

4 Applications of PHAs in Medicine and Pharmacy

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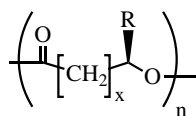
HA	hydroxyapatite
mcl-PHA	medium chain-length PHA
M_w	molecular weight
PCL	polycaprolactone
PGA	polyglycolic acid
PHA	polyhydroxyalkanoate
PLA	polylactic acid
poly(3HB)	poly- <i>R</i> -3-hydroxybutyrate
poly(3HB-co-3HV)	poly- <i>R</i> -3-hydroxybutyrate-co- <i>R</i> -3-hydroxyvalerate
poly(3HB-co-4HB)	poly- <i>R</i> -3-hydroxybutyrate-co-4-hydroxybutyrate
poly(3HO-co-3HH)	poly- <i>R</i> -3-hydroxyoctanoate-co- <i>R</i> -3-hydroxyhexanoate
poly(3HP)	poly-3-hydroxypropionate
poly(4HB)	poly-4-hydroxybutyrate
Poly(5HV)	poly-5-hydroxyvalerate
poly(6HH)	poly-6-hydroxyhexanoate
T_g	glass transition temperature
T_m	melting temperature

1

Introduction

Polyhydroxyalkanoates (PHAs) are a class of naturally occurring polyesters that are produced by a wide variety of different microorganisms (Steinbüchel, 1991). Although they are derived biologically, the structures of these polymers bear a fairly close resemblance to some of the synthetic absorbable polymers currently used in medical applications. Owing to their limited availability, the PHAs have remained largely unexplored, yet these polymers offer an extensive range of properties that extend far beyond those currently offered by their synthetic counterparts.

At the last count there were well over 100 different types of hydroxy acid monomers that had been incorporated into PHA polymers, and the list is continuing to grow (Steinbüchel and Valentin, 1995). These monomers include hydroxyalkanoate units ranging from 2- to 6-hydroxy acids substituted with a wide range of groups including alkyl, aryl, alkenyl, halogen, cyano, epoxy, ether, acyl, ester, and acid groups (see Figure 1). By no means will all of these monomers be useful or suitable for medical use; however, they provide a set of materials with properties that range from rigid and stiff to flexible and elastomeric, including polymers that degrade relatively quickly *in vivo* and others that are slow to degrade. In



Typical values of x , n and R

$x = 1$ to 4

$n = 1,000$ to $10,000$

$R =$ alkyl group (C_mH_{2m+1})

or functionalized alkyl group

Fig. 1 General chemical structure of the PHAs.

addition, the PHA polymers are thermoplastic in nature, with a wide range of thermal properties, and can be processed using conventional techniques (Holmes, 1988).

2

Historical Outline

As a class of polymers, the PHAs are relative newcomers, with many of the different types having been discovered during only the past 20 years. One of the simplest members of the class, poly-*R*-3-hydroxybutyrate, poly(3HB), is an exception as it was first identified in 1925 and is the most well-known PHA polymer. It should be noted however, that the properties of poly(3HB) are not representative of the polymer class as a whole.

During the 1980s, the British company, Imperial Chemical Industries (ICI), developed a commercial process to produce poly(3HB), and a related copolymer known as poly-*R*-3-hydroxybutyrate-*co*-*R*-3-hydroxyvalerate, poly(3HB-*co*-3HV). These polymers were sold under the tradename of Biopol®, and were developed primarily as renewable and biodegradable replacements for petroleum-derived plastics. As a result of these activities and others (Lafferty et al., 1988), both polymers became widely available, which in turn provided opportunities for their evaluation as medical biomaterials. While these efforts have resulted in several promising clinical trials, and development efforts continue, products containing these materials have yet to be approved for *in vivo* medical use.

In 1993, ICI transferred its biological division to Zeneca, which continued to develop PHAs for commodity applications under the tradename Biopol. Zeneca, however, sold its Biopol assets to Monsanto in the mid-1990s. In 2001, an American company,

Metabolix, Inc. acquired the Biopol assets from Monsanto, and is developing transgenic approaches to the large-scale manufacture of PHAs through fermentation and agricultural biotechnology.

More recent interest in the use of PHA polymers for medical applications has arisen primarily in response to the needs of the emerging field of tissue engineering, where a much wider range of absorbable polymers are being sought for use as tissue scaffolds. In fact, in the past two years PHAs have become one of the leading classes of biomaterials under investigation for the development of tissue-engineered cardiovascular products because they can offer properties not available in existing synthetic absorbable polymers.

