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# Antimicrobial metal–organic frameworks incorporated into electrospun fibers

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## HIGHLIGHTS

- Cobalt-based metal–organic frameworks were included in composite electrospun mats.
- Antibacterial activity assessed with *Pseudomonas putida* and *Staphylococcus aureus*.
- Viable cells and colony forming microorganisms decreased with cobalt content.
- Up to 60% reduction in the number of colony forming microorganisms.
- Viable but non culturable bacteria were observed in biocidal mats.

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## ABSTRACT

The objective of this paper is to present a new class of polylactic acid (PLA) fibers containing cobalt-based metal organic frameworks (MOF). The material used was Co-SIM-1, a cobalt-based substituted imidazolate. Composite mats were prepared by electrospinning PLA with a suspension of polyvinylpyrrolidone-stabilized Co-SIM-1. MOF particles formed aggregate of a small number of primary particles that, after electrospun, became completely embedded inside polymeric fibers. The dispersion of particles was better for lower loadings, for which the relative amount of metal released to culture media was also higher. The antimicrobial activity of composite mats was assessed using SEM images, fluorescence microscopy, direct plate reading of fluorescent stains and plate count of colony forming units among other. The microorganisms used in this study were *Pseudomonas putida* and *Staphylococcus aureus*. Fluorescence techniques allowed recording viable and damaged cells directly on mat surface and in the culture media embedding the fibers. The results showed higher sensitivity of *S. aureus* to cobalt-containing fibers, with a reduction in colony forming units of up to 60% with respect to PLA mats. The results also showed the presence of viable but non-culturable microorganisms, which fail to form colonies but yield a positive signal to viable cell staining. Cobalt-based MOF included in electrospun mats provide antibacterial activity suitable to be used to prepare membranes for various biomedical applications.



## 1. Introduction

Metal–organic frameworks (MOF) are a remarkable class of materials in which organic bridging ligands are connected by metal ions to form one-, two-, and three dimensional coordination networks [1]. The key advantages of MOF compared to inorganic microporous structures such as zeolites, is their highly tunable composition, which can be achieved by using different metals or changing the organic linker. The initial interest on MOF came from their very high surface areas and hence an extremely high capacity to capture gases in energy-related technologies [2,3]. Many other potential applications have been proposed for MOF in fields like heterogeneous catalysis, gas purification and sensing [4,5]. The focus on gas-related processes has been recently complemented with novel biological applications based on the capacity of coordination polymers for the controlled release of bioactive molecules, either physisorbed within the pore structure or behaving themselves as linkers in pro-drug form [6]. New developments

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are increasingly focused on the development of materials of complex chemical functionalization in order to impart tailored chemical and physical properties [7].

The release of metal ions contained in the structure of MOF makes them attractive antimicrobial materials for applications in which a tunable antibiotic is required. Silver ion releasing compounds are a well known family of antimicrobials and several silver containing MOF have been reported up to date to this end. Berchel et al. reported a silver-based MOF with a 3-phosphonobenzoate ligand that acts as a source of silver ions and showed antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa* [8]. Liu et al. reported another silver-based metal–organoboron framework with antibacterial activity against Gram-negative and Gram-positive human pathogens [9]. Hindi et al. [10] and Slenters et al. [11] prepared other silver-based coordination compounds with proved or suggested biocidal capacity. Silver, however, is an expensive metal and the indiscriminate use of it in many consumer goods is suspected to promote not only a general toxic risk but also bacterial resistance [12]. Silver was also incorporated in different types of nanofibers in order to incorporate antimicrobial effect. Sheikh et al. prepared silver doped polyurethane nanofibers via electrospinning in which silver nanoparticles were obtained from silver nitrate by *in situ* reduction with N,N-dimethylformamide [13]. Shi et al. prepared silver nanoparticle-filled nylon 6 nanofibers by electrospinning, the electrospinning solvent behaving as reducing agent for *in situ* conversion of $AgNO_3$ into silver nanoparticles. [14]. Several groups reported the use of electrospinning to prepare hybrid nanomaterials for antimicrobial applications by loading chitosan-based fibers with silver nanoparticles [15,16].

In a recent paper Aguado et al. reported high antibacterial activity of a cobalt imidazolate MOF [17]. The results showed that the controlled release of cobalt gave rise to a long-lasting antibacterial activity with the advantage with respect to silver that it is a relatively inexpensive element, still active for bacteria but less toxic than silver [18]. Zhuang et al. also proposed a cobalt MOF with tetrakis[(3,5-dicarboxyphenyl)-oxamethyl] methane acid as ligand, which demonstrated activity for the inactivation of *E. coli* [19]. Concerning other metals, Sancet et al. prepared surface-anchored copper MOF with dual functionality, intended to combine sensing and controlled release of an antimicrobial metal ion [20]. The material was tested using the marine bacteria *Cobetia marina*, and displayed good surface response to adhering microorganisms. It is interesting to point out that in most cases, the linker used in the preparation of MOF reservoirs was not commercially available, the synthesis of it requiring several reaction-separation steps. In this work, we tested a cobalt-based MOF prepared using a simple, relatively cheap and commercially available ligand [21].

