Exploiting Polyhydroxyalkanoates for Tissue Engineering

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Abstract

Petroleum based synthetic plastics are an integral part of our daily life. However, their excessive usage has resulted in environmental pollution. The primary reason for this pollution is due to their non-biodegradable nature. On the other hand, polyhydroxyalkanoates (PHAs) are biodegradable polymers, which have been shown to be produced by a wide range of bacteria. The unique feature of this bioplastic production is that they can be produced from renewable substrate materials through a unique metabolic route. These PHAs have the potential to replace petroleum based synthetic plastics. PHAs have high commercial value which make them suitable agent for industrial and medical applications. Although simpler and monomeric forms of PHAs have limited biotechnological applications, however, modified forms of PHA can be used in various medical applications such as, drug delivery, biodegradable implants, anticancer agent, and tissue engineering etc. Among all, tissue engineering has emerged globally to improve the current therapeutic approaches, entailing a revolution in clinical practice. PHAs offer several benefits in tissue engineering. These chemically modified biopolymers can be used in tissue repair, regeneration of tissue, scaffolds preparation etc.

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10.1 Introduction

Polyhydroxyalkanoates (PHAs) are biodegradable biopolymers, produced by a diverse range of bacteria under nutrient limiting conditions (Reddy et al. 2003; Porwal et al. 2008; Patel et al. 2011, 2012, 2015a, b, 2016; Kumar et al. 2016; Ray and Kalia 2017a, b). In general, under conditions here high carbon concentration is accompanied by limited quantities of nitrogen, microbes regulate their metabolic pathway in a way that acetyl-CoA gets in to PHAs production pathway rather than going towards TCA cycle (Kumar et al. 2013, 2014, 2015; Singh et al. 2009, 2013, 2015; Ray et al. 2018). These PHAs have gained attention due to the following properties, such as (i) biocompatibility (ii) biodegradability (iii) non-toxicity (iv) cytotoxicity, and (v) non-carcinogenicity as compared to synthetic plastic. Thus, PHAs can be serve as an attractive target for tissue engineering biomaterials (Peppas and Langer 2004; Ray and Kalia 2017c, d; Kalia et al. 2019). Tissue engineering is an emerging field which combines biology, material science and surgical re-construction to help in maintenance and improvement of tissue function through repairing and surgical procedures. Generally, there are three different steps which are being used in engineering of new tissues such as (i) cell substitutes, (ii) materials use to induce tissues, and (iii) use of scaffolds for implantation of cells. Several PHAs, such as poly (3-hydroxybutyrate) P(3HB), poly (3hydroxybutyrate-co-3hydroxyvalerate) P(3HB-co-3HV), poly (4-hydroxybutyrate) P(4HB), poly (3hydroxybutyrate-co-3hydroxyhexanoate) P(3HB-co-3HHx), and poly(3-hydroxyoctanoate) P(3HO) are employed for tissue engineering. The applications involve sutures, wound dressings, scaffolds preparation, bone tissue engineering, subcutaneous tissue engineering, nerve tissue engineering, maxillofacial treatment etc.

10.2 Scaffolds

Tissue engineering involves the scaffold preparation, which helps in the repair and regeneration of defective tissues (Martina and Hutmacher 2007). They provide support for cells to adhere and undergo proliferation process to form an extracellular network (ECM). These scaffolds are composed of bioactive molecules like biodegradable polymers, which play a major role in tissue engineering (Jagur-Grodzinski 2006; Armentano et al. 2010). Scaffolds can be prepared by several methods such as solvent casting, foaming, electro-spinning etc. P (3HB-co-3HV) with pearl powder was prepared for nanofiber scaffold by electrospinning method which promotes cell proliferation (Bai et al. 2015). Curcumin entrapped with polyaniline was conjugated with PHBV for the preparation of scaffold. It was employed in the tissue engineering process. The PHBV scaffolds were characterized by UV−vis and ATR/FT-IR spectrophotometry,