An American company, Tepha, Inc., is currently engaged in the development of a

range of tissue-engineered products based on PHA polymers, and is expanding the number available for medical research to meet both the needs of tissue engineering and the development of more traditional medical devices. As a result of these efforts during the past two years, the number of materials currently under evaluation has expanded and now includes three additional PHA polymers, namely, poly-*R*-3-hydroxyoctanoate-*co*-*R*-3-hydroxyhexanoate (poly(3HO-*co*-3HH)), poly-4-hydroxybutyrate (poly(4HB)), and poly-*R*-3-hydroxybutyrate-*co*-4-hydroxybutyrate (poly(3HB-*co*-4HB)). This brings the total number of PHA polymers currently under investigation for medical application to five (Figure 2).

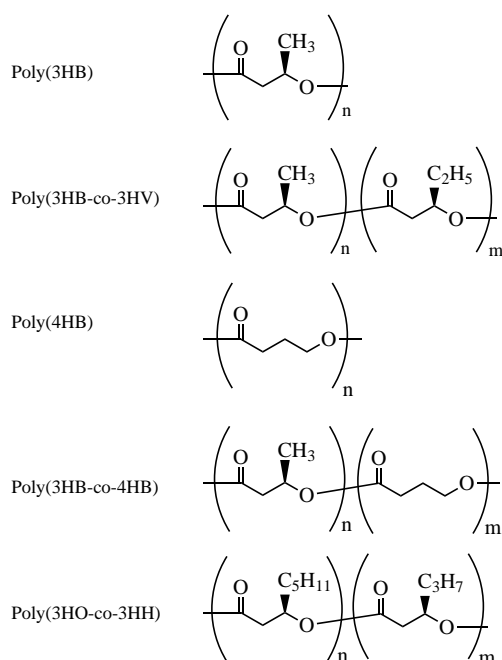


Fig. 2 Chemical structures of PHAs currently under medical investigation.

3 PHA Preparation and Properties: A Primer

3.1

Production

The PHA polymers are accumulated as discrete granules within certain microorganisms at levels reaching 90% of the dry cell mass, and can be isolated fairly readily by breaking open the cells and using either an aqueous-based or solvent-based extraction process to remove cell debris, lipid, nucleic acids, and proteins. Traditionally, these polymers have been produced by fermentation from sugars or oils, often with co-feeds, and the majority of medical studies on poly(3HB), poly(3HB-co-3HV), and poly(3HO-co-3HH), have been based on polymers derived via this route.

During the late 1980s, the genes responsible for PHA production were isolated, and this has led more recently to the development of transgenic methods for PHA production (see Williams and Peoples, 1996, and references therein). This breakthrough has provided a new means of tailoring the properties of PHA polymers to particular applications, and represents a potentially important advance in the development of technologies to produce designer biomaterials for medical use. Poly-4-hydroxybutyrate (poly(4HB)), for example, is produced using this technology. Transgenic PHA production may also prove to be important in the medical field from a regulatory standpoint, since this technology allows the production host to be selected. For example, PHAs may now be produced by fermentation in *Escherichia coli* K12, a well-characterized host used extensively by the biotechnology industry.

In general, PHA polymers are produced with relatively high molecular weights (M_w) *in vivo*. Commercial grades of poly(3HB) copolymers typically have M_w that are at least

500,000, although PHAs with much longer pendant groups (known as medium chain-length PHAs, mcl-PHAs), such as poly(3HO-co-3HH), typically have M_w that are closer to 100,000. Polydispersity is typically around 2.0. By isolating the enzymes responsible for PHA production, namely PHA synthases, researchers have also been able to produce PHA polymer *in vitro* with ultra-high M_w exceeding several million (Gerngross and Martin, 1995), and also *in vivo* in transgenic organisms (Kusaka et al., 1997; Sim et al., 1997).

3.2

Mechanical and Thermal Properties

As a class of polymers, the PHAs offer an extensive design space with properties spanning a large range, and usefully extending the relatively narrow property range offered by existing absorbable synthetics (Engelberg and Kohn, 1991). The mechanical properties of the five PHAs currently being investigated for medical use are shown in Table 1. The homopolymer, poly(3HB), is a relatively stiff, rigid material that has a tensile strength comparable with that of polypropylene. The introduction of a comonomer into this polymer backbone, however, significantly increases the flexibility and toughness of the polymer (extension to break and impact strength), and this is accompanied by a reduction in polymer stiffness (Young's modulus). This is evident in the poly(3HB) copolymers, poly(3HB-co-3HV) and poly(3HB-co-4HB) (Doi, 1990; Sudesh et al., 2000).

