrospinning, to mimic collagen fibres found in articular cartilage. All 4 blend fibres of P(3HO)/P(3HB) demonstrated high cell viability of human articular chondrocytes and high expression levels of type II collagen, concluding that the (PHB/P3HO) 1:0.25 blend fibres most closely mimicked articular cartilage. Blends of PHAs with other materials has also been investigated for cartilage regeneration [50]. Another study blended PHAs with collagen type I to form solvent cast films and 3D printed structures. Cell viability assays, using C-20/A4 chondrocytes, confirmed samples containing PHAs did not exhibit any toxic effects and provided a 'supportive environment for chondrocyte activity' [51]. PHAs can be processed to closely mimic the native cartilage due to their mechanical properties, their ability to encourage cell activity and very importantly the production of collagen making PHAs the next generation material for this area.

Hard tissue engineering

Hard tissue applications are focused on bone implants, where the emphasis is on SCL-PHAs (e.g. P(3HB)), as they provide the mechanical stiffness required. A large variety of PHA blends and composites have been developed to produce viable biodegradable scaffolds with suitable physical and mechanical properties.

Bone implants.

Bone is a highly vascularised tissue with high regeneration capability, able to heal small fractures without surgical intervention [52]. However, large bone defects, especially those obtained from bone tumour resections and severe fractures, require surgical intervention, with the use of autografts, allografts, xenografts or biomaterials-based bone implants [53]. PHAs have been widely investigated for bone tissue engineering applications due to their biocompatibility, biodegradation rates and enhanced mechanical properties [54].

Recently, antimicrobial PHA films for bone regeneration applications have been developed. Blends of P(3HB)-based and P(3HO-*co*-3HD-*co*-3HDD) were loaded with Selenium-Strontium-hydroxyapatite, to have antimicrobial properties, without the use of antibiotics, and hydroxyapatite to support tissue integration into bone and support osteoblast adhesion and proliferation. Samples had 'high antibacterial activity against *S. aureus* 6538P and *E. coli* 8739 and produced a range of films with varying mechanical properties [55]Fibrous scaffolds, of Poly(3-hydroxybutyrate) (P(3HB) and Poly(3-

hydroxyoctanoate-*co*-3-hydroxydecanoate) (P(3HO-*co*-3HD), using pressurised gyration for bone, nerve and cardiovascular applications were manufactured in another study. Composite P(3HB)/Hydroxyapatite (HA) fibres were assessed for valuation of the osteoinductive properties using a Chorioallantoic Membrane (CAM) *in vivo* model, and 'implanted subcutaneously *in vivo* within immunodeficient mice to assess the degree of bone tissue formation, angiogenesis, and host tissue invasion'. P(3HB) fibres containing HA and seeded with Stro-1+ human bone marrow stromal cells (HBMSCs), had the highest level of vascularization, and a significantly higher number of blood vessels present compared to P(3HB) fibres seeded with (HBMSCs), as well as greater amounts of collagen deposition [56]. These positive results show that for hard tissue engineering applications, PHAs composites can also be utilised due to their osteoinductive properties and promotion of angiogenesis.

Drug delivery

Another application of PHAs is for drug delivery, where they hold many advantageous properties [57-60]. They can be tailored, by production methods, to release the chosen therapeutic for specific time periods required, and can also be modified to reach, and target, chosen areas in the body [61]. Drug delivery systems vary, from the use of nanoparticles, often injectable, transdermal materials and devices, oral and pulmonary administration and drug delivery implants [62]. PHAs have been used for the production of drug delivery devices, consisting of several approaches such as micro/nanoparticles, patches, films and prototypes [61], for these applications their tunable biodegradability is particularly useful.

Micro and Nanoparticles.

Polymer micro, and nano, particles are used as carriers in drug delivery applications. Containing the selected therapeutic, they offer advantages over traditional drug carrier systems by providing protection from chemical and enzymatic degradation systems *in vivo* [63]. Further, particle size and porosity can be tailored so that the selected therapeutic can cross physiological barriers, such as the blood brain barrier, and access target sites [64]. PHAs have been processed into nano/micro particles and assessed for their potential in drug delivery applications. For example, tetracycline, an antibiotic used to treat a number of different applications, has successfully been encapsulated into P(3HB) microspheres, coated onto Bioglass® scaffolds in bone tissue engineering applications.

