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Efficiency of surface modified Ti coated with copper nanoparticles to control marine bacterial adhesion under laboratory simulated conditions

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Abstract. Titanium (Ti) used as condenser material in nuclear power plants encounter severe biofouling in marine environment which in turn affects the efficiency of the metal. To reduce the biofouling by marine microorganisms, surface modification of the Ti was carried out by anodization process to obtain nanotubes (TiO$_2$-NTs). The electrolyte solution containing 1% of ammonium fluoride resulted in uniform growth of TiO$_2$-NTs. TiO$_2$-NTs were further coated with chemically synthesized copper nanoparticles (NT-CuNP) using 3-amino propyl triethoxy silane as a coupling agent. NT-CuNP was characterized by field-emission scanning electron microscopy (FE-SEM), energy-dispersive spectroscopy and X-ray diffraction. The stability of the coating was determined by the amount of Cu$^+$ ions released into the surrounding using AAS. The microbial adhesion on the surface of Ti, TiO$_2$-NTs and NT-CuNP coupons were evaluated by sea water exposure studies using total viable count method and also characterized by FE-SEM for any morphological changes. The NT-CuNP coupons show a 60% reduction in microbial adhesion when compared to control Ti coupons.

Keywords. Titanium; surface modification; copper nanoparticles; 3-amino propyl triethoxy silane; microbial adhesion.

1. Introduction

Titanium (Ti) is widely used as heat exchanger material in many nuclear power plants because of its characteristic properties like light weight, high strength to weight ratio and its corrosion resistant properties which makes it an edge over other metals. Conversely, the inertness of Ti surface and its excellent biocompatibility makes it highly susceptible to biofouling when used as condenser material in aquatic environments [1]. According to Satpathy [2], because of the attachment of biofoulants the performance of heat exchangers declines. The speed of water flow and their carrying capacity were reduced significantly owing to biofouling growth along the pipe line systems. Unfortunately, the inner wall of all cooling system remains an appropriate substrate for marine growth. Therefore a common solution for biofouling problem is not reasonable relating to the complexity of the nature of the aquatic life, which colonizes a submerged surface. The combination of mechanical and chemical treatments like sponge ball cleaning, back washing and chlorination is commonly used as the fouling control strategies in cooling water systems [3]. However, the practical experience has shown that no routine treatment regime can successfully keep the condenser system clean over a period of years [4]. According to Vishwakarma *et al* [1] surface modification of the materials is an enviable process for controlling the microbial attachment, since the initial adhesion and further growth

of microbial cells on the materials is influenced by the surface properties of the substratum. Hence the surface modification of Ti was carried out in this study to decrease the microbial attachment.

Several methods like sol–gel transcription [5,6], deposition on a nanoporous alumina template [7–10], seeded growth mechanism [11] and hydrothermal processes [12–14] were adopted for surface modification of Ti which results in TiO$_2$ nanotube (TiO$_2$-NT) arrays growth on its surface. Among these, the electrochemical anodization method has attracted the most attention owing to its ability to produce integrative, vertically oriented and highly ordered nanotube arrays with controllable dimensions in an economical and efficient manner [15–19] has achieved self-organized porous TiO$_2$-NTs by anodizing Ti-based alloy in an acidic and fluoride-based electrolyte. TiO$_2$-NTs have wide range of applications from water and air purification to tissue engineering and molecular filtration [20]. In addition TiO$_2$-NTs reduces bacterial adhesion remarkably which in turn helps to decrease the effect of biofouling [21].