A progressive and substantial change in the mechanical properties of poly(3HB) also occurs when the pendant groups are extended from the polymer backbone. The mcl-PHA, poly(3HO-co-3HH), for example, shares the same backbone as poly(3HB), but in contrast is a highly flexible thermoplastic

elastomer with properties comparable with those of commercially produced materials (Gagnon et al., 1992).

Extending the distance between the ester groups in the PHA backbone can also have a dramatic impact on mechanical properties. The homopolymer poly(4HB), for example, is a highly ductile, flexible polymer with an extension to break of around 1000%, compared with poly(3HB), which has an extension to break of less than 10%. Combining these different monomers to form copolymers, as in poly(3HB-co-4HB), produces one series of materials with a wide range of useful mechanical properties that can be tailored to specific needs. Interestingly, at levels of around 20–40% 4HB, the poly(3HB-co-4HB) copolymers actually behave like elastic rubbers.

The thermal properties of PHAs also span wide ranges (see Table 1). Typical melting temperatures (T_m) range from around 55 °C for poly(3HO-co-3HH) to about 180 °C for the poly(3HB) homopolymer. Glass transition temperatures (T_g) span the range from about –55 °C to about 5 °C. In general, T_m values decrease as the pendant groups become longer. This is particularly important in the melt processing of poly(3HB), which is unstable at temperatures just above its melting point. Incorporation of other monomers into the poly(3HB) polymer backbone yields lower-melting poly(3HB) copolymers that can be more readily pro-

cessed. T_g values are also depressed by the incorporation of monomers with longer pendant groups, the depression being relatively modest in the poly(3HB) copolymers, but pronounced for the mcl-PHAs.

3.3

Sterilization of PHA Polymers

For medical use, most PHAs have been sterilized using ethylene oxide, without causing any significant changes to the physico-chemical properties of the polymers. However, low-melting PHAs such as poly(4HB) and poly(3HO-co-3HH) are generally sterilized using a cold cycle, particularly if the polymer has been fabricated ready for use. Residual ethylene oxide levels in poly(3HO-co-3HH) after cold sterilization with ethylene oxide for 8 h at 38 °C with 65% humidity have been reported to be < 1 ppm after one week (Marois et al., 1999a).

Several studies have described the effects of γ -irradiation on PHA polymers derived from 3-hydroxy acids, such as poly(3HB), poly(3HB-co-3HV), and poly(3HO-co-3HH). It has been reported that poly(3HB), unlike polyglycolic acid (PGA), can be sterilized by γ -irradiation doses on the order of 2.5 Mrad (Holmes, 1985), although it is likely that some reduction in molecular weight results from this treatment. At higher doses (10–20 Mrad) the mechanical integrity of both

Tab. 1 Mechanical and thermal properties of some representative PHAs

PHA	Poly-(3HB)	Poly(3HB-co-20%3HV)	Poly(4HB)	Poly(3HB-co-16%4HB)	Poly(3HO-co-12%3HH)
Melting temperature [T_m , °C]	177	145	60	152	61
Glass transition temperature [T_g , °C]	4	–1	–50	–8	–35
Tensile strength [MPa]	40	32	104	26	9
Tensile modulus [GPa]	3.5	1.2	0.149	n.d.	0.008
Elongation at break [%]	6	50	1000	444	380

poly(3HB) and poly(3HB-co-3HV) are significantly compromised (Miller and Williams, 1987). Luo and Netravali (1999) have also reported significant changes in the mechanical properties and M_w of poly(3HB-co-3HV) after exposure to γ -irradiation at doses of 10–25 Mrad.

Exposure of poly(3HO-co-3HH) to γ -irradiation at a dose of 2.5 Mrad at room temperature has also been reported to result in a loss of molecular weight on the order of 17%; this is caused by random chain scission, accompanied by some degree of physical cross-linking (Marois et al., 1999a). Thus, while γ -irradiation is generally recognized as a desirable alternative to ethylene oxide for sterilization, care must be exercised in its use on PHA polymers, and the procedures carefully validated.

