

**20**  
**Applications of PHAs in**  
**Medicine and Pharmacy**

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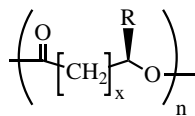
HA	hydroxyapatite
mcl-PHA	medium chain-length PHA
$M_w$	molecular weight
PCL	polycaprolactone
PGA	polyglycolic acid
PHA	polyhydroxyalkanoate
PLA	polylactic acid
poly(3HB))	poly- <i>R</i> -3-hydroxybutyrate
poly(3HB-co-3HV))	poly- <i>R</i> -3-hydroxybutyrate-co- <i>R</i> -3-hydroxyvalerate
poly(3HB-co-4HB))	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.

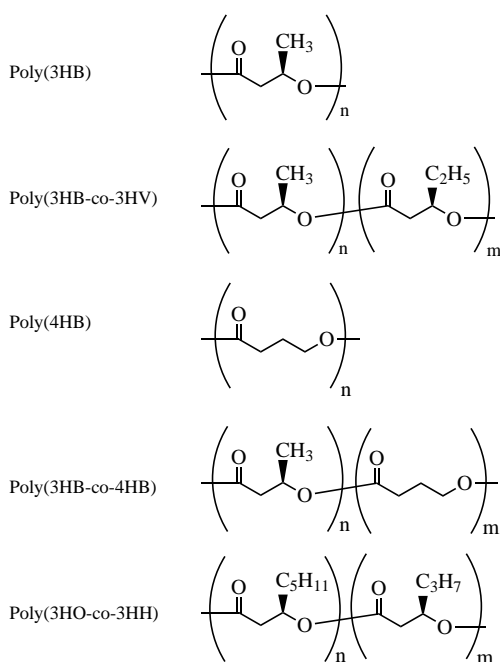
In 1993, ICI transferred its biological division to Zeneca, which continued to develop PHAs for commodity applications under the tradename Biopol. Zeneca, however, sold its Biopol assets to Monsanto in the mid-1990s. In 2001, an American company,

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 sort 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(3-HO-*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  $\gamma$ -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  $\gamma$ -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	53	152	61
Glass transition temperature [ $T_g$ , °C]	4	–1	–48	–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  $\gamma$ -irradiation at doses of 10–25 Mrad.

Exposure of poly(3HO-co-3HH) to  $\gamma$ -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  $\gamma$ -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  $\mu\text{m}$ ), 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  $\mu\text{m}$  thickness developed after one month, and these increased to 200  $\mu\text{m}$  by three months, but then thinned to 100  $\mu\text{m}$  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 LD<sub>50</sub> being greater than 5 g kg<sup>-1</sup>.

## 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 <sup>14</sup>C-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  $\beta$ -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  $\gamma$ -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

replaced with a nonwoven poly(3HB) patch (Malm et al., 1992a). Patches were then harvested between 2 and 30 months after the operation, examined for adhesions, infection, and inflammatory response, and compared with controls where native pericardium had been removed and left open. Moderate adhesions were present in all the controls, whereas no adhesions developed in 14 of the animals receiving poly(3HB) patches. Interestingly, the pericardium was regenerated in all animals receiving a patch, with the surface of the patches being completely covered with mesothelium-like cells after two months, and a dense underlying collagen layer developing over 12 months. A pronounced tissue response to the patch was observed, with the polymer being slowly phagocytosed by polynuclear macrophages – a finding which has led others to question the biocompatibility of the patch (Tomizawa et al., 1994). Polymer remnants were still present after 24 months, and some macrophages were still found at 30 months, but no platelet aggregates were detected.

Following animal studies with the poly(3HB) patch, a randomized clinical study of 50 human patients admitted for bypass surgery and/or valvular replacement was undertaken (Duvernoy et al., 1995). Using computed tomography (CT), 39 of these patients (19 with the patch and 20 without) were examined for the presence of adhesions at 6 and 24 months, and a lower incidence of postoperative adhesions was reported for the group receiving PHA patches, based on the presence of fat located between the patch and the cardiac surface.

In contrast to these studies, Nkere et al (1998) found no significant difference in a short-term study of adhesion formation among calves undergoing bypass surgery with and without the poly(3HB) patch, as well as calves not undergoing bypass surgery

but with their pericardium left open. It was noted however that there might be a species variation, and also that the duration of the studies was different. Also, in comparison with Malm's sheep study, the calves in this study had been subjected to bypass, which was considered more clinically relevant.

#### 7.1.2

##### **Artery Augmentation**

Non-woven patches of poly(3HB) have been evaluated in the augmentation of the pulmonary artery as scaffolds for the regeneration of arterial tissue in low-pressure systems (Malm et al., 1994). A total of 19 lambs was used for the trial, with 13 receiving poly(3HB) patches, and six receiving Dacron patches as a control group. No aneurysms were observed in either group, and the pores of the non-woven poly(3HB) were small enough to prevent bleeding. All the patches were harvested between 3 and 24 months, and endothelial layers were found on both patch materials. Beneath the endothelium-like surface, the configuration closely resembled native artery, with smooth muscle cells, collagen and elastic fibers in the poly(3HB) explants. By contrast, a thin collagenous layer had formed under the endothelium lining of the Dacron implants due to the well-known inflammatory reaction to Dacron fiber, and a dense infiltration of lymphocytes was present. As in the case of the pericardial studies, the nonwoven poly(3HB) patch was phagocytosed by polynucleated macrophages, and macrophages persisted, even at 24 months. However, no platelet aggregates were found on the luminal surface.

Highly porous foam patches of poly(4HB) seeded with endothelial, smooth muscle cells, and fibroblasts have also been evaluated in artery augmentation, and with good results (Stock et al., 2000a). A total of six cell-seeded patches, and one unseeded control

patch were implanted into the pulmonary artery of sheep. Echocardiography and examination of the cell-seeded explants at 4, 7 and 20 weeks indicated that progressive tissue regeneration had occurred, but with no evidence of thrombus, dilation, or stenosis. Examination of the control patch at 20 weeks revealed slight bulging at the site of implantation, and less tissue regeneration.

#### 7.1.3

##### **Atrial Septal Defect Repair**

Malm et al. (1992c) also tested the efficacy of the nonwoven poly(3HB) patch in the repair of atrial septal defects created in six calves. Implants were evaluated between 3 and 12 months, and complete endothelial layers facing the right and left atrium were observed, with a subendothelial layer of collagen and some smooth-muscle cells. As before, the patch was degraded by polynuclear macrophages, with small particles of polymer still present at 12 months, and a foreign body reaction persisting. Nonetheless, the patches prompted the formation of regenerated tissue that resembled native atrial septal wall, and had sufficient strength to prevent the development of shunts in the atrial septal position.

