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Surface modification of biomaterials based on high-molecular polylactic acid and their effect on inflammatory reactions of primary human monocyte-derived macrophages: Perspective for personalized therapy

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ABSTRACT

Polylactic acid (PLA) based implants can cause inflammatory complications. Macrophages are key innate immune cells that control inflammation. To provide higher biocompatibility of PLA-based implants with local innate immune cells their surface properties have to be improved. In our study surface modification technique for high-molecular PLA (MW = 1,646,600 g/mol) based biomaterials was originally developed and successfully applied. Optimal modification conditions were determined. Treatment of PLA films with toluene/ethanol = 3/7 mixture for 10 min with subsequent exposure in 0.001 M brilliant green dye (BGD) solution allows to entrap approximately 10^{-9} mol/cm^2 model biomolecules. The modified PLA film surface was characterized by optical microscopy, SERS, FT-IR, UV and TG/DTA/DSC analysis. Tensile strain of modified films was determined as well. The effect of PLA films modified with BGD on the inflammatory reactions of primary human monocyte-derived macrophages was investigated. We developed in vitro test-system by differentiating primary monocyte-derived macrophages on a coating material. Type 1 and type 2 inflammatory cytokines (TNFα, CCL18) secretion and histological biomarkers (CD206, stabilin-1) expression were analyzed by ELISA and confocal microscopy respectively. BGD-modified materials have improved thermal stability and good mechanical properties. However, BGD modifications induced additional donor-specific inflammatory reactions and suppressed tolerogenic phenotype of macrophages. Therefore, our test-system successfully demonstrated specific immunomodulatory effects of original and modified PLA-based biomaterials, and can be further applied for the examination of improved coatings for implants and identification of patient-specific reactions to implants.

1. Introduction

Bioinert biomaterials are widely used in various clinical applications such as dental, orthopedic, cardiovascular, and reconstructive surgery. Nevertheless, the implantation process can cause medical complications and initiate a foreign body response and inflammation [1]. Macrophages are key innate immune cells that coordinate local inflammatory responses in the tissues [2]. Two major types of macrophage activation have been described by us and others: classically activated M1 and alternatively activated M2 [3]. M1 phenotype can be induced by IFNgamma that strongly stimulates TNFalpha expression, while M2 phenotype can be induced by IL-4 that stimulates expression of CD206 and CCL18. M2 phenotype definition covers a broad range of macrophage subtypes found in sites of healing, tumorous and chronic inflammation, where stabilin-1 was shown by us to be expressed on most of M2 subtypes [4,5]. Wear particles have been shown to induce activation of macrophages and the production of pro-inflammatory cytokines [6]. Exposure of macrophages to titanium particles resulted in an increased secretion of TNF-α, IL-1 and IL-6 [7]. Macrophage inflammatory proteins CCL3 and CXCL2 were also linked to implant-associated inflammation and to bone degradation [8]. Moreover, in aseptic loose periprosthetic tissue macrophages represent approximately 70% of all cells

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[9]. Thus, reaction of macrophages to the implant materials is indicative for the acceptance of this implant by the patient and is predictive for the inflammatory responses to implant leading to the implant intolerance. However, the reliable test-system to examine individual patient innate immune reaction to implant materials and coating was not available to date.

To provide higher implant biocompatibility synthetic biodegradable polymer coatings and materials can be used [10]. For these purposes polylactic, polyglycolic acid, trimethylene carbonate and their mixtures with different ratios of components are applied for implant production [11]. Polylactic acid (PLA) is the most promising one due to its advantages: biocompatibility, processibility and good mechanical properties [12]. In the human body PLA can degrade into lactic acid, which is incorporated then into the tricarboxylic acid cycle and excreted [13]. For these reasons polylactic acid has been widely used in resorbable sutures, clips, plates and screws and in drug delivery devices [14]. The inflammatory complications arising from biodegradable orthopedic implants of lactic acid polymers occur at a rate of less than 10% [15]. It happens if the surrounding tissue cannot eliminate the acid by-products of a rapidly degrading implant [16] and depends on implant size and implantation site.

However, such properties of PLA as poor toughness, hydrophobicity and, what is the most important, lack of reactive side-chain groups can limit its further application in tissue engineering. Thus, PLA chemical inertness makes its surface and bulk modifications a challenging task [12].

Bulk-modification methods are usually used to improve mechanical properties (toughness) [17], degradation behavior [18] and crystallinity of PLA [19]. Stereochemical and processing manipulation, copolymerization and blending are main bulk-modification methods [12].

Surface functionalization is another advanced PLA modification method, which makes it possible to improve physical and chemical surface properties of PLA and, moreover, to create cell-recognition domains on its surface [20]. It includes covalent attachment methods of "wet" chemistry [21–23], the use of UV irradiation [24] and non-covalent attachment by plasma treatment [25–27], the introduction of migrating additives [28], and coating of the PLA surface [29–32]. One of the perspective modification methods is the entrapment of biologically active molecules into the PLA surface layer due to its swelling in solvent/non-solvent mixture. In this way substances such as polyethylene glycol, polylysine [33], chitosan, gelatin, and sodium alginate [34] were entrapped to the PLA film surface. However, average molecular weight of PLA used for the entrapment technique had not exceeded 10^5 g/mol.