A few PHA polymers may also be sterilized by steam (Baptist and Ziegler, 1965), particularly if they have T_m over 140 °C, and are thermally stable at this temperature. Holmes (1985) has reported that poly(3HB) powders can be sterilized in this manner.

4

Biocompatibility

Without doubt, the biological response to PHA polymers *in vivo* represents the most important property of these biomaterials if a medical application is being contemplated. Most of the information currently available relates to poly(3HB) and poly(3HB-co-3HV), and has been recently reviewed (Hasirci, 2000). A small amount of information on poly(3HO-co-3HH), poly(3HB-co-4HB), and poly(4HB) has also been published. Care should be exercised in interpreting these data however, since most studies have been based on the use of industrial rather than medical grades of PHA polymers. Notably, Garrido (1999) has described the presence of

cellular debris in industrial samples of poly(3HB-co-3HV), Rouxhet et al. (1998) detected a number of contaminants on the surface of these samples by X-ray photoelectron spectroscopy, and Williams et al. (1999) reported that an industrial sample of poly(3HB) contained more than 120 endotoxin units per gram. Two methods to remove endotoxin have been reported recently, one being based primarily on the use of peroxide (Williams et al., 1999) and the other by use of sodium hydroxide (Lee et al., 1999).

4.1

Natural Occurrence

Some of the monomers incorporated into PHA polymers are known to be present *in vivo*, and both their metabolism and excretion are well understood. The monomeric component of poly(3HB), R-3-hydroxybutanoic acid, for example, is a normal metabolite found in human blood. This hydroxy acid is a ketone body, and is present at concentrations of 3–10 mg per 100 mL blood in healthy adults (Hocking and Marchessault, 1994). This monomer has been administered to obese patients undergoing therapeutic starvation to reduce protein loss (Pawan and Semple, 1983), and also evaluated as an intravenously administered energy source in both humans (Hiraide and Katayama, 1990) and piglets (Tetrick et al., 1995). There is also interest in the use of this monomer in ocular surgery as an irrigation solution to maintain the tissues (Chen and Chen, 1992).

The monomeric component of poly(4HB), 4-hydroxybutanoic acid, is also a naturally occurring substance that is widely distributed in the mammalian body, being present in the brain, kidney, heart, liver, lung, and muscle (Nelson et al., 1981). This 4-hydroxy acid has been used for over 35 years as an

intravenous agent for the induction of anesthesia and for long-term sedation (Entholzner et al., 1995). It is also one of the most promising treatments for narcolepsy (Scharf et al., 1998), although unfortunately as with many hypnotics there has been some illegitimate use of this compound. However, since the half-life of the acid is short (35 min), and relatively high doses (several grams) are required to obtain any hypnotic effect, small implants of poly(4HB) could not induce general sedation, for example.

In addition to the known presence of certain PHA monomers in humans, low molecular-weight forms of poly(3HB) have also been detected in human tissues. Reusch and colleagues first identified poly(3HB) in blood serum ($0.6\text{--}18.2\text{ mg L}^{-1}$) complexed with low-density lipoproteins, and with the carrier protein albumin (Reusch et al., 1992). The oligomers have also been detected in human aorta (Seebach et al., 1994), and are known to form ion channels *in vivo* when complexed with polyphosphate (Reusch et al., 1997).

4.2

***In vitro* Cell Culture Testing**

Relatively few studies have attempted to characterize the tissue response of PHAs caused by leachables such as impurities, additives, monomers, and degradation products. Chaput et al. (1995) evaluated the cytotoxic responses of three poly(3HB-co-3HV) compositions (7, 14, and 22% hydroxyvalerate) using direct contact and agar diffusion cell culture tests, and reported that the solid polymers elicited mild to moderate cellular reactions *in vitro*. However, the cytotoxicity of extracts from these polymers varied with the medium, surface-to-volume ratio, time and temperature. Dang et al. (1996) also evaluated an extract from an industrial sample of poly(3HB-co-3HV) in

an *in vitro* cell culture test method with mouse fibroblasts, and reported that the extract appeared slightly to suppress cellular activity.