thermogravimetry, fluorescence microscopy, and X-ray diffractometric analysis (Pramanik et al. 2016). P (3HB-co-3HV-co-2,3-dHB) produced by recombinant *Ralstonia eutropha* was exploited for scaffold material by utilizing glycolate as a sole carbon source (Insomphun et al. 2016). Scaffold prepared from P(3HB-co-3HHx) can be used as support material for cartilage tissue engineering (Ye et al. 2009). P (3HB-co-3HHx) can be used as scaffold material for fibroblast growth and capsulation. These scaffolds were also found to be favourable for tarsal repair (Zhou et al. 2010). P(3HB-co-3HHX) scaffold blended with hydroxyapatite (HAP) promoted osteoblast growth, chondrocytes proliferation, migration and cartilage repair (Wang et al. 2005, 2008). P(3HB-co-3HHx) enhances smooth muscle cell proliferation and attachment (Qu et al. 2006a, b) (Table 10.1). P(3HB-co-3HV) when grafted with chitosan or chitooligosachharide showed better antibacterial activity against *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa.* The scaffold helped in fibroblast attachment and adsorption of protein and cell proliferation (Hu et al. 2003). The utilization of P(3HB-co-3HHx) in the preparation of scaffolds helped in liver tissue engineering. P(3HB-co-3HHx) fibres and tubes were used to treat Achilles tendon injury in rats (Xu et al. 2010; Webb et al. 2013). These scaffolds proved effective in tissue remodelling. Scaffolds prepared from co-polymerization of PHB with bacterial cellulose improved condition of artificial ligaments and tendon repair with high biocompatibility and biodegradable properties. Co-polymerization of PHB and PHO resulted in scaffold formation, which have been used in cartilage repair (Ching et al. 2016). Similarly, co-polymerization of PHB with poly (ethylene glycol) (PEG) was used in scaffolds preparation which repaired defective bone tissue (Bonartsev et al. 2016).

10.3 Subcutaneous Tissue Engineering (TE)

PHAs can be used in subcutaneous tissue engineering. P(3HB) was used as the suture material in skin tissue, which showed anti-inflammatory properties (Volova et al. 2003). PHO another class of PHA, when used as grafts in soft tissue reduced inflammation (Stock et al. 2000; Hazer et al. 2009). PHBV, poly (L-lactic acid) and poly (glycerol sebacate) were exploited for the preparation of 3D microfibrous material. As a result, a thick myocardial patch was developed to replace myocardial infarctions (Kenar et al. 2010). Scaffold containing PHB/PHBHHx was seeded along with stem cells derived from differentiated human adipose could produce cartilage -like tissue when it was implanted into the subcutaneous layer of the nude mice (Ye et al. 2009). These scaffolds have also been found effective *in vivo* tendon repair model (Webb et al. 2013). PHA copolymers of medium chain length (mcl) size, were biosynthesized from frying oil. Combination of mcl-PHA (at more than 10% by wt) and P(3HB) proved effective in improving the brittle properties of P(3HB). Such blended materials have application in soft TE, which requires a material having desired mixture of tensile strength, stiffness and ductility. The soft and flexible blended biopolymer showed higher biocompatibility as evident from the high viability and proliferation of C2C12 mouse myoblast cells (Lukasiewic et al. 2018).

Table 10.1 Applications of polyhydroxyalkanoates and their composites in tissue engineering

Bio-products	Source	Particles	Method	Applications	References
Gram-positive					
PHA	*Bacillus* sp.	Scaffold	SLP	Enhance growth of new proliferative cells.	Getachew et al. (2016)
P(3HB)	*B. cereus* SPV	3D-scaffold	PLT	Cartilage tissue engineering	Akaraonye et al. (2016)
PHB + BG + Vitamin E	*B. cereus* SPV	Scaffolds preparation	SCM, PLT	Bone tissue engineering	Misra et al. (2010)
P(3HB-co-3HV)	*Alkaliphilus oremlandii* OhiLas	3D Porus scaffolds	SLP	Electroconductive material for tissue engineering	Pramanik et al. (2016)
Gram-negative					
PHB + nHA	*Escherichia coli*	Scaffolds preparation	SCM	Enhances porosity	Meischel et al. (2016)
P (3HB-co-3HHx)	*Aeromonas hydrophila* 4AK4	Scaffold		SMCs-scaffold fabrication	Qu et al. (2006a)
P(3HO)	*Pseudomonas mendocina*	Cardiac patches	SCPL	Replacement of infarcted cardiac tissue	Bagdadi et al. (2016)
P(3HO)	*P. mendocina*	Polymer nano composite	SCM	Keratinocyte regeneration	Rai et al. (2017)
				Skin tissue engineering, wound healing	
Commercial					
P(3HB)-collagen blends	Commercial	Composite fibrous meshes	ES	Anisotropic tissue engineering	Salvatore et al. (2018)
PHBHHx	Commercial	Scaffolds preparation	SLP	Support cell attachment and proliferation of osteoblasts	Wang et al. (2005)
P(3HB-co-3HV)	Commercial	Scaffold	SCM	Tarsal repairing in eyelid	Zhou and Yu (2014)
P(3HB-co-3HHx)	Commercial	3D-scaffold	WPS	Pre-osteoblast proliferation and differentiation	Mota et al. (2017)

