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PEGylation and BioPEGylation of Polyhydroxyalkanoates: Synthesis, Characterisation and Applications

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1. Introduction

Chemical conjugations with poly(ethylene glycols) (PEGs) are established procedures to facilitate solubilisation of hydrophobic compounds. Techniques for PEGylation are currently applied to various hydrophobic pharmaceutical agents for drug delivery. More recently PEGylation has been applied to members of a novel family of microbial biopolyesters: the 'Polyhydroxyalkanoates' (PHAs). Complementing these chemical techniques, PEG modulation of bioprocessing protocols for the production of PHAs has shown that the strategic addition of certain PEGs not only supports hybrid synthesis, but may provide a technique for control of PHA composition and molecular mass. Furthermore, the addition of PEGs to the microbial cultivation systems can also result in end-capping of the hydrophobic polymer chains; a process termed 'bioPEGylation'. In this chapter we review the processes of PEGylation and bioPEGylation of PHAs as well as the influence they exert on the physiochemical, material and biological properties of the resulting natural-synthetic hybrid copolymer materials.

2. Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) are a family of microbial polyesters synthesized under conditions of environmental stress with excess of available carbon and one or more limiting nutrients (Doi, 1990; Steinbüchel & Fuchtenbusch, 1998; Lenz & Marchessault, 2005;). These biopolyesters are intracellularly sequestered as readily visible white refractile inclusion bodies and are considered to act as carbon reserves and possibly also serve as ion sinks; (Figure 1), (Anderson and Dawes, 1990; Foster, 2000).

First reported by Lemoigne in 1925, Poly(3-hydroxybutyrate), (PHB) is the most studied member of the PHA family and has a track record of commercial success. PHB is a homopolymer comprised of monomer 3-hydroxybutyric acid units (HBA). As one of the ketone bodies, HBA plays the vital role of carbon supply in mammalian systems responding to periods of prolonged starvation or suffering from diabetes mellitus (Bondy et al. 1949). Foster and Tighe have shown that microbial HBA is chemically identical to its mammalian counterpart and is recognised and processed by mammalian enzymes (Foster and Tighe 1995). Martin et al. subsequently patented its use, and that of its oligomers, as therapeutic agents for the treatment of insulin resistant states and appetite control as well as

neurodegenerative disorders and epilepsy (Martins et al. 1999). In addition, Reusch et al. have reported that mammalian cells also synthesise trace quantities of PHB which is proposed to serve a membrane transport function (Reusch et al. 1992). In fact, such low molecular weight PHB is widely distributed in cells and found in representative organism of nearly all phyla. Complexation of this PHB with a range of other macromolecules modifies the physical and chemical properties and as a consequence, this complexed PHB can also be found in cytoplasm (Reusch et al. 1992).

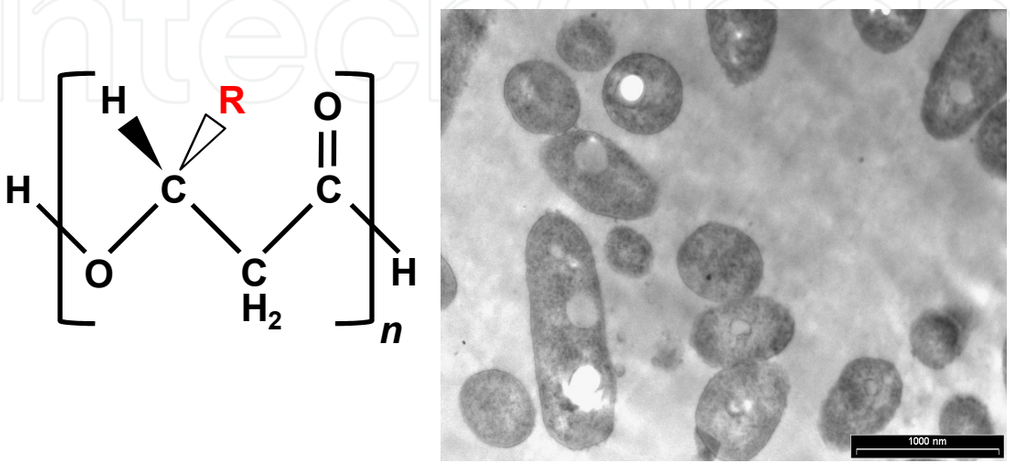

Fig. 1. Chemical formula for poly(3-hydroxyalkanoate)s and transmission electron micrograph PHA refractile inclusion bodies in *Pseudomonas oleovorans* (bar = 1,000nm).