Electrospinning is a simple method for generating nanofibers from a wide variety of materials, including many dissolved or melted polymers, using a high-voltage power supply. Electrospun fibers have been proven useful in a number of fields such as water filtration, the design of sensors, the manufacturing of special clothing and many biomedical applications such as wound dressings or scaffolds for tissue engineering [21,22]. Electrospun fibers have attracted considerable attention due to their remarkable properties, which include small diameter and relatively high surface-to-volume ratio, even though the preparation of porous polymer nanofibers with high surface areas is still a challenge [23]. The incorporation of particles into electrospun polymer nanofibers has also been explored by researchers working in drug delivery applications and in water treatment technologies [24,25].

The purpose of this study was to prepare and test a biocidal composite material consisting of a cobalt-based MOF embedded in an electrospun polymeric matrix based on polylactic acid (PLA). PLA is derived from renewable resources and displays higher natural hydrophilicity than conventional thermoplastic polymers as a result of better access of water to the polar oxygen linkages in the backbone. This fact has been shown to improve water fluxes and reduce the biofouling tendency of membranes made of PLA [26]. PLA also displays a good spinnability. The composite Co–MOF–PLA material is intended for use in antimicrobial applications such as the preparation of antibacterial tissues or the production of membranes for water treatment.

## 2. Materials and methods

### 2.1. Materials

Transparent PLA (trade name: 'PLA Polymer 2002D') was purchased in the form of pellets from NatureWorks LLC, UK with a melt index of 5–7 g/10 min (at 210 °C/2.16 kg), a molecular weight of ~121,400 g/mol, a melting temperature of 160 °C and a D-content of 4% (96% L-lactide content). Polyvinylpyrrolidone (PVP), molecular weight 360,000 from Sigma–Aldrich was used as dispersant. Dichloromethane (DCM, 99.5%), used as solvent for PLA, and acetone (99.8%) were reagent grade, obtained from Sigma–Aldrich and used as received. The components of culture media were biological grade reagents acquired from Conda-Pronadisa (Spain). Fluorescein diacetate (FDA, CAS Number 25535-16-4) and propidium iodide (PI, CAS Number 596-09-8) acquired from Sigma–Aldrich were used as cell viability stains. Live/Dead BacLight Bacterial Viability Kit was acquired from Molecular Probes.

Co-SIM-1 (cobalt-based Substituted Imidazolate Material) is a novel analog of its zinc-based parent SIM-1 [27,28]. It consists of $CoN_4$ tetrahedra linked by methyl-carboxyaldehyde-imidazolate, belongs to the class of ZIF or ZMOF materials and is isostructural to ZIF-8 and ZIF-67. Co-SIM-1 was synthesized by solvothermal procedure reported elsewhere [17,27,29]. Briefly, 0.199 g (0.68 mmol) of $Co(NO_3)_2{\cdot}6H_2O$ and 0.301 g (2.7 mmol) of 4-methyl-5-carboxyaldehyde-imidazole were dissolved in 5 mL of DMF, the resulting solution being heated to 358 K for 72 h. The resulting powder was washed three times with DMF and then with ethanol and dried at 373 K overnight.

### 2.2. Electrospinning

A NANON-01A electrospinning unit (Mechanics Electronic Computer Corporation, MECC Co., Ltd., Japan) was used to prepare composite mats. 7 wt.% PLA solution was prepared by dissolving the required amount of PLA pellets in DCM under constant magnetic stirring for 24 h. Co-SIM-1 was dispersed in a 2.5 wt.% PVP solution in DCM by mechanical stirring followed by sonication for 3 min in pulsed mode (30 s pulses with 30 s delay after every pulse in order to avoid sample overheating) using an ultrasonic processor VC505 (500 W, 20%) from Sonics & Materials Inc. The concentration of dispersed particles was typically 30% over the desired final concentration in the mat. Subsequently, Co-SIM-1dispersion and PLA solution were mixed at 1:1 ratio. Co-SIM-1 loadings were 2, 4.5 and 6 wt.% of the final weight of electrospun composite mats. (From ICP measurements, the true Co-SIM-1 content of the electrospun fibers used in this work was 2.06 ± 0.07, 4.63 ± 0.17 and 6.04 ± 0.10.) After vigorous magnetic stirring the resulting dispersion fed the electrospinning machine with the following parameters: voltage ~21 kV; feed rate ~0.9 mL/h; distance between the tip of the needle and drum collector ~15 cm; and drum rotation speed ~500 rpm.

### 2.3. Analytical methods

The morphology of fibers after was examined using a scanning electron microscope (SEM) from Carl Zeiss (EVO MA15) at an