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# **Sodium chloride assists copper release, enhances antibacterial efficiency, and introduces atmospheric corrosion on copper surface**

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## **Abstract**

Sodium chloride (NaCl) is commonly found in the physiological buffers. This study found out its additional but important role in antibacterial efficiency test by better corroding metallic copper surface. Combined with multiple characterisation methods including scanning electron microscope, grazing incidence X-ray diffractometer, and inductively coupled plasma mass spectrometry, 0.9% saline was observed to cause localised corrosion attacks on copper surface, enhancing release of copper content. The 1 h killing rate against *Escherichia coli* was thus promoted from 22% (when re-suspended in pure water) to 98%. By a long-term observation (3-week), residual NaCl crystals on 0.9% saline treated copper surface were found partially disappeared in atmospheric environment, contributing to an additional Cu<sub>2</sub>O layer forming above the treated surface. Besides, oxygen-containing species were observed even on fresh copper surface exposed by focused ion beam after saline treatment, suggesting a chloride-assisted atmospheric corrosion process.

Keywords: copper, cuprous oxide, chloride, corrosion, *E. coli*, antibacterial surface

## 1. Introduction

Antibacterial surfaces are drawing attentions in the recent years [1-3]. They can be applied as touched surfaces, having enormous potential demands in those heavily microbial burdened healthcare environments [4-6] as well as in our daily lives [7, 8]. In order to verify and assess the antibacterial ability of newly fabricated surfaces, antibacterial efficiency tests were designed and carried out.

Nowadays, there are different types of evaluation methods such as dry plating [9], wet plating [10], agar disk diffusion [11], etc. Limited by the incubation of bacteria and transfer process of bacterial suspension, a certain medium must be introduced during these evaluations. This medium is usually called buffer, which provides a suitable aqueous environment for bacterial survival without further growth. Despite the wide selection of buffers, in most of the cases, two main requirements should be satisfied: (1) a manageable pH range and (2) a suitable osmotic pressure for the microbes. Take phosphate-buffer saline (PBS) as an example [12], it contains a pair of acid and its conjugate base so as to compensate the pH fluctuation around 7.4. For the second purpose, it is sodium chloride (NaCl) that plays the part.

As can be seen, the design of buffers mainly considers the needs of microbes. But when they are to be applied in antibacterial surface research, a question needs to be raised: should the potential interactions between buffers and surfaces also be considered? The answer may be “certainly”. For example, copper-containing surfaces have been recently developed for antibacterial applications in various occasions [13-15]. Their antibacterial capability originates from the multifunctional antibacterial effects of copper ions [16]. To strongly interfere the survival of harmful microbes, copper ions should be efficiently released from the surfaces. As metallic surfaces, this release process is, most of the time, achieved by corrosion. The composition and the properties of buffer could become decisive, as they could facilitate or retard the corrosion process. Therefore, the solution and the copper surfaces together display how effective the antibacterial activity would be shown in antibacterial efficiency test.

Back to the example of PBS or other saline solutions, without a doubt, NaCl is expected to play an extremely important factor in revealing the antibacterial efficiency of copper surface. Electrochemical study has investigated the roles of chloride ions ( $\text{Cl}^-$ ) in formation and transformation of corrosion product ( $\text{CuCl}$ ,  $\text{Cu}_2\text{O}$ ,  $\text{Cu}(\text{OH})_2$ , etc.) [17], which could further affect the diffusion of copper ions [18]. In general, the presence of  $\text{Cl}^-$  also leads to a relatively corrosive environment for copper, depending on the concentration of  $\text{Cl}^-$  [19]. Meanwhile, it easily promotes pitting corrosion and further deterioration on the surface [20]. This could, on the contrary, contributes to the copper antibacterial efficiency. Because whenever the corrosion is enhanced, a faster dissolution process of the antibacterial substance (copper ions) is anticipated. For example, a latest study that evaluated antibacterial property of copper-silver surface has discovered the constructive effect of chloride addition in dissolving antibacterial copper ions [21]. Other experiments also suggest that Cu-Fenton chemistry, as one of the antibacterial mechanism, could be accelerated by  $\text{Cl}^-$  [22], resulting in a better biofilm removal. On the whole, a different

56 antibacterial efficiency is therefore highly expected in Cl<sup>-</sup>-containing environment.

57 Other than affecting the corrosion behaviour in media, it is noteworthy that NaCl deposits could induce atmospheric  
58 corrosion on dry surfaces. At high humidity levels, for instance, deliquescence of NaCl could happen, initiating  
59 micro-droplets formation on metallic surfaces and introducing further corrosion [23]. In lower humidity circumstances  
60 (below than 79% RH, deliquescence point of NaCl), where NaCl does not dissolve though, adsorption of water on the  
61 surface could form several layers that act as bulk water [24]. Diffusion of Cl<sup>-</sup> could thus occur in this quasi-aqueous  
62 condition resulting in corrosion attack on copper [25]. Either way, tracking this atmospheric corrosion effect after  
63 antibacterial efficiency test may have significant implication for long-term product design [26].

64 Lately, we compared PBS and Na-4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Na-HEPES) in terms of their  
65 impacts on evaluating the antibacterial performance of metallic copper and Cu<sub>2</sub>O surface [27]. Corrosion behaviours  
66 introduced by PBS caught our attention, therefore further experiments were designed to reveal the roles of bacteria were  
67 revealed [28] and to discuss the corrosion attacks and oxide growth [29]. Since PBS contains NaCl, this study narrows  
68 down to it, to find out if NaCl plays an important factor as expected, in respect to corrosion. The primary objective is to  
69 explore how 0.9% saline affects the antibacterial efficiency test on metallic copper surface. Afterwards, the corrosion  
70 behaviours were compared among saline with different Cl<sup>-</sup> or *Escherichia coli* (*E. coli*) concentrations. Finally, the  
71 following aging effects resulting from the residual NaCl crystals on copper surface were also characterised.

## 73 **2. Materials and Methods**

### 74 **2.1. Materials**

75 Copper (99.99%, K09, Wieland) coupons were ground with a silicon carbide sandpaper (stepped down to grit number P600),  
76 then cleaned with ethanol in an ultrasonic bath, finally dried by air.

### 78 **2.2. Solutions**

79 Saline with various final concentrations (0.45%, 0.9%, and 1.8%) was prepared with NaCl (VWR, Germany) and pure  
80 water for analysis (Merck, Germany), sterilised in an autoclave after preparation. PBS was prepared with NaH<sub>2</sub>PO<sub>4</sub> · 1H<sub>2</sub>O  
81 (Merck, Germany, final concentration 0.01 M), NaCl (VWR, Germany, final concentration 0.14 M) and pure water for  
82 analysis, and its pH value was adjusted by adding NaOH to 7.4. Buffers were sterilised in an autoclave after preparation.

