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Pathology behavior of different binary Polymer blends and blends composites against bacteria

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ABSTRACT

Background: Reduction the risk of pathogen diffusion and severe diseases, need for antibacterial surface has been urgent. Antimicrobial polymer cab be defined as polymers with antimicrobial activity, or have the ability to prevent the growth of microorganisms such as bacteria, fungi or protozoans. Typically, this type of polymers are produced by inserting or attaching an active antimicrobial agents onto the backbone of polymer via an alkyl or acetyl linker, therefore; this material can be candidate to be used in medical areas as tools to fight infection, more over in the food industry to inhibit bacterial contamination, and in water purification to inhibit microorganisms growth in drinking water. **Objective**: In this work, it has been employed two methods for improvement antibacterial of poly methyl methacrylate (PMMA), high-density polyethylene (HDPE) and ultra-high molecular weight polyethylene (UHMWPE) using both blending technique and reinforcement with 1.5wt.% of Nano powders (zirconia and hydroxyapatite). Samples tested against two types of bacteria positive gram staphylococcus and negative gram pseudomonas to assay the efficiency as antibacterial. **Results:**Results showed that blending technique strongly discourages growth of bacteria colonies; the same result was in polymer blends composites reinforced with zirconia and hydroxyapatite, except in case of PMMA-UHMWPE blend at which the effect of antigrowth was negative. Results of CFU of polymer blends composites showed improvement of the antibacterial with the addition of 1.5 wt. % of zirconia and Hydroxyapatite nanoparticles. Scanning electron microscope of polymer blends shows two different systems, homogenous and heterogeneous at which two distinct phases exist, a continuous PMMA matrix and dispersed globules of the second polymer phase, but with some discontinuous phase structure or so called "phase inversion". SEM of blends Nano composites showed that the dispersion of ZrO_2 and HAp nanoparticles was relatively good and uniformly throughout the entire polymer blend at low ratio of concentrations, but the opposite occurs at high weight ratios(i.e.1.5wt%). **Conclusion:**The positive effect of blending polymers on prevent the growth of colonies and the addition of both reinforcements that indicate to good compatibility between fillers and polymer blend matrix.

KEYWORDS: Polymer blends,bacterial adhesion, blend composites, antibacterial activity and Nano composites.

INTRODUCTION

Lately, severe threats to the public health have been raised as consequences to the spread of pathogens across the population so that there was an urgent need to enhance the antibacterial activity of materials especially those used as bio implants. Polymethyl methacrylate (PMMA), known commonly as bone cement, and is widely used for implant fixation in various orthopedic and trauma surgery, although recently, there was shortcomings, side effects and toxicity of the bone cement have been recognized [1]. UHMWPE is used in prosthetic at which Up to 80% of the total hip and knee replacements incorporate UHMWPE[2].Researches in

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this field may include B. P. Chang et.al whom studied the effect of zinc oxide to improve antibacterial activity of UHMWPE, and in dead results showed that ZnO-filled UHMWPE has active inhibition zone towards *E. coli* (Gram-negative bacteria), while slight inhibition zone for *S. aureus* (Gram-positive bacteria)[3]. More over Delle et.al used ion implantation technique to treat the surface of UHMWPE with Titania which improved the antibacterial activity and discourage surface bacterial colonization [4].

Based on kenaway etal, [5], antimicrobial agent usually kills bacteria through various methods depending on the type of bacteria. One of these methods is to kill the bacteria immediately through the contact, causing burst of bacteria cell, or by depleting the source of bacteria's food to prevent bacterial reproduction. In usual antimicrobial polymer kills bacteria through the first method, at which the polymer adsorbs onto the cell wall of the bacteria and the antibacterial agents diffuse through the cell wall into the cytoplasmic membrane. Suaad et.al prepared fiber of polyvinyl alcohol reinforced with different (1 wt. % and 3 wt. %) TiO2 , PVA–TiO_2 fibers diameter range from 100 to 200 nm. Antibacterial results showed great effect of titania in increasing the inhibition zone as compared with neat fiber [6].

In the present research, it has been used used two ways for improvement antibacterial activity of bomaterials, firstly via blending of chemically different polymers as a tool for tailoring characteristics of products with optimized material properties, secondly via reinforcing the blends with two different nano ceramic materials.

Material and Experimental Procedure:

Ultra-high mw polyethylene was acquired in pellet form Jiujiang Zhongke Xinxing New Material Co., Ltd China, model No. XH-804M has (Mw = 4000000) and density (0.93-0.95 g/cm^2). PMMA white crystals a pellet form supplied by HI Media Laboratories/ India. Product code GRM1746 with average molecular weight about 15,000. High density polyethylene was also purchased in a pellet form Jiujiang Zhongke Xinxing New Material Co., Ltd China. Nanoparticles zirconium oxide and hydroxyapatite powder needle-like supplied from Xian real in Biotechnology Company /China, Purity: 99.5% with average particle size about 20nm.

Blending and pressing:

Each of pure Polymers (PMMA,UHMWPE and HDPE), binary blends (PMMA: HDPE and PMMA:UHMWPE), (HDPE:PMMA and UHMWPE-PMMA) mixed in [95:5] wt.% and blends composites (PMMA-5%HDPE PMMA-5%UHMWPE) reinforced with +1.5 ZrO_2 and Hydroxyapatite) has been prepared via melt processing using single screw extruder machine (general chemical industries company-Baghdad/Iraq) to form long strips (almost 1.5mm thickness) with a screw L/D of 30:1 at temperature (160 -165 ºC) and screw speed 90 r.p.m.

Biological tests:

Antibacterial activity test by zone inhibition method:

Bacterial strains (*S. aureus*) and (*Pseudomonas*), used in our tests were selected from the collection of the laboratory of Biotechnology branch/ Nano technology center, University of Technology /Baghdad- Iraq, The bacteria were sub cultured on nutrient agar (N. agar) and incubated for 24 h at 37^o C. The cells were suspended in 50 ml of normal saline to yield a bacterial suspension of 10^7 (CFU/ml) using plate count. A 100 ml bacterial suspension equal to McFarland solution has been used as a control equal to 10^7 CFU/ml and spread on a nutrient agar plate. Polymer, blends and composites samples have been inserted to the plates to determine the inhibition zone. Finally, these plates were incubated at 37 °C. After 24 h of incubation, zones of inhibition were calculated**.**

Antibacterial Activity test by diffusion method:

Different polymer blend samples and composites were dispersed in 9 ml normal saline, and sterilized by autoclave for 15min at 121^{o}C and 15Psi, then 1 ml of the diluted cell suspensions was added, then the mixture was incubated at 37°C in a shaking incubator (160 rpm) for 24h.Over a period (overnight), the suspension was serially diluted 10 fold in normal saline (100mlspread out on a solid nutrient agar medium) using spread plate technique. The plates were incubated overnight at 37°C, and then the number of viable organism colonies in the plates was determined by multiplication the number of colonies with the dilution factor and compare with control, which represented bacterial suspension, then the percentage reduction was calculated because of the initial count according to the following equation [7]:

Colonies forming unit [(CFU)/ml] = (Number of colonies for each dilution).sample volume / (dilution 10^2 factor)

Also, estimated the percentage reductions of bacteria using bactericidal rate [8]

$$K = \frac{(A-B)}{A} x100\%$$

Where:-

A and B represented the numbers of control bacteria colonies and the number of bacterial with prepared blend and composites samples.