#### 7.1.4

##### **Cardiovascular Stents**

One of the main problems with the use of metallic stents in cardiovascular applications is the subsequent restenosis that can result from excessive growth of the blood vessel wall. This is believed to be due (at least in part) to irritation caused by the metallic stent on the vessel wall. A potential solution to this problem may lie in the development of an absorbable stent that can prevent reocclusion of the vessel in the short term, but then be absorbed so that it does not cause any persistent irritation of the vessel wall.

Attention is beginning to focus on the use of PHAs in absorbable stents as well as coatings, often in combination with drug delivery systems. Van der Giessen et al. (1996) evaluated several bioabsorbable polymers, including poly(3HB-co-3HV), as candidate biomaterials for cardiovascular stents. Strips of polymer were deployed on the surface of coil wire stents, and implanted in porcine coronary arteries of 2.5–3.0 mm diameter. After four weeks, most of the materials tested, including poly(3HB-co-3HV), had provoked extensive inflammatory responses and fibrocellular proliferation. However, these results were shown to be inconsistent with *in vitro* results, and that other factors such as implant geometry, implant design, and degradation products may have been responsible for some of the observed response. It was also noted that the polymers were not sterilized prior to implantation.

The homopolymer, poly(3HB), has also been fabricated into a cardiovascular stent (Schmitz and Behrend, 1997), and tested in a rabbit model (Unverdorben et al., 1998). It was also reported that poly(3HB) stents plasticized with triethyl citrate (Behrend et al., 2000b) and fabricated by laser cutting of a molded construct had an average elastic recoil of about 20–24% immediately after dilation, and of 27–29% after 120 h *in vitro* (Behrend et al., 1998). After implantation into the arteries of rabbits, the poly(3HB) stents instigated a temporary intimal proliferation, and were observed to degrade fairly rapidly *in vivo*.

#### 7.1.5

##### **Vascular Grafts**

Vascular grafts are currently inserted to repair or replace compromised blood vessels in arterial or venous systems that have been subjected to damage or disease, for example atherosclerosis, aneurysmal disease or trau-

matic injury. For the larger-diameter vessels, synthetic grafts are frequently employed, and these can be impregnated with protein to make them completely impervious to blood, and thereby ready for anastomotic procedures. Several studies have shown, however, that when protein substrates are impregnated into grafts they may promote undesirable immunological reactions. In order to try and develop an improved sealant for a synthetic graft, Noisshiki and Komatsuzaki (1995) investigated the possible use of poly(3HB-co-4HB) as a graft coating, the coated grafts being implanted into dogs and examined at 2 and 10 weeks. Subsequently, it was noted that degradation of the polymer had already started after two weeks.

Marois et al. (1999c, 2000) investigated the use of poly(3HO-co-3HH) as an impregnation substrate. Polyester grafts impregnated with poly(3HO-co-3HH) were implanted in rats, and compared with both protein- and fluoropolymer-impregnated grafts for periods ranging from 2 to 180 days. No infiltration of tissue into the poly(3HO-co-3HH)-impregnated graft occurred because of the presence of the polymer; moreover, polymer degradation was found to be very slow with just a 30% reduction in molecular weight after six months. Tissue response, after an initial acute phase seen in all grafts, was reported as generally mild, and additional investigations with this biomaterial were recommended.

The elastomeric polymer, poly(3HO-co-3HH), has also been evaluated as a component of an autologous cell-seeded tissue-engineered vascular graft in lambs (Shum-Tim et al., 1999). Tubular conduits (7 mm diameter) comprising a nonwoven PGA mesh on the inside and layers of poly(3HO-co-3HH) outside were prepared, and seeded with a mixed cell population of endothelial cells, smooth muscle cells, and fibroblasts, obtained by the expansion of

sections of carotid artery harvested from lambs. After seven days, the seeded conduits were used to replace 3–4-cm abdominal aortic segments in lambs. The lambs were sacrificed at 10 days and 5 months after surgery, and the conduits compared with unseeded controls. All control conduits became occluded during the study, but the cell-seeded tissue-engineered grafts remained patent (open to blood flow), except for one stricture, and no aneurysms were observed. This contrasts sharply with results obtained using a polyglactin-PGA composite that was limited by high porosity, stiffness, and a relatively short degradation time, and developed aneurysms within a few weeks. Histologic analysis of the poly(3HO-co-3HH)-PGA grafts was reported to reveal an insignificant inflammatory response, with increased cell density, collagen formation and mechanical properties that were approaching those of native aorta.

It has also been proposed that poly(3HB) might be used to repair severed blood vessels by the insertion of a tube of this material (Baptist and Ziegler, 1965).

#### 7.1.6

##### Heart Valves

Perhaps the most remarkable results with PHA polymers have been obtained in the development of cell-seeded tissue-engineered heart valves. These valves promise to provide unique solutions to the deficiencies of mechanical and animal valves currently in clinical use, such as the need for anticoagulant therapy and repeat surgery to replace defective or even outgrown valves in young children. In initial studies in this area, scaffolds seeded with autologous vascular cells and based on porous PGA and PLA had been used to replace a single pulmonary valve leaflet in lambs, but attempts to replace all three pulmonary valve leaflets had failed due to the relatively high stiffness and

rigidity of the PGA-PLA biomaterials. However, when the leaflets were replaced with porous poly(3HO-co-3HH), and sutured to a conduit composed of a poly(3HO-co-3HH) film sandwiched between layers of PGA mesh, these difficulties were overcome (Stock et al., 2000b). Echocardiography of the seeded constructs implanted in lambs indicated no thrombus formation with only mild, nonprogressive, valvular regurgitation up to 24 weeks after implantation. Histologic examination revealed organized and viable tissue, without thrombus formation. Notably, thrombus developed on all leaflets in the unseeded control scaffolds after four weeks. After six weeks *in vivo*, no PGA remained, but poly(3HO-co-3HH) was still substantially present with a slight reduction in molecular weight (26%). A variant of this scaffold, with leaflets derived from poly(3HO-co-3HH) sandwiched between PGA mesh, has also been evaluated under pulsatile flow *in vitro* (Sodian et al., 1999). Under these conditions, it was shown that vascular cells attached to the scaffold, proliferated, and oriented in the direction of flow after four days.