To increase the efficiency of TiO$_2$-NTs against bacterial adhesion, nanoparticles having bactericidal effects can be incorporated into it. According to Morones *et al* [22] the bactericidal activity of the metal nanoparticles is not only due to the release of metal ions into the solution rather it interacts closely with microbial membranes with their attributes of small size and high surface to volume ratio. Many surfaces can be coated with immobilized metal nanoparticles possessing bactericidal activity and can find application in

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various fields like medical instruments and devices, water treatment and food processing. For better utilization of antimicrobial activity the metal nanoparticles may be combined with polymers to form composites [23]. Our previous study also proved the anti-biofouling property of surface modified Ti coated with silver nanoparticles [24]. Viswakarma *et al* [1] has confirmed that thin films of Cu on Ti surface decrease the bacterial adhesion and also Macak *et al* [25] have showed that using the self-doping and electrode-position technique TiO_2-NTs can be filled with copper for antibacterial purpose. Hence the present study deals with the surface modification of Ti which includes the growth of nanotubes and the subsequent coating of CuNPs on its surface to reduce the effect of biofouling and to increase the efficiency of the metal.

2. Experimental

2.1 *Synthesis of TiO₂-NTs*

The commercially pure Ti coupons (99.9% purity) of size 30 mm × 20 mm × 3 mm were purchased and polished initially with 600 and finally with 1200-grit paper, and then washed with acetone and distilled water in an ultrasonic cleaner. Initially the Ti coupons were etched in a solution containing HNO_3 : HF : H_2O (60 : 25 : 15) for 10 s to remove the air-formed oxide layer. The polished Ti coupons were anodized in an electrolyte solution containing 0.2–1.2% ammonium fluoride using direct-current power-supply unit. The voltage and current density were 20 V and 30 mA, respectively, applied for 10 min at room temperature. The Ti coupon was used as anode and stainless steel was used as cathode. Finally, the anodized Ti was annealed at 500°C for 2 h in order to convert amorphous form of titanium to crystalline form [26]. The nanotubes formed on the Ti surface (TiO_2-NTs) were characterized using FE-SEM (FEI Quanta FEG-200). The phase change from amorphous to crystalline anatase phase during anodization was observed using XRD (PANalytical Instruments) operating with $CuK\alpha$ radiation, λ = 1.5406 Å at a scan rate (2θ) of 0.05° 2 s^{-1} with the accelerating voltage of 45 kV at the applied current of 40 mA.

2.2 *Synthesis of CuNPs*

Copper nanoparticles (CuNP) were synthesized by the chemical reduction method using sodium borohydride. Briefly, to the solution of 0.02 M ascorbic acid 0.01 M $CuSO_4 \cdot 5H_2O$ was added and allowed for strong stirring. Polyvinyl chloride (PVP) was added to the above solution keeping the molar ratio of $[PVP]/[Cu^{2+}]$ = 0.15. One molar NaOH solution was used for adjusting the pH of the solution up to 12. The solution was stirred at room temperature for an hour and finally 0.1 M $NaBH_4$ was added [27]. The surface plasmon resonance peak exhibited by as-prepared CuNPs was examined using UV–VIS spectrophotometer (Amersham Biosciences) in the wavelength range of 300–700 nm.

2.3 *Coating of CuNPs on TiO₂-NT surface*

TiO_2-NTs coupons were silanized in 2% ethanolic solution of 3-aminopropyl tri ethoxy silane (APTES) for about 1.5 h followed by incubation in CuNP solution for 2 h in order to get CuNP-coated TiO_2-NTs (NT-CuNP) [28]. After which the coupons were rinsed and dried. The distribution of CuNPs on TiO_2-NTs was analysed using FE-SEM and EDS.

2.4 *Copper ions release rate*

To determine the release rate of Cu^+ ions, the NT-CuNP coupons were immersed in seven conical flasks individually containing 50 ml of sea water for a week in a shaker at 50 rpm to induce the natural tide effect as in sea. Each day a coupon was taken out and the solution was diluted enough to analyse the concentration of Cu^+ ions released from the Ti coupon using AAS. The experiment was done in triplicates.