RESULTS AND DISCUSSION

Anti-bacterial tests results:

Antimicrobial polymer cab be defined as polymers with antimicrobial activity, or have the ability to prevent the growth of microorganisms such as bacteria, fungi or protozoans. Typically, this type of polymers are produced by inserting or attaching an active antimicrobial agents onto the backbone of polymer via an alkyl or acetyl linker,therfore; this material can be candidate to be used in medical areas as tools to fight infection, more over in the food industry to inhibit bacterial contamination, and in water purification to inhibit microorganisms growth in drinking water. Based on [kenaway et.al.,[5] antimicrobial agent usually kills bacteria through various methods depending on the type of bacteria. One of these methods is to kill the bacteria immediately through the contact, causing burst of bacteria cell, or by depleting the source of bacteria's food to prevent bacterial reproduction. In usual antimicrobial polymer kills bacteria through the first method, at which the polymer adsorbs onto the cell wall of the bacteria and the antibacterial agents diffuse through the cell wall into the cytoplasmic membrane[5]. Results of optical Micrographs approved that method of killing as shown in Fig.(1 and 2) that represent photos of PMMA:5HDPE as one of the polymer blends that tested at two different locations also there was no growth of bacterial underneath each of samples that have been incubated with pseudomonas and aureus bacteria respectively.

Contrary to researches [6,9] that has been used inhibition zone that formed around samples as a way to determine the antibacterial activity, this method was not clearly useful with samples of this work since, Results of showed that there is no inhibition zone formed around the inserted samples in augur plates as shown in Fig. (1) against Pseudomonas and Staphylococcus aureus (S. aureus) bacteria. Moreover, optical photographs showed the adhesion of bacteria at the boundaries of without any growth of microorganisms on or under samples as shown in Fig. (2) and Fig.(3) for both Pseudomonas and Staphylococcus respectively.

Another method has been used to study the anti-bacterial activity which is diffusion method at which colonies of viable organisms been counted. Results of Bactericidal rate for polymer, blends and composites against the two types of bacteria are shown in Fig. (4) and (5). It has been found that all polymers, blends and composites have good antibacterial activity to positive gram bacteria (i.e. Staphylococcus) since the ratio of bactericidal ratio was almost 100% in all samples, while for the negative type (psedo), the situation was different since the behavior changeable between polymers ,blends and composites, UHMWPE showed about 50% of the killing ability so that it is not very effective but improved significantly when blended with PMMA, also PMMA:5HDPE shows about 65% still, its anti-activity has been also improved with 1.5 wt. % of ZrO_2 and HAp, however this positive effect of zirconia and hydroxyapatite was not the same for PMMA:5UHMWPE at which bactericidal rate recorded slightly decrement.

Contrary to researches that has been used inhibition zone that formed around samples as a way to determine the antibacterial activity, this method was not clearly useful with samples of this work, since results showed that there is no inhibition zone formed around the incorporated samples in augur plates, against (A) Pseudomonas (which have orange color) and (B) Staphylococcus aureus (S. aureus) bacteria (which have gold color) as shown in Fig. (3) for (a) polymers, (b) polymer blends and (c) polymer blend composites.

According to above result it has been used, another method determine the anti-bacterial activity which is diffusion method at which colonies of viable organisms been counted. Results of bactericidal rate for polymer blends and their composites as shown in Fig. (4) and (5) respectively against the two types of bacteria staph and psedo. It has been founded that the anti-bacterial activity of polymer blends (PMMA: 5%HDPE), (HDPE: 5%PMMA), (PMMA: 5%UHMWPE) and (UHMWPE:5%PMMA)higher than the neat polymers (PMMA, HDPE and UHMWPE). Moreover, one of the most significant factors that control antimicrobial activity is the molecular weight at which optimal activity can be achieved when the polymer has a molecular weight in the range of $1.4x10^4$ to $9.4x10^4$.Polymers with larger molecular weights show a decrease in activity because their weight may be attributed to the sequence of steps required for biocidal action. Very large molecular weight of polymers will suffer from diffusion through the cell wall of bacteria and cytoplasm [10].

As a result to the above, UHMWPE recorded the lowest anti-bacterial activity about 50% against staph type of bacteria compared with other neat polymers and polymer blends (PMMA: 5%HDPE), (HDPE: 5%PMMA), (PMMA: 5%UHMWPE) and (UHMWPE:5%PMMA as shown in Fig.(4).

Polymer blends composites including ((PMMA:5HDPE): 1.5% HAp) , ((PMMA:5HDPE): 1.5% ZrO_2), ((PMMA:5UHMWPE): 1.5% HAp) and ((PMMA:5UHMWPE): 1.5% ZrO_2) have been examined against to two types of bacteria pseudomonas and *S. aureus* and bactericidal rate as shown in Fig. (5), results indicated that there is no bad effect of nanoparticles on the activity of polymer blends against pseudomonas. Moreover,

nanoparticles of hydroxyapatite and zirconia has positive effect on (PMMA: 5%HDPE) since there was increment in the bactericidal rate (Fig. (5)) since both types of nanoparticles have good antibacterial activity.

Moreover, antibacterial efficiency results of neat polymers (PMMA, HDPE and UHMWPE) and (PMMA: 5%HDPE), (HDPE: 5%PMMA), (PMMA: 5%UHMWPE) and (UHMWPE:5%PMMA)polymer blends is shown in Fig.(6). Results showed that UHMWPE has higher number of living microorganisms (i.e. CFU) as compared with other neat polymers and polymer blends followed by (PMMA: 5%HDPE) polymer blend against pseudomonas, while the opposite recognized against staph bacteria.

Results of CFU of polymer blends composites are manifest in Fig.(7) at which the addition of 1.5 wt. % of zirconia and Hydroxyapatite nanoparticles improved the anti-bacterial activity of (PMMA: 5%HDPE) against both types of bacteria while the contrary occur for (PMMA: 5%UHMWPE) against Pseudomonas.

Fig.(8) indicate to the living colonies of bacteria after incubation with samples for 24 hr. Fig. (9), at which it was approved that Pseudomonas gram negative bacteria has a rod shape and can be visualized the burst on the bacteria as an indication to be killed, Fig (4.102 (a)) is SEM of colonies of killed for Pseudomonas bacteria's adhered on polymer blend (PMMA: 5%HDPE) as approved by kenaway et.al[5]. While the shape of gram positive staph is almost around as shown in Fig. (9 (b and c)) for S. aureus bacteria's adhered on polymer blend composite samples ((PMMA: 5%HDPE):1.5wt. %ZrO_2)) (b) and S. aureus bacteria's adhered on polymer blend (PMMA: 5%HDPE) (c).

