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1 **Polyhydroxyalkanoates and their advances for biomedical applications**

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7

8 **Keywords:** Polyhydroxyalkanoates (see glossary); Biomedical applications; Sustainable biomaterials;

9 **Biocompatible; Bioresorbable;** Bacterial Fermentation

10

11 **Abstract**

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

21

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

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

45 **PHA-based Biomedical Prototype Development:**

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

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

58 ***Soft tissue engineering***

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

65 Cardiac patches.

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

76

77 Stents and vessels.

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

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

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

92 Sutures.

93 The skin constantly regenerates, and under normal conditions, minor skin injuries often heal by
94 themselves [29]. However, depending on factors, such as depth and severity of the defect, location of
95 the wound, amount of microbial invasion and health of the patient, material intervention may be
96 required in the form of sutures, wound dressings, and in the case of large defects, skin grafts and
97 tissue engineered skin [30]. Sutures can be absorbable and non-absorbable however, the material
98 used as the suture must be biocompatible, antibacterial, have high tensile properties, easily
99 sterilisable, and be tied easily with a good grip [31]. Polyhydroxyalkanoates are advantageous
100 materials for use as sutures of which P(3HB), P(4HB), P(3HB-co-3HV) and P(3HB-co-3HHX) have all
101 been investigated for their potential [32]. To highlight their suitability for this in 2007, the FDA
102 approved P(4HB) for use as the suture material SCL-PHA (commercial name TephaFLEX®) P(4HB) is an
103 attractive material for absorbable sutures, as it's degradation product is less acidic than PGA and PLLA,
104 and it degrades faster than PLLA, PCL and other PHAs such as P(3HB) [33]. Since 2007, two further PHA
105 based suture materials have been FDA approved; Phantom Fiber™ (Tornier Co.) and MonoMax®
106 (Braun Surgical Co.), which are both manufactured using P(4HB) [31]. Current suture research,
107 involving PHAs, focuses on further modifying P(3HB-co-3HV) and P(3HB-co-3HHx) for use as suture
108 materials [32]. Blending of the two low molecular weight polymers P(3HB-co-3HHx) and PLLA (ratio
109 20:80) in a film improved mechanical properties, increased toughening and increased degradation
110 rate, in which the blend was confirmed as an excellent choice of material to manufacture 'resorbable
111 medical sutures' [34]. This research has shown that PHAs perform better than other commonly used
112 polymers for wound healing, promoting important aspects such as cell proliferation and ultimately

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

115

116 Wound dressings.

117 Wound dressings are materials used to promote wound healing of acute and chronic wounds, whilst
118 covering and protecting the wound [35]. The material chosen as the wound dressing, must be suitable
119 for the wound type by being non-adherent, easy to use, sterile, non-toxic and prevent bacterial
120 infection. It is chosen based on promoting angiogenesis, providing/maintaining a moist environment,
121 allowing gas exchange, allowing keratinocyte, fibroblast and epidermal cell proliferation and
122 migration . PHAs show much potential in their use as wound dressings, and in skin tissue engineering
123 as previous studies have shown that keratinocytes and fibroblasts adhere and proliferate better on
124 PHA based materials compared to other synthetic polymers, such as PLLA [36]. *In vitro* studies, using
125 murine fibroblasts, showed P(3HB-co-4HB) nanofibers, containing collagen peptides, supported cell
126 adhesion and proliferation, and *in vivo* studies, using a full thickness open excision-type skin wound
127 rat model, showed that P(3HB-co-4HB)/collagen nanofibers was significantly better at promoting
128 wound closure (98%) compared to the control treatment using gauze (63% wound closure) [37].
129 Blending P(3HB-co-3HV) with guar gum powder improved its mechanical properties for wound healing
130 [38]. Samples were also loaded with curcumin, a drug known for its antibacterial and wound healing
131 properties. Studies confirmed samples loaded with curcumin had significant bactericidal activity,
132 compared to those samples without curcumin, and *in vitro* studies using NIH 3T3 fibroblasts,
133 confirmed all samples were not **cytotoxic**. *In vivo* studies, using mice wound defects, also confirmed
134 that P(3HB-co-3HV)/guar gum films loaded with curcumin promoted 90% of wound contraction,
135 whereas the control (commercial gauze), only supported 55% wound contraction and scar tissue
136 formation [38]. This research indicates that PHAs perform better than other commonly used polymers
137 for wound healing applications, promoting important aspects such as cell proliferation and ultimately
138 improving wound closure.

