Answers to Reviewer’s comments

We are grateful for your comments on the manuscript. Your comments have been carefully examined and replied as following. All modified parts have been clearly marked in this revised manuscript.

Reply to Reviewers' comments:

Referee: 1

1. The manuscript by Dave W, Chen et al pertaining the development of PEEK-based materials coated with ZnO nanorods as antibacterial materials in wound treatment is of significant scientific quality. However, serious presentation issues make it non-suitable for publication in its present form. There are several points that need to be addressed so that the manuscript can be understandable, properly-structured and coherently-written. In addition, there is a serious reservations about some of the experimental data presented (see points 9 and 10). Part of the reluctance to accept could be due to the language issues that make the text hard to understand.

Ans.: We really thank the reviewer’s comment on the typing and grammar errors, coherently written of our manuscript. Your comment has been examined carefully and all points that you suggested are check and re-written carefully. All modified parts in the revised manuscript have been clearly marked in this revised manuscript according your comment.

2. The manuscript requires extensive language editing. It is suggested that it is proof-read by a native speaker before resubmission. There are several parts of the text that contain grammatical and syntax mistakes and other parts that the text is very hard to read and comprehend.

Ans.: We really thank your important comment on our manuscript. Indeed, we agree that this manuscript is required extensive language editing and proof-read by a native speaker. According to your comment, we have checked this revised manuscript about the writing, spelling and the corrections of typing and grammar errors. We really thank your comment on our manuscript. We hope that you can satisfy about our efforts about this revised manuscript.
3. The abstract contains too many technical details and does not allow the reader to understand what the manuscript is all about. It must be rewritten in its entirety in order to demonstrate the novelty of the work, its highlights and its general conclusions.

**Ans.** We really thank your important comment on our manuscript. According to your comment, the abstract of this revised manuscript has been modified and re-written to demonstrate the novelty of this work. Here shows the abstract in the revised manuscript:

“In this study, zinc oxide (ZnO) nanorod arrays as the antibiotic agent carriers were grown on polyetheretherketone (PEEK) substrates using chemical synthesis method. With the concentration of ammonium hydroxide in the precursor solution kept at 4 M, ZnO nanorod arrays with the diameter in the range of 100-400 nm and loading density of 1.7 mg/cm$^2$ can be grown onto the PEEK substrate. The drug release profiles and their antibacterial properties of the samples in the buffer solution were investigated. The results showed that the concentrations of antibiotic agents (ampicillin or vancomycin) released from the samples into the buffer solution were higher than the minimum inhibitory concentration of 90% for *Staphylococcus aureus* within the 96 hours test. The bioactivities of ampicillin and vancomycin on substrate also showed the result of around 40 % and 80 % on the *Staphylococcus aureus*, respectively. In the antibacterial activity test, sample with loading suitable antibiotic agent had the good inhibitory effect on the growth of *Staphylococcus aureus.*”

4. The introduction is disproportionately lengthy and unnecessarily wordy. The presentation of the state-of-the-art and the related literature resembles a review article and is not suited for an article. It is recommended to reduce this part (lines 43-84 and lines 95-101) and keep only the information that is mostly relevant to the manuscript's content (e.g. the technical details of various methods of ZnO synthesis are superfluous). In addition, despite the length introduction, the novelty of the work and the importance of choosing PEEK are not stressed enough.

**Ans.** We really thank your important comment about the introduction for our manuscript. According to your comment, the introduction in the revised manuscript has been rewritten and deleted some parts in order to meet the standard requirements of manuscript. Please find them in the revised manuscript with the marked red words. Also, we added the comments about the importance of choosing
PEEK sample in the revised manuscript. Please find them in line 74-80 in the revised manuscript.

“For the orthopedic applications, metal-based biomaterials such as titanium alloy or stainless steels are often used in the clinical treatments. The possibility of metal ion release caused by corrosion and mismatched elastic moduli between these metal (110 GPa for titanium alloy) and human bone (18 GPa) always caused the failure of metal based implants [1]. PEEK is a semicrystalline polymer with several excellent properties such as hard tissue implant material, good elastic modulus (3-4 GPa), good thermal and chemical properties that can avoid the degradation caused by corrosion [1]. It indicates that the PEEK material is an optimal implant candidate for the orthopedic applications”

5. A short description of the 3d-printing method of PEEK would be nice. The bare referral to [29] and complete lack of description is in stark contrast to the superfluous details provided for the work of other references in the introduction.

Ans.: We really thank your important comment. A short description about the 3D printing and the detail parameters about the 3D printing PEEK samples is given in the line 98-104 and line 114-120 in the revised manuscript.

