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Immobilization of bacteriophage in wound-dressing nanostructure

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Abstract

Opportunistic bacteria that cause life-threatening infections are still a central problem associated with a healthcare setting. Bacteriophage capsid immobilization on nanostructured polymers maximizes its tail exposure and looks promising in applications toward skin-infections as alternative to antibiotics standardly used. The main goal of this work was to investigate the covalent immobilization of vB_Pae_Kakheti25 bacteriophage capsid on polycaprolactone (PCL) nanofibers (non-woven textile), as a potential effective antimicrobial, laundry resistant and non-toxic dressing for biomedical use. Surface analyses showed that the immobilization of vB_Pae_Kakheti25 bacteriophage capsid on PCL nanofibres oriented bacteriophage tails to interact with bacteria. Furthermore, antimicrobial assays showed a very effective 6 log bacterial reduction, which was equivalent to 99.9999%, after immediate and 2 hours of contact, even following 25 washing cycles (due to covalent bond). The activity of PCL-vB_Pae_Kakheti25 against *P. aeruginosa* was immediate and its reduction was complete.

Key words: Bacteriophages; Antimicrobial agents; Surface immobilization; Electrospinning; *Pseudomonas aeruginosa*

The skin of patients with inflammatory skin-diseases alongside with chronic or burn wounds and exit-sites of catheters is particularly susceptible to infection by different microorganisms. Opportunistic pathogens are the cause of skin diseases, infections, and the inability of chronic wounds to heal.[1–4] They are capable of producing virulence factors, including enzymes that promote tissue invasion and extracellular polymers, which form the biofilm that contributes to the perpetuation of skin inflammation, even in normal-appearing skin. Fortunately, the majority of our resident skin microorganisms are non-pathogenic and many of these contribute to maintaining health.[1] Accordingly, skin-disease/injury management demands an integrated approach aimed not only at diminishing infection but also at regulating the skin microbiome.[2,5]

P. aeruginosa is the most common infectious agent among *Pseudomonas* spp. As a versatile and opportunistic microorganism it can colonize the skin, soft tissue, gastrointestinal tract, armpits, eye and ear.[6–8] *P. aeruginosa* is the agent responsible for the most common infections under hospital settings, through catheter and ventilator contaminations leading to nosocomial infections, such as pneumonia, urinary tract and wound burn infections, as well as bacteremia, especially in patients with diabetes or immunodeficiency.[8] The major concerns about the control of nosocomial infections vary from the problems of drug safety associated with a high human toxicity, the long-term and large scale application of broad-spectrum antibiotic drugs, to the increased resistance to conventional therapies. These infections tend to chronicity and may fail to be treated with almost any combination of antibiotics, showing mortalities up to 61%.[9] The combination therapy to fight *P. aeruginosa* infections is very difficult to achieve, due to the compromised immune system of the majority of infected patients, and the intrinsic resistance of microorganisms to various antibiotics.[10]

Recent research has been conducted on the three groups of naturally occurring antimicrobials as novel alternatives to antibiotics: bacteriophages, bacterial cell wall hydrolases (BCWHs), and

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antimicrobial peptides (AMPs).[11] Among them, bacteriophages are the most highly specific toward both Gram-positive and Gram-negative bacteria and they are also highly efficient and relatively cost-effective. In contrast, AMPs have a broad-spectrum against bacteria and fungus, low level of induced resistance, but may cause toxicity at high doses in order to be efficient, and are more costly to produce.[11] BCWH has limitations toward Gram-negative bacteria, as a result of the presence of the outer membrane, and important Gram-positive pathogens like *S. aureus* are already resistant to lysozymes. To overcome the changing tide of nosocomial diseases and increasing reports of microorganism-acquired resistances, recently the United States, Canada and European countries have started to take a close interest in bacteriophage-based therapies,[12,13] in which they act without mechanism-based host toxicity.

The bacteriophage vB_Pae_Kakheti25 has a potent activity against *P. aeruginosa* and appears as an alternative approach to conventional treatments, especially as an antimicrobial agent for dressing. It is representative of siphoviral family, has dsDNA as its genome, icosahedral capsid, and long non-contractile tail. vB_Pae_Kakheti25 undergoes a lytic cycle through which it self-replicates and lyses a broad range of *P. aeruginosa* strains in order to spread copies of itself.

Polycaprolactone (PCL), an hydrophobic polyester, can be explored as a substrate for skin regeneration due to its high elasticity and slow biodegradability.[14,15] Furthermore, it is not broken down by enzymes and microorganisms.[14] These features seem worthy of dressing applications.[16]

The textile and medical industries continue to look for eco-friendly processes that may replace the currently used toxic textile chemicals and the use of antibiotics, respectively.[17–19] The demand for medicinal products alternative to antibiotics has increased considerably and our proposed application seems promising due to its versatility, low content of impurities, antistatic properties and good mechanical properties. Furthermore, as PCL is unfavorable for the development of microorganisms, its shelf life and users' health status are also ensured.