Furthermore, Poly(3-hydroxyoctanoate-*co*-3-hydroxyhexanoate) nanoparticles were investigated for pulmonary drug delivery, looking at their interactions with lung surfactant (a mixture of phospholipids and four surfactant proteins present in the lungs) [65]. PHA nanoparticles interacted with surfactant proteins and lipids, with results suggesting nanoparticles would cross 'the surfactant monolayer reaching the alveolar lining fluid', the target site [65]. PHAs microspheres can also be used as carrier particles, to contain therapeutics, bioactive agents and drugs for drug delivery applications. P(3HB-*co*-3HV) microspheres were used to carrier bioactive glass nanoparticles, containing curcumin, a natural inflammatory reagent, for bone regeneration [66]. The average composite particle size was 2.1 µm, with particles exhibiting a uniform spherical shape, and cell viability *in vitro* experiments, using Human osteoblasts like MG-63 cells, showed increased cell viability in culture with the composite microparticles, compared to Bioglass® nanoparticles on their own [66]. P(3HB) microparticles have also been shown to carry Ceftriaxone, an antibiotic, and release the antibiotic in a more controlled way, in which particles manufactured by spray drying had higher antibacterial activity, releasing the antibiotic faster than other manufacturing methods [67]. ®

Drug delivery prototypes.

In a further development incorporating drug delivery into structured devices, PHAs have also been tailored to elute drugs to aid in the healing, regeneration, or prevention of damage to the area of interest. A good example of these are drug-eluting stents [68] (**Figure 2G**) which prevent restenosis, a condition that follows the introduction of a metallic stent and leads to further complications in patients Furthermore, the PHAs, P(3HB) and P(3HO) have been investigated as the base material for drug eluting stents to further prevent arterial blockage. P(3HB-*co*-3HV) rods and discs have also been investigated as drug eluting implants to reduce post-operative infections [64]. More recently, PHAs have been investigated as drug delivery prototypes, in the form of films, scaffolds and patches.

P(3HO-*co*-3HD-*co*-3HDD) solvent casted films can be modified to produce a polydopamine layer, which has been shown to increase surface free energy, and improve cell viability of human fibroblast cells and promote neo-vascularization when implanted *in vivo* [69]. PHAs can be blended with other materials to form composite materials, to carry drugs into the body. The anti-inflammatory drug Diclofenac, can be carried into the body by PHA composite scaffolds of tricalcium phosphate (TCP) and P(3HO), which can be used to reduce inflammatory effects after invasive bone surgeries [70]. The scaffolds demonstrated excellent biocompatibility, using MC3T3-E1 mouse pre-osteoblast cells, but the addition of P(3HO) to TCP scaffolds was shown to improve the compressive strength of scaffolds, required for bone tissue engineering applications, as well as shown to sustain and control the release of diclofenac from the scaffolds [70]. Fibres can also be used as drug delivery prototypes.

Glycyrrhetinic acid, which possess anti-microbial and anti-inflammatory properties, could be incorporated into chitin/P(3HB)/P(3HO-*co*-3HD) films and fibres, which promoted HaCaT cell metabolic activity and viability, upregulated HBD-2 (antimicrobial peptide) cell expression and down regulated pro-inflammatory cytokines, IL-1, IL-6, IL-8, and TNF-α, indicating strong anti-inflammatory activity [71]. As previously discussed, PHAs can be utilised for a vast range of biomedical applications, and in addition to that their ability to be used successfully for drug delivery in those applications in combination makes PHAs a very promising material for a plethora of biomedical uses.

In vivo Studies

Many *in vivo* studies of constructs, such as those shown in **Figure 2**, have been carried out in a number of different mammalian organisms, ranging in size from small rodents such as mice [38,56,72,73] and rats [74], to rabbits [10], and onto larger mammals such as pigs [9,27,75], sheep [9,76-78], and even primates [79]. The implantation of a multitude of PHA constructs into these animal models has shown that PHA devices result in minimal immune responses, and have non-toxic degradation products [57].