2.5 *Lab scale sea water exposure studies of control and experimental Ti coupons*

Ti-C, TiO_2-NTs and NT-CuNPs coupons were tied with Ti frame and exposed in a tub containing sea water with small pump attached to make sure the continuous water flow on the coupons to study the antibiofouling effect of the nanoparticles-coated coupons. Sea water was changed for once in 2 weeks and the exposure study was conducted for about 2 months. The source of sea water was the coastal water belonging to the Bay of Bengal. The coupons were taken out at 1st and 2nd month of exposure and sonicated in waterbath sonicater for 10 min in 15 ml of sterile phosphate buffer (KH_2PO_4 0.0425 g l^{-1}, $MgCl_2$ 0.19 g l^{-1}). The sonicated buffer was treated as bacterial suspension. The bacterial suspension was then serially diluted and 0.1 ml of each dilution was plated on Zobell's Marine agar (ZMA; Hi Media M384). The plates were incubated at 37°C for 24 h and the total viable count (TVC) was estimated [29]. The sonicated Ti coupons were also analysed using FE-SEM for any morphological changes.

2.6 *Statistical analysis*

Cu^+ ions release rate study and seawater exposure experiments with the coupons were carried out in triplicates to validate the reproducibility of the experiments and the data presented are the means of three independent experiments. The data were analysed statistically by Student's *t*-test to calculate *p*-value and $p < 0.01$ were taken as significant and standard errors of the mean was calculated.

3. Results and discussion

3.1 *Characterization of TiO₂-NT surface by FE- SEM analysis*

Figure 1a–f shows the nanotube growth on Ti surface by anodization in varying concentration of NH_4F (0.2–1.2%).
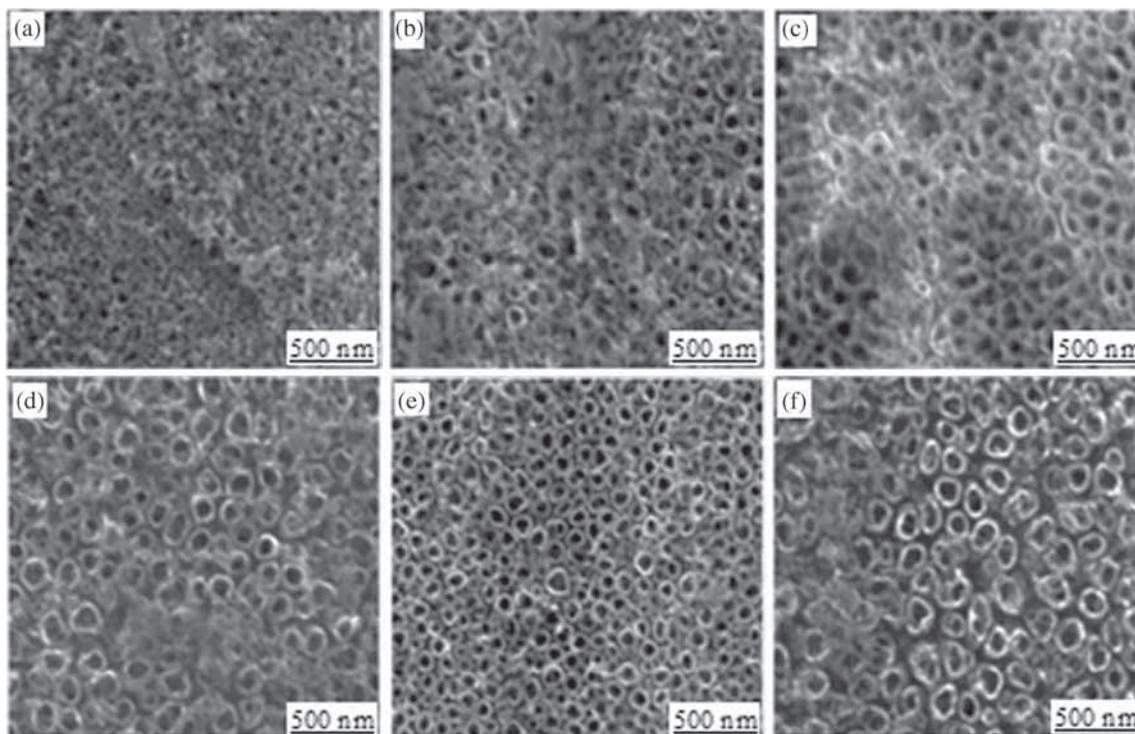
Figure 1. FE-SEM images of Ti surface anodized in varying concentrations of NH$_4$F: (**a**) 0.2, (**b**) 0.4, (**c**) 0.6, (**d**) 0.8, (**e**) 1.0 and (**f**) 1.2%.
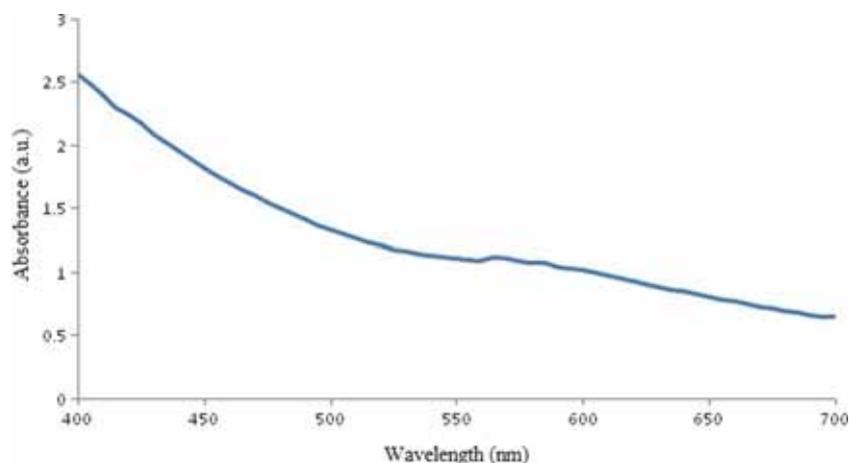