139

140 Nerve conduits.

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

145 lack associated extracellular matrix tissue, topographical cues, and cellular features that autografts
146 possess [41]. However, a required second surgery, as well as donor site morbidity, is associated with
147 autograft use and so research strategies focus on improving NGCs, such as the inclusion of cellular
148 therapies, surface modifications, improved topography and physical guidance cues, for critical gap
149 injury use [42]. MCL-PHAs, such as P(3HO), exhibit mechanical properties close to that of native nerve
150 tissue, whereas SCL-PHAs exhibit excellent biocompatibility, processability and bioresorption profiles
151 [43]. As a result, both SCL and MCL-PHAs have been investigated for use as outer tube materials as
152 well as internal guidance scaffolds, on their own or in blends with other PHAs, as well as blends with
153 other polymers and proteins. Solvent casted films from blends of P(3HO)/P(3HB) 25:75 and 50:50
154 significantly supported NG108-15 neuronal cells adhesion, proliferation and differentiation compared
155 to P(3HO)/P(3HB) 75:25 blend and P(3HO) films[44]. Further work manufactured the P(3HO)/P(3HB)
156 25:75 blend into electrospun fibres, with varying diameters, for use as an intraluminal guidance
157 scaffold. Small ($2.4 \pm 0.3 \mu\text{m}$), medium ($3.7 \pm 0.3 \mu\text{m}$) and large ($13.5 \pm 2.3 \mu\text{m}$) fibres were
158 manufactured and the large fibres significantly supported increased NG108-15 neuronal cell
159 attachment and differentiation [45]. PHAs have also been blended with other materials for nerve
160 tissue engineering applications, such as integrating graphene/gold (RGO/Au) into Poly(3
161 hydroxybutyrate-co-12 mol% hydroxyhexanoate), P(3HB-co-12 mol%-3HHx) fibres to investigate
162 electrically conductive materials [46]. *In vitro* analysis, using Schwann cells, confirmed all scaffolds
163 (PHAs, PHA/RGO/Au and PHA/RGO/Au plus electrical stimulation) supported Schwann cell attachment,
164 proliferation and migration, and the study concluded the addition of the RGO/Au to the PHA scaffolds,
165 and the use of electrical stimulation, further improved Schwann cell attachment, proliferation and
166 migration [46]. Integrating 1% Bioactive glass® into PHA blends of 25:75 P(3HO)/P(3HB) significantly
167 supported NG108-15 neuronal cell adhesion, differentiation and exhibited **tensile strength** (10.0 ± 0.6
168 MPa) similar to that of rabbit peroneal nerve [47]. PHA properties such as cell adhesion are vital in
169 nerve conduits, enabling cells to bridge the gap where injury has occurred, and therefore they have
170 been shown to be a very promising material for this particular application.

171 Cartilage implants.

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

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

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

190

191 ***Hard tissue engineering***

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

196 Bone implants.

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

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

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

219

220 **Drug delivery**

221 Another application of PHAs is for drug delivery, where they hold many advantageous properties [57-
222 60]. They can be tailored, by production methods, to release the chosen therapeutic for specific time
223 periods required, and can also be modified to reach, and target, chosen areas in the body [61]. Drug
224 delivery systems vary, from the use of nanoparticles, often injectable, transdermal materials and
225 devices, oral and pulmonary administration and drug delivery implants [62]. PHAs have been used for
226 the production of drug delivery devices, consisting of several approaches such as micro/nanoparticles,
227 patches, films and prototypes [61], for these applications their tunable biodegradability is particularly
228 useful.