In other *in vitro* testing, Rivard et al. (1995) showed that porous poly(3HB-co-9%3HV) substrates (Selmani et al., 1995), when seeded with canine anterior cruciate ligament (ACL) fibroblasts, sustained a cell proliferation rate similar to that observed in collagen sponges for around 35 days, with maximal cell density occurring after 28 days. Interestingly, the poly(3HB-co-9%3HV) substrates maintained their structural integrity during the culturing, whereas the collagen foams contracted substantially and produced significantly less protein. In evaluating poly(3HB) as a potential drug delivery matrix, Korsatko et al. (1983a) also reported no significant differences in cellular growth with mice fibroblasts.

Saito et al. (1991) evaluated poly(3HB) sheets in an inflammatory test using the chorioallantoic membrane of the developing egg, and reported that the polymer did not cause any inflammation.

Several reports have described the effects of small, low molecular-weight, crystalline particles of poly(3HB) on the viability of cultured macrophages, fibroblasts, co-cultures of Kupffer cells and hepatocytes, and osteoblasts (Ciardelli et al., 1995; Saad et al., 1996a,b,c). These particles represent one of the degradation products expected to arise *in vivo* from the absorption of poly(3HB) and DegraPol®, a phase-segregated multiblock polyesterurethane copolymer. At low concentrations, the small poly(3HB) particles were found to be well tolerated by macrophages, fibroblasts, Kupffer cells and hepatocytes. Macrophages, Kupffer cells, and to a lesser extent fibroblasts and osteoblasts, were all found to take up (phagocytose) the small particles of poly(3HB) (1–20 μm), and evidence of biodegradation by macrophages

was also found (Ciardelli et al., 1995). Hepatocytes, in contrast, demonstrated no signs of poly(3HB) phagocytosis. At high concentrations ($> 10 \mu\text{g mL}^{-1}$), phagocytosis of poly(3HB) particles was found to cause cell damage and cell activation in macrophages and to a lesser degree in osteoblasts, but not in fibroblasts (Saad et al., 1996a,b,c). Separately, the chondrocyte compatibility of a DegraPol foam was also evaluated *in vitro*. Rat chondrocytes were found to attach to about 60% of the foam compared with a polystyrene control, and proliferated at comparable rates (Saad et al., 1999), leading to the conclusion that the DegraPol foam had acceptable chondrocyte compatibility.

Of particular interest in the evolving field of tissue engineering was a report by Rouxhet et al. (1998) on the effect of adhesion and proliferation of monocytes-macrophages to a poly(3HB-co-8%3HV) film when modified by hydrolysis or coated with different proteins. As anticipated, the cells were found to have a greater affinity for the polymer surface after it had been hydrolyzed to liberate additional carboxylate and hydroxyl functions. However, it was also found that adhesion of this cell type increased significantly when fibronectin was adsorbed to the polymer surface, but not when collagen or albumin were pre-absorbed.

Cellular attachment to porous tubes made from poly(3HO-co-3HH) under different seeding conditions has been evaluated (Stock et al., 1998). Although dynamic cell seeding techniques were found initially to result in a higher rate of ovine smooth muscle cellular attachment compared with static seeding, higher attachment was not sustained under simulated blood flow conditions. Cell attachment to a composite material of PGA and poly(3HO-co-3HH) has also been reported (Sodian et al., 1999). After seeding with myofibroblasts and endothelial cells, these composites were incu-

bated in a bioreactor under pulsatile flow. After eight days, near-confluent layers of cells were observed with the formation of extracellular matrix. Sodian et al. (2000a) also studied cellular attachment to porous samples of poly(4HB), and compared the results to those obtained with a porous poly(3HO-co-3HH) material and a PGA mesh. After seeding and incubating these materials with ovine vascular cells for eight days, there were significantly more cells on the PGA, although after exposure to flow no significant differences were found. A considerable amount of collagen development was noted for each sample, with the highest amounts present in the PGA meshes. Cellular attachment to a composite of poly(4HB) with a PGA mesh has also been evaluated *in vitro* recently, and compared with the mesh alone and a poly(4HB) foam (Nasseri et al., 2000). Better cell migration into the composite, and better shape retention were observed.

4.3

***In vivo* Tissue Responses**

Some of the earliest investigations of the *in vivo* tissue responses to PHA polymers were made by W. R. Grace and Co. in the mid-1960s (Baptist and Ziegler, 1965). In these early studies, film strips of poly(3HB) were implanted subcutaneously and intramuscularly in rabbits, and removed after eight weeks. Examination of the implant sites revealed granulomatous foreign body reactions, but these did not affect the underlying area.