Figure 2. UV–Vis spectrum of copper nanoparticles.

Irregularities of nanotube structure were seen in below and above 1% of NH$_4$F concentration. The reason behind the nanotube formation are (i) formation of thin oxide layer at the metal surface due to the interaction of the metal with O^{2-}/OH$^-$ ions; (ii) electric field associate dissolution of the oxide layer resulting in the formation of pits; and (iii) chemical dissolution of these pits into nanotube array by F ions present in the electrolyte solution [26]. It was clear from the FE-SEM image that the growth of nanotube arrays of even size was achieved in 1% NH$_4$F concentration.

3.2 *Characterization of CuNPs*

Figure 2 shows the UV–VIS absorption spectra of synthesized CuNPs in the region of 300–700 nm. The absorption spectra shows a prominent symmetric peak around 570 nm, which is due to the strong interaction of the copper nanoparticles with light occurs because of the conduction electrons on the metal surface undergo a collective oscillation when excited by light at this specific wavelength [30]. This is the characteristic surface plasmon resonance of copper nanoparticles prepared. The synthesized CuNPs were coated on TiO$_2$-NTs surface with the help of coupling agent silane.

3.3 *Characterization of NT-CuNPs coupons*

Deposition of CuNPs on TiO$_2$-NTs surface is represented by FE-SEM image in figure 3c, whereas figure 3a and b represents FE-SEM image of control Ti coupon and anodised Ti in 1% NH$_4$F solution, respectively. The covalent bond between TiO$_2$-NTs and as-prepared CuNP is achieved by silane which serves as a coupling agent. The presence of CuNP on TiO$_2$-NTs surface was also confirmed by EDS analysis and the result shows the presence of silane and copper of remarkable concentration along with Ti (figure 5).