229

230 Micro and Nanoparticles.

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

239

240 Furthermore, Poly(3-hydroxyoctanoate-co-3-hydroxyhexanoate) nanoparticles were investigated for
241 pulmonary drug delivery, looking at their interactions with lung surfactant (a mixture of phospholipids
242 and four surfactant proteins present in the lungs) [65]. PHA nanoparticles interacted with surfactant
243 proteins and lipids, with results suggesting nanoparticles would cross 'the surfactant monolayer
244 reaching the alveolar lining fluid', the target site [65]. PHAs microspheres can also be used as carrier
245 particles, to contain therapeutics, bioactive agents and drugs for drug delivery applications. P(3HB-
246 co-3HV) microspheres were used to carrier bioactive glass nanoparticles, containing curcumin, a
247 natural inflammatory reagent, for bone regeneration [66]. The average composite particle size was
248 2.1 μm , with particles exhibiting a uniform spherical shape, and cell viability *in vitro* experiments, using
249 Human osteoblasts like MG-63 cells, showed increased cell viability in culture with the composite
250 microparticles, compared to Bioglass[®] nanoparticles on their own [66]. P(3HB) microparticles have
251 also been shown to carry Ceftriaxone, an antibiotic, and release the antibiotic in a more controlled
252 way, in which particles manufactured by spray drying had higher antibacterial activity, releasing the
253 antibiotic faster than other manufacturing methods [67].[®]

254 Drug delivery prototypes.

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

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

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

280

281 ***In vivo* Studies**

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

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

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

305 woven P(3HB/4HB) membranes have been shown to facilitate healing, reduce inflammation and
306 'enhance angiogenic properties of skin', compared to sterile gauze in a rat skin defect *in vivo* model
307 [82].

308 Larger animal models, such as rabbit and sheep *in vivo* models, are a popular choice of model for
309 investigating bone and cartilage regeneration using PHA based scaffolds. Freeze dried P(3HB)/P(3HB-
310 co-3HX) scaffolds have been implanted into full-thickness rabbit cartilage defects, 4 mm diameter and
311 2 mm depth, with the addition of rabbit chondrocytes, adipose derived stem cells and stromal vascular
312 fraction cell components [83]. Scaffolds containing chondrocytes and stromal vascular fraction
313 components, significantly promoted cartilage regeneration, compared to plain scaffolds, scaffolds
314 containing chondrocytes, and scaffolds containing chondrocytes plus adipose derived stem cells [83].
315 Porous scaffolds of P(3HB) and chitosan have been implanted into 6 mm² sheep cartilage defects in
316 which after 6 months, the scaffolds had fully degraded, and newly formed neocartilage was observed
317 with high levels of glycosaminoglycans and collagen [84]. These results reiterate the promise that was
318 seen through *in vitro* studies, for the successful use of PHAs in a wide range of biomedical applications.

319

320

321 **Clinical Trials and Regulatory Approval of PHA-based devices**

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

329

330 **Concluding remarks**

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

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

342

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

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608

609 **Clinician's Corner**

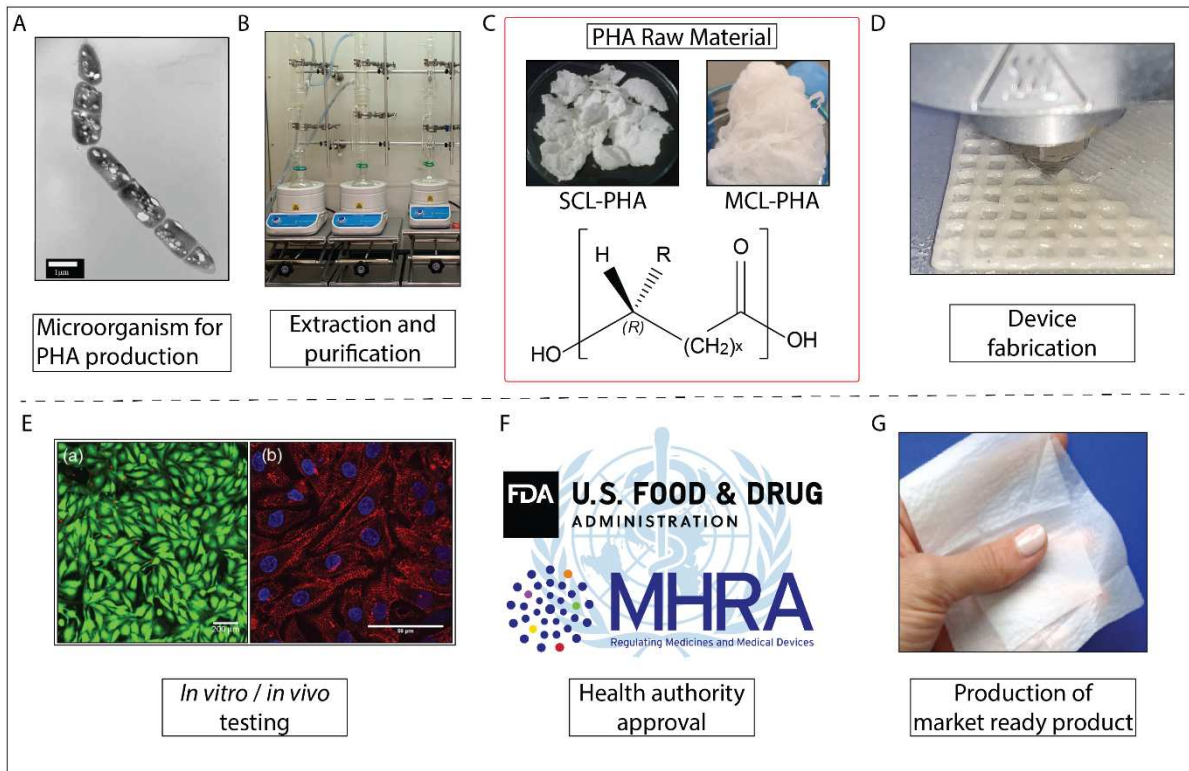
- 610 • Clinical use of biomaterials requires properties to be as close as possible to the native tissue
611 being repaired or replaced. PHAs are non-inflammatory, non-immunogenic, biocompatible
612 and bioresorbable. Their mechanical properties and resorption rates in the human body can
613 be tailored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

- 616 • **The current biomedical applications** in which PHAs have been explored/applied include:
- 617 1. **Sutures** – PHAs based sutures are clinically used due to their biocompatibility,
618 bioresorbability, high tensile strength, and easy ability to be tied with good grip.
- 619 2. **Wound-healing patches/dressings** – *In vitro* testing has shown that PHAs promote
620 better wound healing by promoting angiogenesis, fibroblast and epidermal cell
621 proliferation and migration.
- 622 3. **Cardiac patches** – PHA patches mimic the adult human myocardium enabling them to
623 withstand the repeated contraction and relaxation of the heart muscle, showing
624 promise in heart repair, post myocardial infarction.
- 625 4. **Heart valves** – PHAs used as coatings prevent immune responses observed from the
626 implantation of an allograft/xenograft made using native tissue.
- 627 5. **Stents** – PHA stents have a lower degree of stent restenosis due to bioresorbability.
628 With further refinements, these could replace metallic stents in clinical practice.
- 629 6. **Blood vessels** – Studies have shown that tube structures made via melt extrusion
630 show high feasibility for use as tissue-engineered blood vessels or as blood vessel
631 conduits that can be used in congenital diseases or **aneurysm** repair.
- 632 7. **Bone scaffolds** - PHAs address the challenge with **non-union** in large bone fractures
633 by promoting bone regeneration and tissue integration into the bone by supporting
634 osteoblast adhesion and proliferation.
- 635 8. **Cartilage scaffolds** - Blends of P(3HO)/P(3HB) demonstrated high cell viability of
636 human articular chondrocytes and showed high expression levels of type II collagen,
637 closely mimicking articular cartilage. This can potentially replace the need for joint
638 replacements which is currently the gold standard.
- 639 9. **Nerve guidance conduits (NGCs)** - MCL-PHAs such as P(3HO) exhibit mechanical
640 properties close to that of native nerve tissue, whereas SCL-PHAs exhibit excellent
641 biocompatibility, processability and bioresorption profiles. As a result, both SCL and
642 MCL-PHA- based NGCs have shown promising results for nerve injuries with gaps
643 greater than 30 mm.