“Recently, 3D printing technology is an interesting and fast production technology that can easily prepare the implant for the orthopaedic sufferer. Using the suitable design for the product, a 3D object with the layer-by-layer building by using the 3D printer can be obtained in several hours. The applications of 3D printing have many benefits such as the un-moldable printing, infinite variety of shapes for printing, rapid printing from the digital design and printing at the point-of-care. An important benefit in the treatment of complex musculoskeletal wound for the orthopedic sufferer is the rapid printing and infinite variety of shapes for the printing [2].”

“In this study, we tried to deposit the ZnO nanorod arrays onto the 3D printing PEEK substrates using the chemical synthesis method. The detail apparatus and procedures for the preparation of PEEK disks using 3D printer were similar with those reported in our previous study [2]. However, the melting point and glass transition point for the PEEK are higher than those for the PLA sample. The temperature of nozzle in the 3D printer was changed from 220°C to 340°C in order to meet the requirements of thermal fused 3D printing technology. The temperature of holder for the 3D printing PEEK sample was kept at 45°C. The printing speed was kept at 5 mm/s.”
6. Why did the authors choose the seeding layer formation method of [31]? Why does it have advantages compared to other sol-gel techniques that use simpler solvents (e.g. water or alcohols) and Zn-based precursors? Is it better suited for PEEK? It seems oddly complex with respect to other reports in literature.

Ans.: We really thank your important comment. The reason using the seeding layer formation method has been added into the line 123-131 and line 255-261 in the revised manuscript.

“Kokotov and Hodes [3], the addition of potassium permanganate with the existence of 1-butanol as the reduction agent in solution bath can make the growth of the Mn–hydroxyoxide on the substrate. It can act as the effective seed layer that contribute the formation of ZnO nanorods on the substrate in the alkaline solution. According to the results proposed by Kokotov and Hodes [3], the concentration of ammonium hydroxide in the precursor solution is the important factor that influences the microstructures of ZnO nanorod arrays on glass substrates. High concentration of ammonium hydroxide in the solution results in the high pH value for the solution, which make the decomposition of ZnO nanorod arrays occur. Low ammonium hydroxide concentration in the reaction bath results in the low growth rate of ZnO samples [3-4].

It is well known that the surface properties of substrates influence the crystal phases of metal oxides grown on substrates. [5]. However, an effective seed layer can contribute the formation of ZnO nanorods on the substrate in the alkaline solution, although the XRD pattern of sample showed the polycrystalline hexagonal ZnO phase without (0 0 2) crystal plane prefer orientation [3]. Without the seeding of nucleation centers (Mn or Fe ions) onto non-conductive glass substrates, little or no ZnO films deposited onto the glass or plastic substrates [3]. Seed layers on substrates can also promote columnar growth (prefer orientation growth) although the polycrystalline ZnO phase was observed in the XRD patterns of samples [3].”

7. Why were the samples annealed? What does RTA offer with respect to simple heating (process escribed in lines 140-142).

Ans.: We thank your important comment. In order to get good attachment of ZnO nanorod arrays on the PEEK substrates, an annealing process of samples has to be carried out. Annealing of ZnO composite samples can also improve their chemical resistance abilities on the corrosion in solution bath. However, the high temperature and long- annealing time caused the damage of PEEK substrates. Therefore, the as-prepared samples were annealed in the rapid thermal annealing system...
(ULVAC-RIKO · RHL-P610CP) with the temperature increasing rate of 100°C/min and maintained them at 330°C for 10 min. It has been added at line 142-147 in the revised manuscript.

8. Section 3 contains redundant text of things already described in Section 2 (e.g. lines 213-217 and 275-278). Repetition should be taken care of.

**Ans.**: We thank your important comment. They have been deleted and remodified in the revised manuscript.

9. Lines 219-222 refer to the methods used and should be apart of Section 2.

**Ans.**: We thank your important comment. According your comment, it has been moved to the section 2 in the revised manuscript.

10. Samples solvothermally/hydrothermally grown on different substrates should not be compared or taken for granted that are similar. It is established in literature that the morphology of the nanotextures strongly depends on the nature of the substrate (see for example Procedia Engineering 120 ( 2015 ) 447 – 450). ZnO nanorods grown on glass substrates should not be assumed to be the same as the ones grown on PEEK, hence the analysis based on those cannot and should not be used (Fig.1 and related discussion).

**Ans.**: We thank your comment about the growth mechanism of ZnO samples on various substrates. Indeed, we agree your comment and the writing in the original manuscript caused the misunderstanding for the preparation of our samples. In order to make more focused on the physical properties of ZnO/PEEK samples, we moved the figures 1 and 2 in the original manuscript into the supporting file and added the XRD patterns and SEM images of ZnO/PEEK samples in the revised manuscript. Please find them in line 247-278 in the revised manuscript.

