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# Development of novel electrospun dual-drug fiber mats loaded with a combination of ampicillin and metronidazole

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ABSTRACT

*Objective.* Our study was performed with the aim of preparing electrospun polylactide fibers with a combination of ampicillin (AMP) and metronidazole (MNZ) and investigating their drug release behavior and the antibacterial effect on *Aggregatibacter actinomycetemcomitans* and other oral pathogens.

*Methods.* AMP and MNZ were integrated as a combination in two separate fibers (dual fiber mats – DFW mix) of electrospun PLA fiber mats by means of multijet electrospinning and in a single fiber (single fiber mats – SFW mix). HPLC (high-performance liquid chromatography) was used to measure the released drug quantities. Agar diffusion tests were used to determine the antibacterial effect of the eluates on *A. actinomycetemcomitans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *Enterococcus faecalis*. The neutral red test was made to examine the cytocompatibility of the eluates with human gingival fibroblasts (hGFs).

*Results.* The release of the active agents varied with the antibiotic concentrations initially used in the fiber mats, but also with the distribution of the active agents in one or two fibers. Of the total quantity of MNZ (AMP), the SFW mix fiber mats released >60% (>70%) within a span of 5 min, and 76% (71%) after 96 h. With these drug concentrations released by the fiber mats (≥5 m%), an antibacterial effect was achieved on *A. actinomycetemcomitans* and on all other species tested. Fiber mats and their eluates have no cytotoxic influence on human gingival fibroblasts (hGFs).

*Significance.* Electrospun AMP/MNZ-loaded polymer fibers are a potential drug delivery system for use in periodontal and endodontic infections.



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## 1. Introduction

It is now a well established approach in periodontal therapy to apply adjuvant antibiotics for the suppression of periodontopathogenic species after mechanical removal of biofilms [1–5]. To avoid undesirable systemic side effects on the antibiotics and to achieve a strong effect on the periodontal lesion, the use of electrospun drug delivery systems has recently been discussed in addition, in the context of local antibacterial periodontitis therapy [5–8].

Active substances currently available for local application include, among others, pharmaceutical gel and ointment formulations such as Ligosan® Slow Release (Hereaus Kulzer, Hanau, Germany), Atridox® (TOLMAR Inc., Fort Collins, United States) or Arestin® (OraPharmaInc, Horsham, United States). Each of these systems contains an antibiotic, however their spectrum of activity only rarely includes the important periodontopathogenic species.

The suppression of the species *Aggregatibacter actinomycetemcomitans* must be rated as complicated [1,9,10]. Several studies discuss the absent or distinctly reduced effect on *A. actinomycetemcomitans* if metronidazole or clindamycin is applied alone [11–13]. Within the adjuvant systemic application of antibiotics against *A. actinomycetemcomitans*-associated periodontitis, therefore, use is made, besides metronidazole, of amoxicillin, as its spectrum of activity also comprises facultatively anaerobic bacterial species [14,15]. For local application, however, no suitable form of application is known combining both antibiotics. Such a combined therapy could markedly increase the therapeutic effect of local application systems.

Due to similarly good antibacterial properties, it is possible to use ampicillin as an alternative to amoxicillin [16,17]. Compared to amoxicillin, ampicillin is clearly more soluble, which makes it a candidate for local application in fiber mats. This antibiotic, also of the β-lactam group, is used clinically for treating bacterial Infections of the respiratory tract, the gastro-intestinal tract, the bile and urinary ducts, *etc.* [18,19].

In a previous study, we were able to establish electrospun metronidazole-loaded PLA fibers for periodontitis treatment with good antibacterial properties against *Fusobacterium nucleatum* and *Porphyromonas gingivalis* [8]. However, in this former study, *A. actinomycetemcomitans*, the key pathogen of aggressive periodontitis, could not be suppressed sufficiently.

Meanwhile, the technique of electrospinning has been further optimized, so that a considerable number of synthetic polymers are suitable as matrix materials and can be used with combinations of active components such as antibiotics, hormones or growth factors [20–23]. Especially electrospun micro- and nanomaterials, such as resorbable antibiotic-loaded polylactide (PLA) fiber mats, enable improved, targeted release of the active substances [8,24–26].

Especially with the aid of the advanced technique of multijet electrospinning used in this study, we tried for the first time to load PLA fiber mats with the combination of ampicillin (AMP) and metronidazole (MNZ) in particular to make possible the suppression of *A. actinomycetemcomitans*. We aimed to clarify whether the distribution of the antibiotics in the combination mentioned, in one or both of the separate fibers, influences the release of the drugs. In addition, we sought to rule out a cytotoxic influence of the new drug delivery systems on human gingival fibroblasts

## 2. Methods

### 2.1. Production of fiber mats

For *in vitro* tests we made three different fiber systems of poly(L-lactide-co-D/L-lactide) (70/30, Resomer LR 708, Boehringer Ingelheim, Germany): (i) single-fiber drug delivery system (SFW) with different concentrations of Na-Ampicillinat (AMP, D-(−)-α-aminobenzylpenicillin-sodium salt, A9518 Sigma–Aldrich, Germany); (ii) single-fiber drug delivery system mix (SFW mix) with metronidazole (MNZ, 2-(2-methyl-5-nitro-1H-imidazol-1-yl) ethanol, FARGON GmbH & Co. KG Barsbüttel, Germany) and Na-Ampicillinat combined in a fiber; (iii) dual-fiber drug delivery system mix (DFW mix), in which two single fibers, each with one antibiotic, were spun simultaneously into one fiber mat.

Different concentrations of the antibiotics were added to the PLA, dissolved in acetone. The ampicillin SFW fiber mats were loaded with concentrations of 0.1, 0.5, 1, 5, 10, 20, 30 and 40 m%. The SFW mix fiber mats were loaded with the combination of ampicillin and metronidazole (20/20 m%). In the DFW mix, the active agents were integrated with proportions of 20 m% each per fiber. A fiber mat without antibiotic served as a negative control. Electrospinning was performed in an E-Spintronic apparatus (E. Huber GmbH, Gernlinden, Germany) (Fig. 1a). The electrospinning procedure has been described previously [8]. The morphology of the antibiotic-loaded PLA fiber mats was studied by scanning electron microscopy (SEM) (Supra 55VP; Zeiss, Oberkochen, Germany) (Fig. 1b).

### 2.2. Generation of aliquots

To ascertain the release kinetics and the cytocompatibility of the antibacterial agents, we prepared aliquots of the fiber mat versions in different eluants.

#### 2.2.1. PBS aliquots

Four 2.00 mg (±0.02 mg) specimens of each SFW, SFW mix and DFW mix fiber mats were obtained (Genius ME 215 microbalance; Sartorius, Germany). They were placed into 24-well microplates and UV sterilized for 10 min, then topped with 2 ml PBS each (phosphate buffered saline, Invitrogen, Germany), and kept at 37 °C, 5% $CO_2$ for 28 days. Eluates were obtained at a fixed time pattern: on the first day after 5 min, 30 min, 1 h, 3 h, 6 h, 12 h and 24 h; on days 2–7, and on days 14, 21 and 28. After eluates were taken, the batches were rinsed with 1 ml PBS and kept in fresh PBS. The eluates obtained were aliquoted and kept at −20 °C until they were used for the antibacterial (1 ml) and HPLC tests (1 ml).

#### 2.2.2. DMEM aliquots

To make eluates for the cytotoxicity experiments, two 1.00 mg (±0.02 mg) specimens of each fiber mat were weighed. Eluates were obtained and the samples kept analogously to the PBS