Microbial production of biodegradable polymers and their role in cardiac stent development

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1. Introduction

Biodegradable polymers have attracted a lot of attention in the recent years. These biopolymers are large macromolecules composed of single, repeating monomer units. They are of very high molecular weight and their material characteristics vary according to the nature of their monomer composition. Over the last decade, depletion in the petroleum reserves has resulted in the emergence of biodegradable polymers as a potential alternative to the traditional plastics [1]. Also, increased production of these plastics has proven to be a serious threat to the environment. Being non biodegradable in nature, their disposal has raised major concern. Hence, biodegradable polymers are considered to be a potential solution. Apart from being biodegradable, some of these biopolymers are also biocompatible in nature and can be easily processed to be used for various applications. Hence, their applications range from being used in the packaging industry, chemical industry, agriculture and medicine. Considering these facts, large scale production of these biodegradable polymers and their extensive use is critical both to ensure alternative sources of plastic and also for the environment [2].

These biodegradable polymers can be grouped into two different classes i.e. natural and synthetic polymers. The former are obtained from natural sources and the latter require chemical synthesis. Natural polymers can be further classified into four different classes depending on their sources including agricultural, animal, marine and microbial sources. Those that are derived from the agricultural sources include polysaccharides, proteins and lipids which in turn include starches, lignocellulosic products such as pulp, and pectin. Biopolymers derived from animal sources are gelatin and collagen while marine sources are able to produce chitin which is processed into chitosan. Polymers derived from the microbial sources include polyhydroxyalkanoates (PHA) and polylactic acid (PLA). Polyhydroxyalkanoates are produced completely by microbial fermentation whereas polylactic acid is partially synthesised. The monomer, lactic acid, is produced by microbial fermentation and then polymerised using chemical catalysis. Natural polymers can be completely degraded by the microorganisms and degradation involves enzymatic scission of the polymer chain. The truncated polymer chain is later metabolized. Synthetic polymers on the other hand can be synthesised using bio derived monomers or synthetic monomers (precursors) derived from petroleum products [1]. In this chapter, microbial biopolymers, their production and their role in cardiac stent development will be discussed in detail.

1.1 Microbial polymers

As mentioned earlier, these polymers are produced by a range of microorganisms under various growth conditions. Polyhydroxyalkanoates are produced by the microbes as storage molecules under stress conditions such as excess carbon and limiting nitrogen [3, 4]. Lactic acid is produced by the group of lactic acid producing bacteria by fermenting hexoses into lactic acid. This purified lactic acid is used as the precursor for the chemical synthesis of PLA. Production of these biopolymers in an industrial scale is already well established. They have been produced using cheap carbon sources to achieve economical production [5].

1.2 Polyhydroxyalkanotes (PHA)

Polyhydroxyalkanoates (PHAs), Figure 1, are polyesters of 3-, 4-, 5- and 6-hydroxyalkanoic acids produced by a variety of bacterial species under nutrient-limiting conditions with excess carbon [3, 4, 6]. These water-insoluble storage polymers are biodegradable, exhibit thermoplastic properties and can be produced from renewable carbon sources. Thus, there has been considerable interest in the commercial exploitation of these biodegradable polyesters. PHAs are also known to be biocompatible and hence have the potential to be utilised for a range of biomedical applications [2].
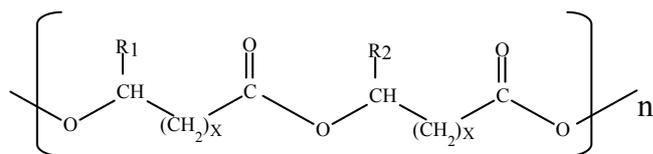
Figure 1: The general structure of polyhydroxyalkanoates (x = 1, 2, 3; n = 100-30000; R_1, R_2 = alkyl groups, C_1-C_{13})

PHAs can be classified into two main types, short chain length PHAs (SCL- PHAs) that have C_3-C_5 hydroxyacids as monomers and medium chain length PHAs (MCL-PHAs) that have C_6-C_{16} hydroxyacids as monomers. The mechanical properties of these PHAs vary from being quite brittle to extremely elastomeric [7]. The composition of the polymer synthesised is governed by the bacterial strain being used and the carbon source utilised to grow the bacteria. Hence, depending on the nature of the biomedical application, one can bioengineer the production of a PHA with suitable material properties. SCL-PHAs are highly crystalline, brittle and stiff whereas MCL- PHAs are elastomers with low crystallinity and low glass transition temperature [8].

1.3 The PHA biosynthetic pathways

There are three different pathways involved in the biosynthesis of polyhydroxyalkanoates. These include Pathway I, observed in *Cupriavidus necator*, which best describes the PHA biosynthetic pathway. In this pathway, 3HB monomers are generated by the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA with the help of the enzyme β-ketothiolase. The acetoacetyl-CoA reductase then acts on the acetoacetyl-CoA molecule to form 3-hydroxybutyryl-CoA. Finally, the PHA synthase enzyme catalyses the polymerisation via esterification of 3-hydroxybutyryl-CoA into poly(3-hydroxybutyrate), P(3HB), as described in Figure 2 [9].

Pathway II which is the fatty acid ß-oxidation pathway is mainly found in organisms such as *Pseudomonas aeruginosa* which leads to the production of hydroxyacyl substrates from carbon sources such as fatty acids. These hydroxyacyl substrates are then polymerised into PHA. This reaction is catalysed by the PHA synthase enzyme as shown in Figure 2 [7].