83 The preparation of bacterial suspension will be described in Section 2.6. Besides, a fivefold concentration *E. coli* NaCl  
84 suspension was simply prepared by re-suspending bacteria in 0.2 mL of NaCl instead of its original volume 1 mL. A tenth  
85 fold diluted *E. coli* NaCl suspension was prepared in the similar manner.

### 87 **2.3. Corrosion protocol**

88 Droplet of 20  $\mu\text{L}$  pure solution or *E. coli* suspension was applied on ground copper surfaces with a pipette. These copper  
89 coupons were placed in a water-saturated atmosphere at room temperature for 3 h. Solutions or suspensions were then  
90 withdrawn with a pipette. These steps are chosen to simulate the antibacterial efficiency test further described in Section  
91 2.6.

92 If not specified, the coupons were then cleaned with ethanol in an ultrasonic bath immediately after withdrawal of  
93 droplet. However, for the tests with various NaCl or bacterial concentrations (Section 3.4), the treated surfaces were firstly  
94 wiped with detergent-dipped cotton pads in order to remove the adhered bacteria. On the other hand, for some tests designed  
95 for atmospheric aging experiments (Section 3.5), the coupons remained unwashed. They were constantly kept in the lab  
96 atmosphere at room temperature, except for the time being characterised.

### 98 **2.4. Surface characterization**

99 Different functions of scanning electron microscope (SEM, Helios NanoLab600, FEI) were employed to observe copper  
100 surfaces after various corrosion experiments. Secondary electron detector (SE) was applied to examine the top-surface as  
101 well as cross-section of the coupons. Meanwhile, with backscatter electron detector (BSE), localised corrosion attacks  
102 covered by corrosion products were explored. Acceleration voltages will be further mentioned in the corresponding figure  
103 captions. The chemical composition of bacteria was obtained by Energy dispersive X-ray spectroscopy (EDS) at 5 kV.  
104 Optical microscope (OM, OLS4100, Olympus) photos were captured in 3D bright field mode. For further confirmation of  
105 the corrosion products covering the surfaces, high resolution grazing incidence X-ray diffractometer (GIXRD, Cu  $K_{\alpha}$  with  $1^{\circ}$   
106 grazing angle, PANalytical X'Pert PRO-MPD) was applied. In the atmospheric aging experiments, focused ion beam (FIB)  
107 was applied to fabricate fresh cross-section of the copper coupon after corrosion treatment, using gallium ions with an  
108 accelerating voltage of 30 kV. Images and spectra were obtained within 24 h after the corresponding corrosion treatments,  
109 except from the atmospheric aging experiments.

### 111 **2.5. Copper content determination**

112 After corresponding surface treatment, 10  $\mu\text{L}$  samples were withdrawn by repetitive pipetting and diluted in 2.990 mL 1 wt%  
113 nitric acid (Merck, Germany). Before being measured by inductively coupled plasma mass spectrometry (ICP-MS, 7500cx,  
114 Agilent), 3  $\mu\text{L}$  of 10 mg/L scandium and caesium internal standard solutions were added to the samples. For calibration,  
115 standards with 0.1, 0.5, 2.5, 10, 50, 250, and 1000  $\mu\text{g/L}$  of copper were used. Original results with a unit of ppb were  
116 converted to the final values with  $\mu\text{mol/L}$  ( $\mu\text{M}$ ), concerning both the fold of dilutions and molar mass 63.55 g/mol for  
117 copper. The average values and standard deviations were obtained by three independent experiments.

## 119 **2.6. Antibacterial efficiency determination**

120 Wet-plating test was used, whose details can be referred to a previous publication [10]. In brief, the *E. coli* K12 strain was  
121 grown aerobically overnight in Lysogeny broth (LB) medium at 37 °C in a water bath with a speed of 220 rpm. The  
122 stationary cells from 1 mL culture were collected by centrifugation for 15 min at 5000×g, washed and centrifuged three  
123 times with the corresponding solutions, and finally re-suspended in 1 mL of the same type of solution. The test commenced  
124 by applying 20 µL of these re-suspended bacteria on the coupons, which were placed in a water-saturated atmosphere at  
125 room temperature. After 1 h, 10 µL samples were withdrawn by repetitive pipetting, serially diluted in PBS and spread on  
126 LB agar plates. Following incubation at 37 °C for 24 h, the colony-forming unit (cfu) on agar plates was counted and  
127 expressed in a way concerning both the fold of dilutions and relative to the original 20 µL samples. The average values and  
128 standard deviations were obtained by three independent experiments.

## 130 **3. Results and Discussion**

### 131 **3.1. Corrosion of copper surface in 0.9% NaCl and pure water**

132 Before analysis of the actual antibacterial efficiency test, this study first focuses on the corrosive effects introduced by 0.9%  
133 NaCl (this concentration is hereafter simply referred to as “saline”) without bacteria. For instance, Figure 1 (a-c) shows the  
134 morphological change and formation of corrosion product on the copper surface after 3 h exposure to this environment.  
135 Uniformly distributed sub-micron corrosion products are evident in SE image. Meanwhile, at some locations, much lower  
136 intensity was recorded in BSE (therefore darker), indicating missing of material underneath the corrosion product. These  
137 cavities signify where localised deterioration took place. Variation of microstructure definitely has impacts on the initiation  
138 of these localised attacks [30]. The distribution of these attacks can be mostly referred to a recent study [29], where how  
139 electropolished copper surface corroded by PBS droplet was investigated in detail.

140 Coupon exposed to pure water for the same duration is shown for comparison purposes in Figure 1 (d and e). Fewer  
141 features can be summarised: no obvious additional product coverage nor localised corrosion sites. Therefore, it can be  
142 preliminarily concluded that, adding NaCl into pure water significantly varies the environmental corrosivity towards copper.

143 The corrosion product formed on copper was further examined by high resolution GIXRD shown in Figure 1 (f).  
144 Crystalline Cu<sub>2</sub>O has been identified as the major product after saline treatment, but not in pure water. Similar observation  
145 was obtained from potentiostatic polarisation [20], highlighting the formation of intermediate product CuCl, followed by the  
146 formation of Cu<sub>2</sub>O and initiation of pits in a chronological sequence. However, CuCl is not identified in the current study.  
147 Meanwhile, the cavities were found being gradually covered by Cu<sub>2</sub>O films, instead of destroying the films. Therefore,  
148 dissolution-precipitation or re-deposition mechanism [27] seems to be more appropriate in explaining the oxide growth: it is  
149 exceeding amount of copper ions gathering near the copper surface and forming oxide.