The design of the heart valve scaffolds have been further refined in subsequent studies, and other PHA polymers have also been evaluated (Sodian et al., 2000a). Sodian et al. (2000b,c,d) also described the fabrication of a functional tissue-engineered heart valve entirely from porous poly(3HO-co-3HH), and seeding of the scaffold with vascular cells from ovine carotid artery which resulted in cellular in-growth into the pores and formation of a confluent layer under pulsatile flow conditions.

Recently, in one of the most astonishing results of tissue engineering described to date, Hoerstrup et al. (2000) succeeded in developing a PHA-based heart valve scaffold in lambs that was completely replaced at eight weeks by a functional trileaflet heart

valve. Two components contributed to this success. First, the use of poly(4HB), which was coated on a PGA mesh to provide a rapidly degrading yet flexible scaffold; and second, the use of an *in vitro* pulse duplicator system. After 20 weeks *in vivo*, the mechanical properties of the valve were reported to resemble those of the native valve, and histologic analysis showed uniform, layered cuspal tissue with endothelium (see Figure 8 of Schoen and Levy, 1999). Echocardiography demonstrated mobile, functioning leaflets without stenosis, thrombus, or aneurysm to 20 weeks, and importantly, the inner diameter of the valve construct was found to have increased from 19 mm at implant to 23 mm at 20 weeks. The latter finding is particularly exciting for the development of a tissue-engineered heart valve that can be used in young children, and will grow with the child.

## 7.2

### Dental and Maxillofacial

#### 7.2.1

##### Guided Tissue Regeneration

In guided tissue regeneration, barrier membranes are used to encourage regeneration of new periodontal ligament by creating a space or pocket that excludes gingival connective tissue from the healing periodontal wound, and also by preventing the downgrowth of epithelial tissue into the wound. Galgut et al. (1991) evaluated the histologic response of rats to poly(3HB-co-3HV) membranes that could be used in this application, and found that the membranes were well tolerated. Compared with Gore-Tex™ (polytetrafluoroethylene, PTFE) membranes, little downgrowth of epithelial tissue was observed with poly(3HB-co-3HV).

During the closure of palatal defects, mucoperiosteal flaps are frequently moved to the midline of the palate, leaving two areas

of denuded bone adjacent to the dentition. These wounds heal by migration of keratinocytes and fibroblasts, as well as by wound contraction. Later, the formation of scar tissue occurs, and it is believed that attachment of this tissue to areas of the denuded bone can disturb subsequent maxillary growth, or might affect the development of dentition. Using beagle dogs, Leenstra et al. (1995) investigated the use of nonporous poly(3HB-co-3HV) films to keep the mucoperiosteum and bone separated until the transition of teeth was completed (at about 24 weeks). At two weeks, it was reported that one of the poly(3HB-co-3HV) films had deformed; however, this was attributed to the method of insertion. At 8 and 12 weeks, the films were unimpaired and surrounded by fibrous capsules, and it was concluded that poly(3HB-co-3HV) films were more suitable for this procedure in terms of mechanical properties and tissue response than was PLA or PCL.

### 7.2.2

#### **Guided Bone Regeneration**

In addition to using barrier membranes to create new periodontal ligament, membranes can also be used to generate new bone in jaw bone defects, as well as to increase the width and height of the alveolar ridge. Kostopoulos and Karring (1994a) reported successful bone regeneration in jaw bone defects in rats using poly(3HB-co-3HV) membranes to create spaces for bone fill. Mandibular defects were produced in rats and either covered with poly(3HB-co-3HV) membranes, or left uncovered. During the following 15 to 180 days, increasing bone fill was achieved with poly(3HB-co-3HV) membranes, whereas in the uncovered control group ingrowth of other tissues occurred, and only 35–40% of the defect area was filled with bone after 3–6 months. Kostopoulos and Karring (1994b) also used

poly(3HB-co-3HV) membranes successfully to increase the height of the rat mandible. In two-thirds of the rats, the space created by the poly(3HB-co-3HV) membrane was completely filled with bone by six months, although in some cases soft tissue migrated through ruptures in the membranes, thereby inhibiting bone formation. In contrast, bone formation was negligible when membranes were not used. It was noted that while this biomaterial might form the basis of a barrier membrane, some modifications to the physical properties would be required.

Barrier membranes of poly(3HB-co-3HV) have been reinforced with polyglactin fibers and used to cover dental implants placed in fresh extraction sockets in dogs (Gotfredsen et al., 1994). However, very poor results were obtained. After 12 weeks, inflammatory infiltrates were seen adjacent to the poly(3HB-co-3HV)-polyglactin membrane that interfered with bone healing, and less bone fill was observed compared with control sites with no membrane. Given the results of Kostopoulos and Karring (1994a,b), and the known inflammatory response of tissues to PGA degradation products, the observed result might be attributed to the polyglactin component of the membrane.

### 7.3

#### **Drug Delivery**

The potential use of poly(3HB) and poly(3HB-co-3HV) in drug delivery has been evaluated in a number of studies, and the field has been reviewed several times (Holland et al., 1986; Juni and Nakano, 1987; Koosha et al., 1989; Pouton and Akhtar, 1996; Nobes et al., 1998; Scholz, 2000). Studies have included investigations of these polymers as subcutaneous implants, compressed tablets for oral administration, and microparticulate carriers for intravenous use.

## 7.3.1

**Implants and Tablets**

In the early 1980s, Korsatko et al. (1983a,b) studied the release of a model drug, 7-hydroxyethyltheophylline (HET), from poly(3HB) tablets prepared by homogeneously compounding and compressing the polymer and the drug. In both *in vitro* and *in vivo* experiments, the drug was released in a linear manner, but the rate of release from subcutaneous implants in mice was found to be about two- to three-fold slower than *in vitro*. At a drug loading of 10%, sustained release was observed for approximately 10 weeks *in vitro*, and up to 20 weeks *in vivo*. At loadings up to 30%, release could be sustained for up to 50 days; however, at substantially higher levels (60–80%) all the drug was released within 24 h. Changes in tablet compaction pressures did not alter the release rates. In subsequent studies, Korsatko et al. (1987) evaluated the influence of poly(3HB) molecular weight on drug release, and found the release of the anti-hypertensive drug midodrin-HCl from compressed tablets to be increased as the polymer's molecular weight increased from 3000 to 600,000. In comparison, when poly(3HB)-coated granules of the beta-blocker, Celiprolol-HCl were prepared using a fluid bed dryer system, it was found that smaller amounts of the higher molecular-weight polymers were required to slow the release of Celiprolol-HCl, presumably because of improved film formation at higher molecular weight.