Pathway III is the fatty acid *de novo* pathway which is used by organisms when grown on sugars such as glucose as the carbon source. The (R)-3-hydroxyacyl intermediates from the fatty acid biosynthetic pathway are converted from their acyl carrier protein (ACP) form to the CoA form by the enzyme acyl-ACP-CoA transacylase (encoded by *phaG*) as shown in Figure 2. Therefore, this enzyme is the key link between fatty acid synthesis and PHA biosynthesis [7].
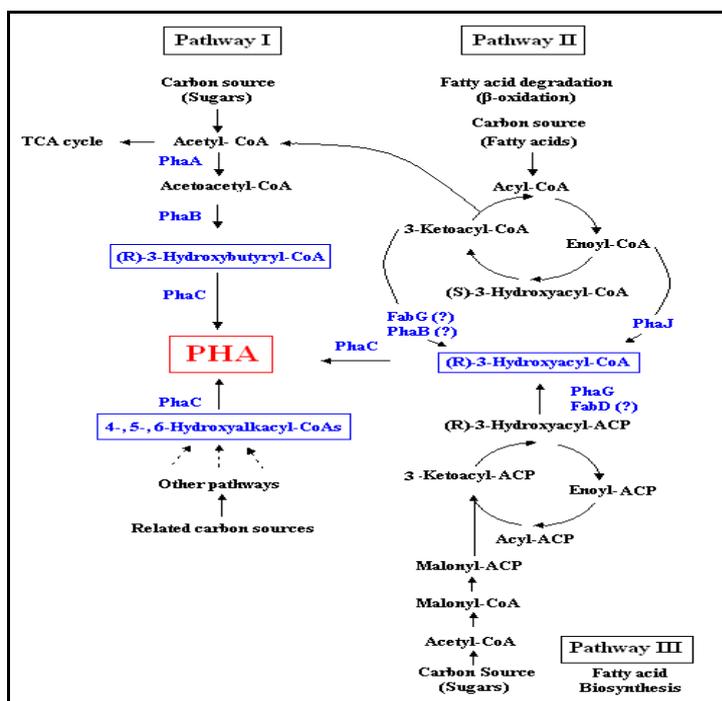

Figure 2: The three biosynthetic pathways that organisms use to produce PHAs [7].

1.4 SCL-PHAs

The most studied short chain length polyhydroxyalkanoate is poly(3-hydroxybutyrate) or P(3HB). This particular SCL-PHA has been extensively investigated since its discovery in 1920s. They were first produced from *Bacillus megaterium* but since then they have been produced using various other bacterial strains including soil bacteria and algae and also recombinant strains [10].

As discussed earlier, P(3HB) is almost 80% crystalline and has an extremely high melting point of 173 – 180 ºC. These polymers cannot be easily processed and are very brittle in nature. P(3HB) is degraded by the hydrolytic cleavage of the ester bonds resulting in surface erosion. P(3HB-co-3HV) is now commercially available under the trade name of Biopol from the American company, Metabolix in 2001 [11].

1.4.1 P(3HB) Production

P(3HB) is produced under specific fermentation conditions such as in the presence of excess carbon and limiting nitrogen. High yields of P(3HB) (80% of dry cell weight) could be achieved under such controlled conditions. Carbon sources such as glucose and sucrose are widely used for the production of P(3HB). Cheap carbon sources such as sugarcane molasses, rapeseed cake and whey are also being used for the economical production of P(3HB). El – Sayed and co-workers (2009) carried out the production of P(3HB) employing three different fermentation strategies. These included batch, fed batch and two stage fermentation processes. They concluded that the two stage batch culture was the most favorable method for the production of P(3HB) by *Cupriavidus necator* [12] .

Somleva and co workers (2007) have successfully produced P(3HB) in switchgrass (*Panicum virgatum*). This is the first successful expression of the multigene pathway in transgenic switchgrass plants [10].

P(3HB) being an intracellular product is extracted from the bacterial cells. Lee *et al.,* (1999) carried out production of P(3HB) from Gram negative bacteria such as *Cupriavidus necator*, *Alcaligenes latus* and recombinant *Escherichia coli* strains. They extracted P(3HB) using two different methods including chloroform extraction and NaOH digestion. The endotoxin level of the P(3HB) recovered using the chloroform method was less than 10 endotoxin units (EU) per g of P(3HB) whereas that recovered by the NaOH digestion method was higher than 104 EU/g of P(3HB). However, the endotoxin level was drastically reduced to 1EU/g by simply increasing the digestion time [10].

The mechanical and thermal properties of P(3HB) are crucial for their applicability. Akhtar and Pouton have determined the glass transition temperature (T_g) and the melting temperature (T_m) of P(3HB) to be -5 ±20 ºC and 160 ºC -180 ºC respectively [11]. P(3HB) can be processed into films, sheets and fibres. The tensile strength of P(3HB) films have been found to 43 MPa. X-ray diffraction studies have confirmed the crystalline nature of P(3HB) with a percentage crystallinity as high as 50-80% [13].

1.5 MCL – PHAs

The MCL-PHAs are more elastomeric than the SCL-PHAs, have low melting temperature and have a much lower level of crystallinity as compared to the SCL-PHAs due to an increase in the length of their side chain. The existence of any other polyhydroxyalkanoate apart from P(3HB) was first reported by Wallen and Rohwedder, 1974 [11]. They observed traces of PHAs containing six and seven carbon atoms alongside P(3HB). These observations were later confirmed by Witholt and coworkers, when they grew *Pseudomonas oleovorans* using octane as the carbon source and produced poly(3-hydroxyoctanoate) [11].