150

### 151 **3.2. Corrosion of copper surface in bacterial suspensions**

152 By adding *E. coli* (hereafter referred to as “bacteria”), solution becomes suspension, some corrosion behaviours on copper  
153 surface thus alter accordingly, as can be found in Figure 2. In saline, localised corrosion attack is still predominant, which  
154 can be easily recognised from the BSE images. SE image usually shows more morphological details, but the  
155 above-mentioned (Section 3.1) sub-micron corrosion product  $\text{Cu}_2\text{O}$  forming in pure solution can hardly be seen. The  
156 missing diffraction peak of  $\text{Cu}_2\text{O}$  (111) in GIXRD also matches this observation. Highly similar phenomena were recently  
157 reported in the case of PBS [28], which could help interpret the current scenario: it is attributed to the bacterial  
158 accumulation effects of copper ions. Copper concentration near surface is thus reduced and oxide growth is inhibited.

159 Without oxide coverage, a considerable amount of cavities, as a result of localised corrosion attacks, become visible  
160 even in SE mode. Those invisible ones, on the other hand, may imply another mechanism of pit initiation: a  $\text{Cu}_2\text{O}$   
161 membrane could be found, separating the inner pitting region from the aqueous environment and promoting further pitting  
162 corrosion under the uppermost copper surface [31]. Similar sites have also been observed in the intermetallic particles  
163 induced crystallographic pitting grown beneath the surface [32], suggesting galvanic couple could be considered in the  
164 current scenario.

165 In the case of pure water, its bacterial suspension has not introduced distinct morphological or chemical changes on  
166 copper surface. These contrasts suggest the important roles of *E. coli*: although its presence inhibits the formation of  $\text{Cu}_2\text{O}$ ,  
167 it does not affect the major corrosion attacks (especially in saline) or cause additional corrosion effect (especially in pure  
168 water). In other words, it is still the properties of solution that determine the corrosion process.

169 From the microbiological perspective, addition of bacteria introduces not only bacteria themselves, but also their  
170 metabolism as well as the products of it. Bacterial respiration, for example, is one of the universal processes frequently  
171 discussed, as it could help reducing oxygen content in the aqueous environment [33]. Meanwhile, dissolved oxygen happens  
172 to play an indispensable factor in the corrosion of metallic copper in aqueous [34] or  $\text{Cl}^-$ -containing environment [35].  
173 However, in the following sections, this aspect will be further ruled out from the present conditions.

174

### 175 **3.3. Copper release and antibacterial efficiency**

176 In the above sections, the corrosion behaviours of copper surface were revealed, in terms of how it varies after being  
177 exposed to different solutions or suspensions. But apart from this, it is also meaningful to investigate the effects of corrosion  
178 on the contacting droplet itself. For instance, the rise of copper content measured by ICP-MS shown in Figure 3 (a) offers  
179 valuable information. First of all, it is evident that the copper content measured from the bacterial suspension is higher than  
180 its corresponding pure solution. This can be again correlated with the role of bacterial addition summarised above: since  
181 bacteria decrease the amount of free copper ions in solutions, it favours a continuous release kinetics. This particularly

182 affects the situation in saline, as it suppresses the formation of  $\text{Cu}_2\text{O}$ , which could have formed as a barrier layer reducing  
183 the metal/solution interface [27] as well as retarding ion diffusion [18].

184 Another marked discrepancy is found between saline and pure water: the amounts of copper presented in saline are, in  
185 both cases, evidently higher than that in pure water. This trend matches the SEM observation: copper surface always suffers  
186 from more detectable localised corrosion attacks in saline. This is likely attributed to  $\text{Cl}^-$ , which has been regarded as an  
187 accelerator of corrosion on metallic surfaces most of the time [36]. With respect to copper, there was also study conducted  
188 on the relationship between  $\text{Cl}^-$  concentration and corrosion rate [19]. It shows that the  $\text{Cl}^-$  attachment allows complex  
189 formation and thereby promotes a faster ion release.

190 Additionally, it is still worth noting that neglectable variations of surface topography does not necessarily represent  
191 similar copper release level. In the case of pure water, although SEM results do not indicate significant difference, its  
192 bacterial suspension does enhance copper release by about three times, from  $75.29 \mu\text{M}$  to  $299.15 \mu\text{M}$  (data listed in the table  
193 in Figure 3). This suggests that it is the uniform corrosion that dominates the copper release in pure water, instead of  
194 localised corrosion.

195 Copper content presented in suspension is also positively related to the surface antibacterial efficiency, as presented in  
196 Figure 3 (b). Bacteria in both suspensions were grown from the same broths and thence share similar original concentration.  
197 Merely in 1 h, their survival already parted ways: around 98% were killed in the saline suspension, while only 22% was  
198 recorded in pure water. Not surprisingly, these numbers match the difference of copper content evaluated in ICP-MS shown  
199 above and that accumulated in the bacterium compared in Figure 3 (c): Stronger  $\text{Cu L}_\alpha$  radiation at 0.930 keV was recorded  
200 from the bacteria in saline.

201 These comparisons demonstrate how corrosion behaviour and antibacterial efficiency are correlated: even though same  
202 type of microbe was tested on same type of metallic copper surface, the composition of the solution can dictate the release  
203 of antibacterial substance and thus result in a different antibacterial efficiency. Saline, a relatively corrosive solution, causes  
204 a higher amount of copper release (at least within a certain period), raising the copper concentration and enabling a faster  
205 killing rate against *E. coli*. A recent study [21] conducted by another research group also found out a similar role of  $\text{Cl}^-$  in  
206 enabling sufficient corrosion on not only copper and but also copper-silver alloy to deactivate *S. aureus*.

207 Two additional remarks may help further interpreting the antibacterial efficiency results. Since the killing rate follows  
208 the same trend of the increasing copper content, it cannot be estimated in the current experiment, how much of the  
209 antibacterial efficiency was assisted by the  $\text{Cl}^-$  accelerated Cu-Fenton reaction [22] or other combined effects [37]. On the  
210 other hand, one may notice that pure water is not a buffer, but a low osmolarity environment in the strict sense.  
211 Hypoosmotic shock does not necessarily lead to cell death [38], but even if it does, the contribution of copper in reaching a  
212 22% killing rate should be considered even lower.