Rodent *in vivo* models, using mice and rats, have been used to show PHA compatibility with a whole range of tissues [80]. Porous MCL-PHA/PCL scaffolds, pre-seeded with cardiac progenitor cells (CPCs), implanted the **anterior** myocardium of mice, demonstrated superior mechanical properties, increased cell proliferation, cell retention on the scaffolds *in vitro* and *in vivo*, highlighting the potential of scaffolds for use as cardiac patches [72]. Bacterial cellulose (BC) modified P(3HB) scaffolds have been implanted into critical size calvarial bone defects (5 mm) in adult CD1 mice and shown to significantly enhance bone matrix production and mineralization [73]. Using 2 wt% BC compared to 1 wt% BC or neat P(3HB) scaffolds, a strong Osterix immunopositivity was observed, 'a transcription factor required for osteoblast differentiation and regulating expression of the main osteogenic factors' [73].

Rat *in vivo* models are commonly used to investigate PHAs for nerve tissue engineering, commonly investigating new nerve growth of the sciatic nerve. Nerve guide conduits, of P(3HO)/P(3HB) 75:25 blend, fabricated by dip molding, were implanted into 10 mm median nerve defects, in which axon diameter and myelin thickness was similar using both PHA conduits and the autograft control, suggesting successful nerve regeneration [26]. A blend of PHAs with PCL, P(3HO-*co*-3HD)/PCL 75:25, was manufactured into NGCs by UV curing and conduits implanted into 10 mm rat sciatic nerve injury model [81]. *C*onduits supported peripheral nerve regeneration, having a larger fibronectin-positive matrix in the whole tube, compared to the Neurolac-TW tube, currently used in the clinic [81]. Rat models have also been used to investigate potential wound healing manufactured from PHAs. Non-

woven P(3HB/4HB) membranes have been shown to facilitate healing, reduce inflammation and 'enhance angiogenic properties of skin', compared to sterile gauze in a rat skin defect *in vivo* model [82].

Larger animal models, such as rabbit and sheep *in vivo* models, are a popular choice of model for investigating bone and cartilage regeneration using PHA based scaffolds. Freeze dried P(3HB)/P(3HB-co-3HX) scaffolds have been implanted into full-thickness rabbit cartilage defects, 4 mm diameter and 2 mm depth, with the addition of rabbit chondrocytes, adipose derived stem cells and stromal vascular fraction cell components [83]. Scaffolds containing chondrocytes and stromal vascular fraction components, significantly promoted cartilage regeneration, compared to plain scaffolds, scaffolds containing chondrocytes, and scaffolds containing chondrocytes plus adipose derived stem cells [83]. Porous scaffolds of P(3HB) and chitosan have been implanted into 6 mm^2 sheep cartilage defects in which after 6 months, the scaffolds had fully degraded, and newly formed neocartilage was observed with high levels of glycosaminoglycans and collagen [84]. These results reiterate the promise that was seen through *in vitro* studies, for the successful use of PHAs in a wide range of biomedical applications.

Clinical Trials and Regulatory Approval of PHA-based devices

Approval has already been gained in the US and Europe for the clinical use of Poly(4-hydroxybutyrate), P(4HB), an SCL-PHA (commercial name TephaFLEX®), in the context of sutures that were cleared by the FDA for marketing in the USA in 2007 [57]. Another product made from P(4HB) that is available for clinical use in the USA is PHASIX™ plug and patch, which is used in the repair of inguinal hernias [85]. An outline of the P(4HB) products which have been approved in the USA and Europe are collated in **Table 1**. The previously discussed *in vivo* studies that have resulted in very positive outcomes indicate that more clinical trials will be undertaken with PHA-based prototypes.

Concluding remarks

In the last two decades PHAs are becoming ever more popular due to their tuneable properties, biocompatibility as well as bioresorbability. Further to this they are environmentally friendly due to their sustainable production, however here more research needs to be done in order to achieve higher yields of PHAs produced from waste materials to further enhance sustainability [86].