MCL-PHAs have different groups such as bromine, chlorine, fluorine, cyano, hydroxyl, branched alkyl, alkoxy, acetoxy, cyclohexyl and alkyl esters in their side chain that are produced by various organisms. These functional groups can be further modified in order to obtain polymers particularly suited for certain applications [14]. The elastomeric nature of MCL-PHAs make them particularly suitable for soft tissue engineering applications such as cardiac tissue engineering and other applications requiring elastic properties. However, the low melting points of MCL-PHAs cause them to be sticky at high temperatures. Also, their low tensile strength leads to some concern in certain load bearing applications. These properties can be modified by making composites and blends with inorganic and organic additives. The thermal and mechanical properties of Poly (3-hydroxyoctanoate), P(3HO), produced by *P. mendocina* is shown in the Table 1. The most commonly studied MCL- PHAs include Poly(3-hydroxyoctanoate), P(3HO) and Poly(3-hydroxyhexanoate-co-3-hydroxyoctanoate), P(3HHx-co-3HO**)** [14]**.**

Table 1: Properties of the MCL-PHA, Poly (3-hydroxyoctanoate), P(3HO) [14].

Property	P(3HO)
1. Melting transition temperature (ºC)	60
2. Glass transition temperature (ºC)	-35
3. Crystallinity (%)	30
4. Tensile strength (MPa)	10
5. Elongation to break(%)	300

1.5.1 Production and characterisation of MCL –PHAs

MCL–PHAs are usually produced using *Pseudomonas sp.* with fatty acids as the carbon source. Chan and co workers confirmed   the accumulation of poly (3-hydroxydecanoate), P(3HD) and poly(3-hydroxyoctanoate), P(3HO) by *Pseudomonas aeruginosa* using different carbon sources such as glucose and a range of fatty acids [10]. Song and Yoon, 1996, observed that *P. putida* when grown on the mixture of 11-phenoxyundecanoic acid and octanoic acid was able to utilise both the carbon sources at the same time and produce co-polyester with 3-hydroxyalkanoate and 3-hydroxy- phenoxyalkanoate. Kim *et al.,* 1999, produced aromatic MCL-PHAs with *p*-methylphenoxy and *m*-methylphenoxy substituents such as 8-*m*-methylphenoxyoctanoic acid and 8-*p*-methyl phenoxyoctanoic acid [15].

1.6 Polylactic Acid (PLA)

Polylactic acid is another biodegradable polymer which has been explored extensively. Lactic acid which is the main precursor in polylactic acid synthesis is produced in large amounts by the bacterial fermentation of the hexoses (carbon source) using lactic acid bacteria. Polylactic acid is used in biomedical applications such as implants, sutures, drug delivery, tissue engineering and stent development due to its biocompatibility properties. PLA can be processed using various techniques and are commercially available in the market in different grades. They are reasonably priced and target only the biomedical market [5].

1.6.1 Production of Lactic Acid

Lactic acid is produced by the bacterial fermentation of carbohydrates. The large scale production of the lactic acid has been carried out using a range of feedstocks including corn, sugar beet, molasses, whey, spent grain, sugar canes and wastepaper as a cellulosic feedstock. Lactic acid contains an asymmetric carbon atom and hence two different configurations, the L and D isomers. Both the isomers can be produced using the bacterial systems.

Fermentation processes involved in the lactic acid production differ based on the kind of bacteria used. Two different methods used in lactic acid production include (a) Homo-fermentative and (b) Hetero-fermentative method. The homo-fermentative method involves the conversion of each molecule of glucose into two molecules of lactic acid. This method is prevalent in most industrial processes due to high yields of lactic acid. Hetero-fermentative method is a type of fermentation in which the lactic acid yield is surpassed by the production of significant amounts of by products such as ethanol, acetic acid, and carbon dioxide. Hence, their use is not widespread due to the low yields of lactic acid. Maximum yields have been achieved by using glucose feedstock as the main carbon source.

Lactobacilli species are the most commonly used microorganisms for the production of lactic acid. Most commonly used lactic acid bacteria include *L.rhamnosus, L. delbrueckii, L. amylophilus, L. bavaricus,* and *L. casei.* Lactic acid can be produced by various other bacteria, fungi, as well as yeast. The production media contains 5% sugar and a nitrogen containing nutrient. Both batch and continuous processes can be used for lactic acid production. Various parameters such as pH, temperature and impeller speed are considered important during lactic acid fermentation. 90 to 99% of lactic acid conversion occurs within two days of fermentation. A continuous fermentation gives higher yield compared to the batch fermentation. Lactic acid fermentations have also been carried out by repeated batch production of the immobilized lactic acid bacteria [5, 16].

After the fermentation is complete, lactic acid has to be separated from the fermentation broth and purified to be used for polymerization process. A traditional method to obtain highly pure lactic acid involves neutralization with a base accompanied by filtration, concentration, and acidification. A Liquid/liquid extraction process is another method used for the recovery of lactic acid. Another process is based on the esterification with alcohols accompanied by distillation and hydrolysis has also been used for lactic acid recovery. Various separation techniques such as ultrafiltration, nanofiltration, electrodialysis and ion exchange can be used in combination with the above mentioned recovery processes for the purification of lactic acid [16].

1.6.2 Polylactic acid Synthesis

The main precursor in the polylactic acid synthesis is lactic acid. There are three different routes involved in the synthesis of polylactic acid. (a) The first route involves condensation of the L and D-Lactic acid isomers to generate low molecular weight pre polymer. These low molecular weight pre polymers can only be used after the addition of the external chain coupling agents whose function is to increase the chain length of the polymer. This would prevent the polymer from being brittle and unusable. (b) The second route involves ring opening polymerization of lactide resulting in high molecular weight poly lactic acid (PLA). The synthesis of lactic acid based polymers by ring opening polymerization is done using a range of solution polymerization, bulk polymerization, and melt and suspension polymerization [5, 16]. (c) The third route involves azeotropic dehydrative condensation of lactic acid to yield high molecular weight polylactic acid. This route does not require addition of chain extenders [16].

Mechanical properties of lactic acid based polymers vary from being flexible to stiff and high strength materials. PLA is a hydrophobic semicrystalline polymer and has a glass transition temperature, T_g, of 55ºC and a melting temperature, T_m of about 180ºC. The tensile strength of PLA is 32.22 MPa and its elongation to break is 30.7%. The melt enthalpy of the 100% pure PLA has been estimated to be 93J/g. The melting temperature and the degree of crystallinity are correlated with the molar mass and the purity levels of the polymer. The solubility of these lactic acid based polymers depends on the molar mass, degree of crystallinity and the monomers that make up the polymer. Enantiomerically, pure poly(lactide) is soluble in chlorinated and fluorinated solvents [5].

1.7 General Applications of natural polymers

Polyhydroxyalkanoates in general have numerous applications. Due to their biocompatibility and biodegradability, they are perfect substitutes for the synthetic plastics. They are used in the packaging industry, agriculture, and also in the medical field. SCL-PHAs being more stiff and tough are being widely used in the orthopaedic applications. They have also been used successfully as drug delivery systems [2]. MCL–PHAs, on the other hand, being more elastomeric is being considered for the use in soft tissue engineering, as adhesives and as coatings. Studies have shown that MCL-PHAs can be processed to form complex shapes and structures [17]. Implants with different physicochemical properties can be made, which can degrade at a tailored rate in a biological media, thereby maintaining their mechanical strength for a tailored period of time. *In-vitro* cell culture and *in-vivo* studies have demonstrated that MCL-PHAs have varying degrees of biocompatibility and biodegradability when they come in contact with cell lines such as fibroblasts, endothelial cells and isolated hepatocytes. Hence, MCL-PHAs have proven to be promising candidates for medical applications. They have been found to be useful in a large number of applications including heart tissue engineering for the manufacture of vascular grafts and heart valves and stent development [18].

Polylactic acid, currently, is the most important commercial bioplastic. They have also been extensively used in the packaging industry including coatings for the paper, fibre for clothing, and also in food services. Their biomedical applications include being used for sutures, screws, pins, prosthetics and drug delivery for controlled release of drugs and in stent development [19].

2. Role of Biodegradable Polymers in cardiac stent development

2.1 Cardiovascular Disease

Heart disease is the biggest killer in England and Wales and also in America where 910,000 people die every year due to fatal cardiovascular disease. Lack of exercise, poor diet, and continuous stress can cause damage to the heart and also to the circulatory system [20]. Coronary artery disease (CAD) is one of the cardiovascular diseases in which the artery gets clogged or narrowed due to the hardening of cholesterol, fats and other components of the blood as shown in Figure 4 [20].

The inner walls of the blood vessels are covered with layers of endothelial cells. The major function of this endothelium layer is to act as an interface between the blood and the other parts of the blood vessel and also to prevent blood platelet accumulation and clotting. Wall shear stress causes the stimulation of the endothelial cells which leads to morphological changes. Hence, platelet deposition is observed in the arteries. This reduces the lumen diameter and affects the blood flow to the cardiac muscle which causes inadequate supply of oxygen rich blood to the heart, leading to heart attack in most cases [21]
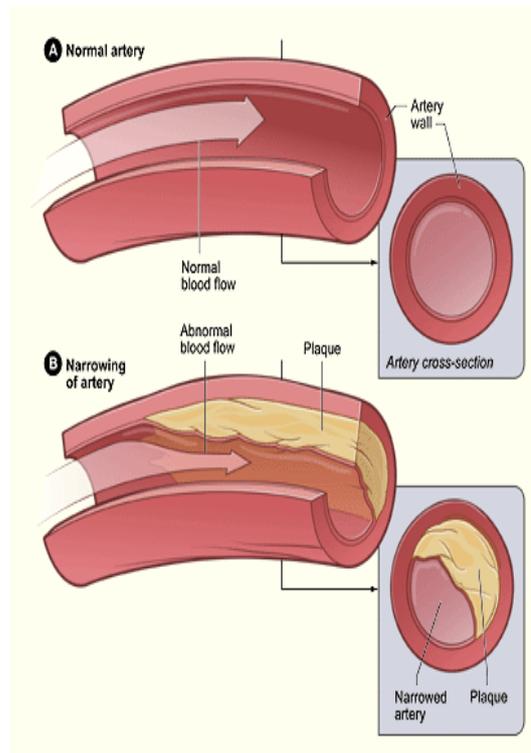
Figure 4: Coronary artery blocked by fatty deposits and blood clot (Taken from: www.heart.org.in) [22]