### 3.4. Effects of NaCl concentration and *E. coli* concentration on copper

Since the effects of NaCl and *E. coli* in this corrosion system have been preliminarily identified, additional experiments were designed to explore these effects. Figure 4 (a-c) compare the effects of saline concentration on the corrosion behaviours of copper surface. For all these selected concentrations, copper was subjected to a similar type of localised corrosion attacks. As the saline concentration increases, the expansion of cavities is observed, identified by imaging contrast in BSE. Besides, contrast can also be found even within a single cavity. This implies the localised attack inside the cavity is not uniform. On the other hand, oxide growth is ignorable in both 0.45% and 0.9% suspensions. But when it added up to 1.8%, huge areas covered by sub-micron oxides are found. Meantime another striking feature is also observed regarding the distribution of oxide on copper surface: they only grew on specific locations.

According to the re-deposition mechanism discussed (at the end of Section 3.1), oxide growth usually corresponds to excessive copper ions. Therefore, the emergence of oxide could be attributed to a faster corrosion kinetics in a high Cl<sup>-</sup> concentration scenario, where the amount of copper ions exceeds the accumulation capacity of *E. coli*. However, as the bacteria presenting in the droplet are supposed to be well-mixed, their copper accumulation effect should be distributed evenly on the surface. That is to say, the localised copper ion concentration is expected similar across the whole near surface. Therefore, other than copper concentration, there must be another factor governing the preferential locations of oxide growth.

As it can be seen in Figure 4 (d), oxide coverage seems to follow the grinding marks. It strongly suggests its correlation with the state of the original copper surface (*e.g.* microstructural or morphological difference introduced by the grinding procedure). In other words, variation of the state of the surface might dominate the distribution of cathodic sites, where oxide growth prevails. To design similar experiments on electropolished copper surface may help, as it did reveal the crystallographic tendency of copper corrosion in PBS [29].

To verify the role of ion accumulation effect introduced by *E. coli*, another corrosion test was conducted with 1.8% NaCl, whereas fivefold *E. coli* was re-suspended. After the same duration, oxide coverage can hardly be seen on copper surface, as shown in Figure 5 (a). Surge of the amount of bacteria boosts both the capacity and efficiency of the ion accumulation, compensating the increase of copper release in high Cl<sup>-</sup> concentration. Besides, higher bacterial concentration did not affect the selective distribution of localised corrosion sites, indicating that the corrosion mechanism stays the same. However, the size of cavities did shrink, and easy to be found connected altogether. Meanwhile, even on the locations that are free from severe localised corrosion, slight degradation can be observed, as the grinding marks can hardly be found. It is unclear at the current stage, but these phenomena could be related to but not limited to the variation of local chemical concentration (Cl<sup>-</sup> or chemicals produced by bacterial metabolism), local fluid flow rate (induced by the mobility of bacteria), etc.

On the other hand, with lower bacterial concentration (tenth fold diluted), oxide coverage can always be found in

246 whole series of NaCl concentration (Figure 5 (b-d)). Oxide particles with bigger size are noticed in lower NaCl  
247 concentration. That is, NaCl concentration has an impact on the nucleation density of oxide. The trend of how NaCl and  
248 bacterial concentration influence oxide growth and corrosion attacks are qualitatively summarised in Figure 5 (e).

### 249 **3.5. Atmospheric corrosion caused by residual NaCl crystals**

250 So far, the impacts of NaCl on copper surface have only been discussed during corrosion test or antibacterial efficiency test.  
251 However, even though the saline will be withdrawn afterwards, some NaCl residues could stay on the copper surface. These  
252 NaCl crystals are found to continue playing a decisive part in the copper aging process.

253 Figure 6 compares the copper surfaces observed 3 weeks after having been exposed to saline. Coupon shown in Figure  
254 6 (a) has been cleaned immediately after the saline was withdrawn in the end of the corrosion test, therefore no NaCl crystal  
255 was remained. In this case, the oxide coverage is found unchanged in 3 weeks. However, coupon that has not been cleaned  
256 properly experienced significant changes, as shown in Figure 6 (b). Firstly, a secondary layer with a slightly different  
257 roughness formed on the previous  $\text{Cu}_2\text{O}$  coverage. Moreover, the reason why some uncovered previous  $\text{Cu}_2\text{O}$  can be located,  
258 is likely related to the NaCl crystals (with brighter colour in SE mode) lying aside: it is assumed that these uncovered zones  
259 were also originally covered by the NaCl crystals. Nevertheless, these crystals gradually disappeared during the 3-week  
260 atmospheric aging.

261 From GIXRD results, two facts have been clearly shown. First, NaCl residues were efficiently cleaned by the current  
262 protocol, as much weak diffraction signals are found on the cleaned coupon. Second, more  $\text{Cu}_2\text{O}$  diffraction peaks with  
263 higher intensity appeared from the coupon without cleaning, confirming the newly grown secondary layer is mainly  
264 composed of  $\text{Cu}_2\text{O}$  as well.

265 Intriguingly, this kind of aging effect does not occur only on the directly treated copper surface. As shown in Figure 7,  
266 a hole on the copper coupon was fabricated by FIB, immediately after the corrosion test without applying cleaning protocol.  
267 This exposes some “fresh” areas beneath the original surface. “Fresh” is here defined as having not directly “met” the saline,  
268 since they were still inside the bulk copper during the corrosion test. OM image taken straightway after FIB operation (*i.e.*  
269 Day 0) recorded some bright zones, not just in the hole, but also around it, as a result of Ga ion beam bombardment. The  
270 areas inside the hole that are darker were due to rougher topography, confirmed by SEM images.

271 However, as the aging experiment proceeds, the bright zones shown in OM turned darker from the boundaries inwardly.  
272 This is related to the morphological changes displayed in Figure 8: submicron particles were forming on top of these  
273 surfaces. Being continuously exposed to atmosphere, part of this region extended, from the cross-section (edge of the hole)  
274 towards the bottom of it. Meanwhile, coarsening can be observed on some of these particles. At the cross-section (invisible  
275 in OM owing to observation angle) which is supposed to be relatively smooth at the original state, similar particle formation  
276 and coarsening phenomenon are also found. Although the exact chemical or phase composition has yet to be determined,  
277

278 these locations do contain an extra amount of oxygen content (Figure 8 (d&e), obvious O  $K_{\alpha}$  radiation at 0.525 keV),  
279 suggesting the formation oxygen-containing species. Served as the control groups, same type of FIB cross-sections was  
280 produced on copper coupon exposed to either pure water or *E. coli* water suspension for the same duration. Neither of these  
281 surfaces shows such changes after 3 weeks (data not included), excluding the potential additional effects brought by Ga ion  
282 implantation during FIB operation.

