บทความวิชาการ

กระบวนการปั่นเส้นใยด้วยไฟฟ้าสถิตของพอลิไฮดรอกซีอัลคาโนเอต สำหรับการประยุกต์ใช้ทางการแพทย์: รีวิว

ELECTROSPINNING OF POLYHYDROXYALKANOATE (PHA) FOR BIOMEDICAL APPLICATION: A REVIEW

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บทคัดย่อ

กระบวนการปั่นเส้นใยด้วยไฟฟ้าสถิตเป็นวิธีที่ง่าย และเป็นเป็นวิธีที่มีราคาถูก ใช้ผลิตเส้นใยขนาดเล็กเพื่อประยุกต์ใช้ด้านชีวการแพทย์เป็นการใช้แรงดันไฟฟ้าสูงในการสร้างเส้นใยจากของเหลว ผ่านหัวฉีดขนาดมิลลิเมตร พอลิเมอร์ที่นิยมใช้ในปัจจุบัน ได้แก่ คอลลาเจน แอลจิเนต ไฮยาลูรอน ไคโตซาน พอลิแลคติคแอซิค และพอลิไกลโคลิคแอซิค อย่างไรก็ตามโครงร่างพอลิเมอร์เหล่านี้มีข้อจำกัดคือ สลายตัวรวดเร็วในร่างกายแม้จะมีความแข็งเริ่มต้นสูง การสลายของพอลิเมอร์บางชนิด เช่น พอลิแลคติคแอซิค และพอลิไกลโคลิคแอซิค จะปลดปล่อยกรดออกมาทำให้เกิดการตอบสนองของเนื้อเยื่อที่ไม่พึงประสงค์ ปัจจุบันมีการใช้ พอลิไฮดรอกซีอัลคาโนเอต เข้ามาแทนที่เนื่องจากมีความเข้ากันได้ทางชีวภาพ และย่อยสลายได้ทางชีวภาพได้ดี ปัจจัยที่มีผลต่อกระบวนการปั่นเส้นใย เช่น แรงดันไฟฟ้า อัตราการไหล ความเข้มข้น น้ำหนักโมเลกุลของพอลิเมอร์ การปรับสัดส่วนของปัจจัยต่าง ๆ จะทำให้ได้เส้นใยนาโนสามมิติที่ซับซ้อน และมีเอกลักษณ์เฉพาะตัว บทความนี้เป็นการรวบรวมข้อมูลเบื้องต้น ได้แก่ การปั่นเส้นใยด้วยไฟฟ้าสถิตของพอลิ-ไฮดรอกซีอัลคาโนเอตการประยุกต์ใช้อิเล็กโตรสปันของพอลิไฮดรอกซีอัลคาโนเอต ทางชีวการแพทย์ และข้อเสนอแนะสำหรับการใช้เส้นใยพอลิไฮดรอกซีอัลคาโนเอตสำหรับทางการแพทย์ในอนาคต

คำสำคัญ: ความเข้ากันได้ทางชีวภาพ วัสดุชีวภาพ กระบวนการปั่นเส้นใยด้วยไฟฟ้าสถิต พอลิไฮดรอกซีอัลคาโนเอต และชีวการแพทย์

ABTRACT

Electrospinning is simple and is a cheap method that can be used to produce small-diameter fibrous structures with potential biomedical applications. The process uses a high-voltage electrical field to form solid fibres from a polymeric fluid jet delivered through a millimetre-scale nozzle. Normally, natural polymers for biomedical applications are collagen, alginate, hyaluronan and chitosan, and the synthetic polymers include poly (lactic acid) (PLA) and poly (glycolic acid) (PGA). However, the polymeric scaffolds show rapid strength degradation *in vivo* even with high initial strength. Degradation of certain polymers (PLA, PGA) creates a local acidic environment that can also cause adverse tissue responses. Currently, polyhydroxyalkanoate (PHA) is used in the electrospinning process, due to its excellent biocompatibility and biodegradability. The electrospinning parameters (e.g., voltage, flow rate, concentration and polymer molecular weight) can be varied in order to produce nanofibres with complex and unique three-dimensional shapes. This review includes electrospinning of PHA and its biomedical uses of PHA electrospun. Moreover, the future prospect of PHA electrospun was also recommended.

