International Journal of Pharmaceutics 486 (2015) 38–51

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

In-situ forming composite implants for periodontitis treatment: How the formulation determines system performance

M.P. Do [a,b], C. Neut [a,c], H. Metz [d], E. Delcourt [b,e], K. Mäder [d], J. Siepmann [a,b], F. Siepmann [a,b,*]

[a] University of Lille, College of Pharmacy, 3 Rue du Prof. Laguesse, 59006 Lille, France
[b] INSERM U 1008, 3 Rue du Prof. Laguesse, Lille 59006, France
[c] INSERM U 995, 3 Rue du Prof. Laguesse, Lille 59006, France
[d] Martin-Luther-University Halle-Wittenberg, Department of Pharmaceutics and Biopharmaceutics, Halle/Saale, Germany
[e] University of Lille, School of Dentistry, Place de Verdun, Lille 59000, France

ARTICLE INFO

Article history:
Received 5 February 2015
Received in revised form 10 March 2015
Accepted 13 March 2015
Available online 16 March 2015

Keywords:
In-situ forming implant
Periodontitis
PLGA
EPR
Doxycycline

ABSTRACT

Periodontitis is the primary cause of tooth loss in adults and a very wide-spread disease. Recently, composite implants, based on a drug release rate controlling polymer and an adhesive polymer, have been proposed for an efficient local drug treatment. However, the processes involved in implant formation and the control of drug release in these composite systems are complex and the relationships between the systems' composition and the implants' performance are yet unclear. In this study, advanced characterization techniques (*e.g.*, electron paramagnetic resonance, EPR) were applied to better understand the *in-situ* forming implants based on: (i) different types of poly(lactic-*co*-glycolic acid) (PLGA) as drug release rate controlling polymers; (ii) hydroxypropyl methylcellulose (HPMC) as adhesive polymer; and (iii) doxycycline or metronidazole as drugs. Interestingly, HPMC addition to *shorter* chain PLGA slightly *slows down* drug release, whereas in the case of *longer* chain PLGA the release rate substantially *increases*. This opposite impact on drug release was rather surprising, since the only difference in the formulations was the polymer molecular weight of the PLGA. Based on the physico-chemical analyses, the underlying mechanisms could be explained as follows: since longer chain PLGA is more hydrophobic than shorter chain PLGA, the addition of HPMC leads to a much more pronounced facilitation of water penetration into the system (as evidenced by EPR). This and the higher polymer lipophilicity result in more rapid PLGA precipitation and a more porous inner implant structure. Consequently, drug release is accelerated. In contrast, water penetration into formulations based on shorter chain PLGA is rather similar in the presence and absence of HPMC and the resulting implants are much less porous than those based on longer chain PLGA.

1. Introduction

Periodontitis is a highly prevalent, chronic inflammatory disease of the periodontium. A recent survey estimates that 47% of the US adults have mild, moderate or severe periodontitis (Eke et al., 2012). Prevalence rates increase to 64% for adults older than 65 years. Periodontitis is characterized by a progressive loss of the alveolar bone and periodontal ligament, leading to the formation of periodontal pockets (Silva-Boghossian et al., 2013; Holt and Ebersole, 2005; Silva-Boghossian et al., 2009). If untreated, periodontitis can lead to the loosening and subsequent loss of the teeth. It is indeed the primary cause of tooth loss in adults (Pihlstrom et al., 2005). The initiating factors of periodontitis are likely to be pathogenic bacteria and bacterial products, which form a biofilm covering the teeth surface in the subgingival area. It seems that the microflora 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 associated with specific microbiota (Moore and Moore, 1994). Recently, Silva-Boghossian et al. (2013) reported that *Streptococcus* strains (such as *Streptococcus sanguinis*) are also 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) the

* Corresponding author at: University of Lille, College of Pharmacy, INSERM U 1008, 3 Rue du Prof. Laguesse, Lille 59006, France. Tel.: +33 3 20964708; fax: +33 3 20964942.
E-mail address: florence.siepmann@univ-lille2.fr (F. Siepmann).

http://dx.doi.org/10.1016/j.ijpharm.2015.03.026
0378-5173/

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.