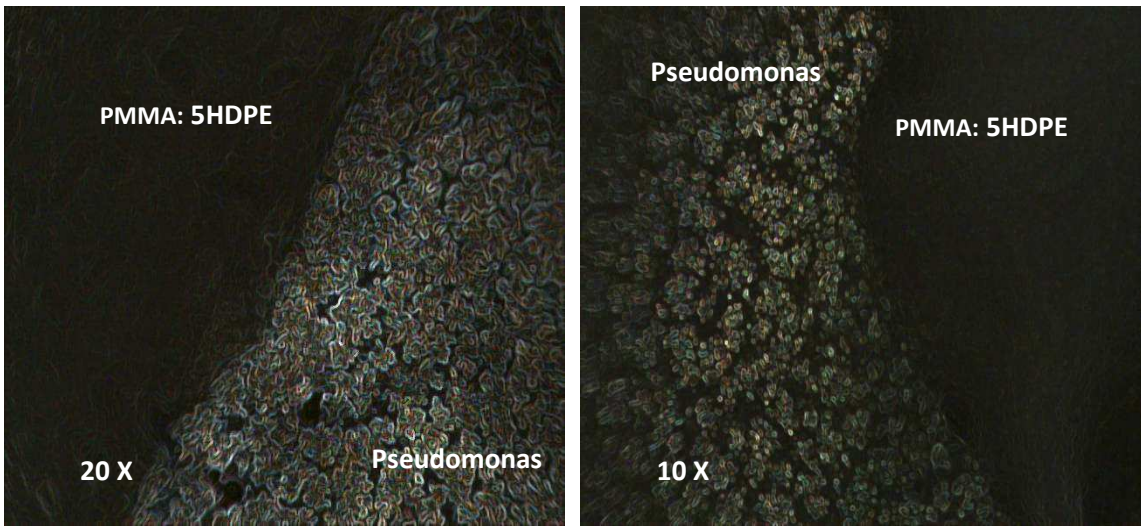


Fig. 1: Optical photographs of PMMA: 5HDPE with Pseudomonas diffusion at the boundaries of the sample after 24 hours of incubation at different positions.

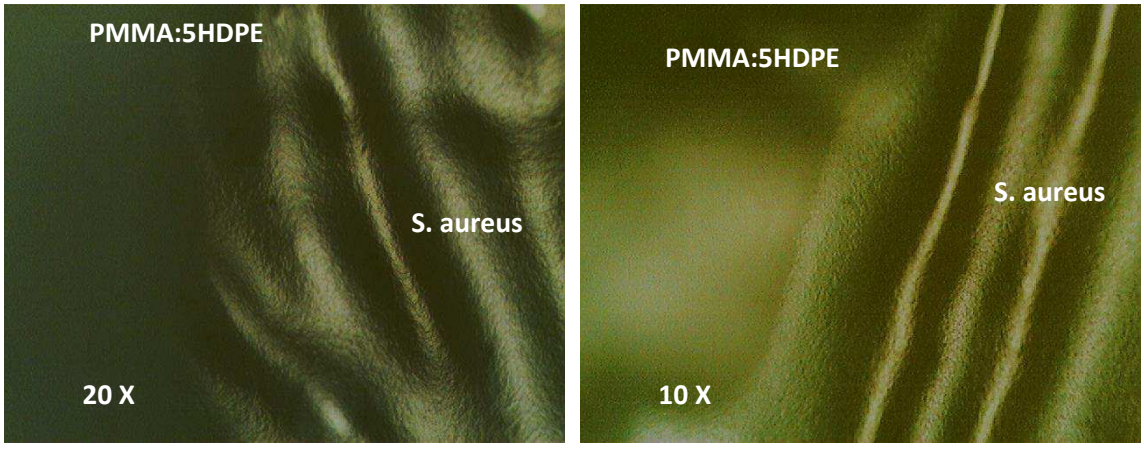
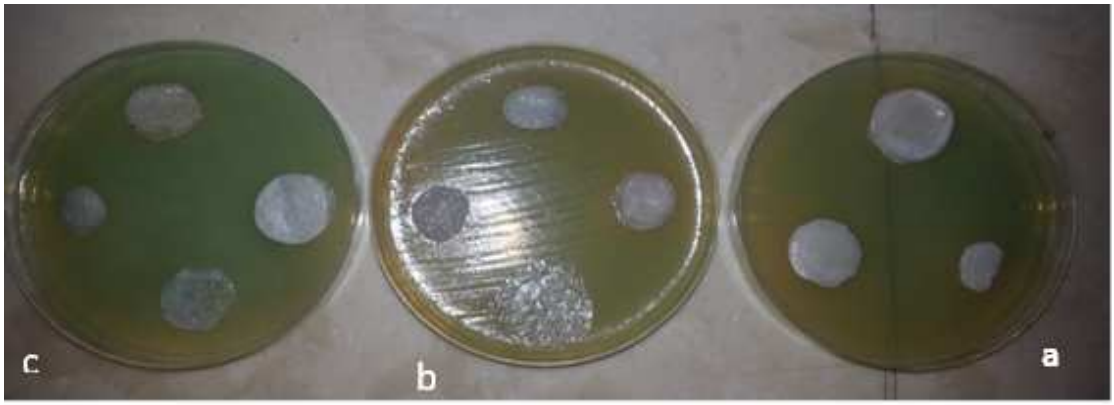
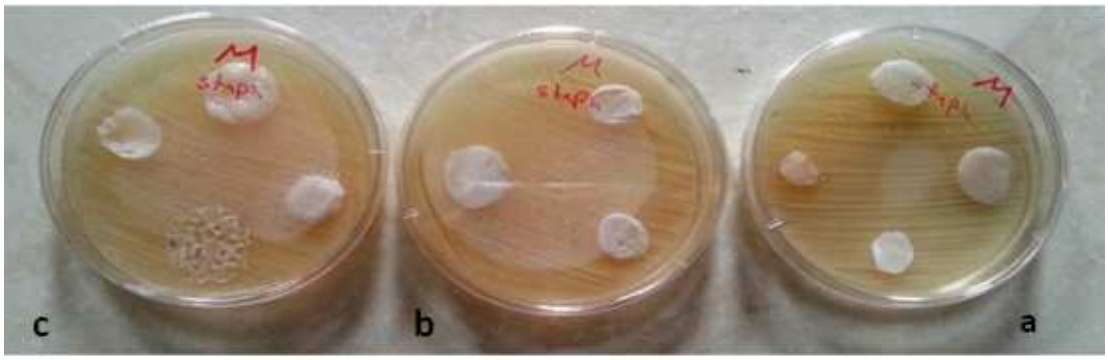


Fig. 2: optical photographs of Staphylococcus aureus (S. aureus) diffusion at the boundaries of samples after 24 hours of incubation.



(A)Tested samples incubated with Pseudomonas bacteria



(B) Tested samples incubated with Staph aureus bacteria

Fig. 3: (A) Pseudomonas and (B) Staphylococcus aureus (S. aureus) after 24 hours of incubation, for (a) polymers, (b) polymer blends and (c) bld composites