283 All these comparisons help to preliminarily pin down the cause of atmospheric aging: NaCl, as it the only varied  
284 environmental parameter. As the solution cannot be withdrawn completely, NaCl crystallised locally on the copper  
285 surface after the remained water evaporated. However, this is not yet the final fate of these crystals. Atmospheric corrosion  
286 continues to play a role. For example, these salts could go through a process so-called deliquescence [23], thereby absorbs  
287 the water from the atmosphere and induce aqueous corrosion. But this process requires a relatively high humidity (79% RH  
288 for pure NaCl), which is usually not fulfilled in the lab environment.

289 In spite of this, NaCl crystals could transform in another way, which is still related to water absorption, not by NaCl  
290 itself, but by the general surface. Because it is common to find monolayers of water forming on surfaces [24]. Therefore, the  
291 copper surface could still be regarded as being exposed to a quasi-aqueous environment. In fact, the properties of the water  
292 layer could already affect the nucleation and formation rate of  $Cu_2O$  in a certain range of relative humidity [39]. Besides,  
293 these monolayers dissolve NaCl and transform itself to electrolyte. Meanwhile,  $Cl^-$  are able to diffuse through these  
294 monolayers, reaching to the other parts of the surface [25]. Considering this circumstance, corrosion related phenomena are  
295 not a surprise. In addition,  $Cl^-$  may not be the only ions that would diffuse through the surface, as copper ions should also  
296 participate a similar process. Take the saline corroded surface as an example (Figure 6 (b)), without copper ion diffusion to  
297 the top of the existing  $Cu_2O$ , it is hard to imagine how the secondary layer of  $Cu_2O$  actually formed on top.

### 299 **3.6. Insight into antibacterial surface research**

300 Firstly, the current results suggest the significance of buffer selection in antibacterial surface research. Buffers are more than  
301 a friendly environment designed for microbes. On the contrary, depending on their components as well as the corresponding  
302 properties (corrosivity in this case), they may react with the investigated surface in different ways, resulting in an enhanced  
303 antibacterial efficiency or vice versa. Evidences for this can also be obtained from other comparisons such as PBS vs  
304 Na-HEPES [27], or nutrient broth (NB) vs minimal media (MM) [40]. For the same reason, design of materials with weaker  
305 corrosion resistance could enhance release of antibacterial substances [41], meanwhile considering that the cytotoxicity  
306 might need to be and definitely can be avoided in specific scenarios [42].

307 Secondly, growth and coverage of passive layer as a result of the subsequent atmospheric corrosion, may not be  
308 beneficial for the long-term antibacterial property of antibacterial copper surface [26]. Therefore, it should particularly  
309 inspire the applied research closely related to touched surfaces, as NaCl is also one of the major residues of perspiration. To

310 evaluate its effect on antibacterial efficiency, cyclic test [43] should be one of the effective ways to be included in the future  
311 work.

## 313 4. Conclusion

314 NaCl as a common component in many popular buffers, was examined in this work in view of its roles in copper corrosion.

315 Some concluding remarks are summarised as below:

- 316 ● Ground copper coupon undergoes severe localised corrosion attacks in the environment of 0.9% saline, regardless of  
317 whether *E. coli* is added or not.
- 318 ● A layer of Cu<sub>2</sub>O grows on copper surface when being exposed to pure 0.9% saline. In the case of its *E. coli* suspension,  
319 formation of Cu<sub>2</sub>O has been inhibited at least in 3 h.
- 320 ● A faster killing rate of ground copper surface against *E. coli* (98% in 1 h) is recorded in 0.9% saline, compared to pure  
321 water (22%), which is attributed to the accelerated corrosion process in the presence of Cl<sup>-</sup>.
- 322 ● Increase of Cl<sup>-</sup> concentration may accelerate copper release, forming Cu<sub>2</sub>O even in *E. coli* suspension. However, it can  
323 be counteracted by increasing the concentration of *E. coli* to fivefold, as this enhances the copper ion accumulation  
324 effect.
- 325 ● Residual NaCl crystals on copper surface promote the formation of additional Cu<sub>2</sub>O as corrosion products. A  
326 Cl<sup>-</sup>-assisted atmospheric corrosion process is revealed by a FIB-assisted 3-week aging experiment.

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335 **Declarations of interest**

336 None.

337

338 **Data availability**

339 The data that support the findings of this study are available from the corresponding author on request.

340

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430

431

## Figure Captions

### Figure 1

Typical SEM images of ground copper surfaces after 3 h exposure to 0.9% NaCl (**a-c**) or pure water (**d, e**). Images were obtained by SE detector (**a, d**) and BSE detector (**b, c, e**) at 20 kV. The dotted lines in (**a, b**) surround a typical localised corrosion zone. Images (**a, b**) correspond to the square dash marked region in (**c**). High resolution GIXRD results (**f**) in the range of (111) planes of  $\text{Cu}_2\text{O}$  (JCPDS#75-1531) obtained from these surfaces.

### Figure 2

Typical SEM images of ground copper surfaces after 3 h exposure to *E. coli* 0.9% NaCl suspension (**a-c**) or *E. coli* pure water suspension (**d, e**). Images were obtained by SE detector (**a, d**) and BSE detector (**b, c, e**) at 20 kV. The dotted lines in (**a, b**) indicate two types of localised corrosion site. Images (**a, b**) correspond to the square dash marked region in (**c**). High resolution GIXRD results (**f**) in the range of (111) planes of  $\text{Cu}_2\text{O}$  (JCPDS#75-1531) obtained from these surfaces.

### Figure 3

Copper content (**a**) in 0.9% NaCl or pure water, with or without *E. coli* after 3 h exposed to ground copper surfaces. Antibacterial efficiency test (**b**) against *E. coli* in two different suspensions in 1 h. The error bars indicate the standard deviations calculated from three independent measurements. Percentages in (**b**) above the bars represent the killing rate in 1 h (compared to the original data). Average values from (**a**) and (**b**) are listed in the tables. Typical EDS results (**c**) collected from the bacterium (*E. coli*) transferred onto silicon wafer after being treated with copper surface in two suspension for 3 h.

### Figure 4

Typical SEM images (BSE, 5 kV) of ground copper surfaces after 3 h exposure to *E. coli* suspension with different NaCl concentration: (**a**) 0.45%; (**b**) 0.9%; (**c**) 1.8%. The dotted lines in (**a, c**) indicate typical localised corrosion cavities. Image (**c**) corresponds to the square dash marked region in (**d**). Orange arrows in (**d**) indicate regions with oxide growth, while white arrows for those without.

### Figure 5

Typical SEM images (BSE, 5 kV) of ground copper surfaces after 3 h exposure to (**a**) fivefold *E. coli* 1.8% NaCl suspension; (**b**) tenth fold diluted *E. coli* 0.45% NaCl suspension; (**c**) tenth fold diluted *E. coli* 0.9% NaCl suspension; (**d**) tenth fold diluted *E. coli* 1.8% NaCl suspension. Dash line in (**a**) separates the severe corrosion zone from the almost intact zone. Table (**e**) summarises the characteristics of oxide growth and corrosion sites in different conditions: more “+” represents

463 bigger particle size; more “-” represents smaller localised corrosion sites; “X” represents situations where particle coverage  
464 was rarely observed.