Keywords: Biocompatibility, Biomaterials, Electrospinning, Polyhydroxyalkanoate, Biomedical

Introduction

Electrospinning is a processing technique to spin polymer fibres with nanometer-scale diameters. This technique, invented in 1934 (Formhals, 1934). uses an electrical field that is applied across a polymer solution and a collector plate to expel a polymer solution jet from a small hole (Hou et al., 2002). As the solution jet travels, the solvent evaporates and leaves a charged polymer fibre that is elongated by an electrical force and attracted to the collection plate with an opposing or zero polarity. To date, many polymers have been successfully electrospun into nanofibres: electrospinning requires a very simple and economical setup, but it is an intricate process that depends on several molecular, processing and technical parameters (Garg et al., 2001). Electrospun scaffolds can be prepared based on a wide range of polymers. There are a variety of natural and synthetic materials with various structures and properties that are potential biomaterials for biomedical uses (Table 1) (Gordzha et al., 2017). However, the limitations and problems of the above mentioned polymers are large diameter and fibre size, weak mechanical strength, fast degradation rate, high viscosity, surface tension, strong water retention ability of the polymer solution and diminished structural strength. Therefore, research has turned to polyhydroxyalkanoate (PHA) in the electrospinning process instead of the other polymers.

For use in medical applications, materials must be biocompatible and nontoxicity, which means they cannot cause severe immune reactions when introduced to the host organisms' soft tissues or blood. Besides that, biodegradable materials are desirable biomaterials, since they break

down, excrete, or are absorbed. They do not need removal or surgical interventions. The porosity of the biopolymer matrix is important for wound healing applications because it allows cell filtration and provides high permeability for the diffusion of oxygen and nutrients. In addition, importantly the properties of the biomedical application of biomaterials include the degradation rate, mechanical strength and elasticity. PHA breakdown inside human cells varies from tissue to tissue and also depends on the dispensation methodology used to generate its various profiles, including PHA nanofibres, thin films or scaffolds (Brigham & Sinskey, 2012). Development of advanced methodologies used to fabricate the surface and PHA properties would certainly open new horizons for their applications in many more medical fields in the coming decades. PHA is a class of biopolyesters that are synthesised intracellularly by microorganisms, mainly different eubacteria genera. These biopolymers have diverse physical and chemical properties (The physical and chemical properties of PHA including crystallinity (%), melting temperature (°C), glass transition temperature (°C), tensile strength (Mpa), young's modulus (Gpa), elongation to break (%), resistance to UV light, resistance to solvents and biodegradability are 40-80, 86-179, -40-4, 5-20, 1-3.5, 40-300, good, weak and good, respectively.) that also classify them as biodegradable in nature and make them compatible to living organisms (Kourmentzna et al., 2015; Mozejko-Ciesielska et al., 2019). Therefore, the versatility of PHA in terms of their non-toxic degradation products, biocompatibility, desired surface modifications, wide range of physical and properties, cellular growth support and attachment without carcinogenic effects have enabled their use as in vivo implants: sutures, adhesion barriers and valves. These structures guide tissue repair and can be used in regeneration devices such as cardiovascular patches, articular cartilage repair scaffolds, bone graft substitutes and nerve guides.

Table 1 Different polymers used in electrospun: advantages and disadvantages for biomedical applications.