These blocked arteries can be widened or unblocked by a technique called as angioplasty as shown in Figure 5. This is a technique where a catheter with an inflatable balloon at its tip is directed to the blocked section of the artery through the groin or arm. The entire procedure is monitored by X-ray screening. On reaching the site of blockage, the balloon is inflated and a stent is placed in the artery which holds the narrowed blood vessel as a result of which the fatty deposits are squashed. This allows normal blood flow into the heart [20].
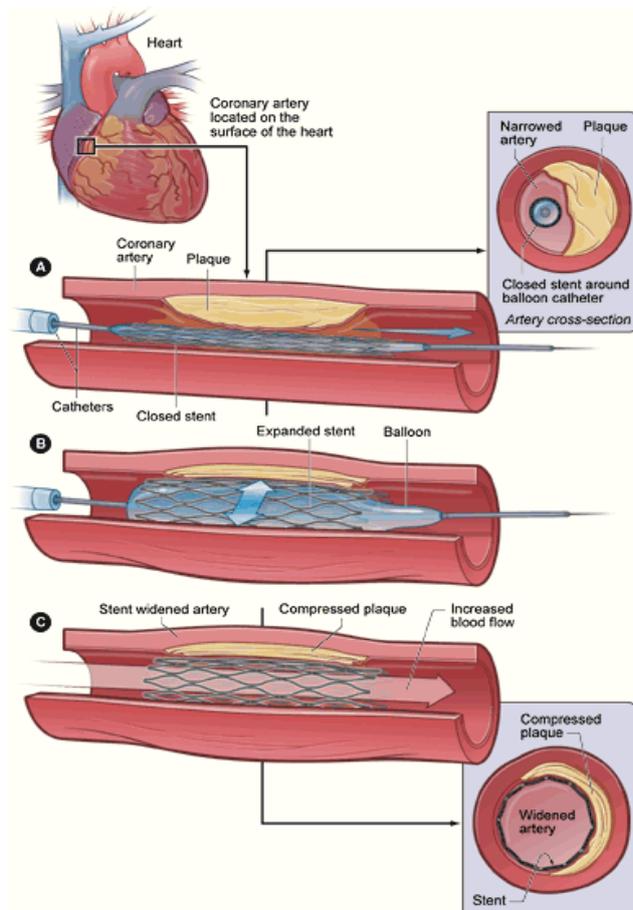
Figure 5: Angioplasty, a process where a catheter with an inflatable balloon carrying a stent is placed in the blocked coronary artery to widen the artery (Taken from: www.heart.org.in) [23]

2.2 Restenosis

Almost one million of angioplasty and stent implantation cases are recorded every year all over the world. In 20 to 30 % of these cases, neointimal growth leads to a condition called as restenosis. This occurs within a period of 3 to 6 months after the stent implantation. Restenosis is the reoccurrence of the blockage in the artery after stent placement as shown in Figure 6. The three main factors that causes restenosis include (a) injury caused to the blood vessel during stent placement (b) interaction occuring between the components of the blood and stent material which sometimes leads to an inflammatory reaction and (c) endothelial cell proliferation caused due to low local wall shear stress [21].
As mentioned earlier, stent placement causes damage to the endothelial cells. This is followed by a trail of physiological responses which can be divided into two stages. The first stage occurring immediately upon implantation is thrombosis. Blood clot formation causes the blockage of the blood vessels hindering the blood flow followed by an inflammatory response. The second stage involves endothelial cell proliferation which occurs 3 to 6 months after implantation. This occurs due to the low wall shear stress in the vessels. The growth rate of the endothelial cells is controlled by releasing substances such as nitric acid and thrombomodulin. When the wall shear stress is high, significant amount of nitric acid is released which inhibits the growth of endothelial cells whereas low wall shear stress leads to the increased production of thrombomodulin and decreased production of nitric acid. Hence, cell proliferation occurs leading to restenosis [21].
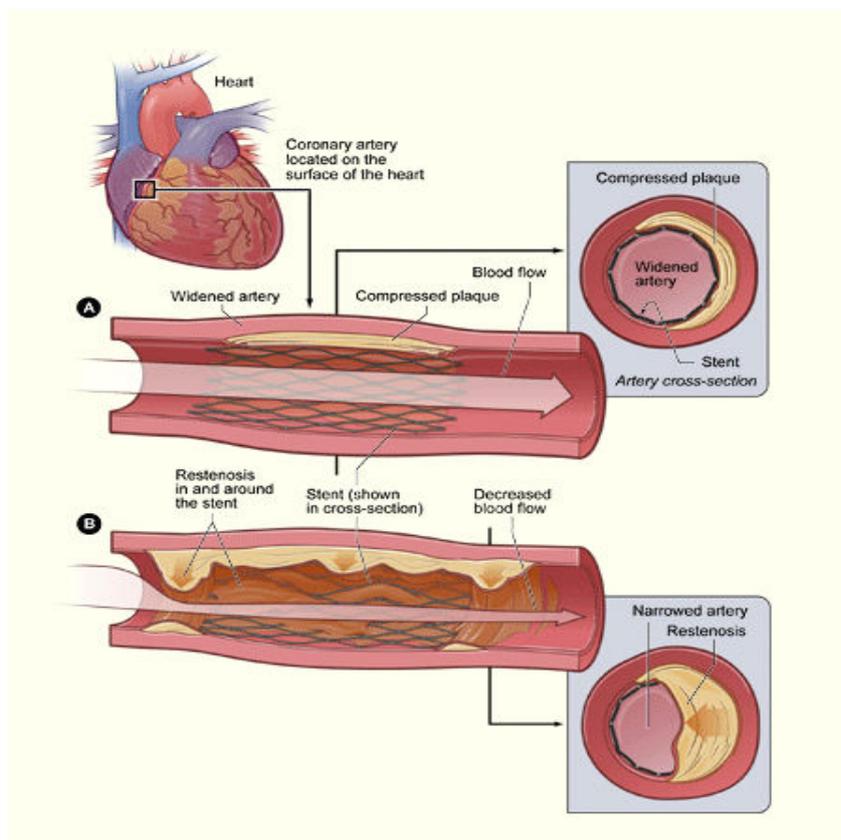
Figure 6: In stent restenosis, the reoccurrence of artery blockage due to lesions created during the angioplasty (Taken from www.heart.org.in) [23].

Drug eluting stents (DES) are being trialled to reduce the chances of restenosis by inhibiting the tissue growth using drugs such as paclitaxel (stabilizing agent causing cell death), sirolimus (immunosuppressive agent) and dexamethasone (anti inflammatory agent) [24].