465

466 Figure 6

467 Typical SEM images (SE, 5 kV) of ground copper surfaces 3 weeks after 3 h exposure to 0.9% NaCl solution. Coupon  
468 shown in (a) has been ultrasonic cleaned by ethanol immediately after the 3 h corrosion experiment, while (b) was kept  
469 without further cleaning. Both images share the same scale bars that presented in (a). The dotted region in (b) indicates the  
470 missing of NaCl crystal as well as the exposed original oxidised copper surface. High resolution GIXRD results (c) obtained  
471 from these surfaces 12 weeks after the original treatment. Cu (JCPDS#04-0836), NaCl (JCPDS#05-0628), and Cu<sub>2</sub>O  
472 (JCPDS#75-1531) were applied to index the patterns. The diffraction peaks marked with hexagonal star (★) represent  
473 diffraction of Cu (111) planes under Cu-K<sub>β</sub> and W-L<sub>α1</sub>.

474

475 Figure 7

476 OM images (a-d) and SEM images (e-k) (SE, 5 kV, coupon being 52° tilted) of fresh section of copper surface prepared by  
477 FIB after 3 h exposure to 0.9% NaCl. OM images and SEM images share the same scale bars presented in (a) and (e),  
478 respectively. This position was repeatedly examined every 7 days. Dark areas surrounded by dotted lines in (a) are the  
479 regions pointed out by arrows in (e). Area marked with dotted line in (d) serves as an example for positions that suffered  
480 colour change compared to the same positions in (a). High magnification images of the square dash marked regions in (f-h)  
481 are further shown in the following Figure 8. A schematic description of the treatment process is shown below the graphs.

482

483 Figure 8

484 High resolution SEM images (a-c) (SE, 5 kV, coupon being 52° tilted) of fresh section of copper surface prepared by FIB  
485 after 3 h exposure to 0.9% NaCl solution. This position was repeatedly examined every 7 days (a-c), whose lower  
486 magnification images are already presented in Figure 7. Zone covered by light blue colour in (a) highlights the cross-section  
487 (edge of the hole). Image (d) (SE, 5 kV) was taken from the Day 21 coupon. The cross shape indicator points out the  
488 position where the EDS spectrum (e) was collected.