In this work, in order to eliminate the growth of *P. aeruginosa*, PCL electrospun nanofibers were threaded and then vB_Pae_Kakheti25 bacteriophages were covalently immobilized by their capsid via acid-amine reactions, forming amide linkages. The effect of anti-*P. aeruginosa* activity of PCL-vB_Pae_Kakheti25 dressing was evaluated under various parameters, so as to produce appropriate applications toward skin-infections, and aiming to further highlight the potential of phage as the "antibiotic" of the millennium by minimizing bacterial resistance and preserving skin-microbiome.

Methods

Materials

Polycaprolactone nanostructure (PCL), average Mn 45,000 (Sigma), was functionalized in a vB_Pae-Kakheti25 bacteriophage solution. PA25 (DSM 25642) clinical isolates of *Pseudomonas aeruginosa* from the Eliava culture collection were used for isolation and subsequent growth of vB_Pae-Kakheti25 bacteriophages. *P. aeruginosa* was grown on Brain Heart Infusion (BHI) agar and then on Brain Heart Infusion (BHI) broth (Sigma) at 30 °C and in shaker at 200 rpm.

Methods

Electrospinning

Nanofibers were produced by NanoSpider (Elmarco s.r.o. Liberec, Czech Republic). PCL 15% (w/v) was dissolved in a mixture of absolute ethanol/chloroform (65:35 vol.%) to prepare a homogeneous solution. Different ratios of ethanol/chloroform solvents were used in order to optimize the final nanostructure. The final concentration of PCL and ratio of solvents were set according to the homogeneity of resulting nanofibers, their easy-detachment from polypropylene-coated collecting electrode and tensile strength. The electrospinning process was done under the following experimental conditions: RH ≈65%, temperature ≈25 °C, electric voltage ≈80 kV, distance between electrodes = 8.98 cm, and electrode spin = 7 r/min (44 Hz). Figure 1 shows the representative images of these nanofibers.

Tensile strength assays

Tensile strength of electrospun PCL was evaluated with resistance-to-rupture assays. These assays were performed in a Dynamometer (Thwing-Albert Instrument Co.) according to Standard EN ISO 2062, at 20 ± 2 °C, under 60% relative humidity. Samples were strip-cut 1×5 cm, with an average thickness of 50.33 μm, grammage of 18.6 g/m^2, and placed between dynamometer tweezers. A defined pre-tension was set in the beginning, and the test ended up with the rupture of samples. Seven replicates were used.

Protein structure modeling – I-TASSER method

The sequence of amino acids (UniProtKB) of the Major capsid protein referred to as H6WTZ9–1 and Major tail tube protein referred to as H6WU05-1 of vB_Pae-Kakheti25 bacteriophage were the following, respectively: MALSDLAV YSEYAYSAFSETLRQQVDLFNTATGGAIMLQSAAHQGD FSDVAFFAKVTGGLVRRRNAYGSGTVAEKVLKHLVDTS VKVAAGTPPVRLDPGQFRWIQQNPEVAGAAMGQQLAV DTMADMLNVGLGSVYSALSQVSDVVYDATANTDAAD KLPTWNNLNNGQAKFGDQSSQIAAWIMHSTPMHKLYG SNLTNGERLFTYGTVNVVRDPFGKLLVMTDSPNLFAAG TPNVYHILGLVPGGVLIGQNNDFDANEETKNGDENIIRT YQAEWSYNIGVKGFAWDKANGGKSPTDAALFTSTNWD KYATSHKDLAGVVVKTN;

MVCEIAKIDSNITGLAFAEEECLKQLPTTPVWYGLEPN SYSDFGGELSTVARAPIDPSRQNKKGTITDLDASGGFNA DFTKTNLARILQGFFFADARELPSTQPLNGASVALTGVT AIDSTYAAASGLGVFGADMLVYATGFANAANNGLKTV VSATAAGVVVAETLIDETPPAGAKLECVGRQLAAADAN IAVTGNVVSLIVTAGDFTTMPELFAGRWVFVGGDATAN RFANNVGYARIKSVAAKALVFDDVTWQAVNETGTGKSI RLFVGTVIKNEKTPALIKRRSYQIERTLGEGLNGTQCEYL EGAVPNEFTLNVPQADKLNADLSFVACDNTYRSGDPGD EQKAGTRVPAPGEDAYNTSSDVYRIKMAVHDAASSNPA ALFGYVSEANVSINNNVTPNKAVGVLGAFDTSAGNFEV GGSITAYFTTVAAVKAVRANADVGLSVISAAKNAGFVF DIPLLGLGGGRLNVEKDAPITVPLEPAGAENANGYTMLY EVFSYLPNLAMPD.