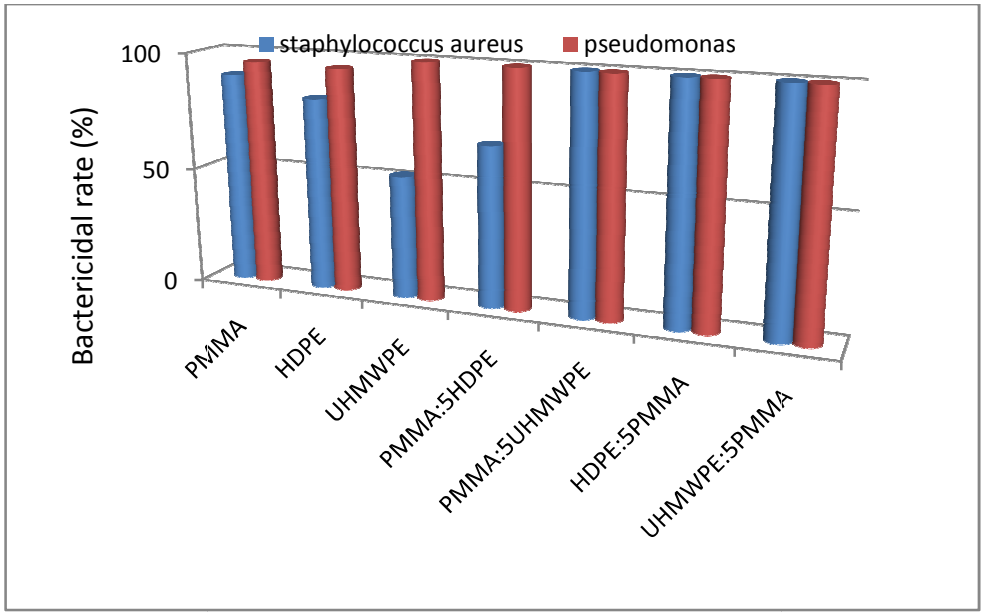


Fig. 4: Bacteriostatic rate (%) of neat polymers and polymer blends against *S. aureus* and pseudomonas microorganisms.

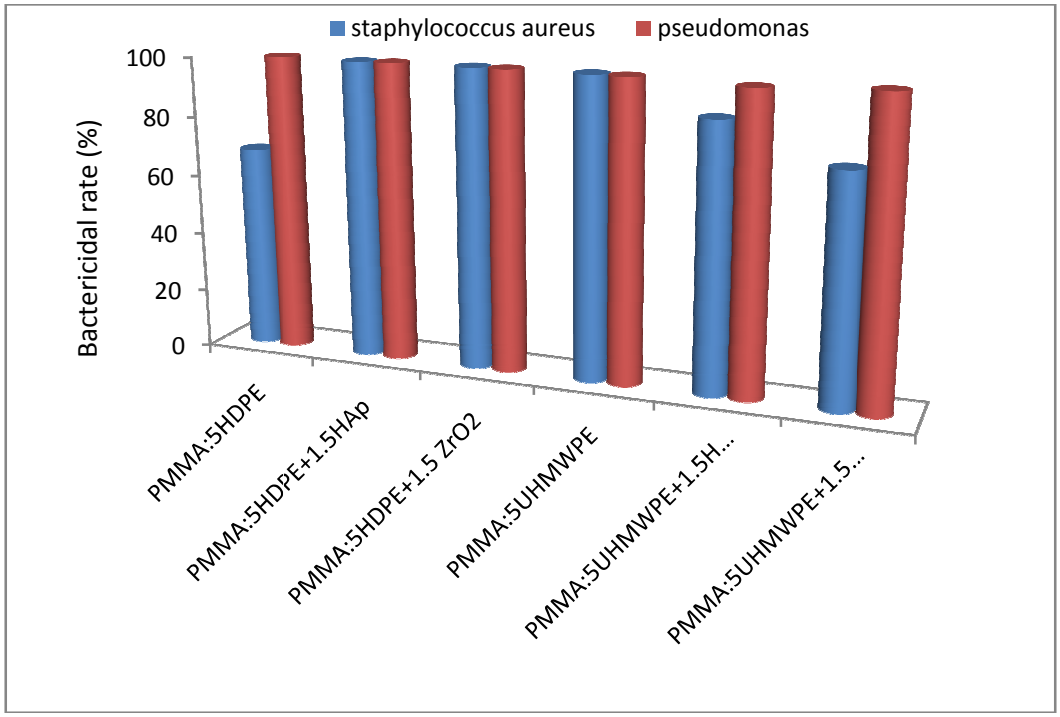


Fig. 5: The bacteriostatic rate (%) of blends and their composites against *S. aureus* and pseudomonas microorganisms.

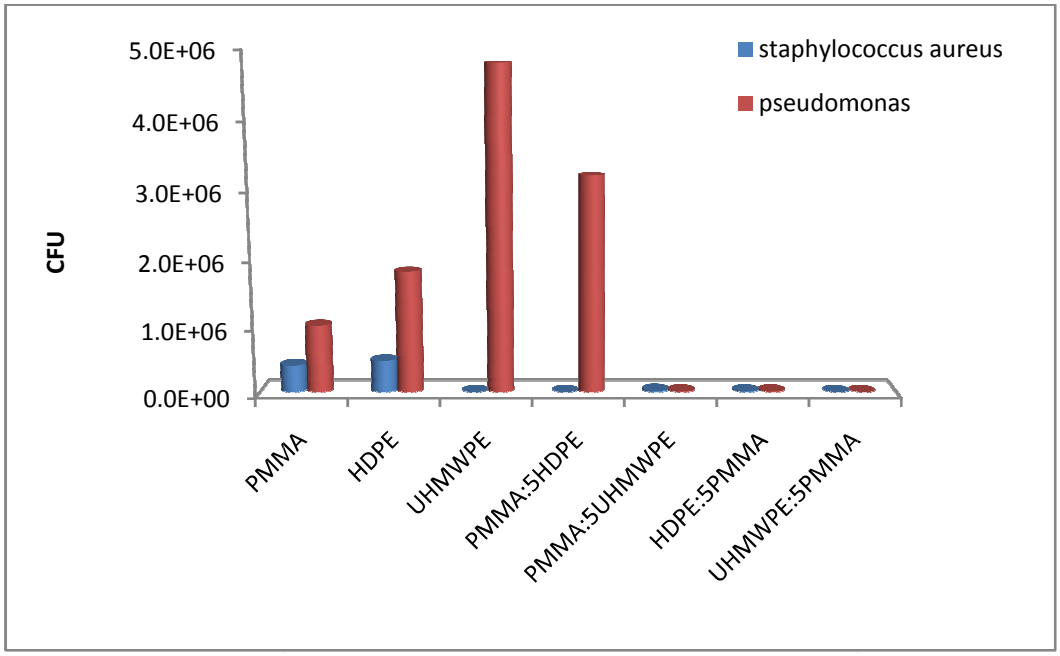


Fig. 6: Changes in the number of live cells counted on nutrient agar (CFU/ml) of neat polymers and polymer blends.

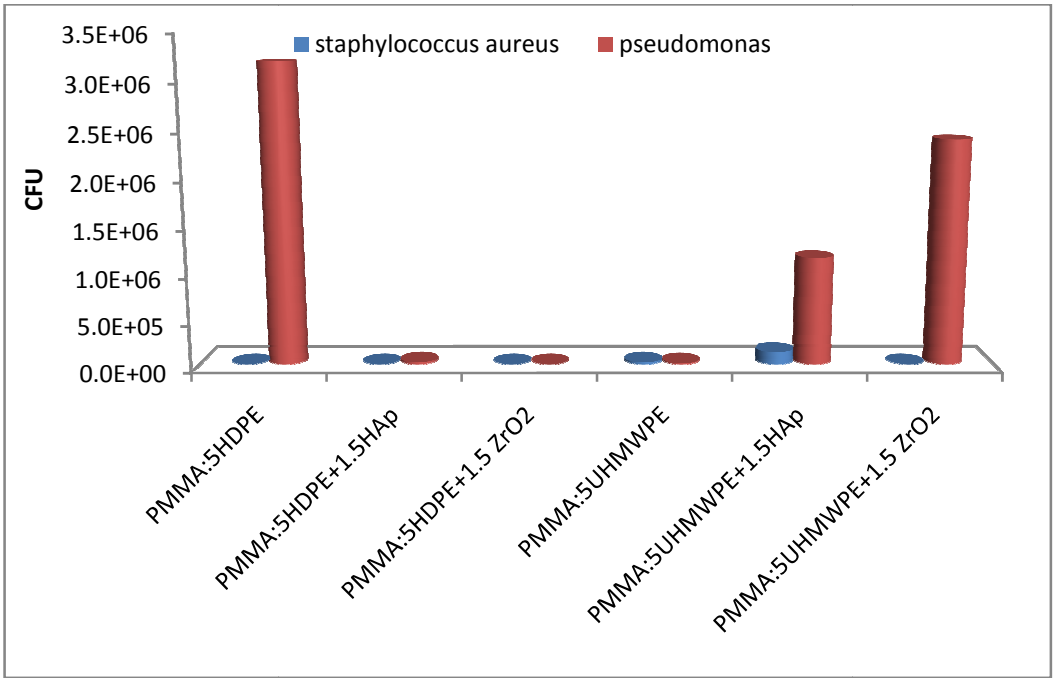
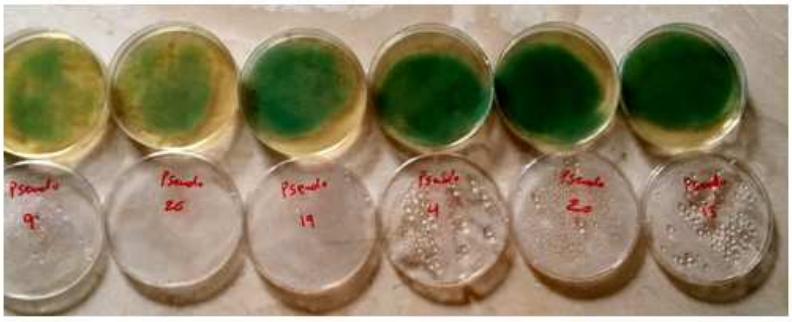
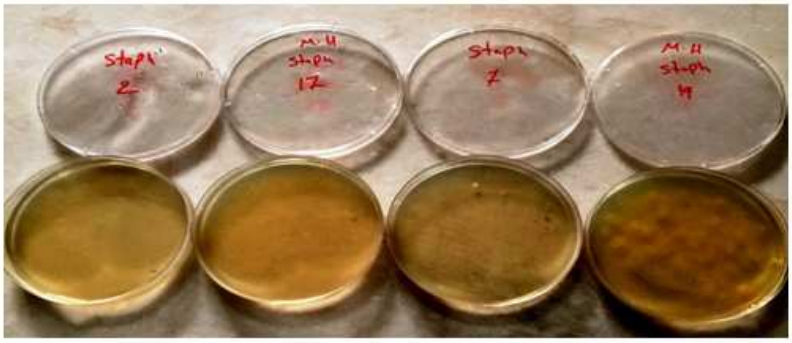


Fig. 7: Changes in the number of live cells counted on nutrient agar (CFU/ml) of polymer blends and their composites.



(A) Living cells of Pseudomonas bacteria after incubation with tested samples.



(B) Living cells of Aureus bacteria after incubation with tested samples.

Fig. 8: Photographs of the living colonies of *S. aureus* and *Pseudomonas* bacteria for different sample

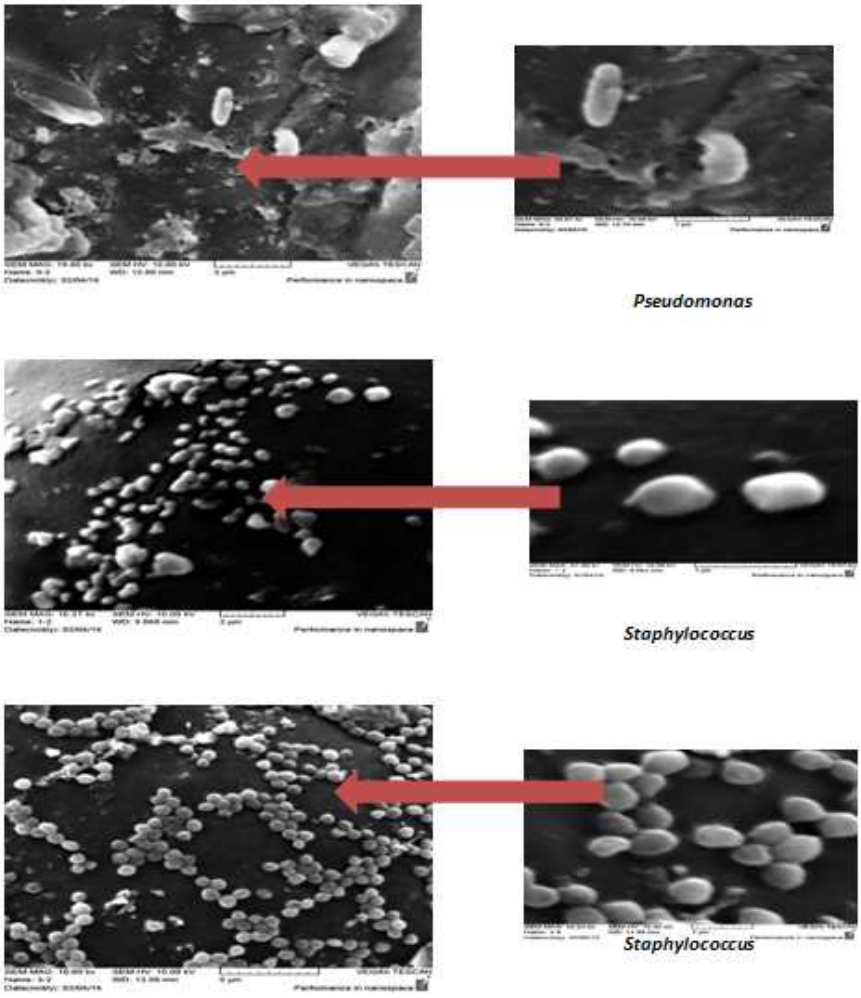


Fig. 9: SEM of colonies of killed *S. aureus* and *Pseudomonas* bacteria's adhered on polymer blends and composites samples

SEM images of polymer blends:

In order to evaluate the changes in surface morphology of the polymer blends, samples were subjected to scanning electron microscopic. The morphology of polymer blends depends on the component, concentrations of the constituents of polymer blend, component melt viscosities and processing conditions. In most heterogeneous system, morphology where by one phase is distributed in another phase is observed [11].

The morphology of the fracture surface for binary polymer blend at different magnification were shown in Figures (10 a and b), (10c and d), (11 a and b) and (11 c and d), respectively. These figures show difference morphology for these polymer blends groups, the fractured surface of polymer blend (PMMA: 5%HDPE) clearly showed two distinct phases a continuous PMMA matrix and dispersed globules of HDPE phase, but with some discontinuous phase structure or so called "phase inversion". As well as it can be observed different sizes of spherical shaped HDPE particles were dispersed randomly in PMMA matrix, as shownin Fig. 10 (a and b), moreover incompatible immiscible blends can be characterized by the domains of one phase pulling away from the domains of the other phase resulting in a droplet-in-matrix morphology. HDPE particles will play as a center of energy dissipating in the PMMA matrix, and as a result, HDPE particles would respond to the triaxial stresses near the crack tip and make the localized shear yielding occurs, followed by bridging mechanism of HDPE at the crack tip zone in the PMMA matrix and plastic void growth initiated by cavitation or deboning of the HDPE particles from the surface [11].