Figure 1

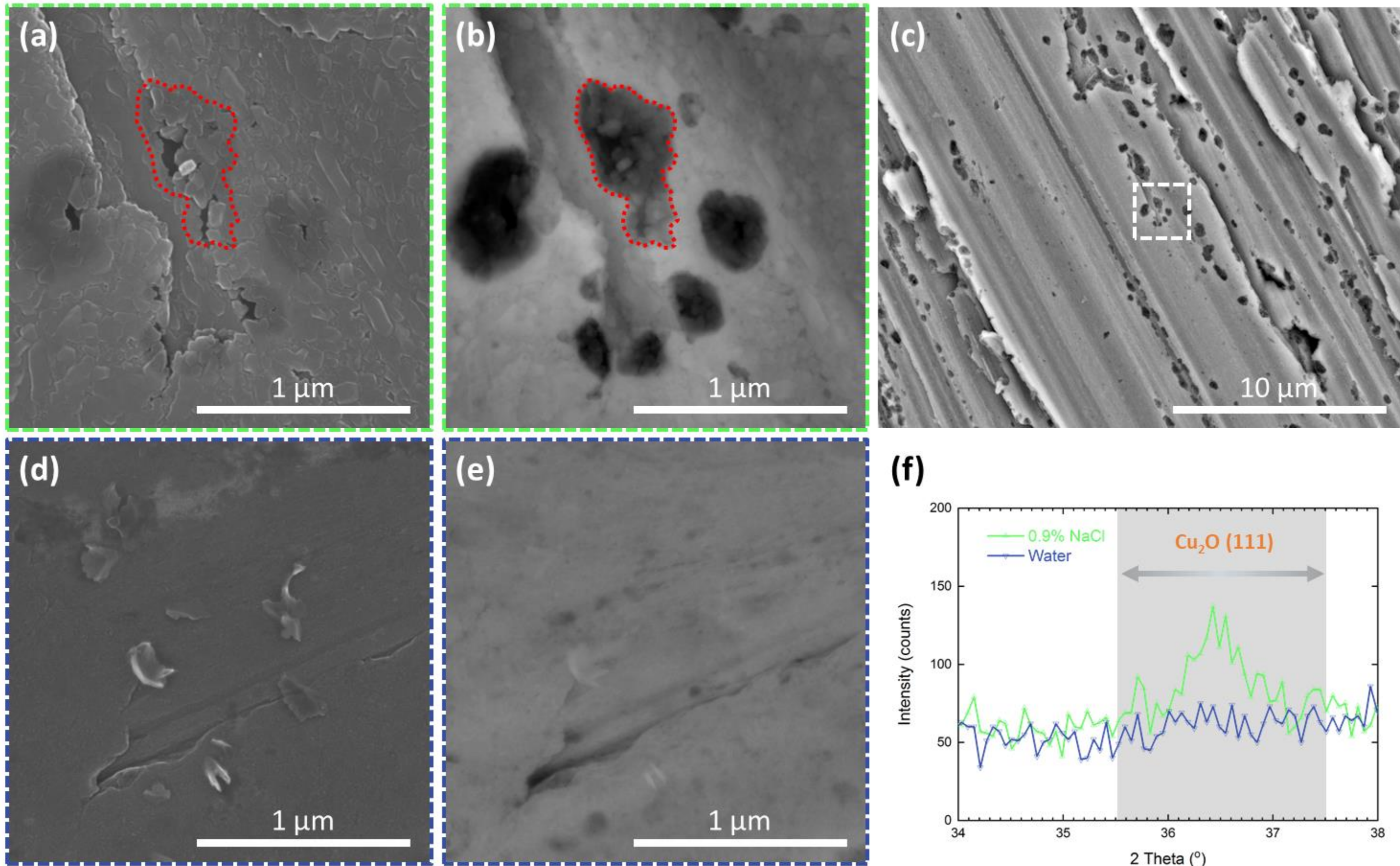


Figure 2

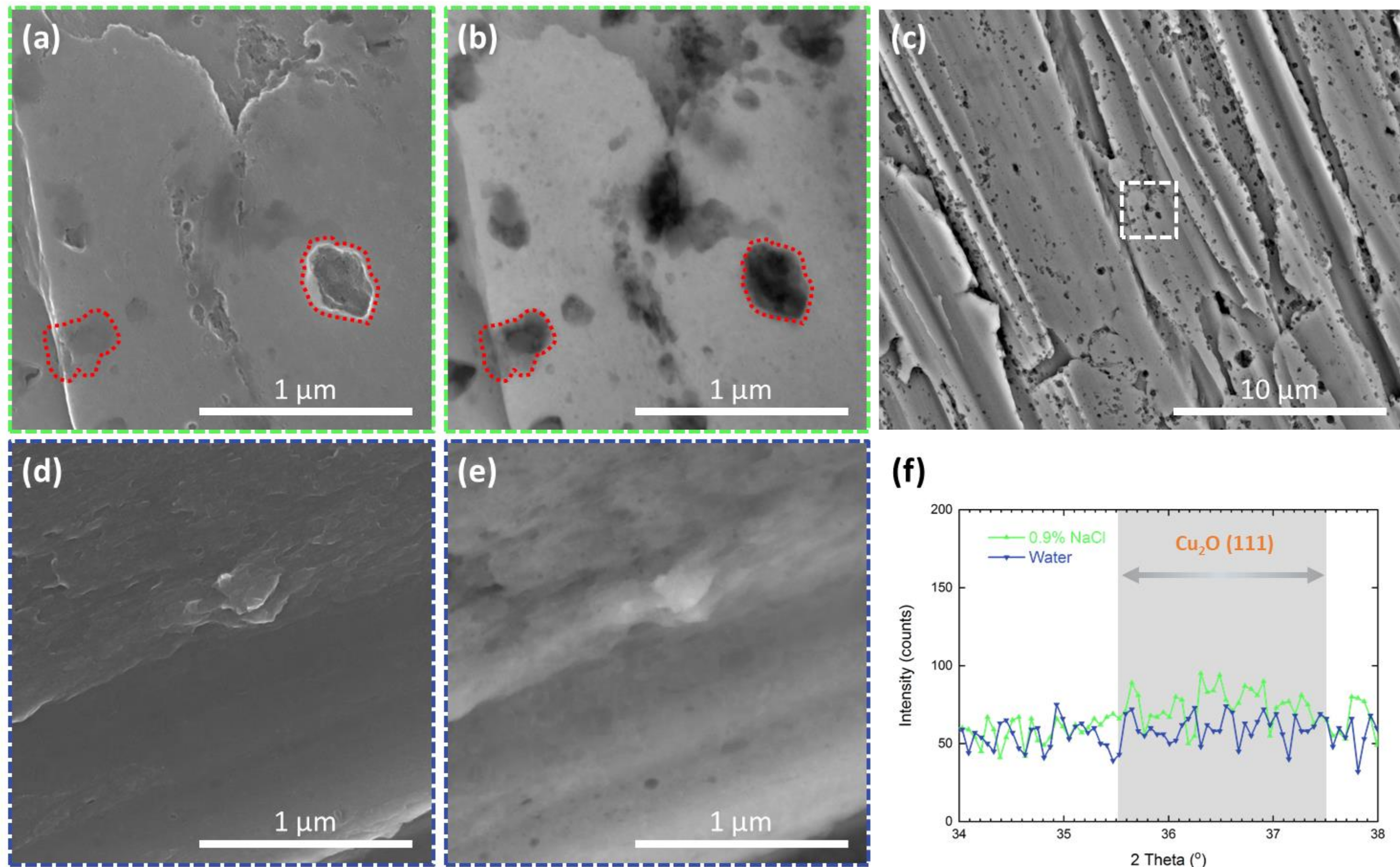
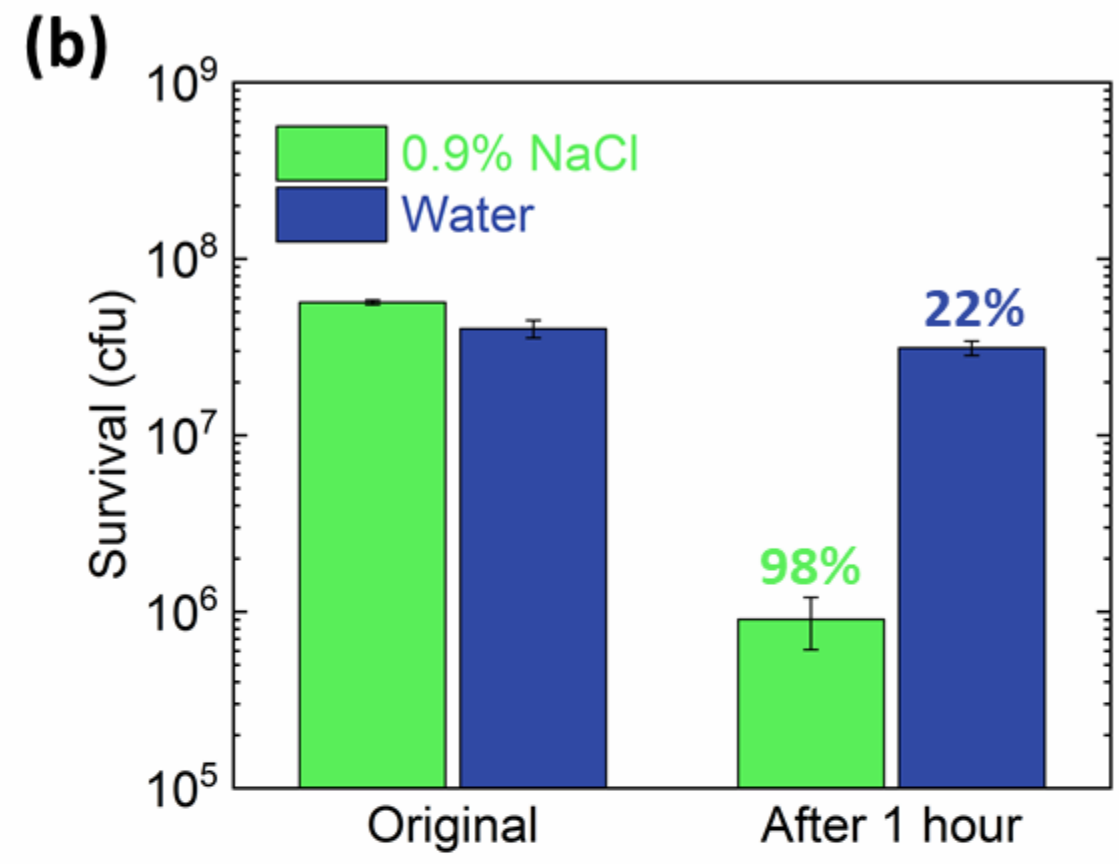
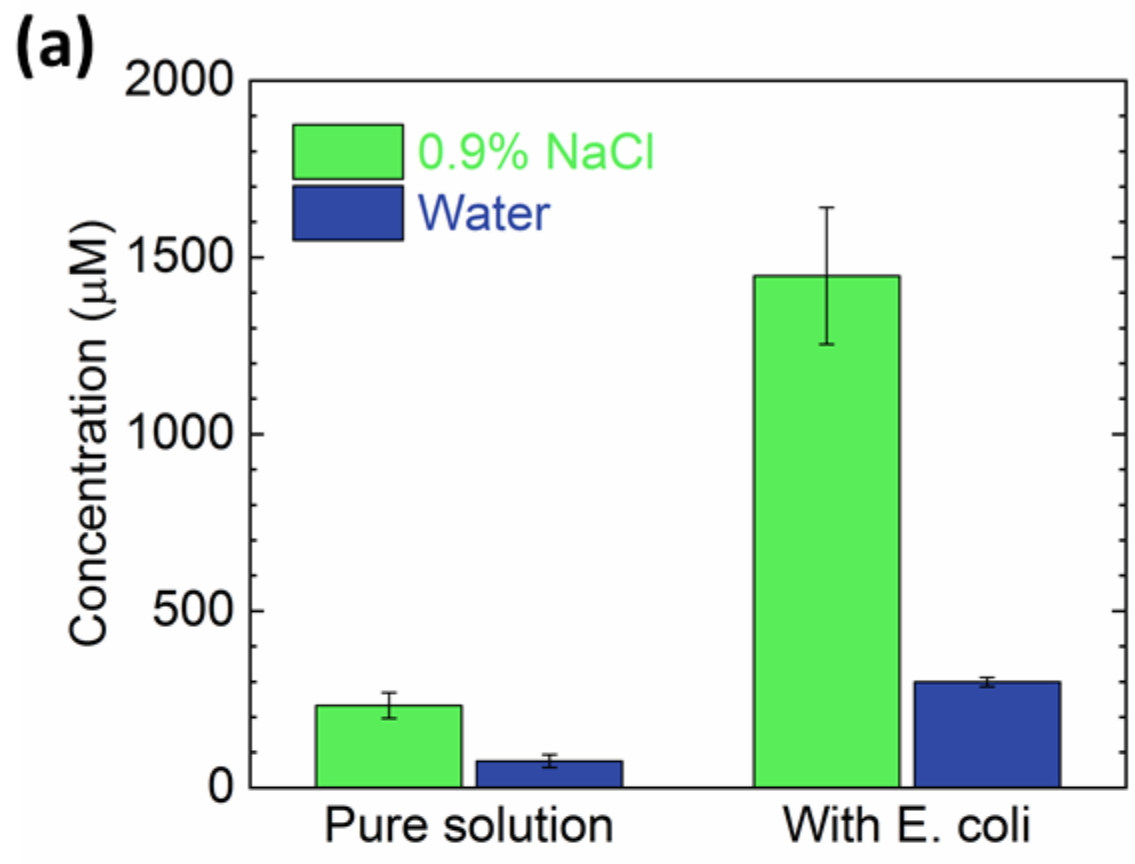


