

# Enhanced Antibacterial activity of eugenol loaded m-PEG/PCL nanoparticles in eliminating resistant bacteria from wastewater

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## Research Article

**Keywords:** Polymeric nanoparticle, Pathogenic bacteria, Eugenol, Hospital Wastewater, antibacterial, Eliminate

**Posted Date:** June 17th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-627508/v1>

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# Abstract

In this study, eugenol loaded m-PEG/PCL nanoparticles were used to make better the anti-bacterial properties of eugenol in an attempt to eliminate the resistant bacteria. m-PEG/PCL copolymer was prepared by ring-opening polymerization of  $\epsilon$ -caprolactone monomer in the vicinity of dry m-PEG and tin (II) octoate catalyst. Polymeric nanoparticles were prepared by nanoprecipitation procedure. The particle size and zeta potential of mPEG/PCL/eugenol were specified to be  $157.23 \pm 3.81$  nm and  $-6.95 \pm 0.19$  mv, respectively. The polymeric nanoparticle structure was identified by AFM, FT-IR, and DSC techniques. To evaluate and compare the anti-bacterial efficiency of m-PEG/PCL/eugenol and free eugenol, a turbidity assay was used in association with gram-positive and gram-negative bacteria. Images of SEM were taken from bacteria before and after exposure to the mPEG/PCL/eugenol. Colony-forming unit per milliliter (CFU/ml) method was considered to follow the effect of mPEG/PCL/eugenol on bacteria growth rate in the original hospital wastewater. The results showed that m-PEG/PCL/eugenol nanoparticles at 40  $\mu$ M concentration show the enormous antibacterial effect at 37°C. In original hospital wastewater, m-PEG/PCL/eugenol in the concentration of 0.125  $\mu$ M at 25 ° C showed the greatest growth decrease of microbial total count.

## 1. Introduction

Water pollution by wastewater contaminants has become harmful for human health and ecosystem and attracted a great deal of public and scientific attention. The main contaminants in wastewater includes nutrients material, heavy metals, hydrocarbons, organic matter, and microbes that lead to harmful effects on human safety and the environment (1). Among the various environmental contaminations, antibiotics have attracted specific interest due to their severe adverse effects. To remove these contaminants, one strategy could be the implementation of the antibacterial effect of metallic nanoparticles. For example, ZnO nanoparticles prevent the growth of *Staphylococcus aureus*, and Ag nanoparticles show antibacterial effect against *Escherichia coli* and *Pseudomonas aeruginosa* (2). In contrast to the high potential, metallic nanoparticles are costly for being used for wastewater treatment. In this regard, it is imperative to take advantage of natural organic materials as low-cost and environmentally-friendly sources. There is little research on usage of non-metallic nanoparticles for wastewater treatment. For instance, carbon nanotube-based nanomaterials, due to special features such as vast surface area, vast chemical stability and mesoporous structure have great potential for wastewater treatment (3). Chitosan nanocomposite has been used for the elimination of heavy metals and microbial agents (4).

Medicinal plants because of having several compounds such as flavonoids, alkaloids, essential oil, polyphenols, and terpenoids show good antimicrobial and antioxidant properties (5, 6). The antimicrobial traits of the variety of plants have been studied. Eugenol has been introduced as an effective ingredient against antibiotic-resistant bacteria (7). Eugenol is a phenylpropene compound and a main component of clove essential oil which play a determinant role against bacterial growth (8). There are many reports on the antibacterial properties of eugenol against pathogenic bacteria. According to the kamtou *et al* report, eugenol inhibits the growth rate of gram-positive (*Bacillus cereus*; *B. subtilis*; *Staphylococcus aureus*) and

gram-negative (*Escherichia coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*) bacteria at a concentration of 1,000 ppm (9). In another study, eugenol showed an obvious antibacterial activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, and *Bacillus cereus* (10). However, water slightly solubility of eugenol components restricts its vast applications. To resolve this issue, a variety of formulations such as microemulsions, nanoemulsions and nanocapsules have been investigated to increase the solubility and effectiveness of eugenol in water (11). Studies have shown that the use of nanoparticles such as liposomes, emulsions and ethosome enhances the antibacterial activity of eugenol because of high permeability and sustainable release properties (12–15).

Nanoparticles composed of mPEG-PCL copolymer are considered as a promising carrier with the privilege of biodegradability, good release properties and simple production process (16). Polymeric nanoparticles loaded with active antibacterial ingredients also show good antibacterial effects. For example, Jaglone-loaded poly-lactic acid glycolic acid polymer nanoparticles show high antibacterial effect on gram-positive bacteria (17). In another study, chitosan-loaded polymeric nanoparticles were more effective against the pathogens *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas* and *Staphylococcus aureus* (18).

The aim of this research was the investigation of the antibacterial efficacy of eugenol-loaded m-PEG/PCL against bacteria isolated from hospital wastewater. The nanoparticles were designed to release the active ingredient slowly to control pathogenic bacteria effectively in the environment and achieve a long-term antibacterial action. In contrast to other methods used to eliminate pathogenic bacteria from wastewater which have many side adverse effects, the most remarkable feature of the nanoparticles is that they have no considerable harmful effects on the environment and its components can be degraded by the environment.

## 2. Materials And Methods

### 2.1 Materials

m-PEG (Mn=5000 Da), Eugenol, and tin (II) octoate catalyst were purchased from (Aldrich, St. Louis, USA).  $\epsilon$ -caprolactone (98% purity) were supplied by Acros (New Jersey, USA).

### 2.2 Synthesis of m-PEG/PCL copolymer

m-PEG/PCL copolymer was prepared by ring-opening polymerization of  $\epsilon$ -caprolactone monomer with the vicinity of dry m-PEG as the molecular initiator and tin (II) octoate catalyst.  $\epsilon$ -caprolactone (4 g) was placed in calcium hydride for dehydration for one week. Briefly, m-PEG (2 g) (with a molecular mass of 5000 g / mol) and  $\epsilon$ -caprolactone (4 g), and tin(II) octoate catalyst (0.01 mmol) was heated to 120°C and the reaction was performed for 12 hrs. After the reaction, the copolymer was solved in chloroform and precipitated in cold diethyl ether, then the precipitate was gathered by filtration and then dried (19).

### 2.3 Characterization of m-PEG/PCL copolymer

Nuclear magnetic resonance spectroscopy ( $^1\text{H}$  NMR) was used to detect the structure of m-PEG/PCL copolymer. The spectra were captured by a device (Bruker, Avance 400) at 25 °C. Deuterated chloroform was consumed as solvent and trimethylsilane was used as an internal standard.