As a consequence of its biocompatibility, PHB was initially pursued as a commercial biomaterial for various medical devices (Foster, 1996). However, its ability to successfully fulfil such a role is hampered by its relatively high crystallinity and brittle nature which also influence in degradation behaviour *in vivo* (Wu & Chen, 2009). These properties can be modified through copolymerisation with hydroxyvalerate to yield P(HB-*co*-HV) (Lenz, 1993; Yasin & Tighe 1993). The physiochemical and material properties of P(HB-*co*-HV) are more commercially appropriate and it is this copolymer which is the focus of much attention as a 'bioplastic'; a biodegradable replacement to conventional thermoplastics (Hänggi, 1995; Steinbüchel, 1996; Lenz & Marchessault, 2005).

Copolymerisation of PHB with random units of HV occurs through manipulation of bioprocessing systems, usually through variations in carbon feedstock. Further investigations in bioprocessing parameters have shown significant metabolic flexibility in a wide range of microorganisms for the synthesis of a spectrum of PHAs with over 105 different monomeric components. The properties of these PHAs range from the brittle and crystalline, as with PHB, to the flexible and elastomeric found in Poly(3-hydroyoctanoate-*co*-undecylenoate), (PHOU), (Steinbüchel, 1996; Steinbüchel, 2001). PHA synthesis is species dependent and while some species such as *Ralstonia eutropha* synthesise PHAs with comparatively short chian length substituents in the side chain (*scl*-PHA), many of the pseudomonads belonging to RNA homology Group I, produce PHAs with medium chain length alkyl groups in the side chain (*mcl*-PHAs) such as Poly(3-hydroxyoctanoate), (PHO). An additional group may be also be classified as PHAs possessing interesting chemically functional groups in their side chains (*fcl*-PHAs), such as cyanophenoxy and phenyl groups (Kim et al. 1995; Foster, 1996; Steinbüchel, 1996; Kim et al. 2000). In addition to variations in

side chain composition, PHAs with elongation of monomer units in the chain backbone have also been reported, such as Poly(3HB–*co*-4HB), (Nakamura et al. 1992).

3. Poly(ethyelene glycol)s (PEGs)

Poly(ethylene glycol)s (PEGs) are oligomers or polymers of ethylene oxide (Figure 2). These synthetic polymers, produced through acidic or base catalysed polycondensation reactions, constitute a family of neutral, water-soluble polyethers with various molecular weights ranging from the oily, viscous liquid of PEG with a molecular weight of 106, also known as diethylene glycol (DEG, PEG-106), to the waxy, crystalline solid of PEG-4000. PEGs with molecular weights around 25,000 contain no hydroxyl end group and are referred to as Polyethylene oxide (PEO), (Harris, 1992).

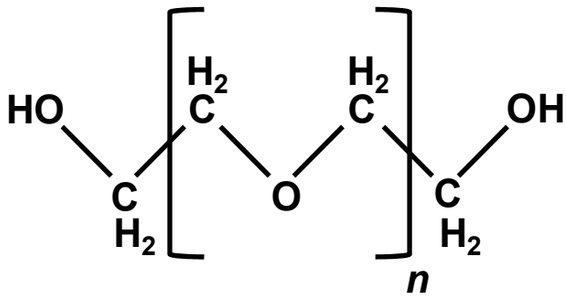

Fig. 2. Chemical formula for poly(ethylene glycol)s.

A variety of PEGs are employed as commodity chemicals in various industrial products ranging from cosmetics and lubricants to plasticisers and antifreeze agents. However some PEGs are biocompatible and utilised in the biomedical field, such as in the preservation of organs and tissues. Because of their hydrophilic nature, PEGs are also applied in the pharmaceutical industry to solubilise hydrophobic components and in protein purification. Protein-PEG conjugates exhibit a longer retention time in blood, this consequently supports their prolonged activity and, in the case of medication, longer dosing intervals. Classic examples include PEG-interferon α for the treatment of hepatitis-C and PEGylated granulocyte colony stimulating factor for neutropenia (Harris, 1992; Harris and Chess, 2003; Steward, 2005). In 1990, Krause and Bittner used PEGs with varying molecular weights to morphologically fuse severed axons in invertebrates and continuing research in this field has demonstrated that PEG can support nerve repair in mammals (Krause and Bittner, 1990; Borgens & Bohnert, 2001; Laverty et al. 2004).

4. PEGylation of PHAs

PEGylation is an established process for attaching or conjugating PEG chains to other molecules, including biomacromolecules such as peptides, proteins and antibody fragments for the production of biopharmaceuticals. PEGylation modifies the physiochemical properties including chain conformation, electrostatic binding and most noticeably, hydrophobicity. Conjugation of PHB with PEGs through chemical routes has been investigated by a number of authors.

Triblock copolymers of PHB-*co*-PEG-*co*-PHB have been synthesised by Kumagai et al. and Shuai et al. using ring-opening polymerisation of [R,S]-β-butyrolactone with PEG based

macroinitiators. These triblocks possessed atatic PHB segments and had good solubility but lacked sufficient crystallinity as solids. However, there are issues with atatic PHB which can have severe implications for biological properties such as biodegradability and biocompatibility (Kumagai et al. 1993; Shuai et al. 2000). Chen used such triblocks to form biodegradable nanoparticles for drug release, which showed better release characteristics than triblocks of PHB with poly(ethylene oxide), (PEO-PHB-PEO), (Chen et al. 2006). Low molecular weight PEGylated PHB triblocks also demonstrated self-assembly and formed strong hydrogels (Kerh et al. 2010).

In contrast to studies using synthetic PHB, Marchessault and coworkers used microbial PHB which are 100% isotatic, highly crystalline and insoluble in water. Diblock copolymers of this bacterial PHB with monomethoxy-PEG (mPEG) were synthesised using the dehydrating agent dicyclocarbodi-imide (DCC) and (dimethyleamino)pyridine (DMAP) as a catalyst (Figure 3). However, yields and reaction times were low (Marchessault and Yu, 1999). Using the same PHB and mPEG-2000 components, but with bis(2-ethylehexanote) tin as a catalyst in a transesterification reaction resulted in yields of up to 77% within 60 minutes. This single-step synthesis of mPEGylated PHB proceeded with a concomitant depolymerisation to lower molecular weights, such that molecular weights of the resulting hybrids were reduced to approximately 2,300 to 7,300 (Ravanelle and Marchassault, 2002).

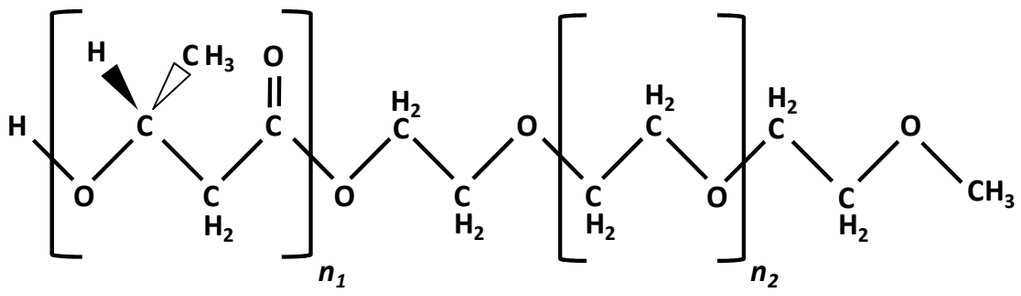

Fig. 3. Chemical formula for diblock copolymer of PHB (n_1) and mPEG (n_2).

As transesterification is a random chain scission process, its application for PEGylation of PHAs can result in hybrids with wide molecular polydispersity. By manipulating the experimental conditions, mPEG in the final diblock ranged from 23 to 53% of the weight and these segments reduced the melting point of the PHB as well as crystallisation behaviour (Ravanelle and Marchassault, 2002). The synthesis of such low molecular weight PEGylated PHB supports their application as soluble therapeutics and for drug delivery.

5. BioPEGylation of PHAs

5.1 Modulation of PHA composition using PEG

While copolymerisation of Poly(3-hydroxybutyrate) with Poly(4-hydroxybutyrate), P(3HB-*co*-4HB), results in copolymer with more favourable physiochemical and material properties than its P(3HB) counterpart, yields are low. PEG is known to associate with phospholipids' head groups in cell membranes, and this promotes an increase in membrane fluidity and consequently ions and small metabolites permeation (Yamazaki & Ito, 1990; Ingram & Buttke, 1984). In 1996, Gross and coworkers working on the theory that adding PEG-200 to the cultivation medium of the microorganism *Ralstonia eutropha* during the production of P(3HB-*co*-4HB) would promote the synthesis of PHAs with an enhanced composition of 4-

HBA units (Shi et al. 1996a). By extension of this reasoning, PEG modulated bioprocessing could also increase the diversity of monomeric units incorporated, potentially leading to the synthesis of new PHAs.

Shi et al. cultivated *R. eutropha* for the production of P(3HB-*co*-4HB) in Erlenmeyer flasks with PEG-200 loadings of 1 to 4% (w/v). When cultivated on 4-hydroxybutyrate as carbon source, *R. eutropha* synthesised a random copolymer with 3-HBA and 4-HBA monomeric units. However, addition of only 2% (w/v) PEG-200 to the bioprocessing significantly reduced the proportion of 3-HBA units from 34 to 11 mol %, with an increase in 4-HBA units from 66 to 86 mol %. Furthermore, this *scl*-PHA also contained approximately 3 mol % 3-hydroxyvalerate units, P(3HB-*co*-3HV-*co*-4HB), (Shi et al. 1996a). Steinbüchel and coworkers have compared the influence of PEG-200 to a variety of chemical inhibitors of fatty acid oxidation and the citric acid cycle to probe the influence of such polyethers on lipid and PHA synthesis in various *Rhodococcus* and *Nocardia* species (Alvarez et al. 1997). In this study they report that 0.2 to 5% (w/v) PEG-200 in the cultivation media caused decreases in *scl*-PHA contents in *R. ruber*, but under the conditions studied also stimulated greater incorporation of 3HV monomer units.

Foster and coworkers report that PHO produced by *Pseudomonas oleovorans* in the presence of 2% (w/v) PEG-106 had a significant change in the relative proportions of its C6, C8 and C10 monomeric hydroxyalkanoate components (Sanguanchaipaiwong et al. 2004; Foster et al. 2005). Bioprocessing using another strain of *P. oleovorans* in the presence of PEG-200 and PEG-400 for the production of a *fcl*-PHA with some degree of unsaturated bonds in the side chain, also saw a change in its composition, with the C8 component increasing from 47 to 56 mol % (Ashby et al. 2002). Results suggest that the presence of relatively low molecular weight PEGs in microbial growth media provided some measure of control over the PHA monomeric composition by promoting the main monomer in the carbon feed source. Therefore, *R. eutropha* cultivated with 4-hydroxybutyrate as carbon source incorporated more of these units in its *scl*-PHA, while different strains of *P. oleovorans* followed this trend for their *mcl*- and *fcl*-PHAs when cultivated with octanoic and oleic acids respectively (Ashby et al. 2002; Foster et al. 2005).

5.2 Modulation of PHA molecular weight using PEG

In addition to influencing PHA composition, the presence of PEGs in the bioprocessing systems also affected the molecular mass of the biopolymers (Figure 4). With the exception of *Azotobacter vinelandii* UWD, PEGs with molecular weights greater than approximately 2,000 had no apparent impact on the molecular weights of the *scl*-PHAs synthesised by a variety of microbial species. However, PEGs with molecular weights below 2,000 supported PHA chain termination and resulted in PHA molecular weights significantly below those for *scl*-PHAs synthesised in the absence of PEGs. The lower the PEG molecular weight the greater the PHA molecular weight reduction (Figure 4).

PHB synthesis by *R. eutropha* with PEG-350 and its methoxy end-capped derivatives in the cultivation media have shown that capping of one hydroxyl group on the PEG chain had no apparent affect on the ability of PEG-350 to interact with polymer biosynthesis and effect molecular weight change. However, when both hydroxyl groups on the PEG were capped molecular weight reduction did not occur (Shi et al. 1996b). These results suggest that PEGs interact with the biosynthetic system, presumably through the PHA synthase, to increase the rate of *scl*PHA chain termination relative to propagation.

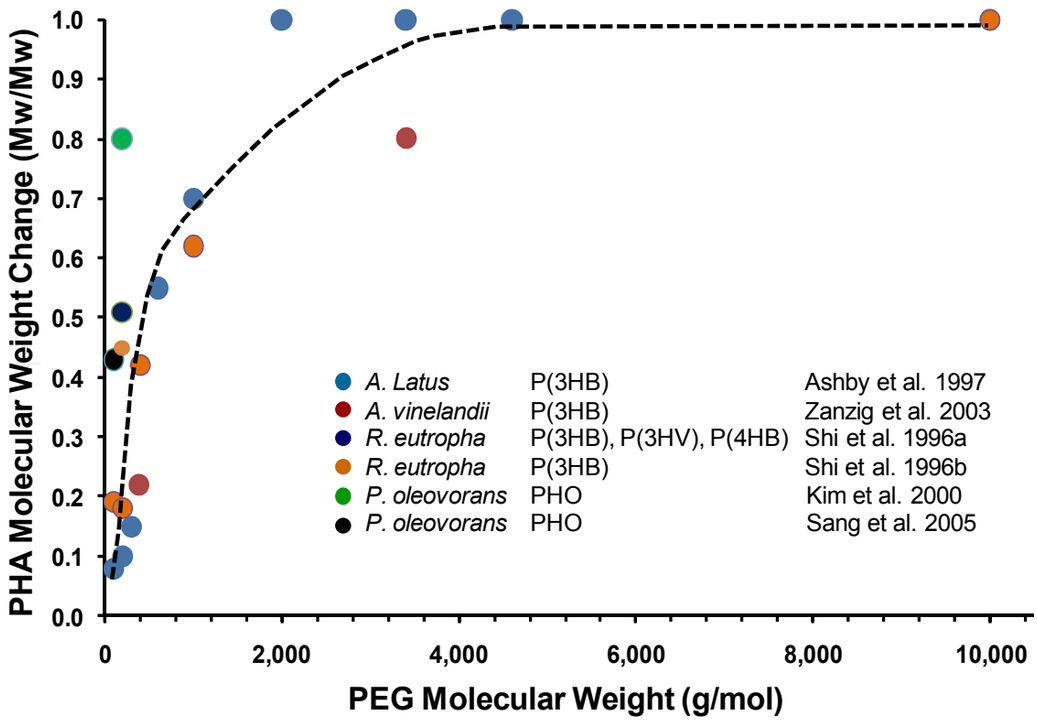

Fig. 4. Graph illustrating the relative change in molecular weight of PHAs due to presence of PEGs in the cultivation media.

Molecular weight is an important factor influencing the biodegradability and physico-mechanical properties of a biomaterial. Consequently, it has been suggested that PEGs may also offer some degree of PHA molecular weight control, a so-called 'tunable switch' (Ashby et al. 1999). The addition of 2% (w/v) PEG-200 to cultivations of *A. latus* and *R. eutropha* at different growth stages showed that the PHB synthesised had bimodal distributions of comparatively high and low molecular weights. In contrast, additions of PEG-200 at the beginning of the cultivations produced PHB with low to high ratios of 1:0.10 and 1:0.19 for *A. latus* and *R. eutropha* respectively; these ratios increased to 1:7.33 and 1:3.17 when the PEG was added during the established growth phases (Ashby et al. 1999).

The addition of comparatively low molecular weight PEGs to bioprocessing systems using P. *oleovorans* and *P. putida* induced manipulation of *mcl*-PHAs molecular weight. Additions of 2% (w/v) of PEG-106, PEG-200 or PEG-400 reduced the molecular weights of PHO synthesised by these species (Kim, 2000; Sanguanchaipaiwong et al. 2004). However, the impact appeared to be greater for *P. oleovorans* that *P. putida* with this *mcl*-PHA reduced to 40 and 80% respectively (Kim 2000).

5.3 Biosynthesis of PHA-PEG natural-synthetic hybrid copolymers

Modification of PHA molecular weight and composition during their biosynthesis is dependent upon the microbial species, the conditions of its cultivation and the molecular weight of PEG utilised, with PEGs possessing a relatively low degree of polymerisation influenced PHA compositions and reduced their molecular weights. Changes in molecular weight suggest that PEG molecules promote PHA chain scission. ^{1}H- and ^{13}C-NMR analysis of PHAs from PEG modulated cultivations support this, with results proving the

termination of PHA chains by the PEG chains (Madden et al. 1994; Shi et al. 2000b; Sangaunchaipaiwong et al. 2004; Foster et al. 2005). Thus, PHA chains are 'end-capped' with PEGs forming a new class of natural-synthetic hybrid block copolymers. This biological end-capping is referred to as 'bioPEGylation' (Foster, 2007).

Foster and coworkers have quantitatively monitored the concentrations of PEG-106 in cultivation media during the synthesis of PHO by *P. oleovorans* and for PHB production in *A. latus*. PEG-106 was also shown to be readily associated with the cell membranes and penetrated into the cytoplasm (Sanguanchaipaiwong et al. 2004; Foster et al. 2005; Sanguanchaipaiwong, 2007). ^{13}C-labelled ethylene glycol (EG) was utilised by Shah *et al.* to probe the influence of these polyethers on the PHB synthetic pathway of *R. eutropha*. The results clearly showed that the EG units acted as PHB chain terminators (Shah et al. 2000a, 2000b).

	EG	DEG	Polyethylene Glycol (PEG)											*reference*
Species	62	106	200	300	350	400	600	1,000	2,000	3,400	4,600	10,000		
***scl* PHA**														
Comomonas testosteroni **	Y	Y												Shah et al. 2000
Ralstonia eutropha *	Y	Y												Madden et al. 1999; Shah et al. 2000
" " **			Y	Y										Shi et al. 1996a
" " *		Y	N	Y	N	Y	N	Y	N				N	N " " 1996b
" " *			Y	N										Ashby et al.1999
Alcaligenes latus *			Y	N										" " "
" " *		Y	Y	Y	Y		Y	Y	Y	N		N	N	N Ashby et al. 1997
" " *		Y	Y			Y	N			N -	N –	N –		Zanzig et al. 2003
Azotobacter vinelandii UWD *		Y	Y			Y	N			N -	N -	N –		" " "
Pseudomonas oleovorans * *B14682*			Y	Y			Y	Y						Ashby et al. 2002
***scl* PHA + *fcl* PHA**			Y	Y			Y	Y						
P. oleovorans B778 *†														Ashby et al. 2002
***mcl* PHA**			Y	N			Y	N						
P. putida KT2442 °			Y	N			Y	N						Kim 2000
P. oleovorans ATCC29347 °		Y	Y	Y			Y	N						" "
" " " °														Sanguanchaipaiwong et al. 2005, 2007
***fcl* PHA**			Y	N			Y	N						
" " *B14683* ‡														Ashby et al. 2002

Table 1. Summary of PHA bioPEGylation and/or molecular mass reduction due to the addition of various molecular weight PEGs to microbial cultivations for the production of PHAs. (**Y** = molecular mass reduction, **Y**= bioPEGylation, **N** = No effect, **-** not measured), *(3HB); **(3HB)(4HB)(3HV); †90 mol% (3HB)+10 mol% fclPHA; °fclPHA, ‡PHO

The influence of PEG molecular weights on PHA hybrid biosynthesis and molecular weight reduction for a number of species is summarised in Table 1. From Table 1 it can be clearly seen that bioPEGylation of PHA chains does not always occur and appears to be favoured in cultivation systems where the PEG molecular weight is 300 or below. However, this is not entirely surprising given the original premise that these polyethers would support small molecule penetration into the cell.

