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Research Paper

Mechanistic analysis of PLGA/HPMC-based *in-situ* forming implants for periodontitis treatment

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ABSTRACT

In-situ forming implant formulations based on poly(lactic-co-glycolic acid) (PLGA), acetyltributyl citrate (ATBC), minocycline HCl, N-methyl pyrrolidone (NMP) and optionally hydroxypropyl methylcellulose (HPMC) were prepared and thoroughly characterized in vitro. This includes electron paramagnetic resonance (EPR), nuclear magnetic resonance (^{1}H NMR), mass change and drug release measurements under different conditions, optical microscopy, size exclusion chromatography (SEC) as well as antibacterial activity tests using gingival crevicular fluid samples from periodontal pockets of periodontitis patients. Based on these results, deeper insight into the physico-chemical phenomena involved in implant formation and the control of drug release could be gained. For instance, the effects of adding HPMC to the formulations, resulting in improved implant adherence and reduced swelling, could be explained. Importantly, the *in-situ* formed implants effectively hindered the growth of bacteria present in the patients' periodontal pockets. Interestingly, the systems were more effectively hindering the growth of *pathogenic* bacterial strains (e.g., *Fusobacterium nucleatum*) than that of strains with a lower pathogenic potential (e.g., *Streptococcus salivarius*). In vivo, such a preferential action against the pathogenic bacteria can be expected to give a chance to the healthy flora to re-colonize the periodontal pockets.

1. Introduction

Periodontitis is a highly prevalent, chronic inflammatory disease of the periodontium [1–3]. It may be defined as "a disease that affects the periodontal structures and, as a result of interactions between periodontopathogens and the host immune response, leads to the destruction of the tooth supporting tissues, periodontal ligament and alveolar bone" [1]. Briefly, microorganisms colonizing the patients' periodontal pockets are considered as a major factor, causing inflammation and tissue destruction [4]. It seems that the bacterial flora in the disease state is different from that in healthy subjects. For example, the number of gram negative anaerobic bacteria is likely to be increased, and certain clinical forms of periodontitis might be related to specific microbiota [5]. For instance, Silva-Boghossian et al. [1] reported that *Streptococcus* strains (such as *Streptococcus sanguinis*) are associated with suppuration in periodontitis subjects. However, up to date, the exact mechanisms underlying this disease are not yet fully understood. It is hypothesized that: (i) suspected periodontal pathogens produce biologically active molecules, which directly attack the host tissue, and/or that (ii) the immune response of the host organism (human body) to these pathogens results in the tissue destruction. The consequence of the tissue loss is the deepening of the periodontal pockets, and once the mechanical anchorage of the tooth becomes insufficient the latter are lost. Periodontitis is in fact the main cause for tooth loss in adults [6]. A recent survey estimates that 47% of the US adults have mild, moderate or severe periodontitis [7]. The prevalence rate even increases to 64% for adults, who are older than 65 years.

At present, the standard treatment method of periodontitis is the mechanical removal of the bacteria (in particular of bacterial biofilms). This is a procedure also called "root planing". But the geometry of the patients' pockets can be very challenging for this type of treatment: Parts of the pockets might be very difficult to access with the dentist's instruments. As the oral cavity is always highly colonized, complete removal of bacteria cannot be expected.

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The remaining pathogenic microorganisms, thus, have a chance to re-colonize the periodontal pockets soon after the treatment. In order to reduce the risk of such pathogen re-appearance, it has been suggested to combine mechanical root planing with drug treatments [4,8,9]. However, appropriate delivery of drugs to the site of action is difficult, since many compounds do not easily partition into the periodontal pockets. In addition, the gingival crevicular fluid (GCF) flow generally rapidly eliminates the drug from its site of action [10]. For instance, it has been estimated that the contents in a 5 mm periodontal pocket are renewed 40 times per hour [11]. Thus, using conventional administration routes, often high systemic drug levels are required, while the drug concentrations at the target site remain low. This leads to potentially severe side effects combined with limited or insufficient therapeutic efficacy, despite the availability of highly potent drugs, able to act against the pathogenic flora and inflammation. Controlled local drug delivery systems offer the possibility to overcome these crucial hurdles of limited drug accessibility to the site of action and rapid elimination, releasing the drug in a controlled manner directly in the periodontal pockets during prolonged periods of time [4,12–14]. In-situ forming implants are particularly promising for this purpose, since these are liquid formulations, which upon injection into the periodontal pockets form customized solid implants: The fluids readily spread within the cavities, assuring that the entire pockets are filled with formulation and that the shape and geometry of the resulting implants are fully adapted to the characteristics of every single patient and each single pocket.