2.3 Biodegradable Drug Eluting Stents

As discussed above, stents are scaffolds that are placed in the coronary artery (using an inflatable balloon on a catheter) to recover the shape of narrowed or diseased arteries caused due to accumulation of fatty deposits, cholesterol and other components of the blood. The two most important functions of a stent are to allow normal blood flow and to prevent restenosis [25]. According to Zidar *et al.*, the following are the characteristics of an ideal stent:

a) They should be biodegradable in nature as this eliminates the need to remove the stent from the treatment site since they degrade in the body.
b) They should be flexible in nature to facilitate insertion.
c) They should possess mechanical properties such as high elasticity, high tensile strength, self expandability, high ductility (to prevent deformation during expansion) and high radial strength (to prevent recoiling after the placement) to perform the function.
d) They should be able to release drugs such as rapamycin (immunosuppressant), or paclitaxel (mitotic inhibitor) or tranilast (anti allergy) or heparin (anti-coagulant) at the treatment site to prevent restenosis [26].

During the last three decades or so, stent design has witnessed a fairly rapid evolution from bare metal stents (BMS) of increasing complexity, through shape memory alloy stents, polymer coated, drug eluting stents to biodegradable (or bioabsorbable) stents made using polymers or corrodible metals such as magnesium [27]. Biodegradable stents have been shown to be less obtrusive than the metallic stents which can cause the arterial vessel to rupture. In addition, the presence of anti-restenosis drugs within the stent is another desirable feature. A biodegradable stent loaded with the anti-restenosis drug minimizes the systemic toxic effects, facilitates the healing of the injured vessel caused due to the stent implantation and most importantly prevents restenosis [28].

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In recent years, drug eluting stents (DES) have witnessed a major increase in popularity following early trials and approvals in 2002-2003, largely due to their effectiveness in reducing in-stent restenosis. At present, US FDA has approved two drug eluting stents namely Cypher[TM] and TAXUS Express[TM]. Cypher[TM] is made up of a stainless steel tube coated with a mixture of (polyethylene-co-vinyl acetate (PEVA) and poly(n-butylmethacrylate) (PBMA) whereas TAXUS Express[TM] is made up of stainless steel coated with poly(styrene-β-isobutylene-β-styrene) [29] . Cypher[TM] delivers sirolimus which is an immunosuppressive agent that causes cells to revert back to the quiescent state by inhibiting the progression of the cell cycle at an early stage. Thus, the proliferation of the endothelial cells in the arteries is inhibited thereby preventing restenosis. TAXUS Express[TM] delivers paclitaxel which is a stabilizing agent that causes cell death or apoptosis, also called programmed cell death. Hence, it prevents restenosis by inhibiting the neointimal growth in the coronary artery. However, these DES products are biostable and not biodegradable which means that these are bare stents made up of metals such as stainless steel and coated with a mixture of (polyethylene-co-vinyl acetate (PEVA) and poly(n-butylmethacrylate) (PBMA) and also poly(styrene-β-isobutylene-β-styrene) [30]. The Cleveland Clinic/Mayo/Thorax center also developed a biodegradable stent made up of Wiktor tantalum with a mixture of bioabsorbable polymer coating. The polymer coatings that were used include poly(D,L-lactide/glycolide co-polymer), polycaprolactone, poly(hydroxybutyrate-hydroxyvalrate) and polyorthoester which led to increased inflammatory response and neointimal growth [31].

The first biodegradable stent was developed by Stack *et al.*, at the Duke University using poly-L-Lactic acid (PLLA)[32]. This stent was designed in such a way that it could withstand 1000 mm Hg of crush pressure and could maintain its radial strength for one month. The degradation period of the stent was 9 months. After the stent implantation into the animals, minimal thrombosis, moderate neointimal growth and a very limited inflammatory response was observed. However, further clinical tests with this device remained limited. Another biodegradable stent made up of polyglycolic acid was developed by the Kyoto University. This stent was associated with thrombus deposition upon implantation in the canine model [33].

A series of experiments were carried out by Lincoff *et al*. to demonstrate that stents made up of PLLA of high molecular mass caused minimal inflammatory response whereas stents made up of low molecular mass caused increased inflammatory reaction. These results vary in different animal models, for example in dogs, minimal cell proliferation occurs, while in the pig model significant tissue growth is observed [33].

Igaki-Tamai *et al*. developed high molecular mass poly-L-Lactic acid (PLLA) stents of zigzag helical design. This was implanted into humans and a report on a 6 month follow up study after stent implantation was published. This design proved to be of great advantage as it reduced vessel wall injury during stent placement. This in turn significantly reduced blood clot and tissue growth [34]. Angiographic follow up data at 1 day, 3 months and 6 months were published. Only 10.5% of restenosis rate per lesion was observed after 6 months of implantation. These results were encouraging as these biodegradable stents did not cause any intimal hyperplasia[33]. Yamakawi *et al*. investigated the incorporation of anti proliferative agent (tyrokinase inhibitor) in the Igaki-Tamai stents. They concluded that there was no tissue proliferation at the site of stent implantation.

P(3HB) biodegradable stents were also developed and implanted into the iliac arteries of the New Zealand white rabbits for 30 weeks. Upon implantation, it was observed that P(3HB) caused inflammatory reactions, stent thrombosis and lumen narrowing. This resulted in a ban on the clinical use of P(3HB) stents [34].
Another bioabsorbable everolimus coronary stent made up of polylactic acid called Absorb is under trial and from the data collected so far, it has showed promising results. After six months of implantation, the stent appeared to be safe and effective and showed no signs of stent thrombosis. Optical coherence tomography and multislice CT was used to follow up this bioabsorbable stent. Physiological function of the stented part of the vessel was fully restored after two years upon implantation. However, this study was conducted using 30 patients which is too small a number to come to a conclusion. Therefore, extend trial studies are now being initiated using Absorb with about 1000 patients from different parts of the world [35].