From the summary in Table 1, we can speculate that PEGs with molecular weights up to 300 facilitate PHA chain scission as well as the synthesis of bioPEGylated hybrids. PEG molecular weights between 300 and 2,000 may support fluidity of the cell membrane but due to their large size, they fail to enter the cell and do not act as chain terminating agents. Finally, PEGs with molecular masses exceeding 2,000 do not appear to influence PHA

properties. While PEGs with molecular weights between 300 and 2,000 supported PHA composition and molecular mass control, these influences may be a function of the additives influence on cell viability.

3.4 Influence of PEGs on PHA bioprocessing parameters

Cell yields for *R. eutropha* for PHB production decreased by 30% as the concentration of PEG-200 in growth media increased from 1 to 4 % (w/v). In addition, the *scl*PHA yield also decreased by 50%. In companion experiments, the viability of this species was also shown to be reduced with the addition of 1 to 10% (w/v) of PEG-200 or PEG-400 (Shi et al. 1996b). Thus, higher concentrations of these comparatively low molecular weight PEGs in the microbial growth medium, the greater the reduction in cell viability. In contrast, increases in PEG molecular weight had comparatively less influence on cell viability. Thus, the addition of similar loadings of PEG-10,000 had no apparent effect on *R. eutropha* cell viability (Shi et al. 1996b). Studies reporting reductions in cell viability during PEG modulated cultivations for PHA production support the theory proposed here that PEGs of molecular weights between 300 and 2,000 influences PHA molecular mass and composition through modifications to cell viability in the bioprocessing system. In contrast, PEGs with molecular weights below approximately 300 modify PHA synthesis by acting as chain terminating agents forming natural-synthetic hybrids.

With the exception of PEG-10,000, the presence of PEGs in the cultivation media reduced the yield of *scl*-PHAs in *R. eutropha*. This was partly a consequence of their influence on cell mass (Shi et al. 1996b). Similar studies using *A. latus* have shown that PEGs with molecular weights between 106 and 600 had a greater impact on polymer synthesis compared to their higher molecular weight counterparts (1,000 - 10,000), with PHB productivity also being reduced depending on PEG concentration in the cultivation medium (Ashby et al. 1997). Thus, while 5% (w/v) PEG-106 in the growth media resulted in no PHA synthesis, 10% (w/v) of PEG-10,000 still showed some biopolymer synthesis, although the impact was greater than that observed when the studies were conducted with *R. eutropha* (Ashby et al. 1997). Similarly, additions to cultivations of *A. vinelandii* UWD of 2% (w/v) of various PEGs from PEG-106 to PEG-3400 also resulted in variable polymer yields (Zanzig et al. 2003). P(HB-*co*-HV) synthesis by *R. ruber* was influenced by the presence of 0.2-5% (w/v) PEG-200, with a reduction in *scl*-PHA yield of up to 50% (Alvarez et al. 1997).

Such variations in polymer yields are obviously due to the influence of PEGs on the bioprocessing systems. However, the utilisation of Erlenmeyer flasks in these studies limits their value. Foster and coworkers have cultivated *A. latus* for the production of PHB in the presence of 2% (w/v) PEG-106 using Braun 5 L bioreactors, which provides greater control over cultivation parameters. In these studies PEG was demonstrated to support an increase in cell mass (12%) as a consequence of the additional carbon, but through reduced microbial viability a reduction in cellular PHA synthesis was measured (9%), (Sanguanchaipaiwong, 2007). This increase in cell mass is consistent with the studies of Shah et al. who reported that EG was metabolised by *R. eutropha* for the production of PHB (Shah et al. 2000a)

Microbial PHA synthesis is species dependent; the diversity of *fcl- and mcl*-PHAs are significantly greater than their *scl*PHA companions. Kim investigated the influence of PEG-200 on the synthesis of *mcl*-PHAs by *P. oleovorans* and *P. putida* and reported that PHO yield was reduced with increasing PEG concentration, while at 8% PEG-200 loading no PHO synthesis was found (Kim, 2000). Using the same strain of *P. oleovorans* cultivated in the

presence of PEG-106, Foster and coworkers reported an increase in cell concentration but a decrease in PHO yield (Sanguanchaipaiwong et al. 2004). Similarly, the addition of PEG-200 or PEG-400 to another strain of *P. oleovorans* (B-778) primarily producing PHB but mixed with 10 mol% of a *fcl*PHA, also showed an increase in cell mass but no change in polymer yield. In contrast, other strains of *P. oleovorans* producing PHB (B-14682) and a *fcl*PHA (B-14683), showed no apparent change in cell mass but did show a reduction in their polymer yields (Ashby et al. 2002). Thus, the influence of PEGs on PHA synthesis also has a degree of species dependence.