P(3HB-co-4HB)	Commercial	Composite fibers	ES	Tissue engineering	Zhijiang et al. (2016)
P(3HB-3HV)::97:3	Commercial	Nerve conduit	SCPL	Regeneration of peripheral nerve	Young et al. (2002)
P(3HB); P(3HB-3HV)::72:28; P(3HB-4HV)::92:8; P(3HB-3HV-4HV) ::73:10:17	Commercial	Film preparation	SLP	Enhances cell attachment and proliferation	Ansari and Amirul (2013)
P(3HB-co-3HV)- chitosan; Chitooligosaccharide	Commercial	Grafted membrane	ES	Antibacterial activity	Hu et al. (2003)
P(3HB)- glial growth factor (GGF) in hydrogel	Commercial	Conduit	ES	Repairing of nerve gap	Mohanna et al. (2003)
P(3HB-co-3HHx)	Commercial	Scaffold	SCM	Cartilage repair, chondrocytes proliferation, migration and differentiation	Wang et al. (2008)
PHB + poly (p-dioxanone) (PPD)	Commercial	Blend preparation	SCM	Enhances thermal stability and mechanical strength in bone tissue engineering	Dias et al. (2008)
PHB +PEG	Commercial	Blend preparation	SCM	Support cell spreading and proliferation	Cheng et al. (2003)
PHBHHx/PHB	Commercial	Scaffolds preparation	SLP	Improve cell proliferation in chondrocytes cartilage tissue engineering	Deng et al. (2002)

SCPL Solvent casting particle leaching, *PLT* Particulate leaching technique, *SCM* Solvent casting method, *WPS* Wet-spinning system, *ES* Electrospining, *SLP* Salt leaching process

10.4 Nerve Tissue Engineering (TE)

Nerve injuries result in axonal disruption which cause degenerative changes. Thus, gap formation occurs between nerves where repair is not possible. In this case, nerve grafts act as a bridge to support axonal growth (Arslantunali et al. 2014). Several synthetic nerve conduits have been prepared for the repair of peripheral nerve faults. PHAs are modified to improve neural prosthesis. A porous and fibrous type of polymer prosthesis is favourable for neural regeneration (Mosahebi et al. 2002; Bian et al. 2009). P(3HB), as a neuronal conduit exhibited axonal regeneration which showed low level of inflammatory infiltration (Hazari et al. 1999; Mosahebi et al. 2002). P(3HB-co-3HHx) was found to be helpful in neuronal regeneration (Bian et al. 2009). PHA coated films were found to improve the survival rate of neural stem cells and neural progenitor cells and differentiated in to neurons (Lu et al. 2013).

(PHBV-P(L)-PLGA) with (PHBV-PLGA) was used as a nerve conduit which showed good mechanical properties. PHB conduit was found helpful in peripheral nerve regeneration. PHB conduit was composed of glial growth factor and alginate hydrogel resulted in a progressive and sustainable nerve regeneration. P (3HB-co-4HB) was exploited for the preparation of composite nanofibrous membrane. This membrane was developed by electro-spinning of P(3HB-co-4HB) and cellulose acetate blend solution (Zhijiang et al. 2016). P(3HB-co-3HHx) produced by microbial fermentation was found to be a suitable candidate for artificial nerve conduit due to their proper mechanical strength and biodegradability. This helps to repair nerve damage. These nerve conduits are prepared by particle leaching method (Bian et al. 2009). Neural stem cells which were grown on PHA scaffolds were reported to be useful for repairing injury to the central nervous system (Xu et al. 2010).

