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Release of metronidazole from electrospun poly(L-lactide-co-D/L-lactide) fibers for local periodontitis treatment

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ABSTRACT

Objectives. We aimed to achieve detailed biomaterials characterization of a drug delivery system for local periodontitis treatment based on electrospun metronidazole-loaded resorbable polylactide (PLA) fibers.

Methods. PLA fibers loaded with 0.1–40% (w/w) MNA were electrospun and were characterized by SEM and DSC. HPLC techniques were used to analyze the release profiles of metronidazole (MNA) from these fibers. The antibacterial efficacy was determined by measuring inhibition zones of drug-containing aliquots from the same electrospun fiber mats in an agar diffusion test. Three pathogenic periodontal bacterial strains: *Fusobacterium nucleatum*, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis* were studied. Cytotoxicity testing was performed with human gingival fibroblasts by: (i) counting viable cells via live/dead staining methods and (ii) by exposing cells directly onto the surface of MNA-loaded fibers.

Results. MNA concentration influenced fiber diameters and thus w/w surface areas: diameter being minimal and area maximal at 20% MNA. HPLC showed that these 20% MNA fibers had the fastest initial MNA release. From the third day, MNA release was slower and nearly linear with time. All fiber mats released 32–48% of their total drug content within the first 7 days. Aliquots of media taken from the fiber mats inhibited the growth of all three bacterial strains. MNA released up to the 28th day from fiber mats containing 40% MNA significantly decreased the viability of *F. nucleatum* and *P. gingivalis* and up to the 2nd day also for the resistant *A. actinomycetemcomitans*. All of the investigated fibers and aliquots showed excellent cytocompatibility.

Significance. This study shows that MNA-loaded electrospun fiber mats represent an interesting class of resorbable drug delivery systems. Sustained drug release properties and cytocompatibility suggest their potential clinical applicability for the treatment of periodontal diseases.

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1. Introduction

For treatment of periodontal disease, there is a need for an optimal local drug delivery system since the widespread systemic administration of antibiotics might cause undesired side effects or favor the development of resistances.

The use of antibacterial biomaterials becomes increasingly important in medical and dental science. Especially in the field of conservative dentistry, the elimination of bacteria and plaque is foundational for effective treatment [1,2]. For instance, the conventional treatment of periodontitis by scaling and root planning is advantageously accompanied by the adjuvant administration of antibiotics [3–5]. Antibacterial drug compounds can be applied by systemic or local administration. Compared to systemic drug delivery the local administration of drugs in periodontology is considered to be more effective, since the pathogen-specific drug can be placed directly in the periodontal pocket achieving effective concentrations. In addition the risk of undesired side effects caused by high systemic doses or resistance development can be reduced [6,7]. For effective elimination of pathogenic bacteria, the antibiotic agent has to be available in the periodontal pocket in adequate concentrations for a sufficiently long period of time. It is therefore necessary to use local delivery systems that control the release of their agents and guarantee lasting drug concentrations in the pocket in spite of high sulcular fluid rates.

Non-resorbable drug carrier systems such as tetracycline-loaded fibers are placed from seven up to ten days in the periodontal sulcus. In this period concentrations up to 1300 μg/ml in the sulcus fluid can be maintained. However, the insertion of such non-resorbable fibers is time consuming and when their removal is required this incurs the risk of tissue damage.

Many resorbable drug delivery systems were developed during recent decades, such as drug loaded hydroxypropylcellulose films [8], which were first described by Noguchi et al. (1984), or drug carrying gels such as Elyzol® (Dumex GmbH, Bad Vilbel, Germany) dental gel, based on melted glycerol mono-oleate [9–12]. However, also for these systems, the periodontal milieu often poses the major problem that the required period of drug exposure (7–10 days) cannot be achieved [9,13]. Also in the field of periodontal surgery – as in the transplantation of a mucous membrane [14] – resorbability of the scaffold material is important to avoid inflammatory effects and surgical removal.

Therefore the aim of this study was to investigate a resorbable drug reservoir, which releases essential amounts of its ingredients within an adequate period of time. A possible drug delivery system based on electrospinning of polylactide was developed whereby mats of electrospun fibers containing the antibiotic metronidazole (MNA) were generated having a large surface area per volume ratio. Fiber mats incorporating different proportions of MNA (from 0.1 to 40.0%, w/w) were created to investigate release characteristics and determine the concentrations necessary for effective antibacterial action when placed in an appropriate host environment.

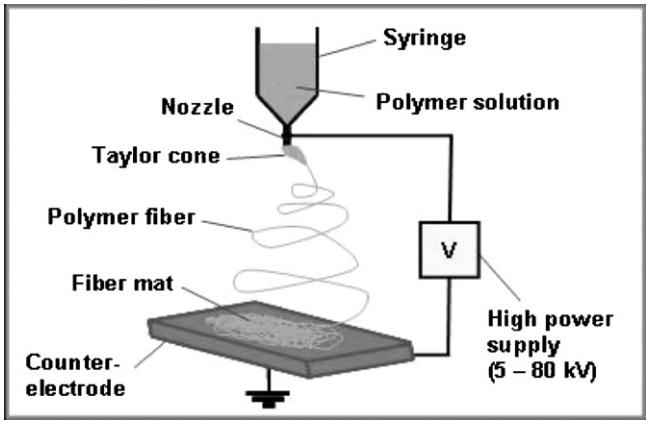

Fig. 1 – The principle of electro-spinning to produce mats of PLA-fibers.

2. Materials and methods

2.1. *Electrospinning*

Poly(L-lactide-co-D/L-lactide) 70/30 (Resomer LR 708, Boehringer Ingelheim, Germany) was used for electrospinning (Fig. 1). A weight-average molecular weight of 1.5×10^6 g mol^{-1} was determined for the polymer by gel permeation chromatography using $CHCl_3$ as solvent and polystyrene as external standard. All solvents used for electrospinning purposes were of HPLC grade (Sigma–Aldrich, Germany). Micronized 2-(2-methyl-5-nitro-1H-imidazol-1-yl)ethanol (Metronidazole, MNA, Ph Eur 6.0 specification) was purchased from FARGON GmbH & Co. KG (Barsbüttel, Germany). Tetrahydrofuran, chloroform, dichloromethane, and acetone were tested for their suitability to dissolve poly(L-lactide-co-D/L-lactide) (PLA) and MNA for its subsequent use for electrospinning. Acetone was chosen because of its good solubilizing of both PLA and MNA, its low boiling point and its established use in dental adhesive applications. We determined that a 3–5% (w/w) polymer solution, depending on the MNA content, was necessary to spin the copolymer under the conditions described below, to obtain similar fiber diameters. A homogeneous solution was prepared by slow stirring of appropriate amounts of PLA and MNA in acetone at room temperature for 3 h using a magnetic stirrer at 250 rpm. The obtained clear and viscous solutions were transferred directly into a 5 ml plastic syringe. The PLA/MNA mixture was then deployed in the electrospinning process using a custom designed electrospinning apparatus. This incorporated an adjustable high-voltage power supply (ESV-100; Ingenieurbüro G. Fuhrmann, Leverkusen, Germany) and an infusion pump (LA-100, Landgraf Laborsysteme, Germany). The syringe was connected by a 35 cm PTFE tube to a stainless-steel straight-end hollow needle (0.4 mm) under conditions adapted from those that have been previously described [15,16]. A mirrored glass surface (20 cm × 20 cm; glass thickness 2 mm) was used as the electrically grounded plate to collect the drug loaded fibrous mat. The needle was connected to the ESV-100 DC power supply adjusted to 20 kV. The syringe was mounted