Gould et al. (1987) investigated the release of fluorescein, and dextrans labeled with fluorescein, from tablets of poly(3HB-co-3HV) prepared by direct compression. Consistent with the observations of Korsatko et al. (1983a,b), the rate of release was found to increase significantly at higher loadings. Faster rates of release were also observed as the percentage of valerate in the copolymer

was decreased, leading to reduced compressibility, and more rapid influx of fluid into the tablet. The higher molecular-weight dextrans also released more rapidly than fluorescein, but this was attributed to the creation of more porous and hydrophilic matrices and led to the testing of porosigen (pore-forming) additives as a means of controlling the rate of drug release. Two additives, microcrystalline cellulose and lactose, were tested, and both were found to enhance the rate of drug release from the modified tablets.

Release profiles of 5-fluorouracil (5-FU) from melt-pressed poly(3HB) disks have been examined *in vitro* over a range of drug concentrations (10–50%) by Juni and Nakano (1987). The rate of release was found to increase with drug loading, and complete release was observed in five days at a 50% loading. Gangrade and Price (1992) also used melt compression to prepare PHA drug delivery systems with lower porosity, but chose the lower-melting poly(3HB-co-3HV) copolymer, and incorporated progesterone at loadings of 5 to 50%. Consistent with the findings of others, increased loadings resulted in faster rates of release, with 85% of the drug being released in three days at a 50% loading compared with 50% at a 5% loading.

The homopolymer, poly(3HB), has been evaluated as a potential candidate for the development of a gastric retention drug delivery device that could extend the absorption period of a drug from the stomach (Cargill et al., 1989). However, further development was not pursued because no degradation of the device was observed in a 12-h period during an *in vitro* dissolution assay. Jones et al. (1994) developed an auricular poly(3HB-co-3HV) implant for cattle containing metoclopramide at a loading of 50% as a prophylactic treatment for fescue toxicosis (which is caused by cattle grazing on

endophyte-infected fescue). The implant was prepared by melt compression, and released metoclopramide at an effective rate of 12 mg per day *in vivo*.

Compression-molded compacts of poly(3HB) loaded with tetracycline have been evaluated in the treatment of periodontal disease (Collins et al., 1989; Deasy et al., 1989). Using *in vitro* studies, poly(3HB) compacts loaded with 50% tetracycline were developed that could deliver therapeutic levels of the antibiotic for eight to nine days. Six patients with gingivitis were treated with these compacts, and their saliva was monitored for the release of tetracycline. Therapeutic levels of tetracycline were detected over the 10-day study period, and an improvement in the gingival condition from moderate to mild inflammation was reported. However, when the treatment was stopped the improvement was not maintained.

Subcutaneous implants of poly(3HB) containing gonadotropin-releasing hormone (GnRH) have been tested for their ability to release this hormone, and stimulate luteinizing hormone (LH) secretion, promote preovulatory follicle growth, and induce ovulation in acyclic sheep (McLeod et al., 1988). In comparison with oil-based formulations, the poly(3HB) implants containing 40–50 µg of GnRH consistently produced elevated plasma levels of LH for periods of two to four days, and a high incidence of ovulation was obtained, particularly when two implants per animal were used.

Akhtar et al. (1989, 1991, 1992) investigated the release of a model drug, methyl red, from both solution-cast and melt-processed films of poly(3HB) and poly(3HB-co-3HV), and examined the effect of varying the temperature during polymer crystallization. It was found that the faster-crystallizing homopolymer, poly(3HB), was better able to trap methyl red than the slower-crystallizing

poly(3HB-co-3HV) copolymers, contributing to a slower release of the drug from the homopolymer *in vitro*.

Hasirci et al. (1998) described the development of poly(3HB-co-3HV) rods containing the antibiotic Sulperazone for the treatment of osteomyelitis. Rods loaded with 20 and 50% of the drug were prepared by adding granules of the antibiotic to poly(3HB-co-3HV) solvent solutions, and molding the resulting pastes into rods. These rods were then introduced into rabbit tibias containing metal implants infected with *Staphylococcus aureus* (obtained from chronic osteomyelitis patients). After 15 days the infection had been eradicated. A similar approach to the treatment of osteomyelitis using poly(3HB-co-3HV) rods containing sulbactam-cefoperazone has also been reported (Yagmurlu et al., 1999). Recently, Korkusuz et al. (2001) evaluated the use of poly(3HB-co-4HB) and poly(3HB-co-3HV) rods as antibiotic carriers for the treatment of osteomyelitis. A bone infection, experimentally induced with *S. aureus*, was effectively treated with solvent-blended rods containing Sulperazone or Duocid. It was noted that the poly(3HB-co-4HB) rods were preferred as they were less rigid and easier to handle than the poly(3HB-co-3HV) rods.

Kharenko and Iordanskii (1999) prepared poly(3HB) tablets containing the vasodilator, diltiazem, with drug loadings up to about 45%, and monitored release rates of these delivery systems *in vitro*. Near-complete release of the drug was observed at the highest loading concentrations, with slower release being observed at lower loadings.

### 7.3.2

#### **Microparticulate Carriers**

A number of groups have examined the potential use of poly(3HB) and poly(3HB-co-3HV) polymers as microparticulate carriers for drug delivery. In general, these systems

have been produced using solvent evaporation techniques, and variables such as drug loading, polymer composition, molecular weight, crystallization rate, particle size, and the use of additives have been investigated. Although there are exceptions, the following observations are fairly typical: (1) Increased valerate content in copolymers of poly(3HB-co-3HV) usually slows the rate of drug release, presumably because the copolymers are less crystalline than poly(3HB). Incorporation of valerate into poly(3HB) also tends to yield microparticles that are less susceptible to physical damage than poly(3HB); (2) Smaller particle sizes decrease loading capacity, but increase the rate of drug release; (3) Small changes in polymer molecular weight have little impact on release rates; however, large changes can increase crystallinity and lead to enhanced release rates; (4) Drug release from poly(3HB) and poly(3HB-co-3HV) is completed well before any significant degradation of the polymers has begun, so drug release is entirely diffusion controlled; and (5) Lower drug loadings reduce the release rate.