Since these early investigations, many reports have been made describing the *in vivo* tissue responses of poly(3HB) and poly(3HB-co-3HV) in both biocompatibility and application-directed studies. Chaput et al. (1995) described one of the longest *in vivo* studies, in which poly(3HB-co-3HV)

films (containing 7, 14, and 22% valerate) were sterilized by ethylene oxide and implanted intramuscularly in sheep for up to 90 weeks. No abscess formation or tissue necrosis was seen in the vicinity of the implants. However, after 1 week *in vivo*, acute inflammatory reactions with numerous macrophages, neutrophils, lymphocytes and fibrocytes were observed in a capsule at the interface between the polymers and the muscular tissues. After 11 weeks, the observed reaction was less intense with a lower density of inflammatory cells present, though lymphocytes were still observed in the capsule and muscular tissues. At this stage, the capsules were reported to consist primarily of connective tissue cells, and were dense and well-vascularized with highly organized oriented fibers and fibroblastic cells aligned in parallel with the polymer surfaces. A large number of fatty cells were also observed in the capsule, as well as at the interface and in adjacent muscles after long-term implantation (at 70 and 90 weeks). Interestingly, few differences were observed between the capsules, tissue characteristics or cellular activity in terms of the compositions of the three poly(3HB-*co*-3HV) polymers.

Similar results were also observed by Goglewski et al. (1993) when poly(3HB) and poly(3HB-*co*-3HV) samples were implanted subcutaneously in mice. Fibrous capsules of around 100 μm thickness developed after one month, and these increased to 200 μm by three months, but then thinned to 100 μm at six months. However, the number of inflammatory cells was found to increase with valerate content, and a few granulocytes were still present around blood vessels near encapsulated implants containing 22% valerate at six months. Separately, Tang et al. (1999) suggested that leachable impurities and low molecular-weight poly(3HB) are at least partly responsible for increased colla-

gen deposition following an *in vivo* study of subcutaneous poly(3HB) implants in rats.

Williams et al. (1999) reported a 40-week subcutaneous implant study of poly(3HO-*co*-3HH) in mice. At two weeks, there was minimal reaction to the implants which had been encapsulated by a thin layer of fibroblasts, four to six cell layers thick, surrounded by collagen. There was no evidence of macrophages, and the tissue response continued to be very mild at 4, 8, 12 and 40 weeks, with the amount of connective tissue surrounding the implants remaining fairly constant. The polymer proved to be particularly inert, and could be readily removed with little tissue adherent to the implants. An extract from poly(3HO-*co*-3HH) was also tested in a standard skin sensitization test (ASTM F270), but no discernable erythema/eschar formation was observed.

Subcutaneous implants of poly(4HB) have also been reported to be well tolerated *in vivo* during the course of their degradation (Martin et al., 1999), with minimal inflammatory responses occurring.

It is worth noting that, *in vivo*, as most PHA polymers break down they release hydroxy acids that are significantly less acidic and less inflammatory than many currently used synthetic absorbable polymers (Taylor et al., 1994). For example, poly(3HB) and poly(4HB), are derived from 3- and 4-hydroxybutanoic acids ($\text{p}K_{\text{a}}$ 4.70 and 4.72, respectively), that are significantly less acidic than the 2-hydroxy acids (glycolic acid, $\text{p}K_{\text{a}}$ 3.83; lactic acid, $\text{p}K_{\text{a}}$ 3.08) found in PGA and poly-lactic acid (PLA). Furthermore, significant differences in the mechanism of degradation of these synthetic polymers, which can degrade autocatalytically from the inside outward, can result in substantial amounts of acidic degradation products being released. In one clinical study, for example, around 5% of the patients receiving PGA screws had an inflammatory

reaction to the implants that was sufficient to warrant operative drainage (Böstman, 1991).

Finally, Holmes (1988) has reported that poly(3HB) shows negligible oral toxicity, the LD₅₀ being greater than 5 g kg⁻¹.