The treatment of periodontitis is highly challenging, since drug partitioning into the periodontal pockets is generally not very pronounced and gingival crevicular fluid flow rapidly eliminates drugs from the site of action (Pascale et al., 1986). For example, it has been estimated that the content in a 5 mm deep periodontal pocket is renewed 40 times per hour (Greenstein and Tonetti, 2000). Thus, using conventional administration routes (such as oral, intravenous, intramuscular, subcutaneous etc.) often very high systemic drug levels are required, while the drug concentration at the target site remains low. This leads to potentially severe side effects and limited or insufficient therapeutic efficacy, despite the availability of highly potent drugs able to act against the pathogenic flora and inflammation. Importantly, the crucial hurdles of limited accessibility of the site of action and rapid elimination can be overcome using advanced local drug delivery systems, releasing the drug in a time-controlled manner in the periodontal pockets during prolonged periods of time (Schwach-Abdellaoui et al., 2000, 2001; Heller et al., 2002; Kempe and Mäder, 2012; Parent et al., 2013; Thakur et al., 2014). Biodegradable *in-situ* forming implants are particularly promising for this purpose (Van Tomme et al., 2008; Kranz and Bodmeier, 2007; Soskolne, 1997): these are liquid formulations, which upon injection into the periodontal pockets form solid implants. The implant formation can be induced by different mechanisms (Kempe and Mäder, 2012; Hatefi and Amsden, 2002; Avachat and Kapure, 2014), for example solvent exchange: briefly, the basic idea is to dissolve the drug and a biocompatible and biodegradable matrix former [*e.g.*, poly(lactic-*co*-glycolic acid), PLGA] in an appropriate organic solvent [*e.g.*,*N*-methyl pyrrolidone, NMP]. This liquid phase can easily be injected into the periodontal cavities. Once injected, the NMP diffuses into the surrounding environment and water from the periodontal pocket penetrates into the liquid formulations. Since PLGA is not soluble in water, it subsequently precipitates and entraps the drug. For information on the biocompatibility of NMP see for instance Kempe and Mäder (2012). Major advantages of this type of biodegradable, *in-situ* forming implants include the fact that: (i) the injection of a liquid formulation is relatively easy (compared to the implantation of "pre-formed" implants). (ii) There is no need to remove empty remnants upon drug exhaust, due to complete biodegradability of the system. (iii) The geometry and size of the implants are adapted to the patient's periodontal pockets. (iv) The incorporated drug is locally released in a time-controlled manner through the slowly degrading polymer network.

However, up to date major challenges remain to be addressed, namely the fact that: (i) the adherence of *in-situ* formed implants to human tissue is yet poor, resulting in pre-mature and uncontrolled expulsion of implant fragments from the periodontal pockets due to the non-negligible flow of gingival crevicular fluid (Greenstein and Tonetti, 2000; Goodson, 2003). This leads to a considerable uncertainty with respect to the amount of drug reaching the target site and the time periods during which therapeutic drug levels are provided. (ii) The 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. In contrast, fully elastic implants force the periodontal pockets to keep their geometry and dimensions, which is also not desirable. Recently, the addition of plasticizers, such as acetyltributyl citrate and dibutyl sebacate, as well as of a second type of polymer, such as hydroxypropyl methylcellulose, has been proposed to increase the adhesiveness of the implants (Do et al., 2014, 2015). However, yet it is unclear how the systems' composition affects the key properties of the *in-situ* formed implants. The aim of this study was to better understand the physico-chemical phenomena involved in implant formation and the control of drug release as well as to evaluate the antimicrobial activity of doxycycline-loaded formulations using gingival crevicular fluid samples from periodontitis patients.

2. Materials and methods

2.1. Materials

Two types of poly(D,L-lactic-*co*-glycolic acid) (PLGA), differing in the average polymer molecular weight: Resomer RG 502H (inherent viscosity of a 0.1% solution in chloroform at 25 °C: 0.16–0.24 dl/g) and Resomer RG 504H (inherent viscosity of a 0.1% solution in chloroform at 25 °C: 0.45–0.60 dl/g) (Evonik, Darmstadt, Germany); acetyltributyl citrate (ATBC; Morflex, Greensboro, NC, USA); two types of hydroxypropyl methylcellulose (HPMC), differing in the average polymer molecular weight: Methocel E5 (viscosity of a 2% aqueous solution at 20 °C: 5 mPa.s) and Methocel E50 (viscosity of a 2% aqueous solution at 20 °C: 50 mPa.s) (Colorcon, Dartford, UK); *N*-methyl pyrrolidone (NMP, 99%), glucose and cysteine chlorhydrate (Acros organics, Geel, Belgium); doxycycline hyclate and metronidazole (Fagron, Colombes, France); 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); Parocline [a "dental gel" consisting of hydroxyethylcellulose, magnesium chloride, Eudragit RS, triacetin, glycerol and minocycline (2%); Sunstar France, Levallois-Perret, France].

2.2. Preparation of the liquid formulations

PLGA (28 or 32% w/w, based on the total liquid formulation without drug) was dissolved in NMP at 25 °C in a glass vial (30 min stirring). Optionally, the plasticizer ATBC (10% w/w, based on the PLGA mass) and/or HPMC (10, 20, 25 or 30% w/w, based on the PLGA mass) was added and the mixture was vortexed for 3 min, followed by standing for 3 h at 25 °C. Subsequently, 5 or 10% doxycycline hyclate or 1 or 10% metronidazole (w/w, based on the total liquid formulation without drug) was added, and the mixture was vortexed for 3 min, followed by standing for 3 h at 25 °C. To eliminate air bubbles, the liquids were ultrasonicated for 10 min. The formulations were stored at −20 °C and protected from light to avoid drug degradation.

2.3. In-situ *implant formation and drug release measurements*

One hundred microliter of the respective formulation was injected at the bottom of an Eppendorf vial using a standard syringe. One and a half milliliter preheated (37 °C), degassed phosphate buffer pH 7.4 (USP 35) was carefully added using a pipette, initiating solvent exchange and implant formation. The vials were horizontally shaken at 37 °C at 80 rpm (GFL 3033; Gesellschaft für Labortechnik, Burgwedel, Germany). At predetermined time points, the bulk fluid was completely withdrawn and replaced with a fresh medium. The drug content in the samples was determined UV-spectrophotometrically (λ = 325 nm for doxycycline and λ = 351 nm for metronidazole; Shimadzu UV-1650PC, Shimadzu, Champs-sur-Marne, France). All tests were performed