PHAs can be applied to a vast array of biomedical applications that include soft, hard tissue engineering, drug delivery applications and medical device development. Results obtained *in vitro*, *ex vivo* and *in vivo* have shown little to no immune response and cell toxicity, as well as demonstrated high cytocompatibility to a multitude of different cell types that readily grow and proliferate on PHAs. PHA material properties allow them to be manufactured using many different techniques, therefore enabling them to be used as a toolbox for countless complex scaffold structures. It is now important to further develop PHA-based prototypes for *in vivo* studies and work towards clinical trials.

Table 2 below emphasises the increased importance that PHA polymers are gaining in the scientific community with an ever-growing number of patents being filled. It is our belief that PHAs are the future biomedical material when it comes to creating new scaffolds for tissue repair and regeneration, as well as implants and even futuristic synthetic organ replacement challenges. The rapidly increasing interest in CAD orientated additive manufacturing techniques to fabricate complex 3D scaffolds shows great promise for the future of PHA-based biomedical implants and scaffolds for regenerative medicine applications. However, much research is still needed to enable the use of PHAs for these technologies as there are a variety of material property limitations during manufacturing that need to be overcome including material degradation (see **Outstanding Questions**). Finally, regulatory approval, scaling up of the production and cost effectiveness of PHAs other than P(4HB) and P(3HB) need to be achieved in order to open up the huge possibilities of this amazing family of sustainable and biocompatible polymers.

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Clinician's Corner

- Clinical use of biomaterials requires properties to be as close as possible to the native tissue being repaired or replaced. PHAs are non-inflammatory, non-immunogenic, biocompatible and bioresorbable. Their mechanical properties and resorption rates in the human body can be tailored by changing their production conditions, creating blends and/or composites. They are highly amenable to 3D processing, allowing the bespoke fabrication of implants/constructs, to meet specific patient needs.

- **The current biomedical applications** in which PHAs have been explored/applied include:

1. **Sutures** – PHAs based sutures are clinically used due to their biocompatibility, bioresorbability, high tensile strength, and easy ability to be tied with good grip.

2. **Wound-healing patches/dressings** – *In vitro* testing has shown that PHAs promote better wound healing by promoting angiogenesis, fibroblast and epidermal cell proliferation and migration.

3. **Cardiac patches** – PHA patches mimic the adult human myocardium enabling them to withstand the repeated contraction and relaxation of the heart muscle, showing promise in heart repair, post myocardial infarction.

4. **Heart valves** – PHAs used as coatings prevent immune responses observed from the implantation of an allograft/xenograft made using native tissue.

5. **Stents** – PHA stents have a lower degree of stent restenosis due to bioresorbability. With further refinements, these could replace metallic stents in clinical practice.

6. **Blood vessels** – Studies have shown that tube structures made via melt extrusion show high feasibility for use as tissue-engineered blood vessels or as blood vessel conduits that can be used in congenital diseases or **aneurysm** repair.

7. **Bone scaffolds** - PHAs address the challenge with **non-union** in large bone fractures by promoting bone regeneration and tissue integration into the bone by supporting osteoblast adhesion and proliferation.

8. **Cartilage scaffolds** - Blends of P(3HO)/P(3HB) demonstrated high cell viability of human articular chondrocytes and showed high expression levels of type II collagen, closely mimicking articular cartilage. This can potentially replace the need for joint replacements which is currently the gold standard.

9. **Nerve guidance conduits (NGCs)** - MCL-PHAs such as P(3HO) exhibit mechanical properties close to that of native nerve tissue, whereas SCL-PHAs exhibit excellent biocompatibility, processability and bioresorption profiles. As a result, both SCL and MCL-PHA- based NGCs have shown promising results for nerve injuries with gaps greater than 30 mm.

10. **Drug delivery** – PHA based microspheres/nanospheres can be used for the encapsulation of a chosen therapeutic agent and be tailored to release these in a controlled manner. They can also be modified to target specific areas in the body.