Figure 1 shows the XRD patterns of PEEK samples before and after the ZnO nanorods growth with the concentration of ammonium hydroxide kept at 4 M (sample (I)) in precursor solution. The XRD pattern of samples indicated that the ZnO sample can be grown onto the PEEK substrate using the simple chemical synthesis. The inset figures in the Figure 1 show the pictures of the PEEK samples before and after ZnO growth. It showed that a white film was covered at the PEEK samples surface. The XRD pattern of sample shows that the crystal phase of ZnO sample agrees well with those reported in the JCPDS card (no. 89-510), which indicates the sample is the polycrystalline hexagonal ZnO phase. However, the (0 0 2) crystal plane prefer
orientation for ZnO sample on PEEK samples is not observed, which may be due to surface property of sample. It is well known that the surface properties of substrates influence the crystal phases of metal oxides grown on substrates. [5]. However, an effective seed layer can contribute the formation of ZnO nanorods on the substrate in the alkaline solution, although the XRD pattern of sample showed the polycrystalline hexagonal ZnO phase without (0 0 2) crystal plane prefer orientation [33]. Without the seeding of nucleation centers (Mn or Fe ions) onto non-conductive glass substrates, little or no ZnO films deposited onto the glass or plastic substrates [3]. Seed layers on substrates can also promote columnar growth (prefer orientation growth) although the polycrystalline ZnO phase was observed in the XRD patterns of samples [3]. Figure 2 shows the FESEM images of ZnO nanorods grown on PEEK substrate with the concentration of ammonium hydroxide kept at 4 M. Figure 2 (I) and (II) show the SEM images of ZnO/PEEK samples using simple chemical synthesis at 10 and 50 k(X), respectively. From the SEM image of sample, it can be confirmed that the morphology of ZnO grown on PEEK substrate is the nano- to submicro- rod arrays. The diameter distribution of ZnO sample grown on PEEK substrate is in the range of 100-400 nm, which is a little larger than the ZnO samples grown on the glass substrates. The specific surface area analyzer was also employed to estimate active surface area of ZnO sample grown on PEEK substrate. The active surface area of ZnO/PEEK sample is around 4.0 m²/g, which is a little lower than that prepared at the glass substrate.

![Figure 1 XRD patterns of (I) PEEK sample and (II) ZnO nanorods/PEEK sample.](image)
Figure 2 SEM images of ZnO nanorods/PEEK sample at (I) 10 k(X) and (II) 50 k(X), respectively.

10. It is not clear in the text whether the SEM images correspond to the nanorods grown on PEEK or on glass. If they are grown on PEEK, then it is OK to present them and use the morphological features in the analysis. If not, then the subsequent analysis is problematic since it is not guaranteed that the nanorods on PEEK share the same features and hence the same functionalities.

**Ans.**: We really thank about your comment. The SEM images (figure 2 in revised manuscript) in the original manuscript are based on the growth of sample on glass substrate due to the evaluation of the optimal parameters for the growth of ZnO samples. In order to compare with the results proposed by Kokotov and Hodes [3], we used the glass substrates for the growth of ZnO sample. After that, we grew the ZnO sample on PEEK substrates using the optimal parameters. The detail discussions about the physical properties of ZnO sample on PEEK substrates are given in line 247-278 in the revised manuscript.

11. Which sample was chosen to be studied with the antibiotics (Table 1) and the subsequent experiments with S. aureus. It is not clear in the text.

**Ans.**: We really thank your important comment. The ZnO sample grown on substrate is based on the sample (I). Please find the discussion at line 241-247 in revised manuscript.

“Sample (I) has the largest number of nanorod arrays although it has the lowest diameter of the ZnO nanorod. The largest number of ZnO nanorod arrays on substrate indicates that it has the largest active surface area. Because sample (I) has the largest active surface area, it seems a good drug carrier for the absorption of antibiotic agent.”
Hence, we used the sample (I) (the concentration of ammonium hydroxide of 4 M in precursor solution for the growth of ZnO sample) as the antibiotic agent carrier for the loading various amounts of antibiotic agents with the directly absorption of antibiotic agents in solution bath.”

12. Comparison between the XRD data of Fig.1 and Fig.5 as well as a more detailed analysis are missing.

Ans.: We thank your important comment on our revised manuscript. In order to avoid the misunderstanding of readers about the growth of ZnO sample on substrates, we moved the figures 1 and 2 in the original manuscript into the supporting file and added the XRD patterns and SEM images of ZnO/PEEK samples in the revised manuscript. Please find them in line 247-278 in the revised manuscript. Also, only the SEM images for the samples before and after test remain in the revised manuscript in order to make more focused on our study.

13. The result analysis between lines 399-437 is very hard to follow -mostly because of the language problems.

Ans.: We really thank your comment. According to your comment, the results analysis in line 339-437 have been rewritten in order to avoid any misunderstanding of the general readers.

Other modification:

“The title of revised manuscript was changed as “Antibacterial Applications on Staphylococcus aureus Using Antibiotic Agent/High Surface Area Zinc Oxide Nanorod Arrays/Polyethylethylketone Composite Samples” in order to make more focused on our study.

Reference:
