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# Design of antibacterial biointerfaces by surface modification of poly (ε-caprolactone) with fusion protein containing hydrophobin and PA-1

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ABSTRACT

Class IIa bacteriocin pediocin PA-1 has broad-spectrum activity and is a well-characterized candidate food biopreservative. Here, a simple approach is designed to extend the application of pediocin PA-1 in improving the antibacterial activity of electrospun poly(caprolactone) (PCL) grafts through combining PA-1 with HGFI, which is a self-assembled protein with characteristics allowing the modulation of surface properties of other materials originated from *Grifola frondosa*. *Saccharomyces cerevisiae* was used as the host for expression of fusion protein PA-1-linker-HGFI (pH) and his-tag purification was used to purify recombinant protein pH. An antibacterial activity assay showed the fusion protein pH retained the biological property of native PA-1. Water contact angle, X-ray photoelectron spectroscopy, immunofluorescence assay and atomic force microscopy indicated the surface properties of HGFI were greatly preserved by the fusion protein pH. Finally, antibacterial activity of pH-modified PCL substrate measurements implied the fusion protein significantly improved the bacterial-resistance of the PCL film through dressing the PCL fibers with the recombinant pH protein. This work presents a new perspective on the application of hydrophobin and pediocin PA-1 in antibacterial medical devices.



## 1. Introduction

Medical devices are widely used in modern healthcare systems. As more medical devices are applied in implant surgery in direct contact with patients which might provide surfaces and environments suitable for colonization by microorganisms, device-related infection remains a challenge to modern medicine. In particular, many bacterial species adhere preferably to hydrophobic materials and many medical devices are made of such material. Thus, the design and fabrication of novel nanostructured surfaces unsuitable for bacterial colonization or that inhibiting bacterial growth are required to improve medical devices.

Poly(ε-caprolactone (PCL) is a biodegradable polymer with a high degree of hydrophobicity that is in general use for biomedical applications; such as bioabsorbable surgical materials, temporary internal fixation of tissue damage and drug delivery systems [1]. Generally, two strategies have been used to increase the antibacterial activity of PCL materials. One is to embed conventional anti-bacterial (antibiotics) agents into PCL scaffolds during the process of electroplating. For example, Ruckh et al. and Jang et al. investigated the antimicrobial effect of PCL scaffolds releasing rifampicin and vancomycin [2,3]. However, at present, multi-resistant bacteria appear because of the abuse and misuse of antibiotics, which makes the destruction of pathogens more challenging. Another strategy uses non-drug antimicrobial agents, for example Ag and $TiO_2$, which are used also to fight against infection. For instance, Tran et al. incorporated silver nanoparticles into PCL scaffolds during the process of electroplating [4]. Although nanoparticles (NPs) were originally considered nontoxic, an increasing number of studies report toxicity associated with exposure to NPs. Therefore, effective therapeutic and highly safe

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agents for the prevention and treatment of device-associated infections are urgently required.

Bacteriocins capable of permeabilizing membranes are mostly cationic, ribosomal synthetized antimicrobial peptides (AMPs) produced by bacteria [5]. Owing to excellent antibacterial activity and safety, bacteriocins have gained some attention in the fields of food preservation, health care and pharmaceuticals [6]. Compared with traditional antibiotics, these peptides are less likely to provide pathogen resistance [7,8]. Although the exact mechanism of action of AMPs is unknown, there is a consensus that they exert their antimicrobial specificity and activity by binding to invariant components of microbial surfaces through specific (target-specific molecules) or nonspecific (electrostatic) interactions and cause membrane leakage/disruption of the target cell [9,10]. As a kind of bacteriocin, pediocin PA-1 is a peptide of 44 amino acids produced by *Pediococcus acidilactici*, which shows a particularly strong activity against the growth of *Listeria* and *Staphylococcus* species and inhibits other Gram-positive bacteria [11,12].

Hydrophobins are surface-active proteins produced by filamentous fungi that have an important role in fungal growth [13,14]. They belong to the group of most surface-active proteins, which have hydrophobic and hydrophilic parts as amphiphiles [15] and can self-assemble on interfaces of air-water, oil-water and water-solid along with changing the properties of the surfaces to which they attach [16–18]. All hydrophobins have eight conserved cysteine residues forming four pairs of disulfide bonds, which are divided into classes I and II on the basis of their hydropathy patterns [19]. In general, films formed by class I hydrophobins are more stable and more difficult to dissolve compared to class II. Until now, they have led to many applications, including formation of emulsions [15], drug delivery [20], biosensor [21], and biomaterials [22–24]. HGFI used in this study is an 8 kDa class I hydrophobin from *Grifola frondosa* [25].

In this study, we aim at immobilizing pediocin PA-1 on PCL using a kind of self-assembled hydrophobins HGFI to design antibacterial interface, which represents a simple, economical and safe method to modify PCL fibers with improving antibacterial ability. Gene engineering was used to express the fusion protein PA-1-Linker-HGFI (pH) containing pediocin, HGFI and the flexible linker GGGGSGGGGS [26] in *S. cerevisiae*. The self-assembly of pH was observed by water contact angle (WCA) measurements, atomic force microscopy (AFM), X-ray photoelectron spectroscopy (XPS) and an immunofluorescence assay. The antibacterial ability of pH was determined by an agar diffusion assay against *Micrococcus luteus* A1 NCIMB 8166 and a 96-well microplate assay against indicator *M. luteus* A1 NCIMB 8166, *Listeria monocytogenes* WSLC 1018 and *Staphylococcus aureus* ATCC 6538. The antimicrobial effect of pH-coated PCL fibers was examined by measuring the ability to inhibit the growth of *L. monocytogenes* WSLC 1018 and *S. aureus* ATCC 6538 and biofilm of *S. aureus* ATCC 6538. Experimental results indicated the fusion protein has the dual functionality of assembling on the surface of PCL fibers, leading to an increased antimicrobial property of PCL fibers.

## 2. Materials and methods

### 2.1. Strains, plasmids and reagents

*S. cerevisiae* strain BY4741 was purchased from EUROSCARF (Frankfurt, Germany). Expression plasmid pSP-G1 was donated by S. Partow and J. Nielsen [27].The PCL films used in this study were donated by Professor Deling Kong (Nankai University, China) [28]. Our laboratory keeps stocks of the indicator strains *Micrococcus luteus* A1 NCIMB 8166, *Listeria monocytogenes* WSLC 1018 and *Staphylococcus aureus* ATCC 6538 [29]. All restriction enzymes, Ex Taq DNA polymerase and DNA marker were purchased from TaKaRa (Dalian, China). The PageRuler™ Prestained Protein Ladder was obtained from Thermo (Fermentas, Lithuania). The anti-His mouse monoclonal antibody was obtained from Roche (Basel, Switzerland) and fluorescein isothiocyanate (FITC)-labeled goat anti-mouse antibody was purchased from TransGen (Beijing, China).

### 2.2. Construction of plasmid pSP-G1- pedA-hgfI

Expression plasmid pSP-G1-*pedA-hgfI* was constructed as described by in vivo recombination-mediated PCR-directed plasmid construction in yeast [27]. A double-stranded DNA (dsDNA) fragment encoding the pediocin PA-1 gene was cloned with primer (P1) and primer (P2). The sequence of *hgf1* was amplified with primer (P3) and primer (P4). Strain *S. cerevisiae* BY4741 transformed with pSP-G1 plasmid was obtained and used as the control strain. The primers used in this study are given in Table S1 (Supplementary material).

### 2.3. Production, purification and identification of fusion protein pH

A single transformed colony containing the recombinant plasmid was inoculated into 5 mL of SC-Ura medium until the absorbance at 600 nm ($A_{600}$) reached 1.0. 1 mL sample of the culture was added to 100 mL of SC-Ura medium then grown for 72 h. The cells were harvested at 5000 *g* for 15 min, washed twice with distilled water and suspended in breaking buffer (1 mM phenylmethylsulfony1 fluoride, pH 6.0) until $A_{600}$ reached 50.0. According to the Invitrogen manufacturer's protocol, the supernatant of cells lysate was collected at 11,000 *g* for 10 min. The expression of pH was determined by Tricine SDS-PAGE stained with Coomassie Brilliant Blue R250 and by Western blot analysis with anti-His monoclonal antibody.

The pH cell lysate was purified by mixing with Complete His-tag Purification Resin (Roche, Basel, Switzerland). The purity of pH was analyzed by Tricine SDS-PAGE (12% (w/v) polyacrylamide), which was stained with Coomassie Brilliant Blue R250. The antibacterial activity against *M. luteus* A1 NCIMB 8166 was assessed by an agar diffusion assay.

### 2.4. Antibacterial assay of fusion protein pH

The antibacterial activity of fusion protein pH was observed using a 96-well microplate assay against indicator strains *M. luteus* A1 NCIMB 8166, *L. monocytogenes* WSLC 1018 and *S. aureus* ATCC 6538 using 1 mg/mL solution of pH with 0.2 mg/mL solution of PA-1 as the positive control. Each well was filled with 100 μL of sample to be measured, which was mixed with 100 μL of the indicator strain ($A_{600}$ 0.1) in 2 x Tryptic soy broth with yeast extract (TSB-YE) medium and incubated at 37 °C. $A_{600}$ was monitored for 8 h at 1 h intervals. The sample with no additional bacteria served as the background. There were five parallel samples in the measurement. The assay was performed three times and the trial was repeated three times.

The antibacterial mode of the fusion protein was observed by scanning electron microscopy (SEM). The indicator strains *L. monocytogenes* WSLC 1018 and *S. aureus* ATCC 6538 were cultured in TSB-YE medium until $A_{600}$ reached 0.1. The cells in 1 mL culture were collected at 4000 *g* for 5 min and washed twice with PBS (pH 7.4). The cells were suspended in 1 mg/mL solution of protein dissolved in PBS (pH 7.4) and incubated at 37 °C for 1 h. Samples measurement were prepared for SEM as described [30]