Figures and Tables:

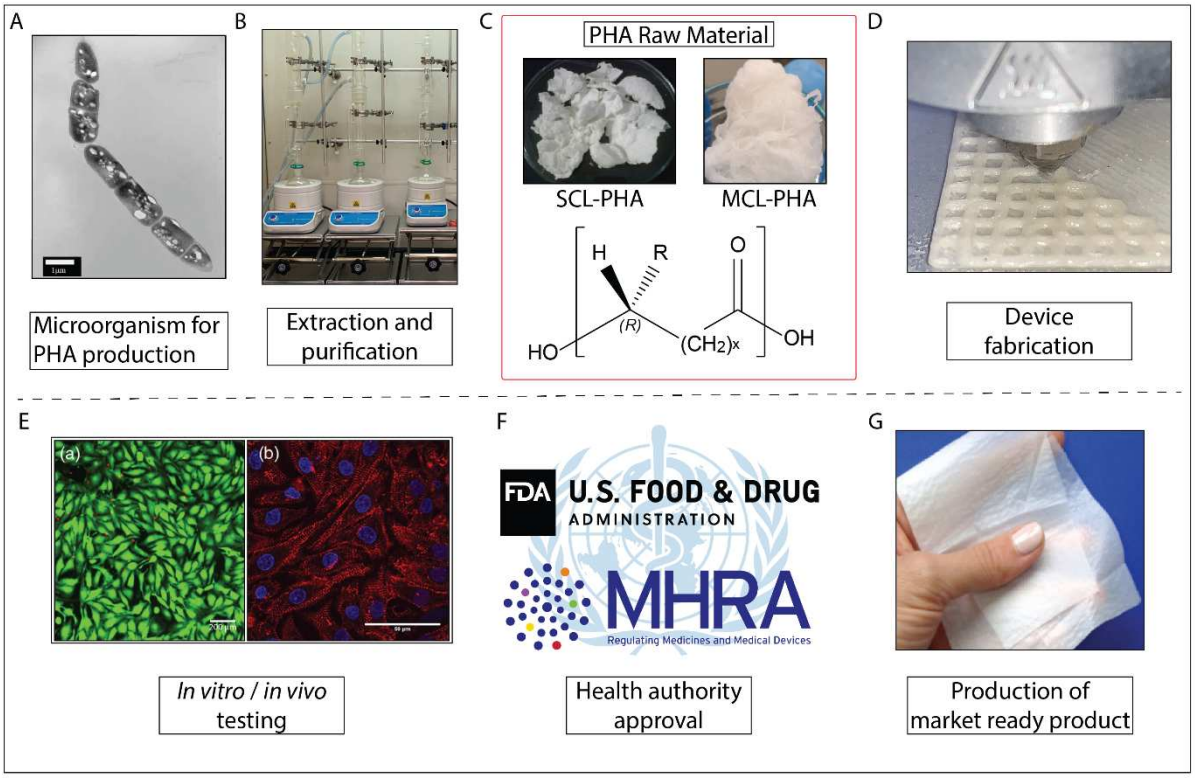
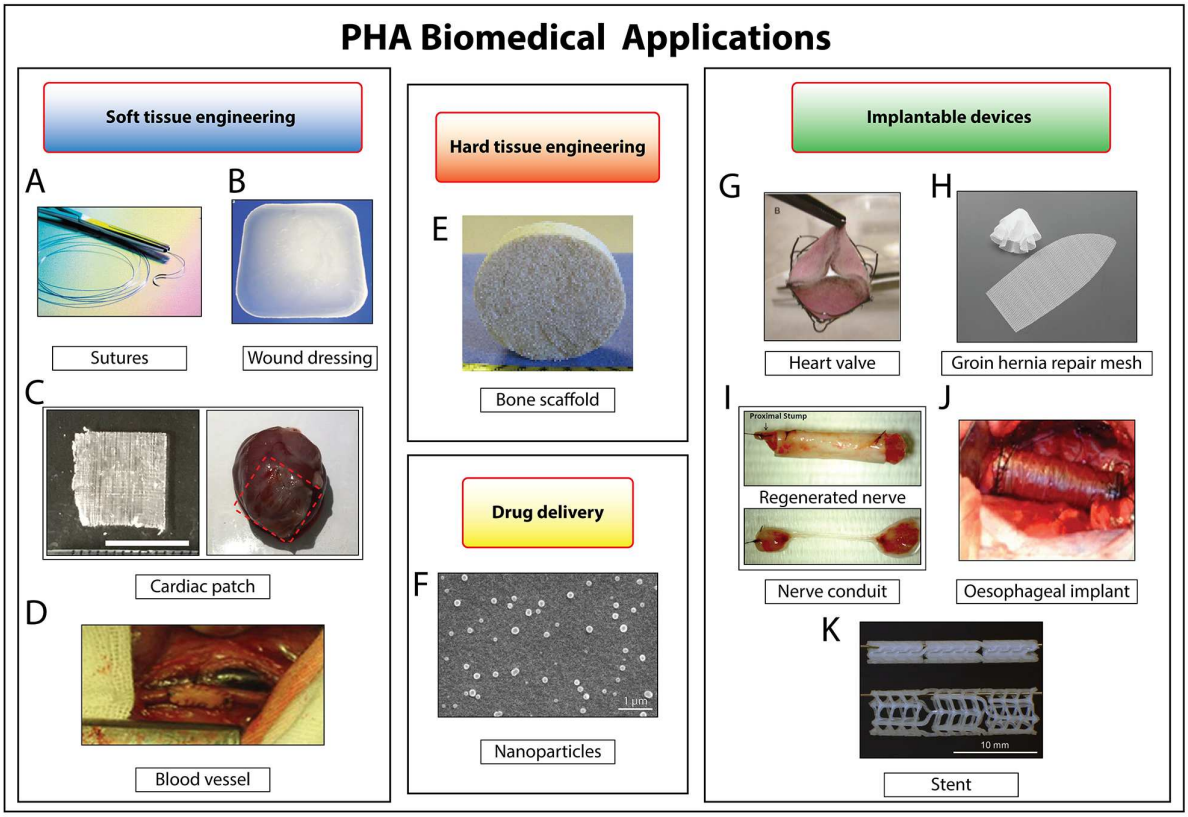