## 2.4 Preparation of polymeric nanoparticles from m-PEG/PCL copolymer

The nanoparticles were synthesized by the nanoprecipitation procedure (20). Briefly, m-PEG/PCL copolymer (10 mg) and (1.74 mg) of eugenol were solved in 1 ml of acetone and added by syringe pump to 25 ml of distilled water. It was also stirred by a magnetic stirrer, until complete evaporation of organic solvent. Blank/m-PEG/PCL was made by the same method but the drug was not added to the formulation.

## 2.5 Characterization of m-PEG/PCL/eugenol

### 2.5.1 Particle shape

Atomic Force Microscope (AFM) was used to determine the shape of the m-PEG/PCL/eugenol (JPK, Berlin, Germany, Wizard 2). m-PEG/PCL/eugenol solution was diluted with distilled water, then a drop placed on the mica substrate (1  $\text{cm}^2$ ) and dried in the air. AFM device was worked through a contact method (21).

### 2.5.2 Determination of m-PEG/PCL/eugenol size

The particle size distribution, polydispersity index (PDI), and zeta potential of the m-PEG/PCL/eugenol was determined by dynamic light scattering (DLS) technology by Malvern Zetasizer *instrument* at 25°C (Zetasizer Nano ZS, Malvern Instruments, UK).

### 2.5.3 Drug-loading efficiency

To define the drug-loading efficiency in the nanoparticles, the drug loading (DL) ratio and entrapment efficiency (EE) were studied. Briefly, 1 mg of m-PEG/PCL/eugenol was dispersed in 1 mL of ethanol, and the drug substance was assayed by a UV-Vis spectrophotometer (JE Norway 6300, Switzerland) at  $\lambda_{\text{max}}=281$  nm. The EE and DL were calculated using the following equations:(22)

$$\text{Equation 1} \quad \text{DL}(\%) = \frac{\text{Amount of drug in nanoparticle}}{\text{Amount of nanoparticle}} \times 100$$

$$\text{Equation 2} \quad \text{EE}(\%) = \frac{\text{Amount of drug in nanoparticle}}{\text{Amount of initial drug}} \times 100$$

Where the amount of the drug in nanoparticles, the amount of nanoparticles, and amount of initial drug show the amount of the entrapped drug, the total amount of polymer added in the preparation step, and the drug added to the formulation, respectively.

## 2.5.4 FTIR analysis

FT-IR analysis was used to observe changes in peak positions and to investigate the interaction between the components in the nanocarrier system. In general, the interaction between the components are studied by the band shift of functional groups. FT-IR analysis by a Fourier transform infrared spectrometer (Bruker Tensor 27, Germany) was done in the range of 500-4000  $\text{cm}^{-1}$ .

## 2.5.5 DSC analysis

To carry out the differential scanning calorimetry (DSC) analysis of m-PEG/PCL copolymer as well as their nanoparticles, the samples were heated at a speed of 10  $^{\circ}\text{C}/\text{min}$  in the range of 25-250  $^{\circ}\text{C}$  by a thermal analyzer (Mettler Toledo, Switzerland) (19).

## 2.5.6 Drug release study

To study the release behavior of eugenol from m-PEG/PCL/eugenol, 1 ml of nanoparticles solution was poured into dialysis bags (Mw 12 kDa) and immersed in water containing 2% (v/v) *DMSO*. The suspension was put in a thermal shaker (Mettler, Germany) at 50 rpm at 37  $^{\circ}\text{C}$  for 48 hrs. *DMSO* was used in the release media to facilitate drug release from the nanoparticles. Then, at regular intervals, 2 ml of the release media was removed and replaced by 2 ml freshly release media.

## 2.6 Antibacterial effect of m-PEG/PCL/eugenol

To evaluate the anti-bacterial performance of m-PEG/PCL/eugenol and free eugenol on bacteria growth, a turbidity assay was used. The colony-forming units per milliliter (CFU/ml) method was used to estimate the m-PEG/PCL/eugenol performance on wild strains in original wastewater. Confirmatory and complementary tests were performed on wild strains isolated from wastewater, which were fully reported in our previous paper (23).

### 2.6.1 Bacteria growth rate in Luria-Bertani in the presence of m-PEG/PCL/eugenol

The antibacterial activity of m-PEG/PCL/eugenol and free eugenol were investigated on gram-positive bacteria (*Staphylococcus aureus*, *Enterococcus faecalis*) and gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*) using the turbidity assay. The bacteria growth rate in Luria-Bertani in the presence of m-PEG/PCL/eugenol and free eugenol at different concentrations (0.125-40  $\mu\text{m}$ ) were measured by a microplate reader (infinite 200, Tecan, Switzerland) at OD (600 nm) for 24 hrs. *DMSO* were used in the bacteria culture media for ease of drug release from the nanoparticles (24).

The reduction percentage of bacteria was examined using the following equation 3 (25):

$$\text{Equation 3} \quad \% \text{ bacteria reduction} = 100 \times \frac{(B - A)}{B} .$$

where A is the OD 600 value of growth rate of bacteria in the culture media containing m-PEG/PCL/eugenol nanoparticles or free eugenol and B is the OD 600 value of growth rate of bacteria in the culture media without an antibacterial agent.

### 2.6.2 Antibacterial properties of m-PEG/PCL/eugenol in real wastewater

The wastewater was sterilized by autoclave for 15 min at a pressure of 200 KP and a temperature of 121 ° C. Then, the optical density value of the wild type bacteria suspension was adjusted to 0.1 and then suspension was diluted with sterilized physiological saline. 100 µl of this dilution, sterilized wastewater, m-PEG/PCL/eugenol, and free eugenol at two different concentrations (0.125 and 40 mM) were poured into microtubes and placed at 4 °C, 25 °C and 37 °C for 24 hrs. The samples were diluted up to 1/1000 with sterilized physiological saline. Then, 10 µl of the samples were cultured on a plate containing Müller Hinton agar, and the plates were heated at 37 °C for 24 hrs. Effect of m-PEG/PCL/eugenol and free eugenol was also performed according to the above method on the original wastewater (before sterilization) to obtain the colony-forming units per milliliter (CFU/ml). All experiments were performed with triplications.

### 2.7 Scanning Electron Microscopy (SEM)

Scanning electron microscopy (SEM) was used to examine the morphological changes in *Staphylococcus aureus* as gram-positive bacteria and *Escherichia coli* as gram-negative bacteria after exposure to m-PEG/PCL/eugenol nanoparticles. SEM analysis was done by AIS 2100 scanning electron microscope (Seron, Korea).

### 2.8 Statistical analysis

Statistical comparisons were expressed by ANOVA analysis, where P-values <0.05 were meaningful. Data were reported as average±SD.

## 3. Results And Discussion

### 3.1 Characterization of mPEG/ PCL copolymer

m-PEG/PCL copolymer was prepared by ring-opening polymerization of ε-caprolactone monomer in the vicinity of dry m-PEG as the molecular initiator and tin (II) octoate catalyst. Chemical structure of m-PEG/PCL copolymer was determined using Nuclear magnetic resonance spectroscopy (1H NMR). In the nuclear magnetic resonance spectrum of the copolymer, the peak in the region of 3.4 ppm (3H, a) and 3.67 ppm (4H, b) corresponds to the methoxy group hydrogens and the polyethylene glycol groups hydrogens. The peak of 1.445 ppm (2H, d), 1.670 ppm (2H, e), 2.33 ppm (2H, c), and 4.085 ppm (2H, f) correspond to the caprolactone hydrogens, respectively Fig. 1 (21)

### 3.2 Characterization of polymeric nanoparticles

The resulting mPEG/PCL/eugenol polymeric nanoparticles were characterized by different methods such as dynamic light scattering (DLS) and atomic force microscopy (AFM). According to the AFM images, mPEG/PCL/eugenol polymeric nanoparticles showed almost spherical shape with the average particle size of  $146.75 \pm 5.39$  nm, Fig. 2. According to the DLS method, the particle size of mPEG/PCL/eugenol and m-PEG/PCL/Blank were determined to be  $157.23 \pm 3.81$  nm ( $n = 3$ ) and  $85.22 \pm 3.26$  nm ( $n = 3$ ), respectively. PDI values of mPEG/PCL/eugenol and m-PEG/PCL/Blank were  $0.21 \pm 1.14$  and  $0.35 \pm 3.81$ , respectively, and zeta potential of mPEG/PCL/eugenol and m-PEG/PCL/Blank were  $-6.95 \pm 0.19$  mv ( $n = 3$ ) and  $-6.25 \pm 3.26$  mv ( $n = 3$ ), respectively. The particle size of mPEG/PCL/eugenol measured by dynamic light scattering method (DLS) was a bit bigger than the one of AFM which can be explained by the fact that AFM determines the particle size of the sample after evaporation of water, while the diameter of the nanoparticles measured by the DLS indicates the hydrodynamic diameter (26). The drug loading (DL) ratio and entrapment efficiency (EE) of eugenol loaded mPEG/PCL nanoparticles were defined to be 13.81% and  $98.33 \pm 0.004\%$ , respectively.

### 3.3 FTIR analysis

To determine the possible interactions between eugenol and the copolymer, fourier transform infrared spectroscopy was performed (Fig. 3). The results show that there is a strong, wide peak in the  $3400 \text{ cm}^{-1}$  region, which belongs to the hydroxyl group in the eugenol chemical structure. The peak in the region of  $1108 \text{ cm}^{-1}$  is related to the carbonyl group's stretching bonds, the peak in the region of  $1730 \text{ cm}^{-1}$  is related to the ester functional group of the mPEG-PCL copolymer. The outcomes indicate that the interaction between mPEG/PCL copolymer and eugenol successfully has been formed.

### 3.4 DSC analysis

Figure 4 demonstrates the DSC thermograms of m-PEG/PCL/Blank, and mPEG/PCL/eugenol (21). The curve for the m-PEG/PCL/Blank displayed a melting peak in the range of  $58.24^\circ\text{C}$ , indicating the melting of the crystalline part of PCL copolymer (19), while the mPEG/PCL/eugenol thermogram shows the melting peak in the range of  $55.33^\circ\text{C}$ . The shift in the melting peak of the copolymer manifests the physical interaction between copolymer and eugenol during the drug loading process in the nanoparticles (27).

### 3.5 drug release study

The eugenol release from mPEG/PCL/eugenol was studied. As shown in Fig. 5, the percentage of eugenol released from polymeric nanoparticles raised over the time and the maximum accessible drug release was 18.54% that was achieved after 24 hrs. The controlled drug release behavior of eugenol loaded polymeric nanoparticles can increase the efficiency of eugenol as a hydrophobic antibacterial agent in the environment. Besides, eugenol entrapment into the nanoparticles increases the dissolution rate and the level of dissolved drugs which in turn causes a higher efficiency for the nanoparticles compared to free eugenol.

### 3.6 Bacteria growth rate

To evaluate the performance of m-PEG/PCL/eugenol and free eugenol on gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) bacteria both standard and wild bacteria strains turbidity assay was used. The characteristics of all bacteria have been reported in our previous article (23). According to the results, the antibacterial effect of m-PEG/PCL/eugenol was greater than the free eugenol at equal concentration and it was different against gram-positive and gram-negative bacteria (28). This finding can be interpreted by the improved properties of eugenol through loading into the polymeric nanoparticles in terms of antibacterial efficiency, stability, and drug solubility (29). The outcomes are consistent with past studies. For example, polymeric nanoparticles loaded with lauric acid showed higher antibacterial activity against peripronium bacteria compared to free lauric acid (30). Poly lactic-co-glycolic acid loaded with clarithromycin show a higher bactericidal effect than free clarithromycin (31). In addition to the higher antibacterial efficiency of the nanoparticles, the most important feature of m-PEG/PCL/eugenol is its long-lasting antibacterial activity due to the slow release of payload drug compared to the free drug as shown in Fig. 6.

There are various reports about eugenol minimum inhibitory concentration (MIC). According to the kamtou *et al* report, eugenol inhibited the growth rate of gram-positive (*Bacillus cereus*; *B. subtilis*; *Staphylococcus aureus*) and gram-negative (*Escherichia coli*; *Salmonella typhi*; *Pseudomonas aeruginosa*) bacteria at a concentration of 1000 ppm. The results demonstrate that the least efficient concentration of m-PEG/PCL/eugenol was 40  $\mu$ M, at which free eugenol did not show any antibacterial effect because it was much lower than eugenol MIC (9). In this study, gram-negative bacteria were more sensitive than gram-positive bacteria and the highest inhibition effect was at 40  $\mu$ M against standard and wild-type of *Escherichia coli* (Fig. 6).

