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Electrosprayed Montelukast/poly (lactic-co-glycolic acid) particle based coating: A new therapeutic approach towards the prevention of in-stent restenosis

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ABSTRACT

Drug-eluting stents (DESs), have shown promising results in prevention of in-stent restenosis after percutaneous coronary intervention (PCI). The elevated level of leukotrienes (LTs) detected in injured arteries after PCI, together with the potential role of LTs in inflammatory cascades and structural alterations in arterial wall provides the rationale for development of therapeutic strategies for prevention of in-stent restenosis using LTs receptor antagonists. Montelukast (MK) is a selective cysLT$_1$ receptor antagonist, with anti-inflammatory and anti-proliferative properties, which has been used for treatment of various diseases. Here, we report on the fabrication of MK/PLGA particles by electrospraying, aiming towards the development of particle based coating of DESs. The electrosprayed particles incorporated with 3% and 6% w/w MK exhibited fairly spherical shape with smooth surfaces and narrow size distribution. Sustained release of MK for up to 40 days was obtained for both formulations, with higher initial burst release and drug release rate for the particles with higher drug loading. The LTD$_4$ induced proliferation and migration of human coronary artery smooth muscle cells (HCASMCs) by 35% and 85%, respectively, which was substantially antagonized using MK incorporated particles. Nevertheless, MK antagonism preserved the normal proliferation and migration of human coronary artery endothelial cells (HCAECs). Moreover, MK antagonism inhibited the LTD$_4$ induced phenotypic transition of HCASMCs from contractile to synthetic type. The electrosprayed MK-PLGA particles can be employed as a coating for DESs to inhibit the formation of neointimal hyperplasia responsible for in-stent restenosis, yet preserve the healing rate of the stented vessel.

Statement of significant

Montelukast (MK) is a selective cysLT$_1$ receptor antagonist, with anti-inflammatory and anti-proliferative properties. The LTD$_4$ induced proliferation and migration of human coronary artery smooth muscle cells by 35% and 85%, respectively, which was substantially antagonized using MK incorporated particles. MK antagonism preserved the normal proliferation and migration of human coronary artery endothelial cells. The MK antagonism inhibited the phenotypic transition of human coronary artery smooth muscle cells from contractile to synthetic one induced by LTD$_4$. The electrosprayed MK-PLGA particles can be employed as coating for DESs to inhibit formation of neointimal hyperplasia, responsible for in-stent restenosis.

1. Introduction

Percutaneous coronary intervention (PCI), commonly named coronary angioplasty, is a nonsurgical treatment for stenotic coronary arteries, involved in cardiovascular diseases such as angina,

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acute myocardial infarction (MI) and multi-vessel coronary artery disease. During this process, a metallic scaffolding mesh, called stent, is usually introduced into the artery, to mechanically support the arterial wall and prop it open. However, due to foreign body reactions and injuries that occur along the endothelial surface, the inflammatory responses are triggered, leading to platelet adhesion/aggregation, thrombus formation, accumulation of inflammatory cells and eventually in-stent restenosis [1]. Among the various processes involved in occurrence of in-stent restenosis, neointimal hyperplasia is considered as the major mechanism. In this process, the proliferation of medial smooth muscle cells (SMCs) as well as their migration into intima is stimulated, due to elevated secretion of different growth factors and cytokines by the recruited inflammatory cells. Thus, the neointimal layer is formed in the stent lumen, increasing the thickness of the arterial wall, which eventually hinders the long-term benefit of the stent implantation [2,3]. Drug-eluting stent (DES), incorporated with effective drugs for prevention of restenosis has gained a lot of interests during the last decades. An ideal DES has to provide a local controlled release of the therapeutic agent with sufficient concentration for a desired period of time. As a result, the first-pass metabolism is bypassed, which results in higher drug availability/efficacy and lower systemic exposure to the drug [4]. Different techniques such as immersion, chemical vapour deposition, ultrasonic atomization and plasma treatment have been used to produce drug incorporated coatings on the stents [5,6]. However, the difficulty to control the thickness and composition as well as the complexity of the coating process hindered the successful use of these techniques to fabricate DESs for clinical applications.