3.4 *XRD analysis of Ti-C, TiO$_2$-NTs and NT-CuNP coupons*

In the XRD analysis, figure 4a shows only the typical refraction peaks for Ti before it was subjected to surface modification. After anodization, upon heat treatment at higher temperature the amorphous TiO$_2$ crystallizes into anatase and further increase in temperature anatase may convert to rutile phase [31]. Thus, the XRD analysis of TiO$_2$-NT coupons annealed at 500°C has shown the peaks at $2\theta = 25.37°$, $35.20°$, $53.12°$, $71.67°$ attributed to (101), (103), (105), (220) lattice planes of anatase, respectively,
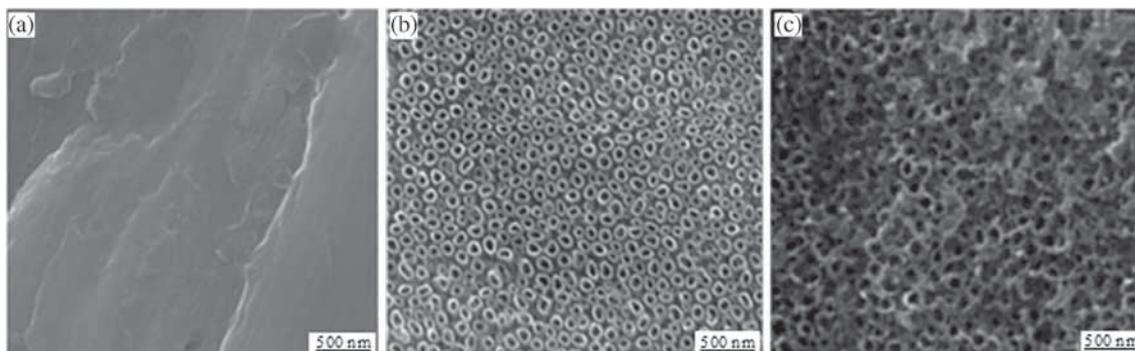

Figure 3. FE-SEM images of (**a**) Ti-C, (**b**) TiO$_2$-NTs and (**c**) NT-CuNPs.
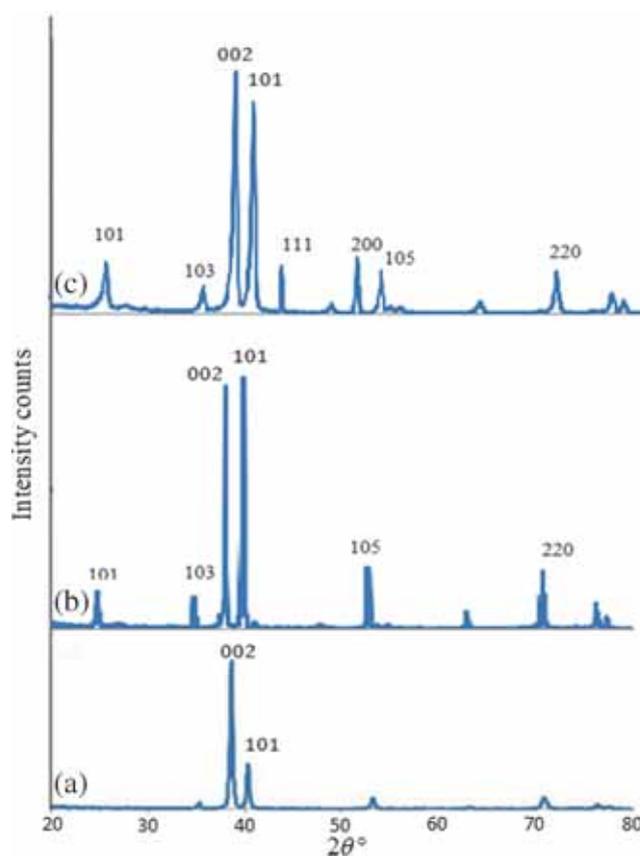