Electrospun Material	Advantages	Disadvantages	Ref.
Silk fibroin	Good biocompatibility, Biodegradability, Minimal inflammatory reaction and Excellent mechanical properties	The selection of appropriate solvents and control of the conformational transitions of fibroin during electrospinning.	(Altman et al, 2003)
Chitosan	High porosity, Mimic skin and Extracellular matrix	Electrospinning of pure chitosan is difficult.	(Croisier & Jerome, 2013)
Collagen	Highly conserved, Relatively non-immunogenic, Support tissue, Tissue maintenance and regeneration	It has relatively weak mechanical strength and a fast degradation rate.	(Bhardwaj & Kundu, 2010)
Gelatin	Biodegradability and Biocompatibility in physiological environments	Gelatin hinders the fibre-forming capacity.	(Bhardwaj & Kundu, 2010)
Hyaluronic acid	Biocompatibility and Biodegradability	It was difficult to form uniformly sized fibres from hyaluronan using electrospinning because of the high viscosity, surface tension and strong water retention ability of the hyaluronan solution.	(Bhardwaj & Kundu, 2010)
Fibrinogen	Easy degradability, Non-immunogenic and Promotes increased cell migration.	Fibrinogen is difficult to construct scaffolds with structural integrity and sufficient mechanical strength over time.	(Bhardwaj & Kundu, 2010; He et al., 2011)
PHA	Non-cytotoxic, Biocompatibility, Biodegradability, Thermo-processability (Melting points ranging from 40 to 180 °C)	Highly crystalline, Extremely brittle (Affect the electrospinning processing; broken and intermittent fibers) and Relatively hydrophobic (lack cell affinity for tissue engineering)	(Brigham & Sinskey, 2012; Bhardwaj & Kundu, 2010)

PHA Electrospinning

Electrospinning creates nanofibres through an electrically charged jet of polymer solution or melting. This technique is applicable to virtually every soluble or fusible polymer and is capable of spinning fibres in a variety of shapes and sizes with a wide range of properties to be used in a broad range of biomedical and industrial applications.

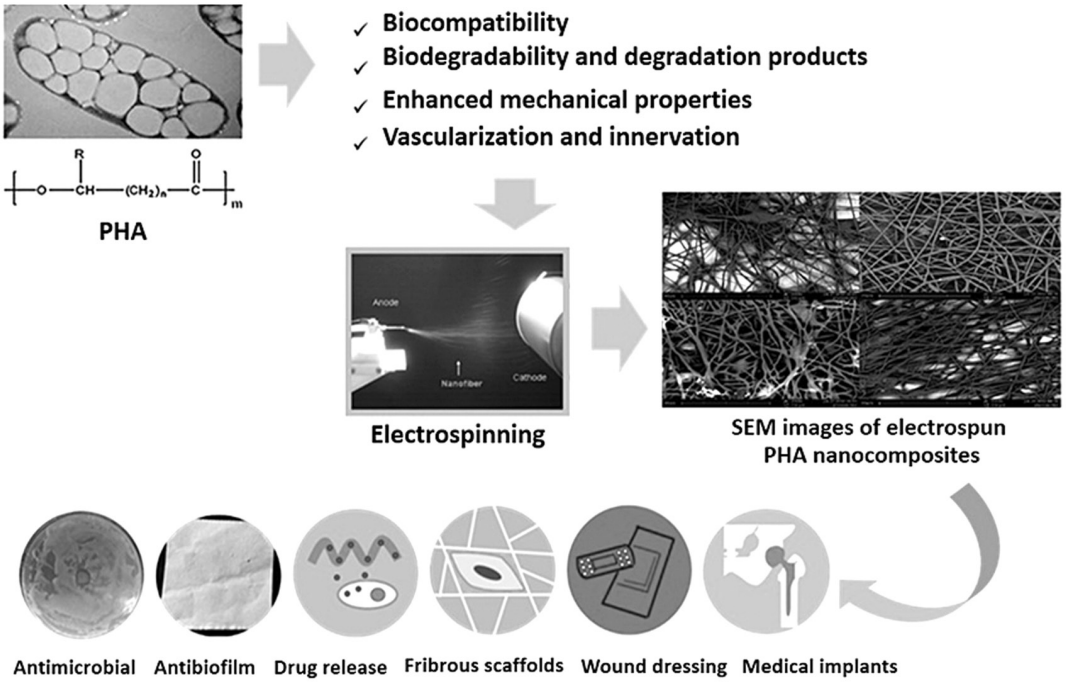

Figure 1 Various applications of electrospun nanofibres in biomedical research.
Abbreviations: PHA; SEM, scanning electron microscopy.
Source: (Weng & Xie, 2015), (with slight modifications).