Figure 1: Overview of the process of PHA fabricated devices for biomedical engineering from production to market ready product: A: Microorganism for PHA production, TEM micrograph showing PHB granules in *Bacillus megaterium uyuni* S29 after 4 hours of fermentation reprinted with permission from [87]; B: Extraction and purification, illustrative photo of the Soxhlet apparatus used for PHA extraction; C: PHA raw material, example extracted SCL-PHA and MCL-PHA polymers and the generic chemical structure for PHAs; D: Device fabrication, FDM 3D printed structure of P(3HB); E: *in vitro* / *in vivo* testing, confocal images of neonatal ventricular rat myocytes (NVRM) grown on poly(3-hydroxyoctanoate), P(3HO), labelled with (a) ethidium homodimer-1(b) immunofluorescent anti-α-actinin and 4',6-diamidino-2-phenylindole (DAPI) [14]; F: health authority approval, representative image showing the FDA/MHRA logos on top of the WHO logo; G: Production of market ready product, example PHA wound dressing adapted from [88]; if not otherwise indicated images are from Prof. Ipsita Roy's group.



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664 **Figure 2:** Overview of current PHA-based biomedical applications: A: Sutures, adapted from [89];
665 B: Wound dressing, adapted from [88]; C: Cardiac patch (scale bar 1 cm, red square indicates the
666 applied patch) Roy Lab; D: Blood vessel, adapted from [90]; E: Bone scaffold, Roy Lab F: Nanoparticles,
667 adapted from [57]; G: Heart valve, adapted from [78]; H: Groin hernia repair mesh, adapted from [85];
668 I: Nerve conduit [26]; J: Oesophageal implant, adapted from [90]; K: Stent, adapted from [27].

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670 **Table 1**: P(4HB) products approved for clinical use in the USA and Europe adapted from [91,92].