Figure 4. XRD image of (**a**) Ti-C, (**b**) TiO$_2$-NTs and (**c**) NT-CuNPs.

with reference to JCPDS card no. 21-1272, figure 4b. For NT-CuNP coupons, the peaks at 43.2° and 50.6° were seen along with the anatase peaks of Ti, were corresponds to the (111) and (200) crystal planes of Cu, respectively, with reference to JCPDS card no. 040836, figure 4c. The calculated particle size of as-deposited copper nanoparticles using the Scherrer formula was around 30 nm. The XRD and FE-SEM result shows that the synthesized CuNPs were coated evenly on the surface of TiO_2-NTs (figure 5).

3.5 *Cu^+ ions release rate analysis*

The silanization technique was adopted for coating CuNPs on the surface of TiO_2-NTs. Since previous research study shows that the surface-grafted polymerization of PVP was achieved on the surface of silane modified Fe_3O_4 nanoparticles [32], in our study stability of the CuNP coating on TiO_2-NTs surface is achieved by silane which acts as a coupling agent between the hydroxyl ends of the metal surface and the polymer surrounding CuNP. At the end of the 7th day, controlled release of Cu^+ ions from the NT-CuNP coupons treated with silane was observed with 76 ± 5.5 ppb. Whereas, NT-CuNP coupons treated without silane shows 145 ± 17.2 ppb of Cu^+ ions release leaching the most (figure 6). Hence, Cu^+ release rate of NT-CuNP coupons treated with silane was significantly very less than without silane treatment ($p < 0.01$). Finally, NT-CuNP coupons treated with silane for coating were used for sea water exposure studies.

3.6 *Sea water exposure studies*

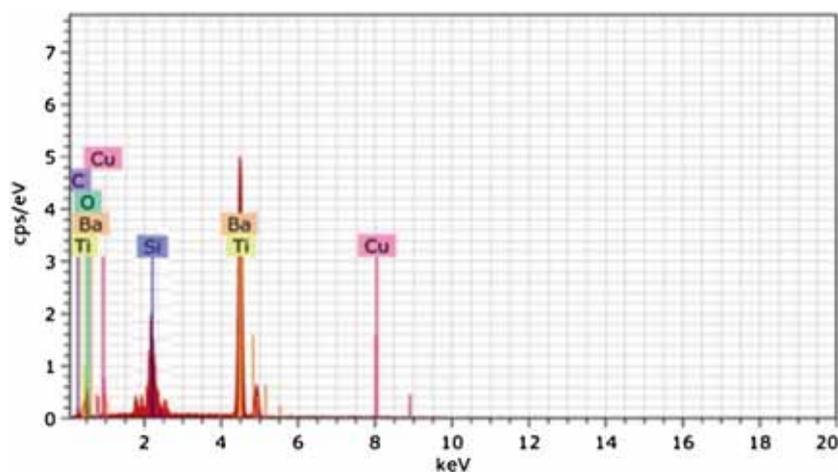Figure 7 shows the total viable count study of Ti-C, TiO_2-NTs and NT-CuNP at 1st and 2nd month of sea water
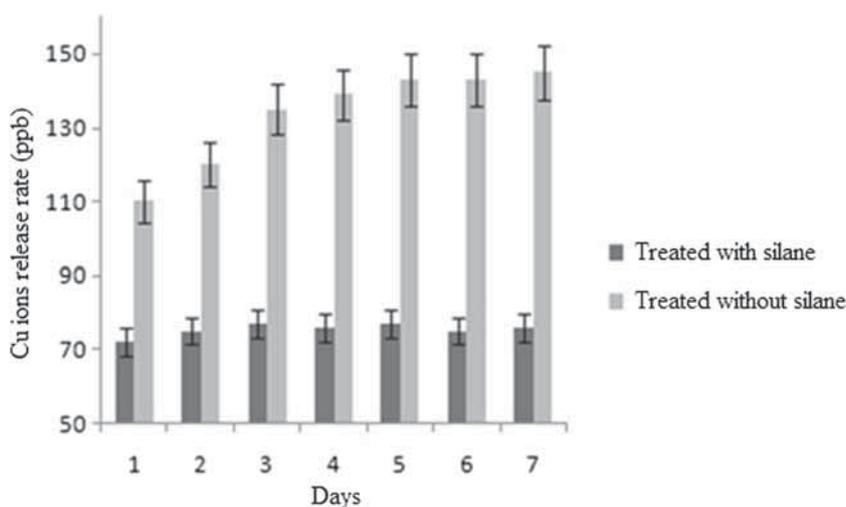
Figure 5. EDS result of NT-CuNPs.

