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# Design, synthesis and evaluation of antibacterial activity of peptide polymer based on branched PEI as a biomimetic polymer

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**Introduction**:Biomimetic means imitating models, systems, and elements of nature to design and build modern systems to solve complex human problems. This has led to the creation of new technologies inspired by biological solutions in macro and nano sizes (Vincent et al., 2006). Antimicrobial peptides are among the compounds found in both prokaryotes and eukaryotes. These peptides are divided into groups based on the type of amino acid and tertiary structure. The common feature of most antimicrobial peptides is their cationic nature, which is related to the presence of positively charged amino acids (lysine, arginine, and histidine) in their structure (Jiang et al., 2008; Zasloff, 2019). Antimicrobial peptides also have limitations; they are degradable, and they have high production prices. Also, they have the potential for cell toxicity and hemolysis (Shen et al., 2018). Therefore, scientists are looking to design and produce a new type of polymer based on antimicrobial peptides without limitations. One of the suitable polymers for this purpose is polyethylenimine, which is a synthetic and biocompatible polymer (Beyth et al., 2010). This study aims to prepare an antibacterial structure by imitating antimicrobial peptides. For this purpose, branched polyethylenimine with a molecular weight of 600-800 Daltons was functionalized with lysine and valine amino acids. As mentioned, antimicrobial peptides have hydrophobic groups with a positive charge; In this structure, valine creates hydrophobic groups, and lysine creates a positive charge. After investigating the structural features, the antibacterial activity of this functionalized polymer was investigated as an antimicrobial polymer.

**Materials & Methods*:*** The experiments of this research are sensitive to humidity and air; therefore, the solvents were dried entirely before experimenting. To activate the amino acids, first the amino acids were dissolved separately in 25 ml of tetrahydrofuran (THF) solvent, then 164 µl of dimethyl carbonate was added and stirred in the presence of nitrogen gas in an oil bath at 55 °C. After 30 minutes and cooling the solution at room temperature, 100 ml of pentane solvent was added to it. After centrifugation, the sediment was washed with pentane twice and finally the sediment was collected and placed in a vacuum oven for 24 h. The optimal amounts of polyethyleneimine polymer were 8 µL, 10.5 mg lysine, and 64.5 mg valine. Amino acids and polymer were dissolved separately in dimethylformamide (DMF)

solvent and the dissolved polymer was added to amino acids solution and stirred under nitrogen gas, after 24 h butanol was added and stirred for one hour. After cooling the solution, diethyl ether solvent was added and centrifugation was performed. Finally, the sediment was dried under a vacuum oven for 24 h (Lam et al., 2016). In order to investigate the structure of the synthesized polymer, nuclear magnetic resonance analysis ($^1$H-NMR), Fourier transform infrared spectroscopy (FTIR-ATR), and zeta potential of polymer was also investigated.

*Staphylococcus aureus* (ATCC 29213), *Bacillus subtilis* (ATCC 168), *Escherichia coli* (ATCC 35218) and *Pseudomonas aeruginosa* (ATCC 10145) were used to investigate antibacterial activity of the modified polymer by disk diffusion and minimum inhibitory concentration (MIC) tests (Shirvany et al., 2021).

The hemolytic activity of the synthetic sample on human red blood cells was investigated. Different concentration of (0.312, 0.625, 1.25, 2.5 and 5 mg/ml) the synthetic sample was dissolved in 1 ml of phosphate buffer saline (PBS). Human blood samples were poured into tubes containing heparin and centrifuged for 10 minutes at 2000 rpm. Then the blood samples were washed three times with PBS. The resulting suspension was diluted 1:20 with buffer, and 100 µL of different sample dilutions and 100 µL of the diluted blood cell suspension were placed in a microtube. The resulting samples were incubated for 1 hour in an incubator with a temperature of 37°C. Then the samples were centrifuged for 5 minutes at 1500 rpm and their supernatant was read at 450 nm, the hemolysis percentage of each sample was calculated (Barman et al., 2019).