In this study, poly(lactic-co-glycolic acid) (PLGA) has been chosen as a matrix former for such in-situ forming implants for periodontitis treatment, due to its biocompatibility and biodegradability. Together with the drug (here minocycline HCl) the polymer is dissolved in N-methyl pyrrolidone (NMP). For information on the biocompatibility of NMP see for instance Kempe and Mäder [14]. Once injected, the organic solvent diffuses into the surrounding environment and aqueous biological fluids from the periodontal pocket penetrate into the liquid formulations. Since PLGA is not soluble in water, it subsequently precipitates and entraps the drug. This type of advanced local drug delivery systems for periodontitis treatment offers various important advantages, including the following: (1) A relatively easy administration (injection of a liquid, compared for instance with the placement of a pre-formed implant); (2) There is no need to remove empty remnants upon drug exhaust, due to complete biodegradability of the system; (3) The geometry and size of the resulting implants are adapted to the patient's dental pockets (personalized medicine); (4) The incorporated drug is locally released in a time-controlled manner through the slowly degrading polymeric system; and (5) Systemic side effects are much less likely, such as impact on other mucosal surfaces (which might increase the emergence of antimicrobial resistance).

However, up to date major challenges remain to be addressed, namely the fact that: (i) The adherence of such in-situ formed implants to human tissue is yet poor, resulting in pre-mature and uncontrolled expulsion of at least parts of the implants from the dental pockets due to the non-negligible flow of gingival crevicular fluid [15]; This leads to a considerable uncertainty with respect to the amount of drug, which really reaches the target site and with respect to the time periods during which therapeutic drug levels are provided; (ii) The balance elasticity/plasticity of the formed implants is generally not adapted to this type of local administration: Systems, which are difficult to deform plastically are not able to adapt their geometry to dynamic changes in the periodontal pocket' size and shape with time. Also, fully elastic implants force the periodontal pockets to keep their geometry and dimensions, which is not desirable. It has recently been proposed to add plasticizers, such as acetyltributyl citrate (ATBC) as well as a second type of polymer, such as hydroxypropyl methylcellulose (HPMC) to the liquid formulations in order to improve the adhesive and mechanical properties of the resulting implants [16]. However, yet it is unclear how these additives affect the underlying physico-chemical phenomena involved in implant formation and the control of drug release, and whether the antibacterial activity of the implants is altered.

It is well documented that the physical and chemical processes in the formation of implants based on such solvent induced phase separation are complex and that the impact of the composition of the systems on drug release is not straightforward [17–22]. For example, McHugh and co-workers reported that the addition of polyvinylpyrrolidone (PVP) accelerates the phase separation and increases the release rate of lysozyme at early time points, but does not significantly affect the water influx rate and implant morphology [23]. Increasing the polymer concentration in the formulation led to a decrease in the phase separation rate, a decreased water uptake rate and significant changes in the implants' porosity. The addition of triacetin also slowed down the phase separation rate and altered the implants' morphology, resulting in decreased drug release rates. Interestingly, the type of release medium (water versus phosphate buffer versus horse serum) did not affect the phase separation and water uptake rates as well as the implants' morphology to a noteworthy extent. In a later study, they also showed that the addition of Pluronic led to faster phase separation and increased water uptake, but decreased lysozyme release rates [24]. Importantly, advanced physico-chemical characterization techniques, such as electron paramagnetic resonance (EPR) and nuclear magnetic resonance (^{1}H NMR) measurements can be expected to be able to provide highly valuable new insight into the underlying mass transport phenomena [25,26].

The aim of this study was to better understand the physico-chemical processes involved in the formation of PLGA-based implants and the control of drug release. EPR, ^{1}H NMR, mass change and drug release measurements under different conditions, optical microscopy and size exclusion chromatography (SEC) were applied. Particular attention was paid to: (i) the impact of adding HPMC to the formulation, which improves the adhesive forces and mechanical properties of the implants, as well as (ii) the antibacterial activity of the systems, using gingival crevicular fluid samples, obtained from periodontal pockets of periodontitis patients.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic-co-glycolic acid) (PLGA, Resomer RG 504 H; Evonik, Darmstadt, Germany); acetyltributyl citrate (ATBC; Morflex, Greensboro, NC, USA); hydroxypropyl methylcellulose (HPMC, Methocel E50; Colorcon, Dartford, UK); N-methyl pyrrolidone (NMP, 99%), glucose and cysteine chlorhydrate (Acros organics, Geel, Belgium); minocycline hydrochloride dihydrate (minocycline HCl; Fagron, Colombes, France); ascorbic acid (Cooper, Melun, France); sodium metabisulfite (Merck, Darmstadt, Germany); dimethyl sulfoxide (DMSO, 99.5%; Grüssing, Filsum, Germany); tetrahydrofuran (THF, 99.99%, analytical reagent grade, stabilized with 0.025% butylhydroxytoluene); acetonitrile (HPLC grade) (Fisher Scientific, Loughborough, UK); oxalic acid (Sigma–Aldrich, Saint-Quentin Fallavier, France); ethylenediamine tetraacetic acid (EDTA; VWR, Haasrode, Belgium); 4-hydroxy-tempo benzoate (TB; Sigma–Aldrich, Seelze, Germany); agarose (GenAgarose LE; Genaxxon BioScience, Ulm, Germany); Columbia agar base and agar (Oxoid, Basingstoke, UK); defibrinated horse blood (E&O Laboratories, Burnhouse, UK);

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