PHA electrospinning has become a widely used and relied-upon method because it allows the production of different alignment scaffolds of ultra-fine fibres that range from nano- to micro-scale in diameter, from a wide range of natural and synthetic polymers. These biopolymers have diverse physical and chemical properties that also classify them as biodegradable in nature and make them compatible to living organisms (Kourmentzna et al., 2015). Therefore, the versatility of PHA in terms of their non-toxic degradation products, biocompatibility, desired surface modifications, wide range of physical properties, cellular growth support and attachment without carcinogenic effects have enabled their use as *in vivo* implants such as sutures, adhesion barriers and valves. These structures guide tissue repair and are part of regeneration devices, including cardiovascular patches, articular cartilage repair scaffolds, bone graft substitutes and nerve guides. This development, in turn,

has encouraged the use of various biodegradable and/or biocompatible polymer materials as fibre matrices for use in drug delivery, wound healing, tissue engineering, biosensors and antimicrobial and antibiofilm applications, among many others (Figure 1).

Biomedical Application of Electrospun PHA

Electrospun PHA is widely used for various biomedical applications, including antimicrobial and antibiofilm applications, drug release and delivery, tissue engineering, wound healing and biosensors, among others.

1. Antimicrobial and antibiofilm applications

(R)-3-hydroxyalkanoic acids (R-3HAs) are chiral compounds that can potentially be used as building blocks for compounds used in the pharmaceutical industry. 3HA can be transformed into 2-alkylated 3-hydroxybutyrate (3HB) and ß-lactones. These compounds can be employed as oral drugs. R-3HAs can be formed by PHA degradation. The most important compounds are carbapenem and macrolide antibiotics. A *Pseudomonas fluorescens* GK13 depolymerase enzyme encoded by phaZGK13 can depolymerise PHA to monomers. These monomeric units can reduce bacterial (e.g., *Staphylococcus aureus*) infection. Furthermore, when they are conjugated with D-peptide, they exhibit anticancer activity. Poly(3hydroxybutyrate-co-4hydroxybutyrate) [P(3HB-co-4HB)] enhances skin angiogenic properties to aid in wound healing (Iftikhar & Jamil, 2016).

Castro-Mayorga et al. (2017) produced electrospun PHA-silver nanoparticles for antimicrobial use. The obtained materials reduce *Salmonella enterica* below the detection limits at very low silver loading (0.002 ± 0.0005% weight).

Kehail et al. (2017) produced electrospun poly(3-hydroxybutyrate-co-3-hydroxyhexanoate) [P(HB-co-HHx)] for antibiofilm use. Maximum loading of 16.1 µg enzyme per 9.5 mm^3 discs effectively inhibit biofilm formation. Based on this study, P(HB-co-HHx) sheets are a suitable material for use as a potential raw material for fabrication of wound dressings for antibiofilm treatments.

2. Drug release and delivery

In order to improve drug efficacy, they should be delivered in a controlled and targeted manner. PHA has desirable physical properties with high biocompatibility. Hence, they could be effectively used as raw material for producing tablets, nano-particles and as drug scaffolds. 3HB monomers are useful in the synthesis of novel polymers, including dendrimers. These polymers are biodegradable and exhibit monodispersity and surface-functional moieties. These features make them potent drug carriers. 3HB and 4HB monomers have been exploited for preparing novel β- and γ-peptides, which are resistant to the action of peptidases. This feature enables them to remain longer in mammalian serum and thus improves their suitability for cargo-drug delivery. 3HB can inhibit glycolysis during haemorrhagic shock. These monomers are helpful in the synthesis of sex

hormones and fragrances (S-citronellol), and can also be used for antibacterial, anti-proliferative and haemolytic activities (Shrivastav et al., 2013).

Lee et al. (2015) produced electrospun poly(3-hydroxybutyrate-co-4-hydroxybutyrate) [P(3HB-co-4HHB)] for drug release and drug delivery. P(3HB-co-4HB) drug-loaded nanofibres could be utilised in the development of biocompatible drug-eluting stents by directly coating a metal stent with a homogeneous layer of electrospun polymer.