Figure 6. Cu^+ ions release rate from NT-CuNP coupons treated with and without silane. Means $\pm$ SE are shown ($n = 3$).
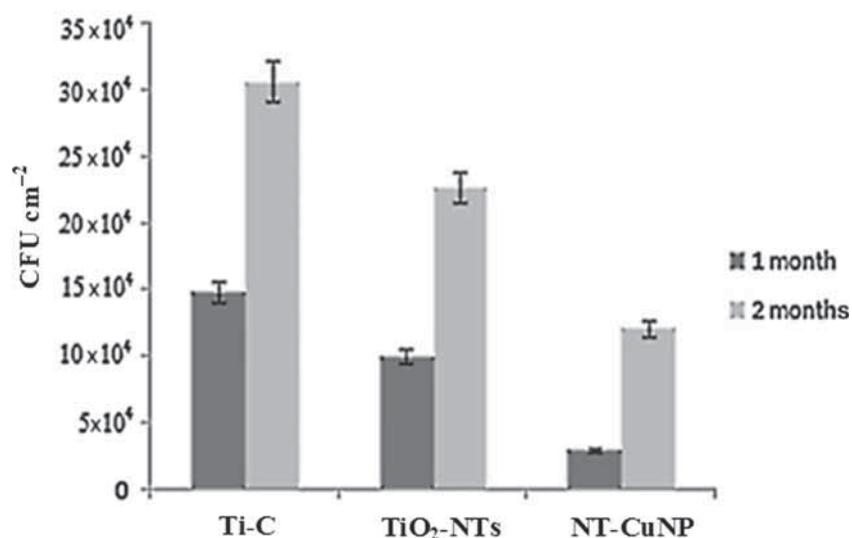
Figure 7. Total viable count data of Ti-C, TiO$_2$-NTs and NT-CuNP coupons exposed in sea water for 1 and 2 months. Means $\pm$ SE are shown ($n = 3$).
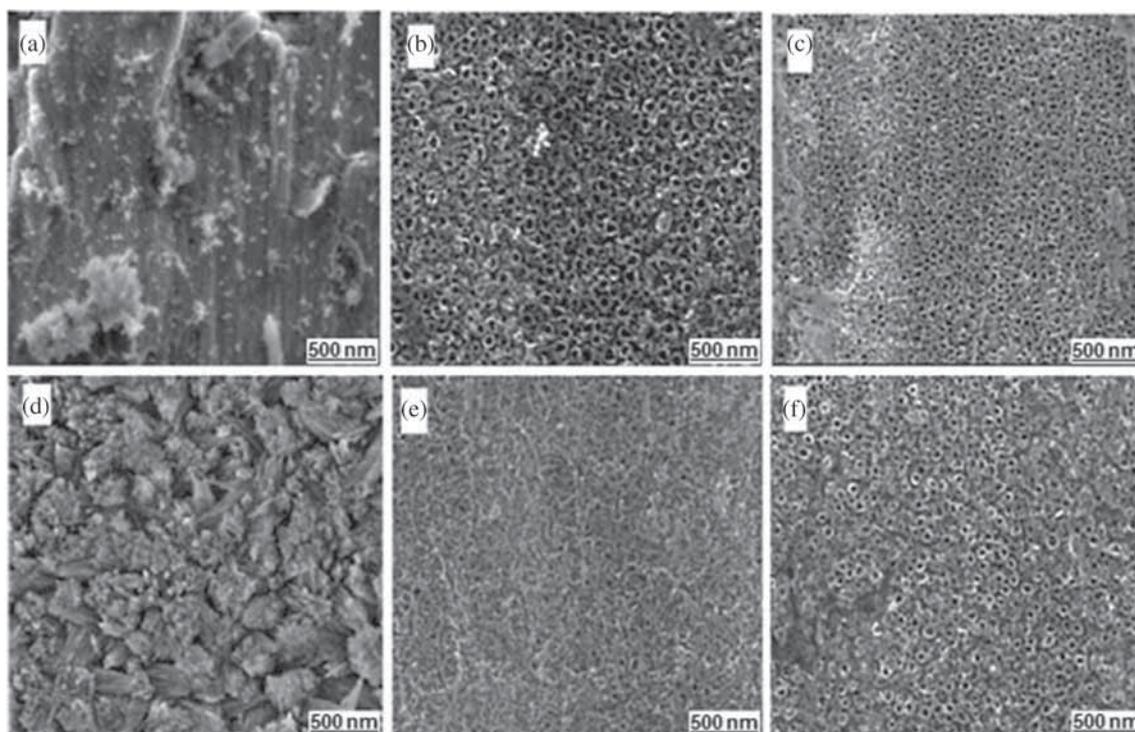