One of the earliest studies of poly(3HB) microspheres in drug delivery was carried out by Bissery et al. (1983, 1984a). Using a solvent evaporation technique,  $^{14}\text{C}$ -labeled poly(3HB) microspheres (1–12  $\mu\text{m}$ ) were prepared and found, as expected, to concentrate primarily in the lungs of mice upon intravenous administration. However, when the microspheres were loaded with an anticancer agent, lomustine [*N*-(2-chloroethyl)-*N'*-cyclohexyl-*N*-nitrosourea; CCNU], and administered to Lewis lung carcinoma-bearing mice, little effect was observed (Bissery et al., 1985). From *in vitro* studies it was found that the drug was completely released from the microspheres in 24 h at a loading of 7.4%, compared with a release time of over 90 h when PLA microspheres were used (Bissery et al., 1984b). Notably, the po-

ly(3HB) microspheres were found to be somewhat irregular in shape, which was attributed to the highly crystalline nature of the homopolymer.

Brophy and Deasy (1986) examined the release profiles of sulphamethizole from poly(3HB) and poly(3HB-co-3HV) microparticles (53–2000  $\mu\text{m}$ ) formed by grinding a solvent-evaporated matrix of these components, and reported that increased rates of release were observed when the molecular weight of poly(3HB) was increased. This observation was attributed to poor distribution of the drug in the highly crystalline poly(3HB) polymer. As anticipated, faster rates of release were also observed as the poly(3HB) particle size was decreased, and as drug loading was increased. Incorporation of valerate into the poly(3HB) polymer chain decreased the release rate, presumably on account of the improved distribution of the drug. Overcoating of the poly(3HB) microparticles with PLA reduced the burst effect (the initial, rapid release of the drug from the delivery device surface), as well as significantly reducing the overall release rate. A sustained release of sulphamethizole was demonstrated *in vivo* when poly(3HB) microparticles (425–600  $\mu\text{m}$ ) loaded at 50% were administered intravenously to dogs. Release was complete in about 24 h, and correlated well with *in vitro* results.

Slower release of several anticancer antibiotics, including doxorubicin, aclarubicin, 4'-*O*-tetrahydropyranyl doxorubicin, bleomycin, and prodrugs of 5-fluoro-2'-deoxyuridine from poly(3HB) microspheres has been reported (Juni et al., 1985, 1986; Juni and Nakano, 1987; Kawaguchi et al., 1992). For example, at a 13% loading of aclarubicin-HCl, poly(3HB) microspheres with a mean diameter of 170  $\mu\text{m}$  were reported to release only 10% of the drug over five days *in vitro*. The observed release rates could, however, be increased by the incorporating fatty acid

esters into the poly(3HB) microspheres. These esters were believed to facilitate drug release by forming channels in the poly(3HB) matrix (Kubota et al., 1988). Abe et al. (1992a,b) also reported faster release of an anticancer agent, lastet, from poly(3HB) microspheres when acylglycerols were incorporated into the microspheres.

*In vivo*, poly(3HB) microspheres containing two prodrugs of 5-fluoro-2'-deoxyuridine were reported to induce higher antitumor effects against P388 leukemia in mice when compared with administration of the free prodrugs over five consecutive days (Kawaguchi et al., 1992).

Koosha et al. (1987, 1988, 1989) reported the use of high-pressure homogenization to produce poly(3HB) nanoparticles containing prednisolone. At drug loadings up to 50%, a biphasic release pattern was observed *in vitro*, with an initial burst effect followed by a slow release of the drug that was complete in one to two days. Similar results were observed with tetracaine. Akhtar et al. (1989) has also prepared smaller poly(3HB) particles (20–40  $\mu\text{m}$ ) by spray-drying, and reported fairly rapid release of a model drug (methyl red) from this matrix.

Microspheres of poly(3HB) have been evaluated *in vivo* as controlled release systems for the oral delivery of vaccines that could potentially protect vaccine antigens from digestion in the gut, and target delivery to Peyer's patches (Eldridge et al., 1990). When a single oral dose of poly(3HB) microspheres containing coumarin was administered to mice, very good absorption of microspheres (of diameter < 10  $\mu\text{m}$ ) was observed in the Peyer's patches 48 h later.

Conway et al. (1996, 1997) have evaluated the adjuvant properties of poly(3HB) microspheres *in vivo*, and observed a potent antibody response to encapsulated bovine serum albumin (BSA), with the highest titer being generated to preparations containing

particles with the smallest sizes. Linhardt et al. (1990) also considered using poly(3HB) and poly(3HB-co-3HV) as vaccine delivery vehicles.

The effect of several different parameters on the release of progesterone (a low molecular-weight model drug) from poly(3HB) and poly(3HB-co-3HV) microspheres prepared with an emulsion solvent evaporation technique has been evaluated by Gangrade and Price (1991). Using scanning electron microscopy (SEM), the use of gelatin as an emulsifier was shown to provide more spherical microspheres than when using polyvinyl alcohol, sodium lauryl sulfate or methyl cellulose, and that smoother surfaces were obtained when the solvent was switched from chloroform to methylene chloride. Generally, poly(3HB) microspheres had very rough surfaces which became smoother as valerate was incorporated. Interestingly, release of the drug from poly(3HB-co-9%3HV) was slower than from either poly(3HB) or poly(3HB-co-24%3HV). Examination of the internal surfaces of these microspheres by SEM revealed fewer cavities and less porosity for the poly(3HB-co-9%3HV) microspheres, and this was consistent with the slower release observed. Porosity was also found to decrease when the microspheres were prepared at increasing temperatures between 25 and 40 °C. Typically, 60–100% of the drug was released from these microspheres in 12 h at drug loadings up to 12%.

Embleton and Tighe (1992a,b) also investigated the effects of increasing the valerate content of poly(3HB-co-3HV), temperature, and molecular weight on microsphere formation, and obtained results consistent with those of Gangrade and Price (1991). When 10–50% PCL was incorporated into these poly(3HB-co-3HV) microspheres, a systematic increase in porosity was observed with increasing PCL content, that was attributed

to the elution of PCL from the hardened microcapsule wall once the poly(3HB-co-3HV) had precipitated during formation (Embleton and Tighe, 1993). Porosity was also found to increase significantly when polyphosphate-Ca<sup>2+</sup> complexes were introduced into poly(3HB-co-3HV) microspheres (Gürsel and Hasirci, 1995).

Atkins and Peacock (1996b) also used PCL to prepare microcapsules (21–200 µm) from a blend of poly(3HB-co-3HV)/PCL(20%) with an inner reservoir of BSA loaded in an agarose core. The protein encapsulation was, however, low (< 12 %) and only slightly influenced by loading. Release from the microcapsules loaded with up to 50% protein was observed *in vivo* for about 24 days.