Conclusion

Biodegradable polymers are likely to play a significant role in building an ecofriendly environment by replacing the widely used non - biodegradable synthetic plastics. However, to make inroads into the thermoplastic dominated market, economical production of the biodegradable polymers is a must. Reduction in the cost of production, resulting in the competitive price of the biodegradable polymers will broaden their range of application. The key properties of these biopolymers such as biodegradability and biocompatibility have made it feasible to envisage the extensive use of these biopolymers in the biomedical field. Their role in cardiac stent development is significant. It is impossible to predict the future with certainty but the datas revealed from the studies so far have been very promising. Hence, biopolymers have emerged as one of the most promising candidates for use in coronary stent development.

References

[1] Kolybaba M, Tabil LG, Panigrahi S, Crera WJ, Powell T, Wang B. Biodegradable Polymers: Past, Present, and Future. The Kolybaba M, Tabil LG, Panigrahi S, Crera WJ, Powell T, Wang B. Biodegradable Polymers: Past, Present, and Future. *The Society for engineering in Agriculture, food and biological systems.* 2003. *Paper Number: RRV03-0007. An ASAE Meeting Presentation.*

[2] Zinn M, Witholt B, Egli T. Occurrence, synthesis, medical application of bacterial polyhydroxyalkanoate. *Advanced Drug Delivery Reviews.* 2001; 53(1):5-21.

[3] Huisman GW, Madison L. Metabolic Engineering of Poly (3-Hydroxyalkanoates): From DNA to Plastic. *Microbiology and Molecular Biology Reviews.* 1999; 63:21-53.

[4] Verma S, Bhatia Y, Valappil, SP, Roy, I. A possible role of poly-3-hydroxybutyric acid in antibiotic production in Streptomyces. *Archives of Microbiology.* 179 (1): 66-69.

[5] Sodergard A, Stolt M. Properties of lactic acid based polymers and their correlation with composition. *Progree in polymer science.* 2002; 27: 1123-1163.

[6] Valappil SP, Bhatia Y, Bucke C, Roy I. Biosynthesis of polyhydroxyalkanoic acid *by S. coelicolor. E-polymers, The Scientific e-only polymer journal.* 2004;049.

[7] Philip S, Keshavarz T, Roy I. Polyhydroxyalkanoates biodegradable polymers with a range of applications. *Journal of Chemical Technology and Biotechnology.* 2006; 233-247.

[8] Anderson AJ, Dawes EA. Occurrence, metabolism, metabolic role and industrial uses of bacterial polyhydroxyalkanoates. *Microbiological Reviews.* 1990; 54: 450-472.

[9] Sudesh K, Abe H, Doi Y. Synthesis, structure and properties of polyhydroxyalkanoates: Biological Polyesters. *Progress in Polymer Science.* 2000; 1503-1555.

[10] Pouton CW, Akhtar S. Biosynthetic polyhydroxyalkanoates and their potential in drug delivery. *Advanced Drug Delivery Reviews.* 1996; 133-162.

[11] Fischer JJ, Aoyagi Y, Enoki M, Doi Y, Iwata T. Mechanical properties and enzymatic degradation of poly([R]-3-hydroxybutyrate-co-[R]-3-hydroxyhexanoate) uniaxially cold drawn films. *Polymer degradation and stability.* 2004; 453-460.

[12] El-Sayed, Azhar A, Abdelhady HM, Hafez AMA, Khodair TA. Production of Polyhydroxybutyrate (PHB) using batch and two stage batch culture strategies. *Australian Journal of Basic and Applied Sciences.* 2009;3 (2): 617-627.

[13] Khanna S, Srivastava AK. Recent advances in microbial polyhydroxyalkanaotes. *Process Biochemistry.* 2005; 40: 607-619.

[14] Kim DY, Kim HW, Chung MW, Rhee YH. Biosynthesis, Modification and Biodegradation of bacterial medium Chain Length Polyhydroxyalkanoate. *The Journal of Microbiology.* 2007; 45: 87-97.

[15] Song JJ, Yoon SC. Biosynthesis of Novel Aromatic copolyesters from insoluble 11-phenoxyundecanoic acid by *Pseudomonas putida* BM01. *Applied and Environmental Microbiology.* 1996;536-544.

[16] Averous L. Polylactic acid synthesis, properties and applications. Available at www.biodeg.net. Accessed on 2nd June 2010.

[17] Freed L. Mending broken hearts with tissue engineering. *Science Daily.*2008.

[18] Williams SF, Martin DP. Applications of PHAs in medicine and pharmacy. Weinheim, Germany, Marchessault RH (Eds) Wiley-VCH. 2005.

[19] Haywood, G.W., Anderson, A.J., Dawes, E.A. A Survey of the Accumulation of Novel Polyhydroxyalkanoates by Bacteria, Biotechnology Letters. 1989; 11(7) 471-476.

[20] Kulick DL, Rahimtoola SH. Acute coronary occlusion after percutaneous transluminal coronary angioplasty: Evolving strategies and implications. *Circulation.* 1990; 1039-1043.

[21] Nouyrigat WK, Szumbarski. Numerical simulation of restenosis in a stented coronary artery. *World Academy of Science, Engineering and Technology.* 2009; 5842-46.

[22] Coronary Heart Disease. Available at www.heart.org.in. Information published by National institute of Health. Accessed 2nd June 2010.

[23] Coronary Angioplasty. Available at www.heart.org.in. Information published by National institute of Health Accessed 2nd June 2010.

[24] Vogt F, Stein A, Rettemeier G, Krott N, Hoffman R, Dahl JV, Bosserhoff AK, Michaelis W, hanrath P, Weber C, Blindt R. Long term assessment of a novel biodegradable paclitaxel eluting coronary polylactide stent. *European Heart Journal.*2004; 1330-1340.