10.5 Bone Tissue Engineering (TE)

Bone TE is developed to eliminate the risk associated with the bone graft transplantation process, supply of a limited quantity of bone grafts, and pitfalls associated with transmission of the disease. It is a complex process with the migration of osteoprogenitor cells (Table 10.1). The process composed of proliferation, differentiation, matrix formation, mineralization and finally the remodelling of the bone. Scaffolds prepared for bone TE should be osteoconductive which helps to attract the stem cells. In the presence of suitable growth factors, scaffolds containing stem cells differentiate into pre-osteoblasts, which in turn get transformed to osteoblasts and ECM. As a result, bone remodelling occurs with osteocytes formation. Biopolymers are exploited for bone tissue repairing, through metallic parts and antibiotic carriers to the infected site of bone tissues (Jagur-Grodzinski et al. 2006). P(3HB-co-3HV) as graft was found to be the best biomaterial for osteoblast attachment, proliferation, and differentiation of bone marrow cells. P(3HB-co-3HHx) also showed better attachment, proliferation and differentiation of osteoblasts.

Combination of Hydroxyapatite (HA) and PHA enhances osteoblastic activity and bone integrity. P(3HB-co-3HV) when conjugated with calcium phosphate-reinforcing phases such as HA, submicron-sized calcined hydroxyapatite (cHA) and submicron-sized (β-TCP) showed anti-inflammatory properties and improved osteogenic properties (Cool et al. 2007). PHB composites fabricated with different quantities of zirconium dioxide and herafill (a bone filler loaded with a antibiotics), when implanted in the femora of growing rats proved to have high strain and tensile strength, which were as good as the actual bone (Meischel et al. 2016). Different PHA scaffolds as blends or as composites with hybrid materials have also been shown to be effective in bone tissue engineering (Lim et al. 2017). Electrospun fiber mesh made up of PHB/PHBV in combination with stem cell derived human adipose tissue proved effective in improving vascularization in engineered bone tissues (Goonoo et al. 2017).

10.6 Cartilage Tissue-Tendon and Ligament Tissue Engineering (TE)

PHAs play a vital role in cartilage tissue engineering. When cartilage tissue is damaged, it results in an osteoarthritis and functional loss of joints. PHA implants help in neocartilage formation. It regenerates hyaline cartilage in the defective site (Hazel Fox and Webb 2013). PHBV matrices cause early cartilage formation. Collagen matrices containing calcium phosphate (Cap-Gelfx) and P (3HB-co-3HV) were designed for novel cartilage by tissue engineering which showed better healing properties (Kose et al. 2004, 2005). P(3HB-co-3HHx) was exploited for producing neocartilage (Ye et al. 2009) (Table 10.1).

10.7 Skin Tissue Engineering (TE)

Skin protects the human body from the surrounding environment by protecting the underlying organs from pathogens. Auto-healing property of skin may be damaged during burns, diabetic wounds etc. Several methods to treat burns have been employed, such as autografts, and allografts. However, these two methods face problems due to the limited availability, disease transmission, risk of donor site morbidity and immune rejection. Thus, there is a need to develop substitutes, which can mimic human skin to replace damaged skin. PHAs and its co-polymers when blended with polysaccharides such as P(4HB) and Hyaluronic acid, increased keratinocyte proliferation rate (Groeber et al. 2011). Electrospun nanofibers have been used as polyvinyl alcohol – PHB scaffolds for engineering skin tissue (Sundaramurthi et al. 2014). In vitro study had shown the use of such scaffolds to enhance the proliferation of keratinocytes and fibroblast cells (Asran et al. 2011).

10.8 Conclusion

PHAs have applications in diverse fields. PHAs and their derivatives are employed in medical purposes which have the highest level of application. PHAs with chemical modifications have great potential in tissue engineering. Various PHA-based tissue engineered products have been employed in several clinical use.

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