Microspheres of poly(3HB) have been evaluated as potential embolization agents in renal arteries (Kassab et al., 1999), and with rifampicin as a chemoembolization agent (Kassab et al., 1997). Release of rifampicin during *in vitro* studies was consistent with other controlled release studies, with near-complete release of the drug being observed at high loadings (40%) in 24 h, and a somewhat prolonged release at lower loadings. Renal angiograms obtained before and after embolization with poly(3HB) microspheres (120–200 µm) using a contrast agent showed that 10 mg of the microspheres was sufficient to slow renal arterial blood flow, with subsequent partial occlusion of the pre-capillaries in two adult dogs. When a second injection was given, complete embolization was achieved. Histopathologic examination of the kidneys revealed changes consistent with renal artery obstruction and blockade of the blood supply to the kidneys.

In addition to using poly(3HB) compacts, microspheres of poly(3HB-co-3HV) have been used to deliver tetracycline and its hydrochloride salt for the treatment of periodontitis (Sendil et al., 1998, 1999).

Depending upon valerate content, between 42 and 90% of the tetracycline was released at 100 h, with loadings up to about 11%. Interestingly, it was found that molecular weight variation in the poly(3HB-co-3HV) compositions tested might have influenced the observed release of tetracycline. Encapsulation efficiency of the tetracycline hydrochloride salt was significantly less than the neutral form of the antibiotic.

A novel approach for preparing PHA drug delivery systems (Nobes et al., 1998) involves encapsulating a given drug during the *in vitro* enzymatic formation of poly(3HB) granules (Gerngross and Martin, 1995). Using this technique, it was possible to encapsulate approximately 4.5% of a model drug compound, Netilmicin, compared with between 4.1 and 17% using solvent evaporation.

Wang and Lehmann (1999) recently prepared poly(3HB) microspheres containing levonorgestrel with an average particle size of 64 µm, and found that this system prolongs the release of the drug by 1.8-fold compared with administration of the drug alone. The microspheres were also found to be effective *in vivo*, inducing a contraceptive effect in mice. Chen et al. (2000) recently described microspheres (30–40 µm) loaded with diazepam, and reported the characteristic biphasic release pattern with an initial burst effect.

Andersson et al. (1999) disclosed the use of supercritical fluid technology to incorporate the water-insoluble *Helicobacter pylori* adhesion protein A (HpaA) into poly(3HB) particles. This was achieved by preparing an emulsion with the protein, polymer, and methylene chloride, and then extracting the organic solvent from the emulsion with supercritical carbon dioxide to induce particle formation. Particles containing 0.6% protein were produced, with sizes of 1–3 µm.

## 7.4

**Prodrugs**

In 1999, it was discovered that poly(4HB) could be used as a prodrug of 4-hydroxybutyrate (Williams and Martin, 2001). In this study, rats were dosed (by gavage) with low molecular-weight polymers of poly(4HB) ( $138 \text{ mg kg}^{-1}$ ), and the serum was assayed for the presence of monomer. The serum concentration of 4-HB increased to  $\sim 86 \text{ }\mu\text{M}$  within 30 min, and remained elevated at approximately three- to five-fold the baseline value ( $9 \text{ }\mu\text{M}$ ) for about 8 h. In contrast, administration of the monomer resulted in a characteristic rapid increase to  $182 \text{ }\mu\text{M}$  within 30 min, followed by a rapid decrease to baseline within 2 h. The prolonged release of the monomer from poly(4HB) might potentially be beneficial in the treatment of narcolepsy, alcohol withdrawal, and several other indications. The therapeutic potential of poly(4HB) was also alluded to recently (Sudesh et al., 2000).

## 7.5

**Nerve Repair**

Partly on account of the piezoelectric properties of poly(3HB), interest has arisen in the use of this polymer for nerve repair (Aebischer et al., 1988). The basic approach involves aligning severed nerve ends within a small tube of poly(3HB), thus avoiding the need for sutures. Hazari et al. (1999a) and Ljungberg et al. (1999) evaluated the use of a nonwoven poly(3HB) sheet as a wrap to repair transected superficial radial nerves in cats for up to 12 months. Axonal regeneration was shown to be comparable with closure with an epineural suture for a nerve gap of 2–3 mm, and that the inflammatory response created by poly(3HB) was also similar to that found in primary epineural repair. In a subsequent study, the same

material was used to bridge an irreducible gap of 10 mm in rat sciatic nerve, and the results were compared to an autologous nerve graft (Hazari et al., 1999b). Good axonal regeneration in the poly(3HB) conduits with a low level of inflammatory infiltration was observed over 30 days, although the rate and amount of regeneration in the poly(3HB) conduit did not fully match that of the nerve graft.

## 7.6

**Nutritional Uses**

## 7.6.1

**Human Nutrition**

Several groups have evaluated oligomeric forms of the ketone body, R-3-hydroxybutanoic acid, as an alternative to the sodium salt of the monomer, for potential nutritional and therapeutic uses. Use of these polymeric forms might provide controlled release systems for the monomer and, importantly, overcome the problems associated with administering large amounts of sodium ion *in vivo*. Tasaki et al. (1998) reported the results of infusing dimers and trimers of R-3-hydroxybutyrate into rats, as well as exposure of these compounds to human serum samples and liver homogenate. Although mixtures of these compounds were not hydrolyzed by human serum, the monomer was liberated upon exposure to the liver homogenate as well as after infusion in rats. *In vitro*, the monomer was also liberated after incubation with the enzyme carboxylesterase.

Veech (1998, 2000) and Martin et al. (2000) have evaluated oligomers and oligolides of R-3-hydroxybutyrate *in vivo*, and observed release of the ketone body over prolonged periods. Potential uses of these delivery systems might include seizure control, reduction of protein catabolism, appetite suppression, control of metabolic disease, use in

parenteral nutrition, increased cardiac efficiency, treatment of diabetes and insulin-resistant states, control of damage to brain cells in conditions such as Alzheimer's, and treatment of neurodegenerative disorders and epilepsy.

#### 7.6.2

##### Animal Nutrition

The potential use of poly(3HB) and poly(3HB-co-3HV) polymers as a source of animal nutrition has been evaluated *in vivo*. Brune and Niemann (1977a) initially reported studies in rats, and subsequently described the digestion of poly(3HB) in pigs (Brune and Niemann, 1977b). In the latter work, when whole cells containing poly(3HB) were used as a nutrient source, approximately 65% of the poly(3HB) was excreted. Forni et al. (1999a,b) found the digestibility of poly(3HB-co-3HV) treated with sodium hydroxide to be increased when compared with the untreated polymer, in both sheep and pigs. Holmes (1988) also reported that poly(3HB) is degraded in the bovine rumen, while Peoples et al. (1999) studied the digestion of poly(3HB) and poly(3HO-co-3HH) in broiler chicks, concluding that the available energy from these polymers lies between that provided by carbohydrates and oils.