The aim of our study was to develop an entrapment modification technique for the high-molecular PLA (MW = 1,646,600 g/mol) based biomaterials and to examine the effect of PLA films modified with brilliant green dye on the inflammatory reactions of primary human monocyte-derived macrophages. Brilliant green dye (BGD) was chosen as a modifying agent because it allows us to visualize the results of the modification process. Optimal modification process parameters were revealed and changes in surface properties of PLA films after modification were investigated. Modified materials have improved thermal stability and good mechanical properties. We analyzed the immunomodulatory properties of original and modified PLA films by development in vitro test-system using human monocyte-derived primary macrophages. Analysis of pro-inflammatory and tolerogentic parameters revealed additional detrimental inflammatory reactions on the modified materials. These reactions were donor-specific. Therefore we, for the first time, have analyzed not only general biocompatibility of the PLA-modifications, but an essential for the implant tolerance or rejection activation of key innate immune cells. Our test system opens the perspectives for 1) examination of patient-specific immune reactions to implants and making of personalized therapeutic decisions and 2) testing next variations of implant coating modifications for their interaction with the immune system and improvement of immunotolerogenic properties of implant materials.

2. Materials and methods

2.1. Preparation of PLA films

A weighted sample of PLA PURASORB® PL65 (Purac, The Netherlands) was dissolved in a mixture of dichloromethane and chloroform (40:60 v/v) (Panreac, Spain) to obtain a solution with concentration of 1.7%. Solution homogenization time was 12 h. Then 18 ± 1 g of solution was poured into a dry petri dish 10 cm in diameter, sealed and dried in solvent vapor for 72 h. Thickness of obtain films was 30 ± 2 μm.

2.2. Modification of PLA film surface

To perform surface modification technique dry PLA films were used. To reveal optimal experiment conditions solvent/non-solvent ratio variations and exposure time in stabilizing solution were investigated.

2.2.1. Method of determination of optimal solvent/non-solvent ratio

Films were dipped into toluene/ethanol (x:y v/v) (Panreac, Spain) miscible mixture for 10 min then rapidly transferred into stabilizing solution. The stabilizing solution was a 0.001 M solution of BGD in ethanol/water mixture (1:1 v/v). Films were incubated in a stabilizing solution for x h. Thereafter, films were dried for 1 h, soaked in 5% ethanol solution for 24 h to remove extra BGD and then dried under vacuum for 12 h.

2.2.2. Method of determination of optimal exposure time in brilliant green dye stabilizing solution

Films were dipped into toluene/ethanol (3:7 v/v) miscible mixture for 10 min then rapidly transferred into stabilizing solution. The stabilizing solution was a 0.001 M solution of BGD in ethanol/water mixture (1:1 v/v). Films were incubated in a stabilizing solution for z h. Thereafter, films were dried for 1 h, soaked in 5% ethanol solution to remove extra BGD and then dried under vacuum for 12 h.

2.3. Characterization of modified PLA films

2.3.1. Morphology investigation

The morphology of original and modified PLA films was investigated by optical microscopy (Motic DM-111) using 40× and 100× magnification.

2.3.2. Spectroscopy analysis

Analysis of the surface chemical structure of original and modified PLA films was performed by using SERS (surface enhanced Raman scattering) and FT-IR (Fourier transform infrared spectroscopy). SERS spectra were recorded by using Centaur U HR complex (NanoScanTechnology, Russia) in Raman intensity (a.u.) mode with 6000–100 cm^{-1} Raman shift ranges at 532.5 nm laser wavelength and 5 mW laser power. FT-IR spectra were obtained using Nicolet 5700 system (Thermo Scientific, USA) in transmittance mode (%) with 4000–600 cm^{-1} wavenumber ranges and 4 cm^{-1} resolution.

Quantification of BGD entrapped on the PLA film surface was provided by using ultraviolet (UV) spectroscopy (Specord 250 Plus, Analytik Jena AG, Germany). UV-spectra of modified PLA films were recorded in absorbance mode with 190–700 nm wavelength ranges. Amount of BGD on PLA film surface (mol/cm^2) was calculated according to Beer–Lambert–Bouguer law at a characteristic BGD absorption maximum in the long wavelength region (λ_{max} = 640 nm) using BGD attenuation coefficient. The following equation was used:

$$C = \frac{A \cdot V}{\varepsilon \cdot l \cdot S}, \quad (1)$$

where C is amount of BGD on modified PLA film surface (mol/cm^2), A — absorbance, ε — attenuation coefficient (l $mol^{-1} \cdot cm^{-1}$), l — path length