Antimicrobial electrospun patches for wound care and healing following osseointegrated limb prostheses implantation

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INTRODUCTION: Infections are among the most severe complications connected to the implantation of biomedical devices, including orthopedic prostheses and percutaneous implants[1]. These latter, in particular, are attracting attention because of their application in limb replacement, and are specifically subjected to the risk of infection, both deep and superficial, due to bacterial adhesion processes to either the prosthesis, the surrounding bone/soft tissue or the skin[2]. At the same time, proper healing of the surgical wound and the prevention of leakage of exudates are required[2]. Electrospun patches have proved to be excellent candidates for wound healing, since they permit draining wound exudates and promote skin regeneration[3,4]. In addition, they can adapt to complex shapes and be easily coupled with prostheses/implants[3,4]. In addition, when dealing with infection, the emergence and spread of antibiotic resistance among human pathogens is a key challenge for human health[5].
Here, we propose the use of antimicrobial electrospun patches, to be coupled with percutaneous devices. To address infection, we propose the functionalization of the patches by tea tree oil (TTO), which is embedded in poly(ethylene oxide), PEO, for immediate and burst release, and in polycaprolactone, PCL, to achieve a longer diffusion profile and higher patch stability.

METHODS: The effect of TTO on fibers morphology was initially assessed using PEO (Mw 200 kDa) using 3, 5, 7.5 and 10 w/w concentrations. First, matrix morphology (fibers diameter/distribution and presence/absence of defects) as a function electrospinning parameters was evaluated by SEM to optimize the process in the presence of TTO. Then, the amount of TTO effectively embedded in the electrospun patch and its stability over time (up to 14 days) was assessed by a UV-VIS spectrophotometry and by performing SEM on aged samples. After optimization of the TTO concentration in PEO matrices, PCL (18% w/v solution in glacial acetic acid) was electrospun, using previously optimized parameters.
Antibacterial efficacy of PEO and PCL electrospun patches w/wo TTO (n = 2) was tested on reference *E. coli* and *S. aureus* strains (*Escherichia coli* ATCC® 8739™ and *Staphylococcus aureus* ATCC® 6538P™, medium Luria-Bertani LB), by evaluating the planktonic growth and the capability of bacteria to adhere to the matrices. For planktonic growth, samples were incubated with bacterial suspensions (optical density, OD_{600} = 600 nm). Serial dilutions and enumeration on LB agar plates were performed at 4h and 8h to assess bacterial growth by counting the CFUs. For adhesion, samples were cultured in 100 µl of bacterial suspension (OD_{600} 0.2), rinsed to remove non-adherent cells, then stained with crystal-violet. Statistical significance was determined by one-way Anova test.

RESULTS SECTION: Upon optimization of the electrospinning parameters, PEO patches were electrospun without significant presence of defects and showed a satisfactory morphology and a suitable distribution in the average fibers diameter (Fig. 1). Based on the absence of defects, the higher distance and flow rate are selected. Incorporation of TTO in PEO matrices did not require further optimization of electrospinning parameters and did not significantly modify fibers distribution and average diameter. Instead, it causes the formation of a higher number of beads and a general coarsening of the fibers, as previously reported for PCL-based patches[6] (Fig. 1 c-e). In this case, the formation of beads is not considered detrimental, since they might serve as TTO reservoirs[6] and boost antibacterial efficacy. TTO can be efficiently incorporated in PEO, in spite of its volatility. Increasing the concentration above 5%, however, did not result in an increased amount of TTO in the patches, so 5% w/w was selected as optimal concentration. In any configuration, the patches remained stable for over 14 days, without any loss in the TTO content. All PCL and PEO patches showed high efficacy in inhibiting the bacterial planktonic growth of *S. aureus* (% reduction vs control: 98% PEO, 96% PCL) and a moderate effect on *E. coli* (72% PEO, 81% PCL). The effect is TTO concentration-dependent and is higher for *S. aureus* probably because of a different capability of TTO to permeate the membrane of gram-positive and gram-negative bacteria[7]. Results also show a remarkable effect in inhibiting bacterial adhesion to the electrospun matrices, for both strains, due to the TTO capability to modify proteins responsible for bacterial binding to the substrate, thus inhibiting the formation of permanent molecular bridges[8].

DISCUSSION: Electrospun TTO loaded antibacterial PEO and PCL patches were successfully developed. TTO concentrations up to 5% can be loaded in the electrospun patches obtaining high stability over time and high antimicrobial efficacy. Different polymers having different stability (PEO ad PCL) can be used to obtain different release profiles. Further studies will investigate the capability of the patches to support skin regeneration and will study their performance in vivo. Results obtained in the present study appear promising for application in percutaneous implants and show prospective application in the treatment of surgical wounds.

SIGNIFICANCE/CLINICAL RELEVANCE: Percutaneous implants cause disruption of skin integrity, possibly resulting in both superficial and deep infection. In the scenario of osseointegrated limb prostheses, the onset of infection is extremely challenging, since any revision surgery requires to increase the depth of amputation. To address infection, systemic prophylaxis of antibiotics is the gold standard, but the phenomenon of antibiotic resistance is making them progressively more ineffective. According to the WHO[5], new solutions must be developed, to avoid a 'post-antibiotic era', in which common infections can again kill. Here, we obtained antibacterial patches, functionalized with TTO, that can be adapted to the shape of the implant and can find application in percutaneous implants and, prospectively, in surgical wounds treatment.

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IMAGES AND TABLES:

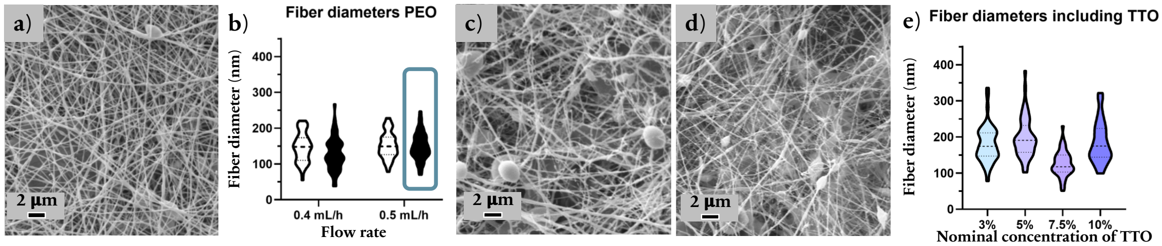

Figure 1: a) Morphology of PCL patches after electrospinning optimization and b) fibers size distribution depending on electrospinning parameters, in the absence of TTO. Morphology of TTO-loaded patches at c) 5%, and 10% concentration. e) Fibers size distribution depending on TTO concentration.