#### 7.7

##### Orthopedic

Several studies of the use of PHA polymers for internal fixation have been undertaken. Vainionpää et al. (1986) described the use of compression-molded T-plates, prepared from poly(3HB) reinforced with carbon fiber (7%), to fix osteotomies of the tibial diaphysis in rabbits. The implants were fixed to the tibia with absorbable PGA sutures, and compared with implants prepared from

reinforced Vicryl®. After 12 weeks, better results were obtained with the reinforced poly(3HB) plates relative to the Vicryl plates, with the latter frequently leading to nonunion of the osteotomies, breakage, and angulation.

Doyle et al. (1991) reported that poly(3HB) can be reinforced with hydroxyapatite (HA) to increase its stiffness to a level approaching that of cortical bone (7–25 GPa). At a HA loading of 40% wt., a poly(3HB)/HA composite had a Young's modulus value of 11 GPa, although strength decreased from ~40 MPa for poly(3HB) to ~20 MPa for the composite. Under *in vitro* conditions in buffered saline at 37 °C it was noted that the modulus of the filled poly(3HB) sample decreased more rapidly, falling from 9 GPa to 4 GPa over four months at a loading of 20% wt. HA. Bending strength of the same filled sample also fell by about 50% over the same time period, from 55 MPa to around 25 MPa. The behavior of poly(3HB) filled with HA was also studied *in vivo*. No significant differences between implants derived from poly(3HB) or poly(3HB) filled with HA were observed when rivets were prepared from these materials and inserted into predrilled holes in rabbit femurs. Over time, increased amounts of new bone were found on the implant surfaces, and by six months the implants were closely encased in new cortical bone. The overall tissue responses were considered favorable, and some indication of osteogenic activity for poly(3HB) was noted. Boeree et al. (1993) and Galego et al. (2000) have also studied the mechanical properties of poly(3HB) and poly(3HB-co-3HV)/HA composites, and concluded that they could serve as alternatives to corticocancellous bone grafts.

Since the homopolymer poly(3HB) is piezoelectric, it might help to induce new local bone formation if used as an implant. The addition of other additives might further

enhance this property, for example bioactive glasses that upon dissolution and deposition at an implant surface may encourage new bone formation. In this light, Knowles et al. (1991) studied the piezoelectric characteristics of poly(3HB-co-3HV) composites with glass fiber at 20, 30 and 40% wt., and found the piezoelectric potential output of these composites to be fairly close to that of bone. In subsequent studies, these composites were evaluated *in vitro* and *in vivo* (Knowles and Hastings, 1993a,b), and have also been studied with the inclusion of HA (Knowles et al., 1992). During *in vitro* studies of the poly(3HB-co-3HV)-glass composites, the glass component was found to be highly soluble in the polymer, and the observed weight loss was attributed to dissolution of the glass from the composites. These studies correlated well with observations *in vivo*. The poly(3HB-co-3HV)-glass composites were implanted subcutaneously, and as nonload-bearing femoral implants in rats. Initially, relatively high cellular activity was observed that was attributed to ions being released from the glass (and causing a soft tissue reaction), though this activity decreased with time. At four weeks in the femoral implants, cells could be seen entering the surface porosity formed by the solubilizing glass, and over time new bone was seen developing on the implant surface (Knowles and Hastings, 1993a,b). However, when composites of poly(3HB-co-3HV)/HA and poly(3HB-co-3HV)/HA-glass were implanted in rabbit femurs, and evaluated using a mechanical push-out test during the first eight weeks *in vivo*, it was found that the former bonded better than the latter. One explanation for this observation was attributed to the release of ions from the poly(3HB-co-3HV)/HA-glass composite inducing a soft tissue reaction that inhibited the formation of hard tissue at the implant surface (Knowles et al., 1992; Knowles, 1993).

In addition to composites with hydroxyapatite and glass, Jones et al. (2000) tested composites of poly(3HB-co-3HV) with tri-calcium phosphate filler in subcutaneous and femoral implants. These implants were compared with composites derived from PLA and tri-calcium phosphate, and found to degrade about four times more slowly *in vivo*.

In an *in vitro* study, Rivard et al. (1996) prepared highly porous foams of poly(3HB-co-3HV) and seeded these scaffolds with chondrocytes and osteoblasts. Maximal cell densities were achieved after 21 days, with cellular diffusion taking place throughout the porous foams.

## 7.8

### Urology

In the mid-1960s it was proposed that poly(3HB) could be used to repair a ureter by inserting a short tube of this material (Baptist and Ziegler, 1965). More recently, Bowald and Johansson (1990) described the use of poly(3HB-co-3HV) in the development of a tube for urethral reconstruction. A solution of the poly(3HB-co-3HV) copolymer was used to coat thin, knitted tubes of Vicryl, and the tubes were then implanted into four dogs to replace the urethra. After six to nine months, it was claimed that a fully functional urethra tissue had been reconstructed in all animals.

## 7.9

### Wound Management

#### 7.9.1

##### Sutures

As early as the mid-1960s it was suggested that poly(3HB) could be used as an absorbable suture (Baptist and Ziegler, 1965). Others have also proposed that poly(3HB-co-3HV) could be used as a suture coating,

and have coated braided sutures of PGA with solutions of this polymer (Wang and Lehmann, 1991).

#### 7.9.2

##### **Dusting Powders**

Holmes (1985) has produced powders of poly(3HB) with small particle sizes, and proposed that these can be used as medical dusting powders, particularly with surgical gloves.

#### 7.9.3

##### **Dressings**

Webb and Adsetts (1986) described wound dressings based on volatile solutions of poly(3HB) and poly(3HB-co-3HV) that could form thin films over wounds; these would be especially useful for emergency treatments. These films could potentially prevent airborne bacterial contamination of the wound, but would still be permeable to water vapor. As the preferred solvents are chlorinated hydrocarbons, however, these solutions might present other health hazards both to the patient and administrator. Steel and Norton-Berry (1986) also described wound dressings based on poly(3HB), their method involving the preparation of nonwoven fibrous materials of poly(3HB) and PHBV that could be used like swabs, gauze, lint or fleece.