4. Properties and potential

4.1 Physiochemical and material properties

The majority of current studies in this emerging field have investigated the influence of PEGs on PHA synthesis, with a focus on their potential to control composition and molecular mass of these biopolyesters. Thus, PEG modulated bioprocessing of PHAs may offer a technique to control PHA physiochemical and material properties. In addition, characterisations of natural-synthetic hybrids of bioPEGylated PHAs suggest that these hybrids may also possess additional properties when compared to their PHA counterparts (Zanzig et al. 2003; Foster et al. 2005). Concurrent cultivations of *A. latus* from the same inoculums have shown that the PHB-*co*-PEG106 hybrid, while possessing a lower molecular weight compared to its PHB counterpart, also had significantly different physiochemical and material properties. In particular the elongation to break increased from 8.4 to 20.6 % (Sanguanchaipaiwong, 2007). In contrast, the change in elongation-to-break for bioPEGylated *mcl*-PHA was not as noticeable, 580 (±9.4) and 540 (±8.3) MPa for PHO and PHO-*co*-PEG106 respectively.

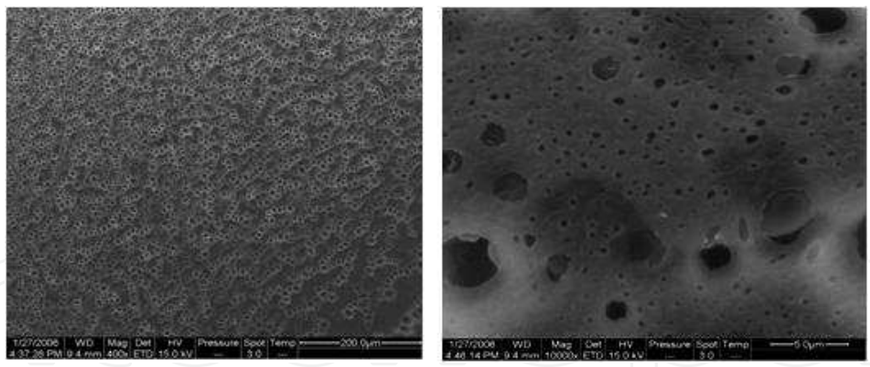

Fig. 5. SEMs of PHO-co-PEG106 films fabricated though solvent casting in the presence of humidity reveal the presence of pores not observed with PHO.

Small angle neutron scattering (SANS) was utilised to probe the chain conformations of protonated and deuterated *mcl*-PHA samples as well as their bioPEGylated hybrids. The comparatively small hydrophilic PEG group terminating much longer hydrophobic PHA chains exhibited no apparent influence on chain conformation when solvated in chloroform (Foster et al. 2008). However, with the addition of microquantities of water to the bioPEGylated PHA solutions, the hybrids formed stable microcrystalline suspensions. Consequently, when processed under humid airflow, bioPEGylated PHAs exhibited a degree of self-assembly resulting in the formation of disordered microporous films (Figure

5), (Foster et al. 2005; Sanguanchaipaiwong, 2007). Microporous and honeycomb films are of considerable interest based on their biotechnological and biomedical potential, such as immobilisation of biomolecules (Stenzel, 2002; Nishikawa et al. 1999).

4.2 Biological properties

Scholz and coworkers investigated the interactions of various bioPEGylated PHB samples with skin melanoma and human breast adenocarcinoma cells (Zanzig et al. 2003). In this study, cell adhesion was significantly affected by the presence of the PEG hydrophilic end groups, with reductions in cell attachments to hybrids ranging from 22 to 74%. The greatest reduction was found with PHB-*co*-PEG106, (Zanzig et al. 2003).