Figure (10 c and d) shows deformation lines tend to propagate through HDPE particles and it proved the ductile fracture did occur. According to Thomas et al.[12] the effectiveness for HDPE particles to act as stress concentrators and dissipate energy can be influenced with increment of these particles size. In small particles size, crack energy propagates through the particle and can be dissipated. Meanwhile, HDPE particles will have less interfacial adhesion when the size of particles became larger. It may cause HDPE particles to be pulled out

due to the crack propagating through the interface between HDPE particles and matrix. Not much crack energy was dissipated because the crack was not propagated through the HDPE particles.

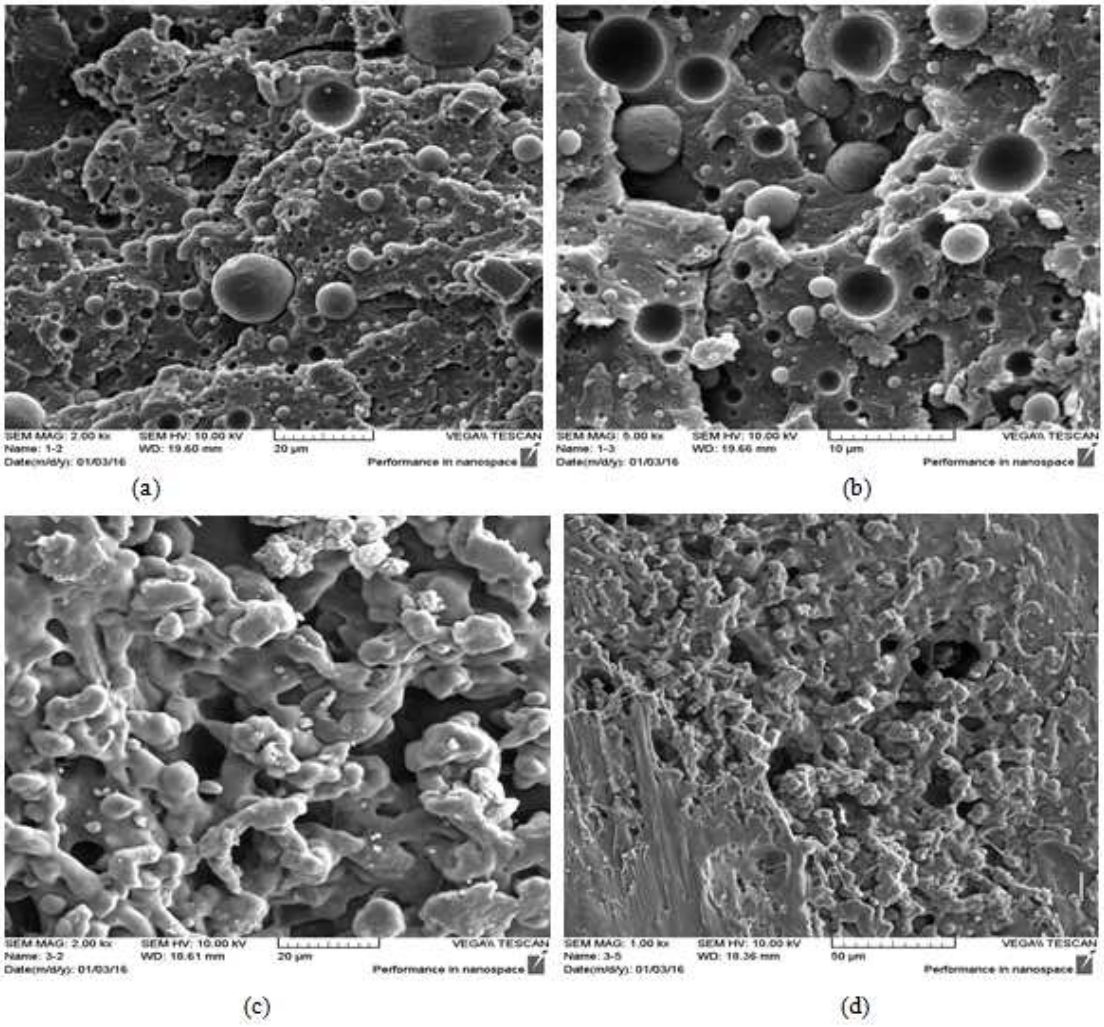


Fig. 10: SEM photographs of (a, b) (PMMA: 5%HDPE)and (c and d) (HDPE: 20%PMMA)polymer blends.(a=2000x, b= 5000x, c=1000x and d=2000x)

The SEM photos of binary polymer blend systems (PMMA: 5%UHMWPE) and (UHMWPE: 20%PMMA) are shown in Figures (11a, b, c and d). Both systems appear to have a co-continuous morphology and as a result, it clearly shows two-phases morphology, one phase identified as droplets (Figures 11 a, b), and the second phase identified as a strips (Fig. 11 c and d). The co-continuous structure shown in the (PMMA: 5%UHMWPE) polymer blend (Fig. 11, a, b) can identified as two phases structure that was broken down into an islands resemble ellipsoidal droplet structure, which may be related to UHMWPE phase in PMMA matrix. A brittle fracture observed in the case of (PMMA: 5%UHMWPE) with micro typical characteristics of a rigid, glassy and much smoother fracture surface, this indicates better interfacial adhesion between two components of binary polymer blend as compared with fracture surface morphology of the other groups of binary polymer blend samples.

Furthermore morphological results of (UHMWPE: 20%PMMA) polymer blend Figure (11 c and d), the co-continuous structure clearly shows two-phases morphology, one phase was identified as a strips these strips transform to micro-fibrils, these micro- fibrils may be act as bridges between other phases of binary polymer blend prepare. The introduction of long fibers has been related to the ductility of the material which indicates the higher tensile strength of polymeric blend (UHMWPE: 20%PMMA), these surface morphologies are similar to those appeared in [13]. So this photo graphics images seems to be have different morphology as compared with their counterparts of the other group (PMMA: UHMWPE) polymer blend (fig a, b), clearly show the fracture surface behavior as a ductile fracture since the fracture surface shows a plastic deformation as shown in Figure (11 c and d).