Davies and Tighe (1995) evaluated the use of poly(3HB) fibers as a potential wound scaffold that could provide a framework for the laying down of a permanent dermal architecture. Using *in vitro* cell attachment assays with human epithelial cells, it was found that pre-treatment of the poly(3HB) fibers with either base or strong acid improved cell attachment and spreading.

Ishikawa (1996) described the implantation of poly(3HB-co-4HB) films in the abdominal cavity of rats between incisions in the skin and intestine to prevent coales-

cence. After one month, the incisions had substantially healed, and no coalescence had occurred. However, no degradation of the film *in vivo* was observed at one year.

#### 7.9.4

##### **Soft Tissue Repair**

In early studies of the potential uses of poly(3HB) it was proposed that this polymer could be used in the surgical repair of hernias (Baptist and Ziegler, 1965).

Patches of poly(3HB) with a smooth surface on one side and a porous surface on the other have been evaluated as resorbable scaffolds for the repair of soft-tissue defects, and specifically for the closure of lesions in the gastrointestinal tract (Behrend et al., 1999). Using *in vitro* cell culture, moderate adhesion of mouse and rat intestine fibroblasts to the patch material was observed. When the patches were sutured over incisions in the stomachs of rats that had been closed with two surgical knots, adhesion of the patch to the gastric wall was found to be better than that seen with Vicryl patches, and good ingrowth and tissue regeneration was evident on the porous side of the implanted poly(3HB) patch.

## **8**

### **Future Directions**

With technology now in place to allow the properties of PHA polymers to be tailored to specific applications, coupled with a significant increase in the need for new absorbable biomaterials, this class of polymers currently appears to have a bright future in medicine and pharmacy. Indeed, a wide new range of applications for PHA polymers has recently been described which includes their use as suture anchors, meniscus repair devices, interference screws, bone plates and bone plating systems, meniscus regeneration de-

vices, ligament and tendon grafts, spinal fusion cages and bone dowels, bone graft substitutes, surgical mesh and repair patches, slings, adhesion prevention barriers, skin substitutes, dural substitutes, bulking and filling agents, ureteric and urethral stents, vein valves, ocular cell implants, and hemostats (Williams, 2000; Williams et al., 2000).

## 9

## Patents

Patents cited in studies described in this chapter are listed in Table 2.

Tab. 2 Patents referenced in this work.

<b>Patent Number</b>	<b>Assignee</b>	<b>Inventor(s)</b>	<b>Title</b>	<b>Date of Publication</b>
WO 88/06866	Brown University Research Found.	Aebischer, P., Valentini, R.F., Galletti, P.M.	Piezoelectric nerve guidance channels	September 22, 1988
WO 99/52507	Astra Aktiebolag	Andersson, M.-L., Boissier, C., Juppo, A.M., Larsson, A.	Incorporation of active substances in carrier matrixes	October 21, 1999
US 3,225,766	W.R. Grace and Co.	Baptist, J.N., Ziegler, J.B.	Method of making absorbable surgical sutures from poly beta hydroxy acids	December 28, 1965
EP 0 349 505 A2	Astra Meditec AB	Bowald, S.F., Johansson, E.G.	A novel surgical material	January 3, 1990
EP 0 754 467 A1	Astra Aktiebolag	Bowald S.F., Johansson-Ruden, G.	A novel surgical material.	January 22, 1997
US 5,116,868	John Hopkins University	Chen, C.-H., Chen, S.C.	Effective ophthalmic irrigation solution	May 26, 1992
EP 355,453 A2.	Kanegafuchi Kagaku Kogyo Kabushiki Kaisha	Hiraide, A., Katayama, M.	Use of 3-hydroxybutyric acid as an energy source	February 28, 1990
GB 2 160 208A.	Imperial Chemical Industries, Plc.	Holmes, P.A.	Sterilised powders of poly(3-hydroxybutyrate)	December 18, 1985
US 5,480,394 WO93/05824	Terumo Kabushiki Kaisha	Ishikawa, K.	Flexible member for use as a medical bag	January 2, 1996 Apr. 1, 1993
WO 99/32536	Metabolix, Inc.	Martin, D.P., Skraly, F.A., Williams, S.F.	Polyhydroxyalkanoate compositions having controlled degradation rates	July 1, 1999
WO 00/04895	Metabolix, Inc.	Martin, D.P., Peoples, O.P., Williams, S.F.	Nutritional and therapeutic uses of 3-hydroxyalkanoate oligomers	February 3, 2000

Tab. 2 (cont.)

<b>Patent Number</b>	<b>Assignee</b>	<b>Inventor(s)</b>	<b>Title</b>	<b>Date of Publication</b>
JP7275344A2	Nippon Zeon Co. Ltd.	Noishiki, Y., Komatsuzaki, S.	Medical materials for soft tissue use	October 24, 1995
WO 99/34687	Metabolix, Inc.	Peoples, O.P., Saunders, C., Nichols, S., Beach, L	Animal nutrition compositions	July 15, 1999
EP 0 770 401 A2.	Biotronik Mess- und Therapiegeräte GmbH & Co.	Schmitz, K.-P., Behrend, D.	Method of manufacturing intraluminal stents made of polymer material	February 5, 1997
US 4,603,070	Imperial Chemical Industries, Plc.	Steel, M.L., Norton-Berry, P.	Non-woven fibrous material	July 29, 1986
WO 98/41200 WO 98/41201	British Tehnology Group Ltd.	Veech, R.L.	Therapeutic compositions	September 24, 1998
WO 00/15216	BTG Int. Ltd.	Veech, R.L.	Therapeutic compositions	March 23, 2000
US 5,032,638	American Cyanamid Co.	Wang, D.W., Lehmann, L.T.	Bioabsorbable coating for a surgical device	July 16, 1991
GB 2,166,354	Imperial Chemical Industries, Plc.	Webb, A., Adsetts, J.R.	Wound dressings	May 8, 1986
WO 00/51662	Tepha, Inc.	Williams, S.F.	Bioabsorbable, biocompatible polymers for tissue engineering	September 8, 2000
WO 00/56376	Metabolix, Inc.	Williams, S.F., Martin, D.P., Skraly, F.	Medical devices and applications of polyhydroxyalkanoate polymers,	September 28, 2000
WO 01/19361A2	Tepha, Inc.	Williams, S.F., Martin, D.P.	Therapeutic uses of polymers and oligomers comprising gamma-hydroxybutyrate	March 22, 2001

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**KeyWords:**

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