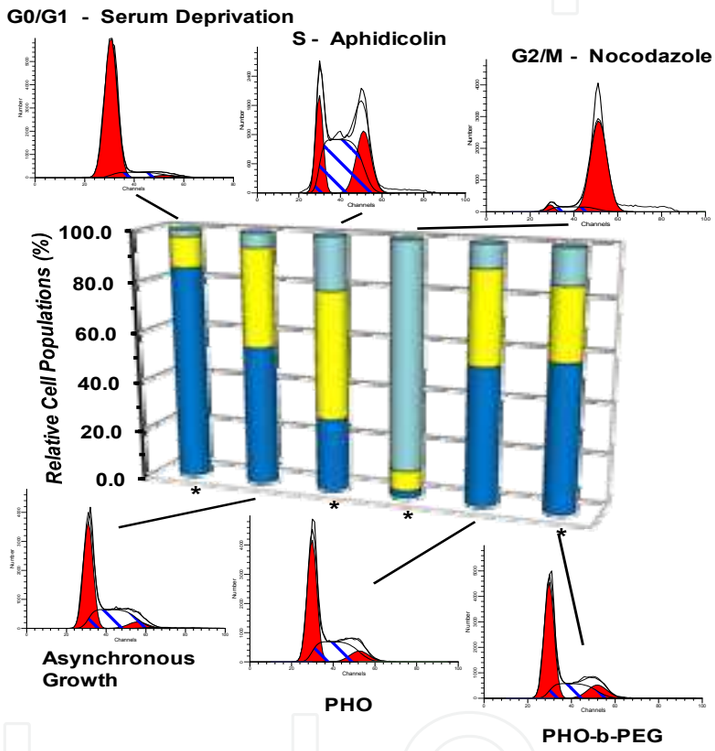

Fig. 6. Summary of relative cell growth populations determined from FACS sorting histograms, expressed as percentages of total populations. BioPEGylated PHO films showed statistically significant differences for cell populations in the G2/M ($p < 0.1$) and S ($p < 0.001$) phases, when compared to the same population cultivated in the absence of biomaterials. (* indicates statistically significant differences from asynchronous growth).

Figure 6 shows the changes in cell cycle when myoblastic satellite stem cells were cultivated in the presence of PHO and its bioPEGylated hybrid. The hybrid showed comparatively small but significant changes in the DNA population distributions for the stem cells compared to PHO, with significant differences between the two PHAs. Hence, the cycle of stem cell differentiation from G0-G1 through S to G2-M phase proceeded in the presence of PHO and its hybrid, but with significant differences in the respective populations of cells at these phases when compared to asynchronous growth, with the PHO-*co*-PEG106 showing the greatest deviation (Figure 6), (Marcal et al. 2008).

5. Conclusions and future trends

Conjugations of the *scl*-PHA, PHB with various PEGs, i.e, PEGylation, have been performed through chemical routes. The resulting diblocks and triblocks of this PHA have a low molecular weight and possess potential as pharmaceutical agents and polymers for injectable hydrogels or nanoparticles for drug delivery.

Strategic additions of PEGs to microbial cultivations for the production of *scl*- and *mcl*-PHAs not only permit a degree of control in PHA molecular mass and composition but may also provide routes for BioPEGylation. While bioPEGylated PHAs have a significantly reduced molecular weight compared to their PHA counterparts produced in the absence of PEG, they are considerable greater than the PEGylated counterparts and retain their material properties. Nevertheless, bioPEGylation permits significant changes in physiochemical and material properties, with subsequent implications for their material processing. Furthermore, the presence of hydrophilic end groups has significant implications for the biological properties of these hybrids, suggesting they may serve as novel biomaterials in their own right.

In general, PEGs with molecular weights below approximately 300-600 apparently increase membrane fluidity, supporting bioPEGylation. While PEGs with molecular masses from 600 to 2,000 facilitate PHA molecular mass and composition control, without bioPEGylation; PEGs above 2,000 appear to have little or no effect. For those PEGs exerting some degree of control over PHA synthesis, polymer yields are significantly reduced. Furthermore, this review suggests that, as with PHA biosynthesis, the influence of PEGs in cultivations systems may be strain specific.

Despite the molecular weight disparities between the hydrophobic PHA chain and it's comparatively tiny hydrophilic PEG terminal in bioPEGylated PHAs, there is sufficient evidence to suggest that the hybrid has subtly different physiochemical and material properties. Thus, work in this field is consistent with current trends to increase the diversification of PHAs and their conjugates (Hazer and Steinbüchel, 2007). Future work requires more detailed study of PHA-*co*-PEG hybrids as biomaterials, as well as investigations in the potential application of PEGs to bioprocessing technology for the production control of PHAs.

While PEGylation and bioPEGylation studies are still in their infancy, results to date suggest that these natural-synthetic hybrids have great potential as medical biomaterials.

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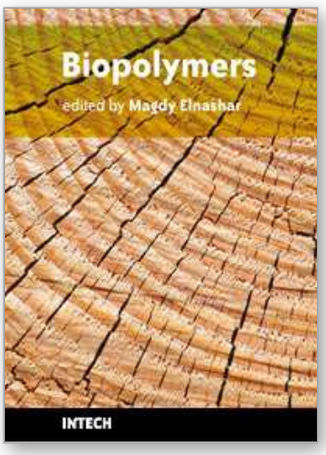

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Biopolymers are polymers produced by living organisms. Cellulose, starch, chitin, proteins, peptides, DNA and RNA are all examples of biopolymers. This book comprehensively reviews and compiles information on biopolymers in 30 chapters. The book covers occurrence, synthesis, isolation and production, properties and applications, modification, and the relevant analysis methods to reveal the structures and properties of some biopolymers. This book will hopefully be of help to many scientists, physicians, pharmacists, engineers and other experts in a variety of disciplines, both academic and industrial. It may not only support research and development, but be suitable for teaching as well.

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