Device name	Generic name	Biomedical application	Company	Year of approval
TephaFLEX® Absorbable suture	Absorbable suture	Suturing	Tepha, Inc.	2007, 2008
TephaFLEX® Surgical Film	Surgical film	Tissue support membrane for a variety of surgical procedures	Tepha, Inc.	2007, 2009
Model 3000 AxyaLoop™ Titanium Bone Anchor	Bone anchor	Attaching soft tissue to bone, e.g. rotator cuff repair	Axya Medical, Inc.	2007

Aesculap® MonoMax® Absorbable Suture	Bioabsorbable suture	Suturing	BBraun, Aesculap®, Inc.	2010
TephaFLEX® Composite Mesh	PHA-composite material mesh	Hernia and vaginal/colon/ rectal prolapse repair. Repair of fascial defects; Reconstructions of the pelvic floor Sacral colposuspension	Tepha, Inc.	2010
TephaFLEX® Mesh	PHA material mesh	Soft tissue reinforcement for use in reconstructive surgery. Hernia repair. Temporary wound support.	Tepha, Inc.	2011, 2012
Tornier® Collagen Coated BioFiber Scaffold	A PHA-based Collagen coated scaffold	Soft tissue reinforcement. Temporary wound support Repair of hernia or other fascial defects. Used in conjunction with sutures/anchors for repair of tendons.	Tornier, Inc.	2012

Phasix™ Plug and Patch	PHA-based plug and patch	Hernia repair, specifically hernias of the groin	C.R. Bard, Inc.	2012
TephaFLEX® Braided Suture	PHA-based suture	Suturing	Tepha, Inc.	2013
GalaFLEX® Mesh	mesh made from PHA	Reconstructive surgeries, e.g. face lift, brow lift, and neck lift.	Tepha, Inc.	2014

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673 **Table 2: Patent applications for PHAs filed from 2017, adapted from [93]**

Patent application No.	Polymer used	Biomedical application	Status	Year of patent application	Counties applied for
2020206067	P(3HB-*co*-3HV) blended with PLGA and PVP.	Tissue regeneration	Active	2019	Worldwide
20210077667	PHA Blends of MCL and SCL PHAs.	Stents	Active	2018	United States
20200261617	PHA Blends of MCL and SCL PHAs.	Nerve Guide Conduit	Active	2018	United States
2019166087A1	P(3HO-*co*-3HD) and P(3HB)	Nerve Guide Conduit	Active	2018	Worldwide
20190167834A1	Poly-4-hydroxybutyrate	plastic surgery implants and meshes	Active	2018	United States
10544301B2	Poly-3-hydroxyalkanoate	biodegradable polyester resin composition	Active	2017	Worldwide

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Glossary:

Allograft: Transplantation within the same species from one individual to another individual but doesn't include identical twins.

Aneurysm: A weakness in a blood vessel leading to a bulge.

Anterior: Front of the body.

Autograft: Transplantation within the same individual of tissue from one location to another.

Avascular: Lack of blood vessels.

Biocompatible: Not harmful to living tissue.

Bioresorbable: Naturally absorbed by the body over time.

Blood brain barrier: This is a highly selective barrier between circulating blood and the central nervous system.

Crystallinity: The amount of structural order in the material.

Cytotoxicity: Substance which causes cell damage or death.

Elastomeric: A material which will return to its original shape once the load applied is removed.

Elongation at break: Is the ratio of change from a material's initial length to its changed length after a load has been applied till it breaks.

***Ex vivo*:** Outside of the body.

***In vitro*:** Outside of their normal environment 'test tube'.

***In vivo*:** Within the body.

Myocardial Infarction: Damage to the heart muscle due to a lack of blood supply.

Myocardium: Heart muscle.

Non-union: A fracture that doesn't fully heal.

Polyhydroxyalkanoates: polyesters produced by microorganisms through fermentation under nutrient limiting conditions.

699 **Polymer:** A very large molecule made up of multiple smaller chemical units called monomers.

700 **Tensile strength:** The maximum stress a material can take when stretched before breaking.

701 **Xenograft:** Transplantation from one species to another.

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