3. Bone tissue engineering

Bone tissue engineering refers to the regeneration of new bone by providing mechanical support while inducing cell growth on PHA scaffolds that have a porous structure for tissue regeneration. One review recently introduced the various PHA scaffold properties that make them suitable for bone tissue engineering, including biocompatibility, biodegradability, mechanical properties and vascularisation. The typical PHA scaffold fabrication techniques include electrospinning, salt-leaching and solution casting. Furthermore, the relatively new technology of using 3D printing in PHA scaffold fabrication is possible. Finally, there is recent progress in using different types of PHA scaffolds in bone tissue engineering by utilising intrinsic PHA/blend forms or as composites with other polymeric or inorganic hybrid materials (Lim et al., 2017). Sukovatiy et al. (2014) produced electrospun P(3HB), P(3HB-co-4HB), poly(3-hydroxybutyrate-co-3-hydroxyvalerate) [P(3HB-co-3HV)] and P(3HB-co-3HHx) for bone tissue engineering. None of the fibrous scaffolds produced by PHA electrospinning had adverse effects on attachment, growth or viability of NIH 3T3 mouse fibroblasts. Additionally, all of them are suitable for tissue engineering applications.

4. Wound healing

Electrospun PHA meshes started gaining high attention in the last decade for potential applications as a wound dressing in skin regeneration. A wide range of PHAs have been used to produce electrospun fiber meshes with different morphology and alignment for wound healing application. For example, Shishatskaya et al. (2016) demonstrated that the 1.4 times faster healing with respect to the wounds under the cell-free membrane and a 3.5 times faster healing than the wound healing under the control when used poly(3-hydroxybutyrate-co-4-hydroxybutyrate); [P(3HB-co-4HB)] membrane. Azimi et al. (2019) produced poly (3-hydroxybutyrate)/poly (3-hydroxyoctanoate-co-3 hydroxydecanoate) [P(3HB)/P(3HO-co-3HD)] fiber meshes surface-decorated via electrospray of chitin-lignin/glycyrrhizin acid (CLA) complexes. These biomaterials showed strong anti-inflammatory activity which is promising in wound healing applications. Kandhasamy et al. (2017) developed a composite scaffold for effective wound healing treatment based on a polyhydroxybutyrate / gelatin/ostholamide (OSA) electrospun blend coated with collagen. The obtained scaffold showed great mechanical stability, stable enzymatic degradation, and efficient antimicrobial activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Future Recommendations

Compared to other materials, there is relatively minimal work currently being performed to investigate the potential of electrospun PHA for biomedical applications. The current research benefits from the fact that the PHA polymer presents a variety of favourable characteristics: biodegradability, elasticity, non-toxicity, biocompatibility, surface modification capabilities, ability to function as nanoparticles and possibility for tailor-made physicochemical properties (Brigham & Sinskey, 2012; Bhardwaj & Kundu, 2010). All PHA produced to date are of bacterial origin, which is a very costly process; hence, they are somewhat actively being exploited in research and development purposes (Ojumu et al., 2014). Microbial PHA production also favours the possibility of obtaining polymers with unique monomer compositions more economically through metabolic engineering approaches. Identification of some novel bacterial strains with the ability to accumulate unique PHA monomers that possess further versatile properties might be helpful in this endeavour. PHA purity, monomer composition, and surface modifications all influence cell responses. These biodegradable polymers certainly deserve a vaunted position, because countless clinical procedures that involve these polymers as medical implants are performed on a daily basis worldwide (Sadat-Shojai, 2016). However, the human body's immune response to the implanted materials must be addressed more precisely, and the use of animal models for testing these implants will help enormously for this purpose. Finally, there are more possibilities to obtain ideal biomedical products in the next few years than previously thought possible (based on the views and expectations in the previous century). Indeed, we have observed massive advancements in the production of non-toxic, biocompatible and versatile PHA in the past decade.

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