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

647

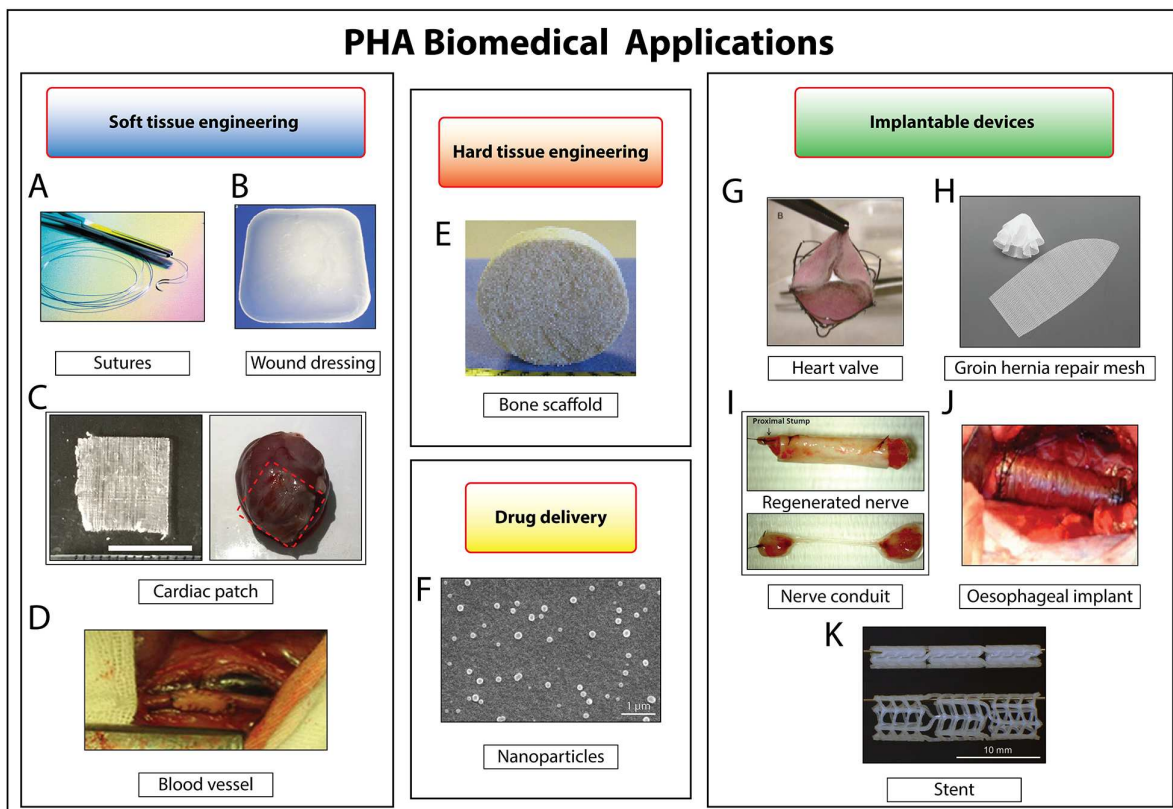
648 **Figures and Tables:**



649

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

662



663

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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675 **Glossary:**

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

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

679 **Anterior:** Front of the body.

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

681 **Avascular:** Lack of blood vessels.

682 **Biocompatible:** Not harmful to living tissue.

683 **Bioresorbable:** Naturally absorbed by the body over time.

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

686 **Crystallinity:** The amount of structural order in the material.

687 **Cytotoxicity:** Substance which causes cell damage or death.

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

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

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

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

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

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

695 **Myocardium:** Heart muscle.

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

697 **Polyhydroxyalkanoates:** polyesters produced by microorganisms through fermentation under
698 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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