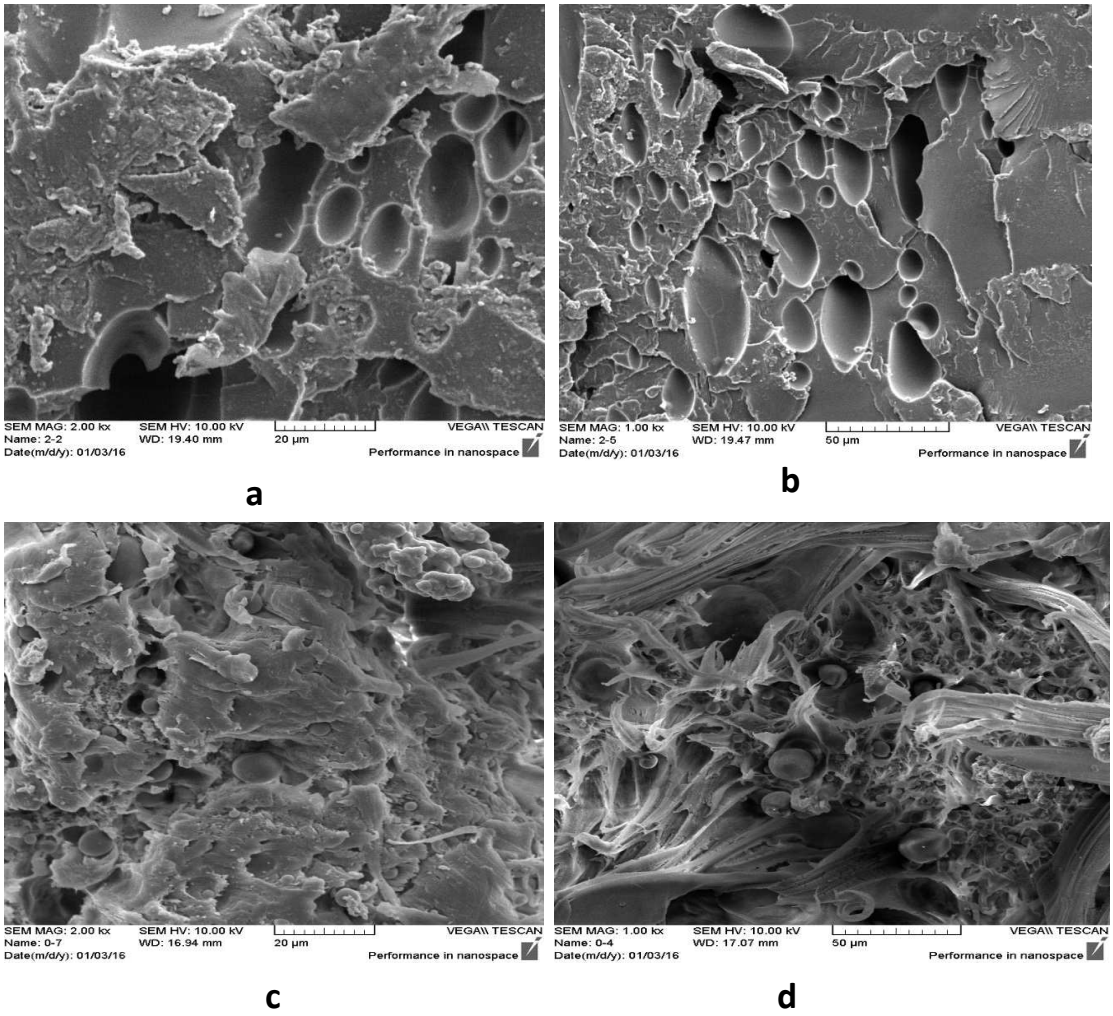


Fig. 11: SEM photographs of (a, b) (PMMA: 5%UHMWPE) and (c and d) (UHMWPE: 20% PMMA) polymer blends. (a=2000x, b=5000, c=2000x and d=5000x).

As for nano polymer blends composites, the morphology depends on the type of composite materials, constituents ratios, component melt viscosities, compatibility between composite constituents and processing conditions. In most heterogeneous system, morphology where by one phase is distributed in another phase is observed [14].

The SEM images of the surface morphology of polymer blend nanocomposites which are ((95%PMMA: 5%UHMWPE): x%ZrO_2) and ((95%PMMA: 5%UHMWPE): x%HAp) are shown in Figures (4.65and 4.66 (a, b, c and d)) respectively, from these figures it is found that there is a difference in the surface morphology of polymer blend ((95%PMMA: 5%UHMWPE) when add different nanoparticle (ZrO_2 and hydroxyapatite).The dark regions are (95%PMMA: 5%UHMWPE) phase while the bright regions are reinforcement phase (ZrO_2 and HAp) nanoparticles, the distribution of nano-zirconia and nano-hydroxyapatite in semi-continuous morphology depending on the constituents of the polymer blend composite and the ratio of each nanoparticle in composites. It was observed from Figures (12 and 13 (a, b, c and d)), that the dispersion of ZrO_2 and Hap nanoparticles was relatively good and uniformly dispersed throughout the entire polymer blend. On the other hand, it was found that the ZrO_2 nanoparticles at low ratio of concentrations are homogeneously dispersed in the polymer blend composites, as shown in Figure (12 a and b). This may belongs to the presence of the ZrO_2 nanoparticles on the polymer blend (95%PMMA: 5%UHMWPE) is likely give strong interfacial and interaction. However, these figures also show a poor dispersion of nanoparticle in some reign of composites, especially at high constituents of HAp and Zirconia nanoparticle; in this case, the nanoparticles may be act as aggregate to each other and become a cluster of HAp and Zirconia, because the nanoparticles have high surface energy. Therefore, the dispersion of these nanoparticles could have an influence on the mechanical properties of ((95%PMMA: 5%UHMWPE): x%HAp or ZrO_2) polymer blend composites.