Electrospraying also known as electrohydrodynamic atomization (EHDA) is a versatile technique for fabrication of micro/nanoparticle based coatings for various applications using electrical charges [7]. This technique enables production of micro/nanoparticle based coatings at a single step process, which is conducted at room temperature. Thus various bioactive reagents can be incorporated into the coating, with minimized risk of deactivation. The size and morphology of the micro/nanoparticles deposited on the device surface can be tailored by varying the solution and processing variables such as solution viscosity and conductivity, flow rate and applied voltage. The morphology of the particles as well as the electrospraying time would enable controlling the physical characteristics of the coating i.e. the thickness, compactness and porosity. Electrosprayed coatings showed great promise towards the fabrication of thin layer coatings for biomedical devices. Acceleration of osteogenesis on the surface of orthopedic implants coated with osteogenic compounds [8–11] and fabrication of anti-bioadhesive surfaces by means of superhydrophobic electrosprayed coatings [12,13] are two of the successful applications of electrosprayed particles as coating for biomedical devices. In spite of the successes in using electrosprayed coatings for other biomedical devices, only few studies have been reported on development of vascular DESs using electrospraying [5,14]. Thus, more extensive investigations are required to explore the potential benefits of this technique for developing vascular DESs.

With regard to the therapeutic agent, various anti-inflammatory, immunosuppressive, anti-proliferative, anti-thrombosis and anti-metabolic agents have been incorporated into DESs to inhibit different mechanisms resulting in restenosis [4]. Many of the employed therapeutic compounds have multiple mechanisms of action to stop progression of restenotic process. Among them, anti-proliferative drugs such as paclitaxel and sirolimus have been widely investigated to block the proliferation of SMCs and neointimal growth, mainly by inhibition of DNA synthesis. Although these anti-proliferative reagents have shown to be beneficial in clinical trials [15,16], the arteries treated with these compounds exhibited delayed healing process due to simultaneous, undesirable prevention of endothelial cells (ECs) cycle progression and, thus, extensive investigations are yet ongoing to find more efficient medications to target neointimal formation [16].

Leukotrienes (LTs) are a group of potent inflammatory mediators with proven role in pathogenesis of bronchoconstriction, mucous hypersecretion, and airway inflammation. The previous studies have provided evidence that PCI can significantly trigger intracoronary formation of LTs [17]. LTs are generally categorized into two different groups, according to their chemical structure: (1) cysteinyl-LTs (cysLTs) consist of LT C_4, D_4, and E_4, containing cysteinyl residues in their chemical structure, and (2) LTB_4 containing hydroxyl groups rather than amino acids [18]. LTs exert their biological actions via interaction with transmembrane G-protein coupled receptors: cysLT receptors defined as $cysLT_1$ and $cysLT_2$, represent affinity for cysLTs, and BLT receptors denoted as BLT_1 and BLT_2, activated by LTB_4 [19,20]. Both cysLT and BLT receptors can be expressed on vascular cells, i.e. SMCs and ECs. However the expression of each specific receptor is dependent on the tissue of origin, species and pathophysiological condition (e.g. the presence of pro-inflammatory cytokines), which in turn determines the dominant subtype of LT, regulating the biological events [21]. Recent evidences suggest that LT signalling has implications in pathophysiological mechanisms such as neointimal hyperplasia [22] and accumulation of leukocytes [23] involved in restenosis. Although the use of specific antagonists for blocking the respective LT receptors has been well-established for inhibition of LT induced airway diseases such as asthma [22,24], the concept of using these antagonists for prevention of inflammatory responses and structural changes in the arterial wall associated with restenosis has not been widely explored [25]. Thus, further studies are required to determine the exact role of each specific LT receptor in progression of restenotic process and the putative antagonist to be employed for treatment of the disease. To the best of our knowledge, no study has been reported on the fabrication of LT receptor antagonist-eluting stent, for prevention of restenosis. Here, we report the fabrication of montelukast (MK) incorporated particle based coatings for prevention of in-stent restenosis after angioplasty. MK was chosen as a selective $cysLT_1$ receptor antagonist frequently used for asthma therapy [26,27]. It has been reported to exhibit anti-inflammatory, anti-proliferative, and antioxidant properties for treatment of various diseases [26,28]. Poly (lactic-co-glycolic acid) (PLGA), an FDA approved polymer served as the biodegradable carrier of this study. The effect of MK-PLGA particles on the proliferation/migration of LTD_4 (a cysLT with high affinity for $cysLT_1$ receptors) stimulated human coronary artery smooth muscle cells (HCASMCs) and human coronary artery endothelial cells (HCAECs) was investigated. The developed MK incorporated particles can be potentially employed as a coating for vascular stents to inhibit restenosis.

2. Experimental section

2.1. Materials

Poly (D,L-lactic-co-glycolic) (50:50, Mw: 57.6–91.6 kDa) was purchased from Lactel Absorbable Polymers (Birmingham, AL, USA). Montelukast (MK) was kindly provided by (Daru Pakhsh, Iran). LTD_4 was purchased from Cayman (Ann Arbor, MI, USA). Dichloromethane (DCM), methanol (MeOH), phosphate buffer saline (PBS) and lipopolysaccharide (LPS, from Escherichia coli) were all bought from Sigma-Aldrich (Singapore).