Figure 8. FE-SEM images of (**a**) Ti-C, (**b**) TiO$_2$-NTs and (**c**) NT-CuNP coupons exposed in sea water for 1 month and (**d**) Ti-C, (**e**) TiO$_2$-NTs and (**f**) NT-CuNP coupons exposed in sea water for 2 months.

exposure. The experiments were done in triplicates. The results show increased bacterial adhesion on the coupons in the order of Ti-C > TiO$_2$-NTs > NT-CuNPs. It is more evident from the result that NT-CuNPs coupons exhibit 60% decrease in bacterial adhesion when compared with control coupons. This is mainly achieved by the presence of copper ions which express the antibacterial activity by attaching to and penetrating the bacterial cell wall and affecting the cellular activity due to their toxicity. Moreover, the negative charge of the surface membrane of bacteria and positive charge of copper ions can easily bind, thereby enhancing the antibacterial activity and in turn reduces the bacterial adhesion on the surface of the coupon [33].

Figure 8 shows the FE-SEM images of control and experimental Ti coupons after the sea water exposure period. It

is clearly seen from the figure 8a and d that the control Ti coupon without any surface modification encountered severe biofouling at the end of 2nd month. Whereas TiO_2-NTs (figure 8b and e) and NT-CuNP (figure 8c and f) coupons shows reduced bacterial adhesion at 1st and 2nd month of exposure in sea water. Since CuNPs were coated onto the nanotubes via silane, a controlled release of Cu^+ ions into the sea water is achieved and the stability of the coating remains stable for longer time to reduce the bacterial adhesion on the surface of the metal, hence the rate of biofouling is also reduced. Also the efficiency of the metal will be increased when used as a condenser material in marine environments.

4. Conclusion

This research study mainly focused on the surface modification of Ti metal to reduce the effect of biofouling mainly for condenser application in sea water environment. In this research work, TiO_2-NTs were grown by the electrochemical anodization technique and to that chemically synthesized copper nanoparticles were coated using silane (APTES). This bonding promotes controlled release of Cu^+ ions into the sea water. The decrease in bacterial adhesion on TiO_2-NT coupon shows that the nanotubes itself has antibiofouling property. In addition the coating of CuNP, which is having bactericidal effect in nature provides longer protection of the metal against biofouling and consecutively increases the lifetime of the metal in the sea water environment.

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