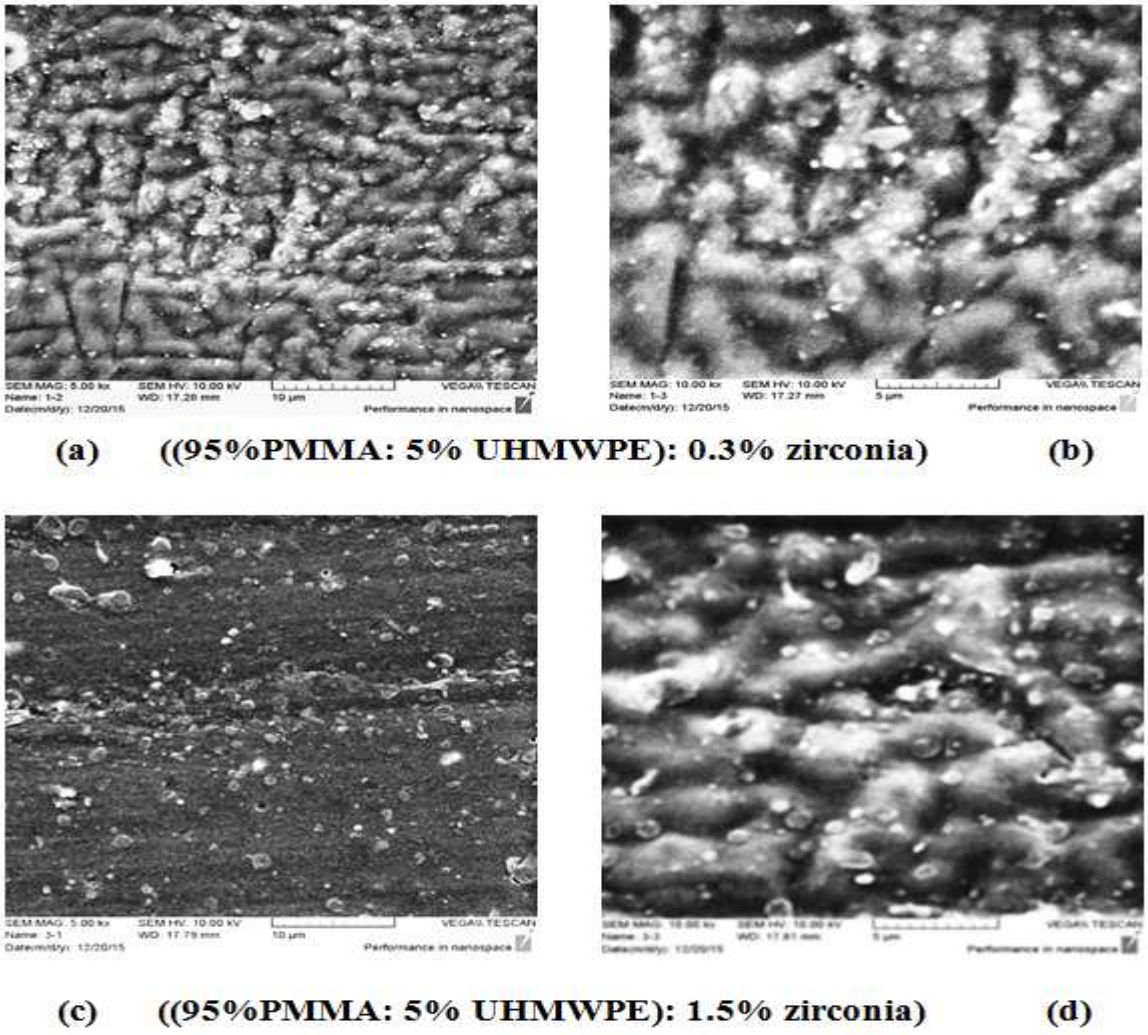


Fig. 12: SEM photos of the surfaces of (95%PMMA: 5% UHMWPE 0.3% zirconia) at different magnification (a) (5000× magnification); (b) (10000× magnification); and (c and d) the surfaces of ((95%PMMA: 5% UHMWPE): 1.5% zirconia) (c) at (5000× magnification); (d) (10000× magnification).

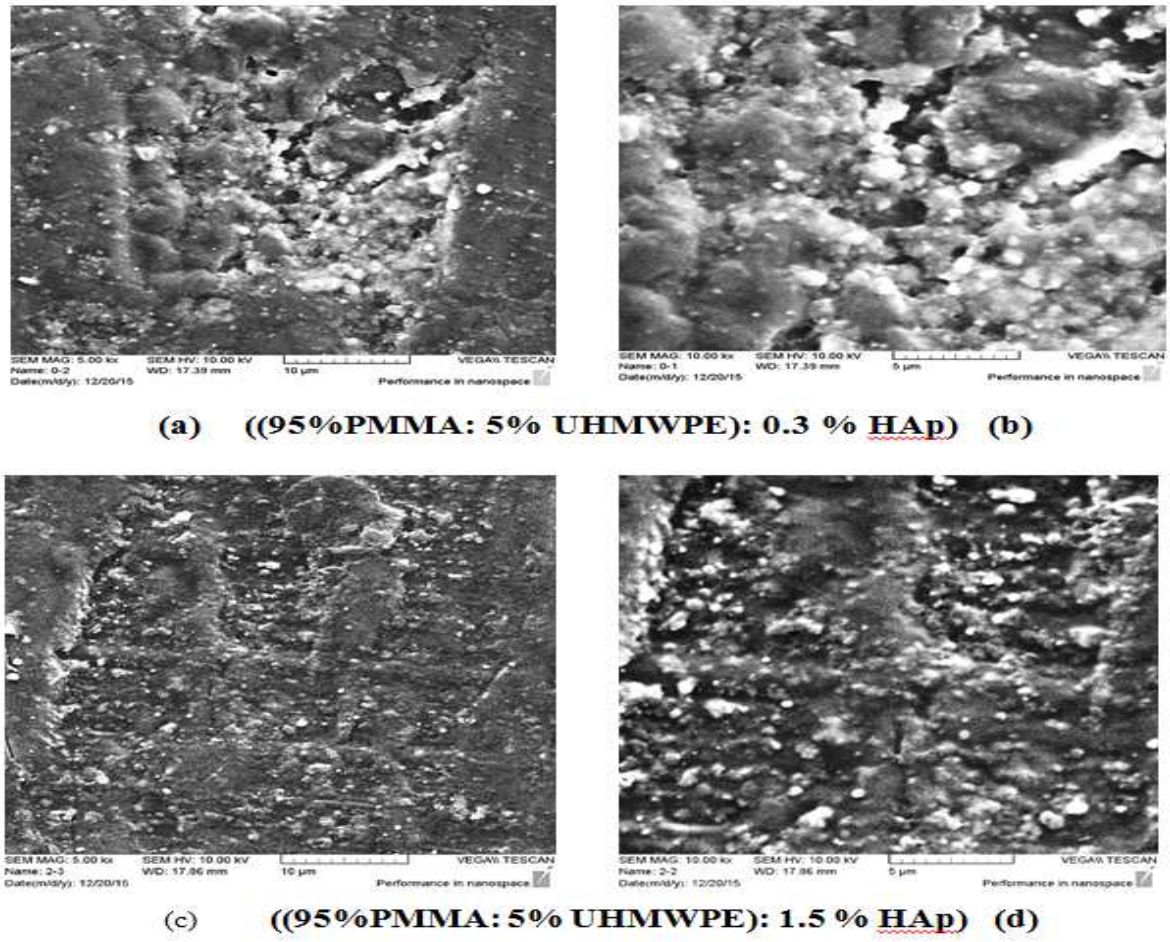


Fig. 13: SEM photos of the surfaces of (95%PMMA: 5% UHMWPE 0.3% HAp) at different magnification (a) (2000× magnification); (b) (5000× magnification); and (c and d) the fracture surfaces of (95%PMMA: 5% UHMWPE 1.5% HAp) (c) at (2000× magnification); (d) (50000× magnification).

Conclusion:

In this study, the results of antibacterial showed that, blending method and reinforcement with nano particles have positive effect in improvement of pure polymers. Moreover, the results of optical photographs indicated to the absence of inhibited zone formed around the incubated samples in augur plates against Pseudomonas and Staphylococcus aureus (S. aureus) bacteria, but instead bacteria adhered to the samples without any growth of microorganisms.

Furthermore, antibacterial efficiency results showed that UHMWPE has higher number of living microorganisms as compared with other neat polymers and polymer blends followed by (PMMA: 5%HDPE) polymer blend against pseudomonas, while the opposite recognized against staph bacteria. Bactericidal rate test for polymer, blends and composites against the two types of bacteria approvedgood antibacterial activity to positive gram bacteria, while the situation was different for the negative type (psedo) in some samples. Moreover, nanoparticles of hydroxyapatite and zirconia have good activity against bacteria so that, there was enhancement in the bactericidal rate as in (PMMA: 5%HDPE) polymer blend.Finally,SEM photographs showed that the outer shape of bacteria doesn't destroyed, but there is some of abrupt, this may leads to, that the bacteria killed by attacking its cytoplasm.

SEM images of fractured surfaces revealed a homogeneous micro structure, and much smoother fracture surface, which seems to indicate better interfacial adhesion between the components of composite sample. As well as, photographs showed, that the size and number of spherical droplets depend on the type and weight ratio of Nanoparticles in the composite, and so the number of spherical droplets increased with the increased of weight percentage of Nanoparticles.

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