**Results & discussion:**Preliminary studies showed that if valine is higher than lysine, the antibacterial activity would be higher; The optimal ratio of valine:lysine was 1:6. FTIR analysis showed that valine and lysine were added to polyethyleneimine polymer. Also, the $^1$H-NMR spectrum confirmed the binding of polyethyleneimine polymer and amino acids. Investigations showed that the antimicrobial properties of amino acid-modified polymers with the branched structure are higher than linear structures. The branched structure of polyethyleneimine polymer modified with valine and lysine allows a better interaction of the polymer (from the positively charged chain region) with the bacterial membrane. Antimicrobial peptides also have this feature (Lam et al., 2016). Polyethyleneimine polymer had a positive zeta potential, and this potential varies according to the polymer's concentration, molecular weight and the environment's pH. The zeta potential of this modified polymer was +5.56 mV. Compounds with positive zeta potential have a significantly better interaction with cell membranes and cause severe destruction of cells. Also, the entry of nanoparticles with positive zeta potential into cells is facilitated. Studies show that the optimal zeta potential value in antimicrobial peptides ranges from +1 to +7 mV. Therefore, the antimicrobial peptide can have a good effect on the bacterial cell membrane, and at the same time, will not cause hemolysis (Lam et al., 2016; Shao et al., 2015).

Studies show that linear and branched polyethyleneimine polymers have antibacterial activity. But the hydrophobicity and the presence of long alkyl groups in the branched form of this polymer cause its interaction with the bacterial membrane and its antibacterial activity to be less; Polymer modification can improve this activity. The MIC test showed that by increasing the concentration of the modified polymer, the inhibition of bacterial growth increases. 5 mg/ml of polyethyleneimine modified with amino acids inhibited the growth of *E. coli* and *P. aeruginosa*, and *S. aureus* by more than 80%.

The disk diffusion test showed that the diameter of the inhibition zone for *B. subtilis*, *P. aeruginosa*, and *S. aureus* bacteria reached about 20 mm at a 5 mg/ml concentration. However, the diameter of the inhibition zone for *E. coli* was 15 mm at the same concentration, and the inhibitory effect of the polymer was significantly lower than the other 3 bacteria ($p<0.05$). In general, gram-negative bacteria are more resistant to antibacterial polymers, and the porous wall of gram-positive bacteria allows the penetration of small molecules (Tegos et al., 2006). Studies show that polymers containing lysine can be used as antibacterial compounds. These polymers disrupt the organization of the bacterial outer membrane and destroy its lipopolysaccharide. The antibacterial effects of lysine have been observed on Gram-positive and Gram-negative bacteria. Also, lysine prevents biofilm formation by S. aureus bacteria (Alkekhia & Shukla, 2019). The antibacterial activity of this polymer was also compared with the antibiotic cefepime at a concentration of 5 mg. This antibiotic inhibits the cell wall synthesis of Gram-positive and Gram-negative bacteria. In

all bacteria, the diameter of the inhibition zone caused by cefepime was significantly greater than the modified polymer ($p<0.05$). The maximum diameter of the inhibition zone was 23 mm for antibiotics and 20 mm for modified polymer in *B. subtilis* bacteria.

As mentioned, one of the limitations of using antimicrobial peptides is the possibility of toxicity and hemolysis of blood cells (Henkelman et al., 2009; Shen et al., 2018). With the increase in polymer concentration, the percentage of hemolysis of cells increased so that at the highest concentration (5 mg/ml), the percentage of blood cell hemolysis was 21%. As zeta potential analysis showed, the modified polymer was positively charged. The interaction of this polymer with the membrane of blood cells causes membrane damage, changes in its structure, and, finally, hemolysis (Chi et al., 2018). Generally, if the hemolysis is less than 10%, the polymer is considered non-toxic (Fischer et al., 2003). Therefore, 0.312 mg/ml of polyethyleneimine polymer modified with valine and lysine was the most suitable concentration.

**Conclusion:***I*n this study, the surface of polyethyleneimine was modified with amino acids. The optimal valine: lysine ratio was 1:6 and 8 µL of polymer. $^{1}$HNMR and FTIR analysis were used to prove the surface modification. The zeta potential of the modified polymer was +5.56 mV. Polyethyleneimine polymer modified with amino acids had concentration-dependent antibacterial activity, and 5 mg/ml of this polymer inhibited the growth of *E. coli* and *P. aeruginosa*, and *S. aureus* more than 80% and was comparable to cefepime antibiotic. The modified polymer was non-toxic and can be used as an antibacterial polymer.

***Keywords: Antimicrobial; Biomimetic; Lysine; Polyethyleneimine; Valine.***

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