5

Biodistribution

The biodistribution of poly(3HB) microspheres in mice (Bissery et al., 1984a), and poly(3HB) granules in rats (Saito et al., 1991) has been investigated using ¹⁴C-labeling and, as anticipated, results have been found to depend upon particle size. In the first study, microspheres of 1–12 µm diameter were injected intravenously into mice, and traced at 0.5, 1, and 24 h, and every seven days thereafter. After 30 min, 47% of the radioactivity was found in the lungs, 14% in the liver, and 2.1% in the spleen. After 1 h, concentrations in the lungs and liver had increased to 62 and 16%, respectively, and by 24 h there was still 60% in the lungs and 24% in the liver. Thereafter, the amounts remained fairly constant, but fell somewhat in the lungs. In the rat study, granules of 500–800 nm diameter were injected through a tail vein into rats and traced at intervals of 2.5 h, 1 day, 13 days, and 2 months. After 2.5 h, approximately 86% of the radioactivity had accumulated in the liver, with 2.5% and 2.4% of the total distributed in the spleen and lungs, respectively. During the following two months, radioactivity levels in most of the tissues decreased slowly, but steadily.

6

Bioabsorption

The rates of bioabsorption of PHA polymers *in vivo* vary considerably, and depend pri-

marily upon their chemical compositions. Other factors such as their location, surface area, physical shape and form, crystallinity, species, and molecular weight can also be very important. While useful information can be derived from *in vitro* studies, results of *in vitro* studies with PHA polymers are not always good indicators of *in vivo* behavior.

6.1

In vitro Degradation

In order to investigate the mechanism of degradation of poly(3HB) and poly(3HB-co-3HV) *in vivo*, a number of studies have been conducted to determine their rates of hydrolysis *in vitro* (see Holland et al., 1987, 1990; Yasin et al., 1990; Knowles and Hastings, 1992; Chaput et al., 1995). These studies have used complementary techniques such as gravimetric and molecular weight analysis, as well as measurements of surface and tensile properties to monitor different aspects of degradation and develop a concept of the overall degradation process. This has led to the following general scheme of *in vitro* behavior for poly(3HB) and poly(3HB-co-3HV). Initially, some surface modification is observed, with water diffusing into the polymer and porosity increasing. Crystallinity also increases, but there is relatively little change in molecular weight in the first few months, and tensile properties remain fairly constant. As the porosity increases, hydrolysis of the polymer chains releases degradation products that can diffuse away more easily. The molecular weight decreases, erosion increases, and both weight and tensile strength begin to decrease more rapidly. At about one year, the initial resistance to degradation is followed by an accelerated degradation with the material becoming more brittle, but not losing its physical integrity. After one year, the most apparent change in the physical appearance

of the polymer is loss of surface gloss and the development of surface rugosity.

Other *in vitro* studies have examined the action of additives such as polysaccharides (Yasin et al., 1989), polycaprolactone (PCL) (Yasin and Tighe, 1992), as well as lipases, PHA depolymerases, and several extracts on PHA degradation. Although PHA depolymerases are abundant in the environment and are responsible for PHA biodegradation in soil, there is currently no evidence that these enzymes are present *in vivo*. Mukai et al. (1993) investigated the action of 16 lipases on five different PHA polymers prepared either by fermentation or synthetically, and found that none of these enzymes catalyzed the hydrolysis of poly(3HB). However, the other four PHA polymers were hydrolyzed by lipases, with the number of lipases capable of hydrolyzing the PHA polymer chains decreasing in the following order: poly-3-hydroxypropionate (poly(3HP)) > poly(4HB) > poly-5-hydroxyvalerate (poly(5HV)) > poly-6-hydroxyhexanoate (poly(6HH)). Interestingly, two lipases have been detected recently in tissue adjacent to poly(3HB) implants in rats, raising the possibility of their involvement in poly(3HB) bioabsorption (Löbler et al., 1999). The copolymer, poly(3HB-co-3HV), formulated as microspheres with PCL, and loaded with bovine serum albumin, has also been incubated with four different extracts *in vitro* (Atkins and Peacock, 1996a). The percentage weight loss decreased in the order newborn calf serum > pancreatin > synthetic gastric juice > Hanks' buffer, and it was speculated that the enhanced biodegradation in newborn calf serum, and surface erosion in pancreatin, must be due to enzymatic activity in these extracts.

The *in vitro* degradation of poly(3HO-co-3HH) has also been examined for up to 60 days (Marois et al., 1999b). When exposed to acid phosphatase and β -glucuronidase for

this time period, no significant surface or chemical modifications were observed, and no significant weight loss was detected. It was concluded that this polymer, which shares a common backbone with poly(3HB), degrades slowly by chemical hydrolysis.