The observed results can be explained by the interaction of nanoparticles with the cell wall of gram-negative bacteria that is more than that of gram-positive bacteria, which in turn may be related to the bulky peptidoglycan layer in the cell wall of gram-positive bacteria (32). On the other hand, drug encapsulation efficiency by polymeric nanoparticles, instability of polymeric nanoparticles due to low zeta potential, and drug release from polymeric nanoparticles can affect their function (33–35). PEG/PCL/eugenol showed a long-term antibacterial effect toward both types of bacteria, particularly gram-negative bacteria, exposing them as a promising materials for wastewater treatment in the future.

Scanning electron microscopy (SEM) was performed to recognize the morphological change on *Staphylococcus aureus* (gram-positive bacteria) and *Escherichia coli* (gram-negative bacteria) after exposure to m-PEG/PCL/eugenol (see Fig. 8). The results showed that the structure of the bacteria cell wall exposed to m-PEG/PCL/eugenol was damaged after 24 hrs. Our previous results have demonstrated that long and slow release of curcumin from nanostructure lipid carriers can eliminate gram-positive and gram-negative bacteria by cell wall degradation, confirmed by SEM images (23). The results are consistent with the previous reports.

### **3.7 Antibacterial properties of m-PEG/PCL/eugenol in real wastewater**

The sterile wastewater containing the bacteria (wild strains of bacteria) was exposed to m-PEG/PCL/eugenol. The effect of different temperatures (37°C, 25°C and 4°C) on the performance of m-PEG/PCL/eugenol was investigated. Colony-forming unit per milliliter (CFU/ml) method and bacteria reduction rate was used to report the m-PEG/PCL/eugenol function. The antibacterial effect of m-PEG/PCL/eugenol was studied in two different concentrations (0.125 µM and 40 µM). The results showed that m-PEG/PCL/eugenol in high concentration (40 µM) had an enormous antibacterial effect on gram-negative bacteria especially *Pseudomonas aeruginosa* (*w*) and *Escherichia coli* (*w*) at 37 ° C. The results obtained for the sterile wastewater were consistent with the results obtained in the in vitro (See Fig. 9). Again, in the same concentration of eugenol, free eugenol showed no antibacterial effect.

To investigate the reduction rate of microbial total count in original hospital wastewater by m-PEG/PCL/eugenol, colony-forming unit per milliliter (CFU/ml) was used, then reduction rate percentage of the microbial total count was followed. The greatest growth decrease of microbial total count by m-PEG/PCL/eugenol (0.125 µM) was observed at 25°C. m-PEG/PCL/eugenol (40µM) showed fewer effect on the growth decreases of microbial total count in original hospital wastewater at 37° C (See Fig. 10).

## Conclusions

This study reports the development of biodegradable and environmentally-friendly nanoparticles in association with the plant active ingredients for wastewater treatment. Eugenol loaded m-PEG/PCL were prepared to improve the solubility of eugenol and provide controlled release properties hoping to increase eugenol antibacterial activity. The size and zeta potential of polymeric nanoparticles ready by the nanoprecipitation procedure were  $157.23 \pm 3.81$  nm and  $-6.95 \pm 0.19$  mv, respectively. The spherical structure of the polymeric nanoparticles was proved by AFM. Turbidity assay was used to evaluate and compare the antibacterial efficiency of m-PEG/PCL/eugenol and free eugenol, on gram-positive and gram-negative bacteria. SEM images of bacteria showed the wall destruction of bacteria after exposure to mPEG/PCL/eugenol. Colony-forming unit per milliliter (CFU/ml) method was used to follow the effect of mPEG/PCL/eugenol on bacteria growth rate in the original hospital wastewater. The results showed that m-PEG/PCL/eugenol nanoparticles at 40 µM concentration show the enormous antibacterial effect at 37°C. However, in original hospital wastewater, the greatest growth decrease of microbial total count by m-PEG/PCL/eugenol was observed in the concentration of 0.125 µM at 25 ° C.

## Declarations

### Funding

The authors declare no funding body for this research.

### Conflict of Interests

The authors express that they have no conflict of benefit.

### Ethics approval

Not applicable for section.

### **Consent to participate**

The authors are completely agree to participate in this research study.

### **Consent for publication**

The authors are completely satisfied with the publication of the article.

### **Availability of data**

The datasets used during the current study are available from the corresponding author on reasonable request.

### **Code availability**

Not applicable for section.

### **Authors' contributions**

Not applicable for section.

### **Acknowledgment**

The authors are most thankful for the assistance of this project by the School of Pharmacy, Zanjan University of Medical Sciences.