[25] Geraghty PJ. Bioabsorbable stenting for peripheral arterial occlusive disease. *Perspect Vasc Surg Endovasc Ther.* 2006;18(4):295-298.

[26] Cantor WJ, Peterson ED, Popma JJ, Zidar JP, Sketch MH, Tcheng JE, Ohman EM. Provisional stenting strategies: systematic overview and implications for clinical decision making. *Journal of the American College of Cardiology.* 2000; 36(4): 1142-1151.

[27] McFadden EP, Stabile E, Regar E, Cheneau E, Ong AT, Kinnaird T, Suddath WO, Weissman NJ, Torguson R, Kent Km, Pichard AD, Satler LF, Waksman R, Serruys PW. Late thrombosis in drug eluting coronary stents after discontinuation of antiplatelet therapy. *Lancet.* 2004; 364:1519-1521.

[28] Kothwala D, Raval A, Choubey A, Engineer C, Kotadia H,. Paclitaxel drug delivery from cardiovascular stent. *Trends in Biomaterials and Artificial Organs*. 2006; 19(2): 89-92.

[29] Venkataraman S, Boey F. Release profiles in drug eluting stents: issues and uncertainties. *Journal of Control Release.* 2007; 120(3): 149-160.

[30] Mani G, Feldman MD, Patel D, Agrawal CM. Coronary stents: A materials perspective. *Biomaterials.* 2007; 1689-1710.

[31] Colombo A. Biodegradable Stents: Fulfilling the mission and stepping away. *Circulation* 2000; 102: 371.

[32] Lincoff AM, Furst JG, Ellis SG, Tuch RJ, Topol EJ. , Sustained local delivery of dexamethasone by a novel intravascular eluting stent to prevent restenosis in the porcine coronary injury model. *Journal of American College of Cardiology.* 1997; 29: 808-816.

[33] Bonan R, Asgar AW. Biodegradable stents - Where are we in 2009? *Interventional cardiology.* 2009; 6.

[34] Valappil SP, Misra SK, Boccaccini AR, Roy I. Biomedical applications of polyhydroxyalkanoates, an overview of animal testing and *In Vivo* responses. *Expert Rev. Med. Devices.* 2006; 3(6): 853-868.

[35] Bruining N, Winter SD, Roelandt JRTC, Regar E, Heller I, Vandomburg RT, Hamers R, Onuma y, Dudek D, Webster MWI, Theusen L, Ormiston JA, Cheong WF, Hebert KM, Veldhof S, Serruys PW. Monitoring *In Vivo* Absorption of a drug eluting stent with intravascular ultrasound derived parameters. *Journal of American college of Cardiology:Cardiovascular Interventions.* 2010; 3: 449-456.

[36] Tamai H, Igaki K, Kyo E, Kosuga K, Kawashima A, Matsui S, Komori H, Tsuji T, Motohara S, Uehata H. Initial and 6-Month Results of Biodegradable Poly-l-Lactic Acid Coronary Stents. *Circulation.* 2000; 102: 399-404.

[37] Fischell TA. Polymer coatings for stents: can we judge a stent by its cover? *Circulation.* 1996; 94:1494-1495.

[38] Otari SV, Ghosh JS. Production and Characterization of the polymer polyhydroxybutyrate-copolyhydroxyvalerate by *Bacillus megaterium* NCIM 2475. *Current Research Journal of Biological Sciences.* 2009; 1(2): 23-26

[39] Durner R, Zinn M, Witholt B, Egli T. Accumulation of poly [(R) -3- hydroxyalkanoates] in *Pseudomonas oleovorans* during growth in batch and Chemotat culture with different carbon sources. *Biotechnology and Bioengineering.* 2001; 72(3): 278-288.

[40] Hidalgo-Bastida LA, Barry JJA, Everitt NM, Rose FRAJ, Buttery LD, Hall IP, Claycomb WC, Shakesheff KM.Cell Adhesion and mechanical properties of a flexible scaffold for cardiac tissue engineering. *Acta Biomaterialia.* 2007;3:457-462.

[41] Hahn SK, Chang YK, Kim BS, Chang HN. Optimization of microbial Poly-β- Hydroxybutyrate recovery using dispersion of sodium hypochlorite solution and chloroform. *Biotechnology and Bioengineering.*1994; 44:256-261.

[42] Martin O, Averous L. Poly (Lactic acid): Plasticization and properties of biodegradable multiphase systems. 2001; 6209-6219.

[43] Stubbe J, Tian J. Polyhydroxyalkanoate (PHA) homeostasis: the role of the PHA synthase. *The royal society of Chemistry.* 2003; 20: 445-457.

[44] Valappil SP, Bhatia Y, Bucke C, Roy I. Biosynthesis of polyhydroxyalkanoic acid *by S. Coelicolor. E-polymers, The Scientific e-only polymer journal.* 2004;049.

[45] Dangas G, Kuepper F. Restenosis: Repeat narrowing of a coronary Artery: Prevention and Treatment. *Circulation.* 2002; 2586-2587.

[46] Vairo G, Cioffi M, Dubini G, Migliavacca F. Drug release from coronary eluting stents: A multidomain approach. *Journal of Biomechanics.* 2010; 1580-1589.

[47] Bhatt R, Shah D, Patel KC, Trivedi U. PHA-rubber blends: Synthesis, characterisation and biodegradation. *Bioresource.* 2008; 99: 4615-4620.

[48] Misra SK, Valappil SP, Roy I, Boccaccini AR. Polyhydroxyalkanoate (PHA) inorganic Ohase composites for tissue engineering applications. *Biomacromolecules.* 2006; 2249-58.