Degradation of poly(4HB) *in vitro* has recently been reported (Martin et al., 1999). The homopolymer is fairly resistant to hydrolysis at pH 7.4, and over a 10-week period very little degradation was observed, although a 20–40% reduction in average molecular mass did occur during this time period.

6.2

In vivo Bioabsorption

In early studies, some confusion arose around the stability of poly(3HB) and poly(3HB-co-3HV) *in vivo*. Korsatko et al. (1983a, 1984) and Wabnegg and Korsatko (1983) evaluated poly(3HB) for use as matrix retard tablets and reported that the polymer was degraded *in vivo* at a rate directly proportional to the elapsed time (a zero-order reaction). However, it was reported later that monofilaments derived from poly(3HB) and poly(3HB-co-3HV) (8 and 17% valerate) showed little, if any, loss of strength when implanted subcutaneously in rats for up to six months (Miller and Williams, 1987), except after γ -irradiation. Many subsequent studies have confirmed that poly(3HB) and poly(3HB-co-3HV) do degrade *in vivo*, albeit slowly (Hasirci, 2000). Typically, poly(3HB) is completely absorbed *in vivo* in 24–30 months (Malm et al., 1992b; Hazari et al., 1999a). During the first four weeks *in vivo*, the degree of crystallinity of a sample of poly(3HB) implanted in the peritoneal cavity was reported to have increased, presumably as a result of the amorphous regions of the polymer degrading more rapidly than the crystalline do-

mains (Behrend et al., 2000a). After four weeks, crystallinity, Young's modulus, and microhardness were each shown to have decreased fairly steadily, this being consistent with a surface process.

Kishida et al. (1989) attempted to develop a method to accelerate the bioabsorption of poly(3HB) and poly(3HB-co-3HV) *in vivo* by adding basic compounds to the polymers. Although *in vitro* the rate of hydrolysis was found to increase, the effect *in vivo* was minimal, presumably because the basic accelerators had leached out.

The mcl-PHA, poly(3HO-co-3HH), also degrades slowly *in vivo*. Williams et al. (1999) reported that the molecular weight (M_w) of subcutaneous implants of poly(3HO-co-3HH) in mice decreased from 137,000 at implantation to around 65,000 over 40 weeks, and that there were no significant differences between the molecular weights of samples taken from the surfaces and interiors of the implants. The latter finding suggests that slow, homogeneous hydrolytic breakdown of the polymer occurs.

While poly(3HB), poly(3HB-co-3HV), and poly(3HO-co-3HH) are generally degraded slowly *in vivo*, consistent with *in vitro* observations, the homopolymer, poly(4HB) is an exception. Martin et al. (1999) found the *in vivo* degradation of this polymer to be relatively rapid, and to vary with porosity. Over a 10-week period it was reported that film, 50%, and 80% porous samples implanted subcutaneously in rats, lost 20%, 50%, and nearly 100% of their mass, respectively. The average molecular mass of the polymer also decreased significantly, but independently of sample configuration. These data suggest that the degradation of poly(4HB) *in vivo* depends in part on surface area, and that the mechanical properties of poly(4HB) implants are likely to undergo a gradual change rather than the more abrupt changes seen with other synthetic absorb-

ables, such as PGA. This might be advantageous, for example, in tissue regeneration applications where a sudden loss of a mechanical property is undesirable, or more gradual loss of implant mass and steady in growth of new tissue are beneficial.

7 Applications

Until recently, only poly(3HB) and poly(3HB-co-3HV) were available commercially, and consequently the majority of investigations into applications have focused on a relatively narrow set of polymer properties within the PHA design space. This situation is beginning to change however, with more recent studies involving poly(3HO-co-3HH), poly(4HB) and poly(3HB-co-4HB).

7.1

Cardiovascular

Without doubt, the major medical use of PHAs has been in the development of cardiovascular products.

7.1.1

Pericardial Patch

One of the most advanced applications of PHA polymers in cardiovascular products has been the development of a regenerative poly(3HB) patch that can be used to close the pericardium after heart surgery, without formation of adhesions between the heart and sternum (Bowald and Johansson, 1990; Malm et al., 1992a,b; Bowald and Johansson-Ruden, 1997). These adhesions represent a significant complication if a second operation is necessary, thereby increasing the risk of rupturing the heart or a major vessel, and prolonging the overall duration of the operation. In an initial study, native pericardium was excised from 18 sheep, and