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## Figures

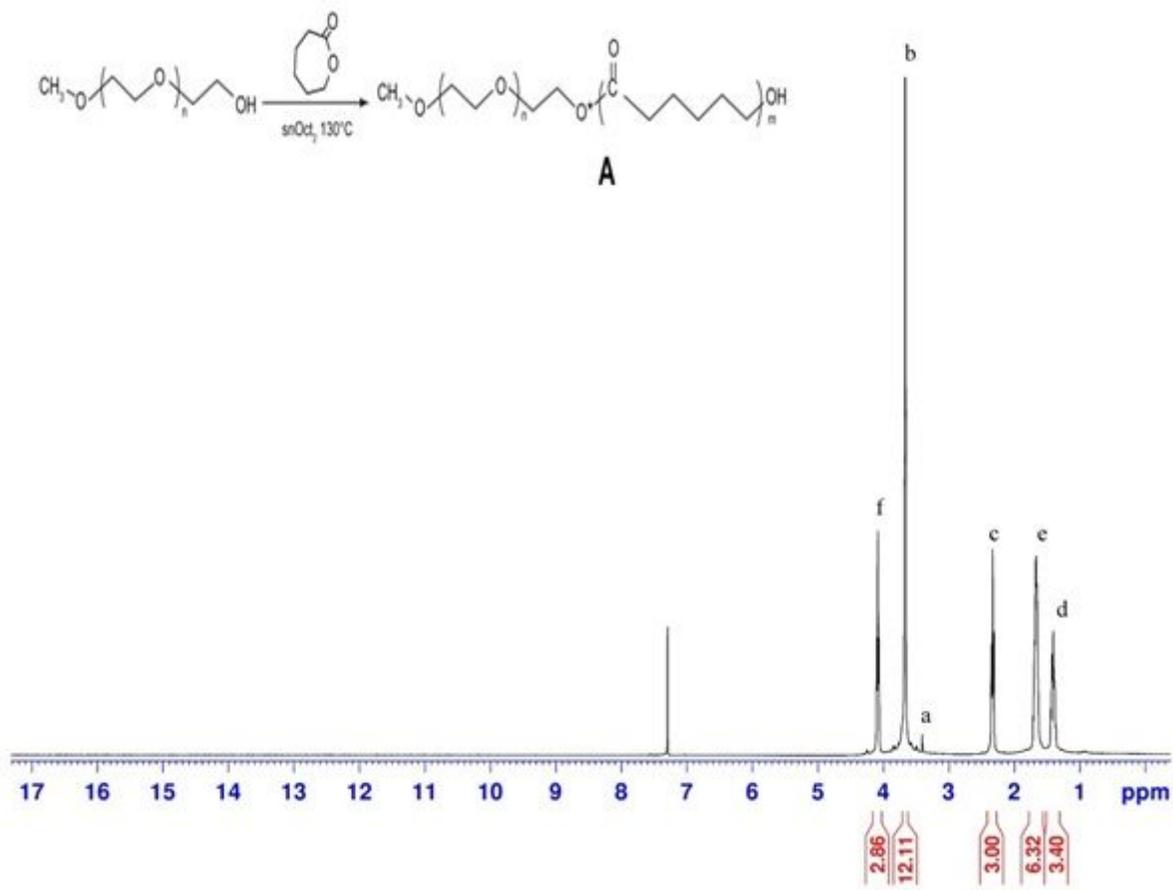


Figure 1

## H NMR spectra of mPEG-PCL copolymer

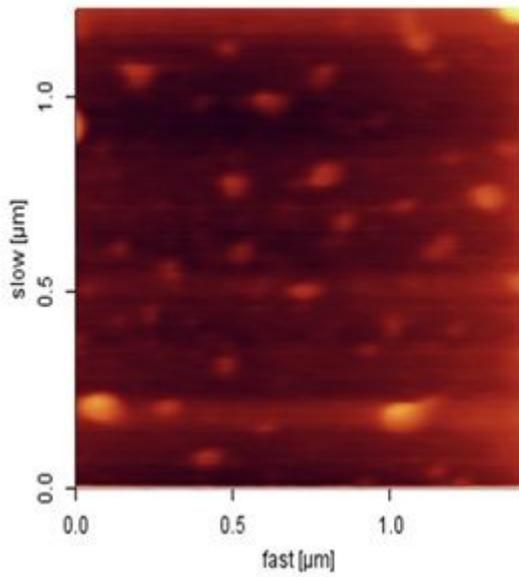


Figure 2

AFM image of mPEG/PCL/eugenol

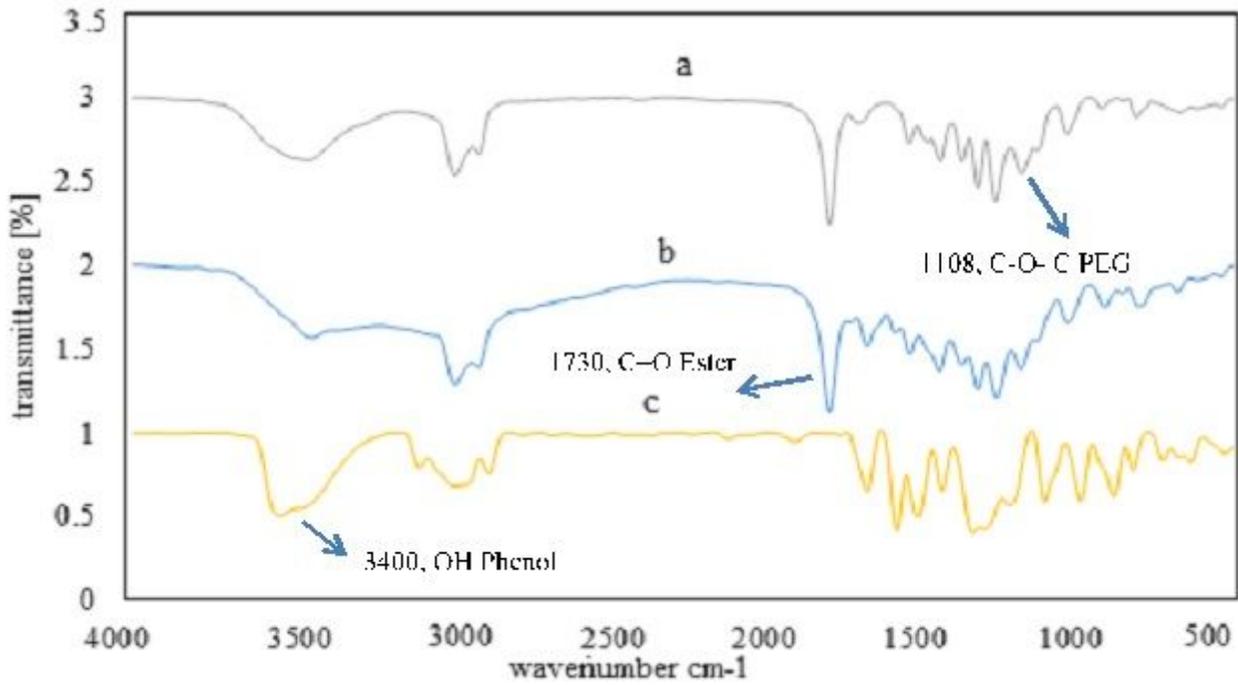
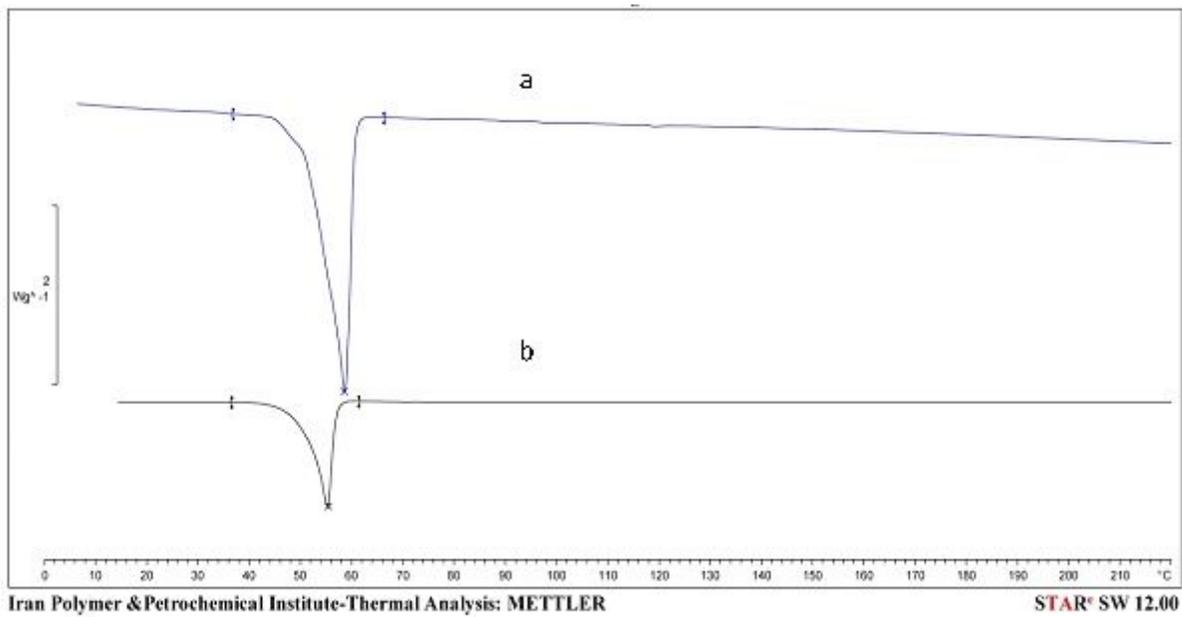


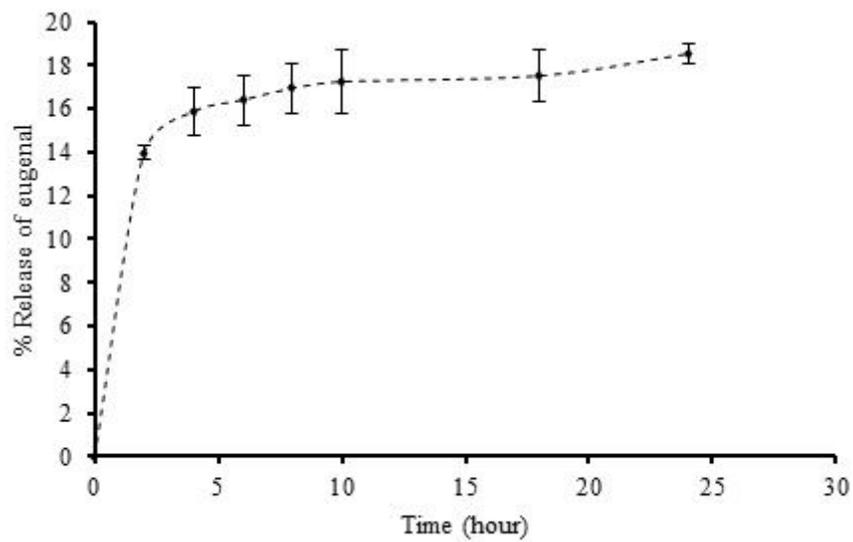
Figure 3

FT-IR spectra of a) mPEG/PCL/eugenol, b) Blank/m-PEG/PCL, c) eugenol



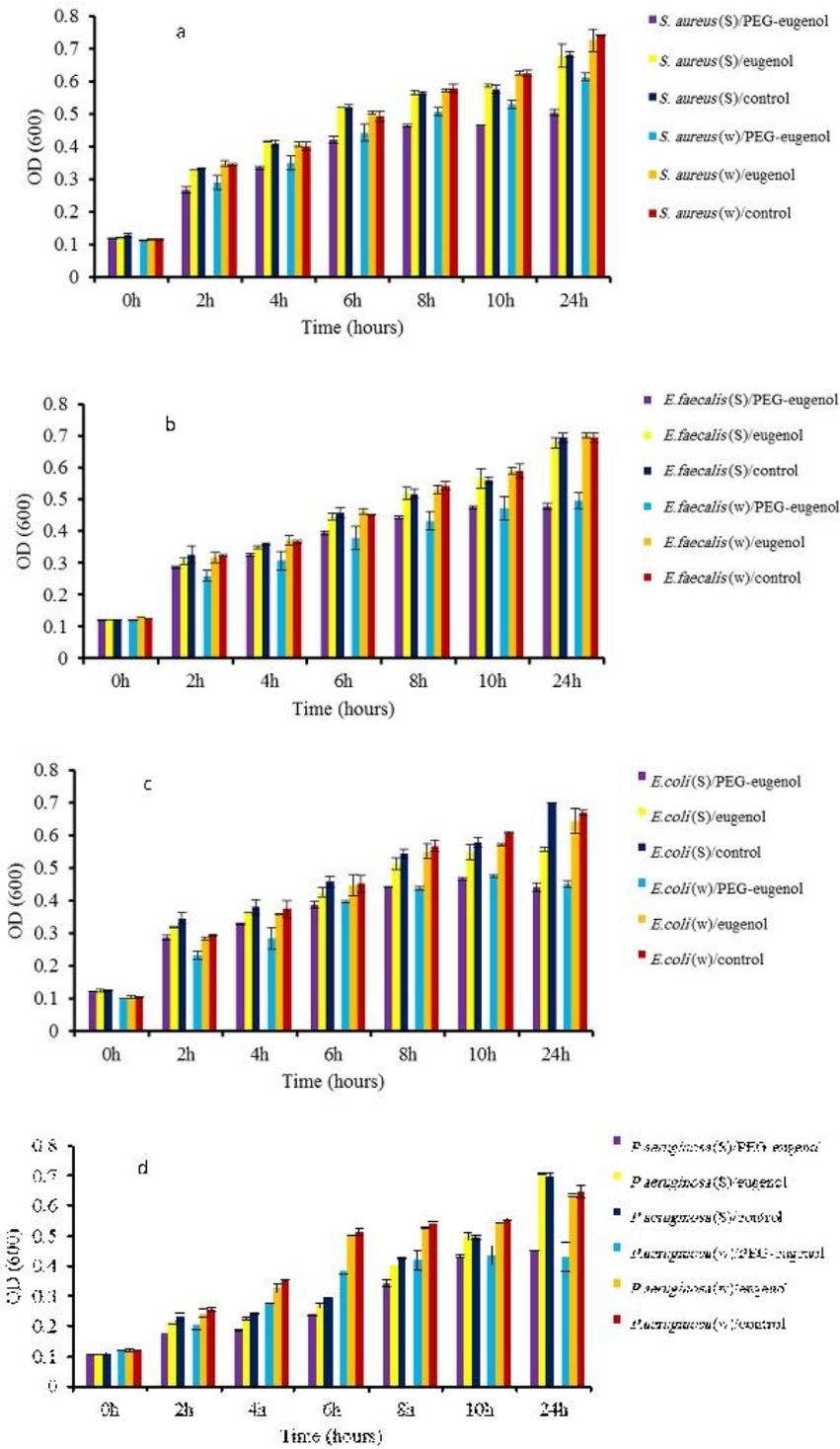
**Figure 4**

DSC thermogram of a) m-PEG/PCL/Blank, b) mPEG/PCL/eugenol



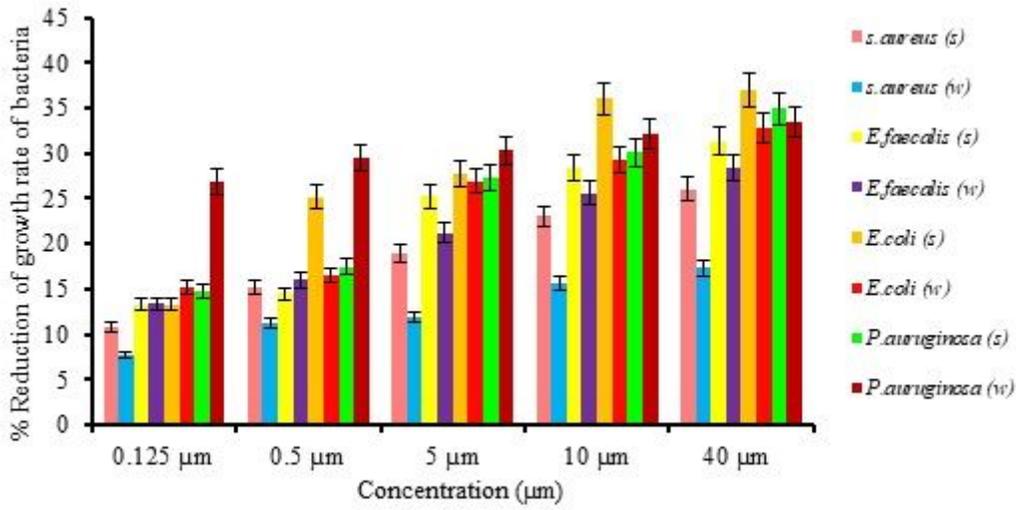
**Figure 5**

The drug release behavior of mPEG/PCL/eugenol. Each data point shows the mean $\pm$ SD (n=3)



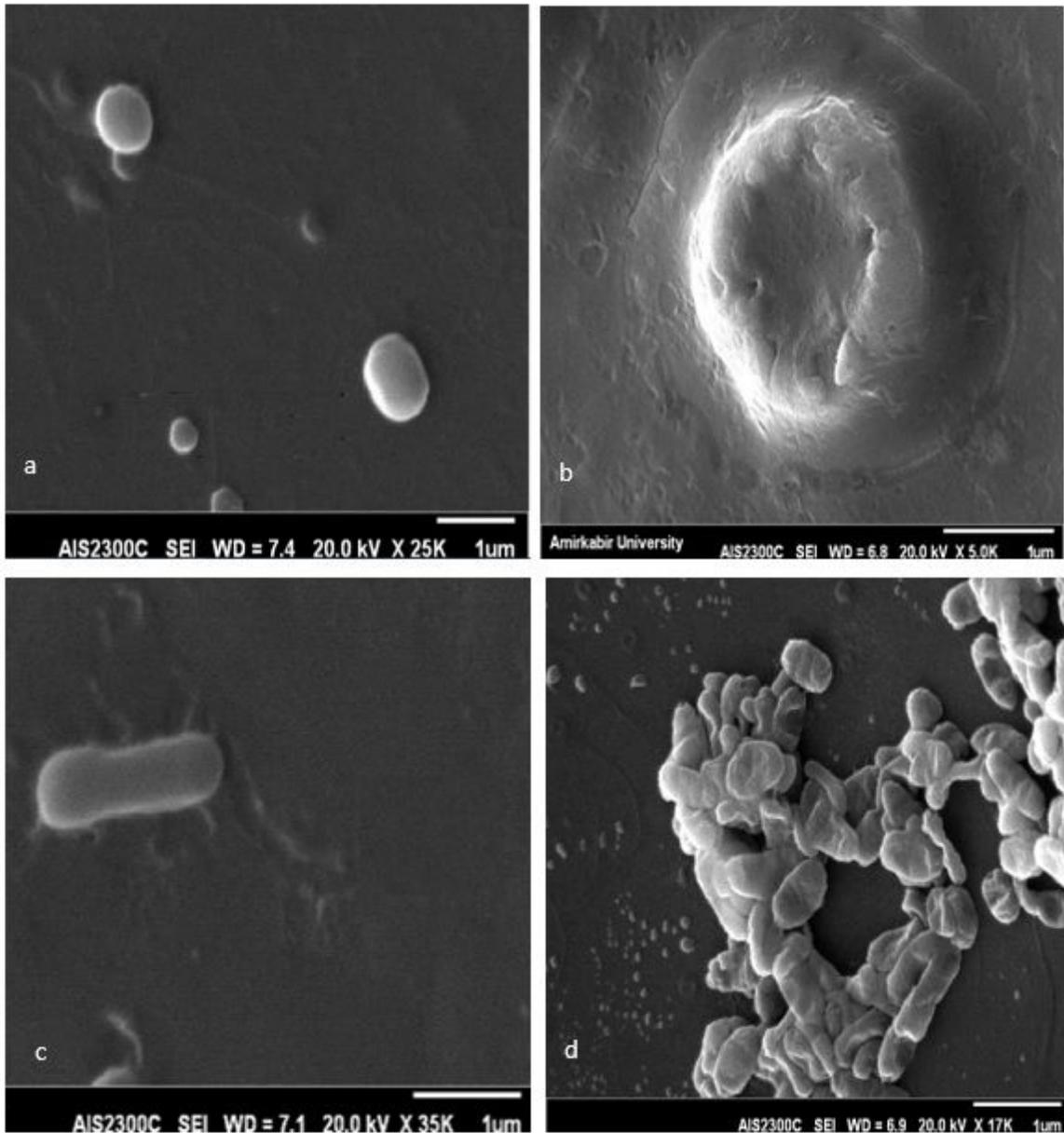
**Figure 6**

Comparison of bacteria growth rate exposed to m-PEG/PCL/eugenol 40  $\mu$ M and free eugenol 40  $\mu$ M: a) Staphilococcus aureus, b) Enterococcus faecalis c) Escherichia coli and d) Pseudomonas aeruginosa (standard and wild strains)



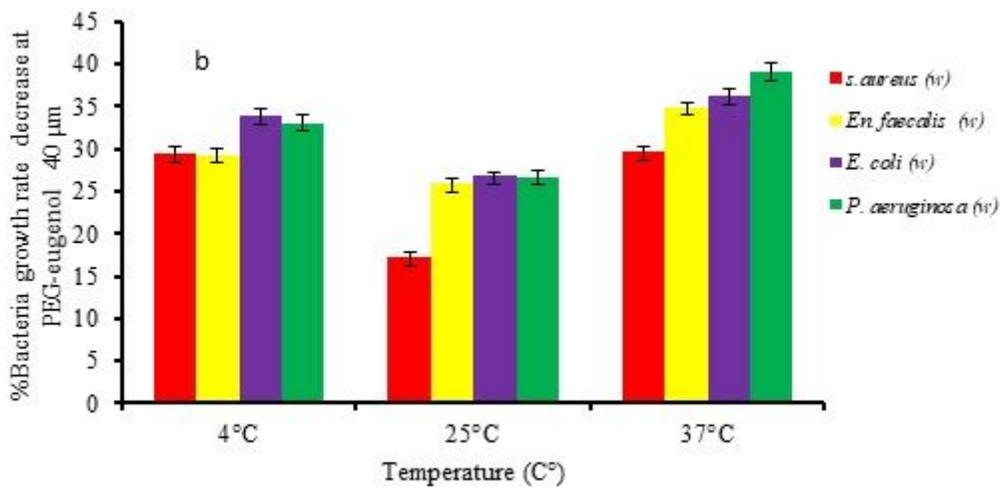
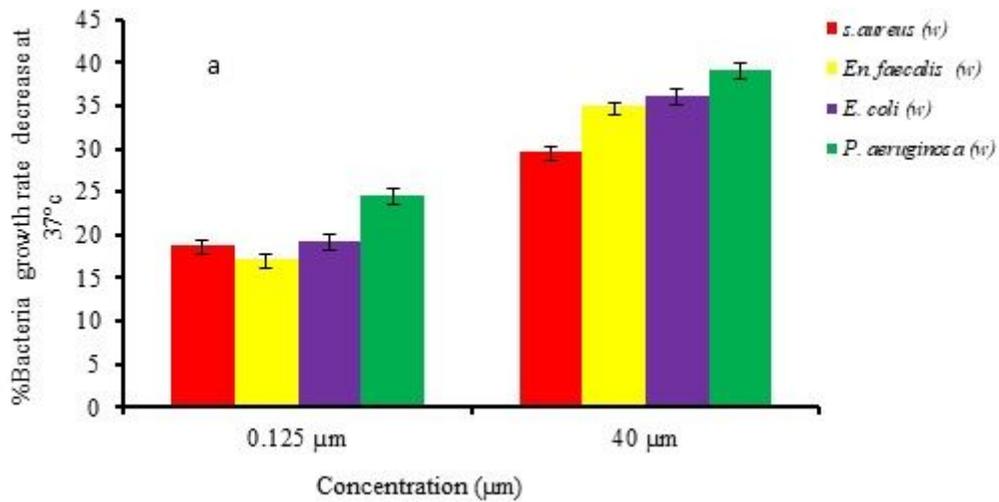
**Figure 7**

The reduction percentage of the bacteria growth in culture media exposed to m-PEG/PCL/eugenol (0.125-40 μM) for gram-positive and gram-negative bacteria (standard and wild strains) after 24 hrs.



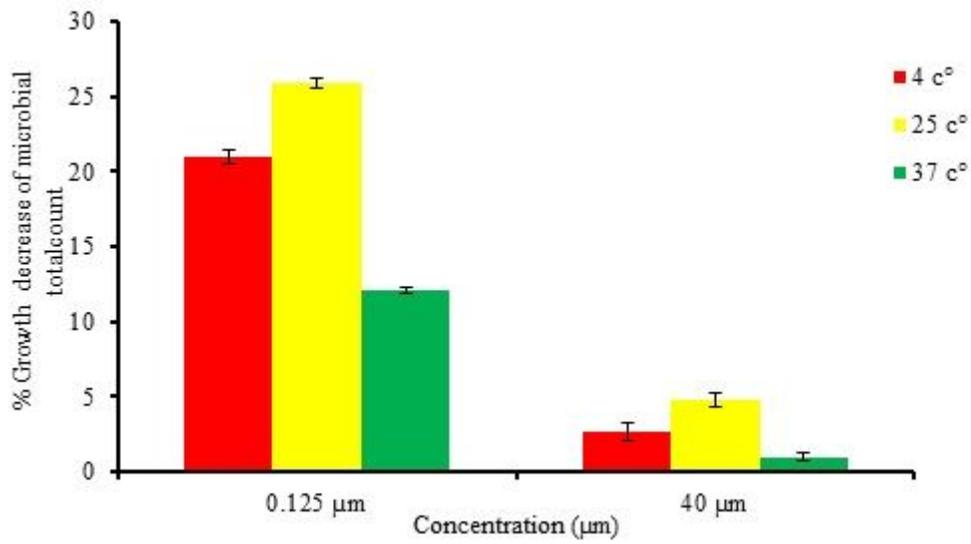
**Figure 8**

Scanning electron microscopy images of *Staphylococcus aureus* (a) without m-PEG/PCL/eugenol, (b) after exposed to m-PEG/PCL/eugenol 40 μM and *Escherichia coli* (c) without m-PEG/PCL/eugenol, (d) after exposed to m-PEG/PCL/eugenol 40 μM



**Figure 9**

a) % Bacteria reduction rate in culture media incubated with various concentration of m-PEG/PCL/eugenol at 37 °C and b) % Bacteria reduction rate in culture media incubated with m-PEG/PCL/eugenol 40 μM at different temperature



**Figure 10**

% Reduction rate of microbial total count in original wastewater exposed to different concentration of m-PEG/PCL/eugenol at different temperature