Figure 3



**Copper concentration (µM)**

Pure solution		With <i>E. coli</i>	
0.9% NaCl	Water	0.9% NaCl	Water
232.64	75.29	1447.60	299.15

**Survival (cfu)**

Original		After 1 hour	
0.9% NaCl	Water	0.9% NaCl	Water
5.66E+07	4.01E+07	9.07E+05	3.12E+07

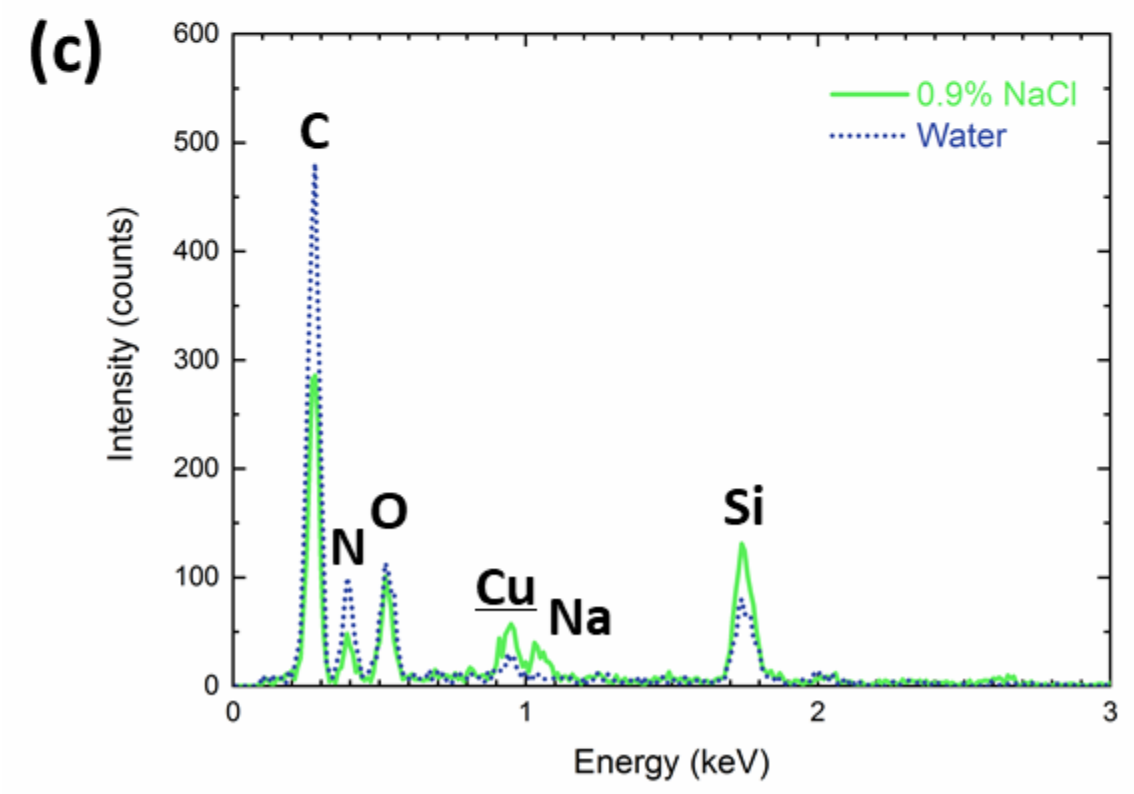


Figure 4

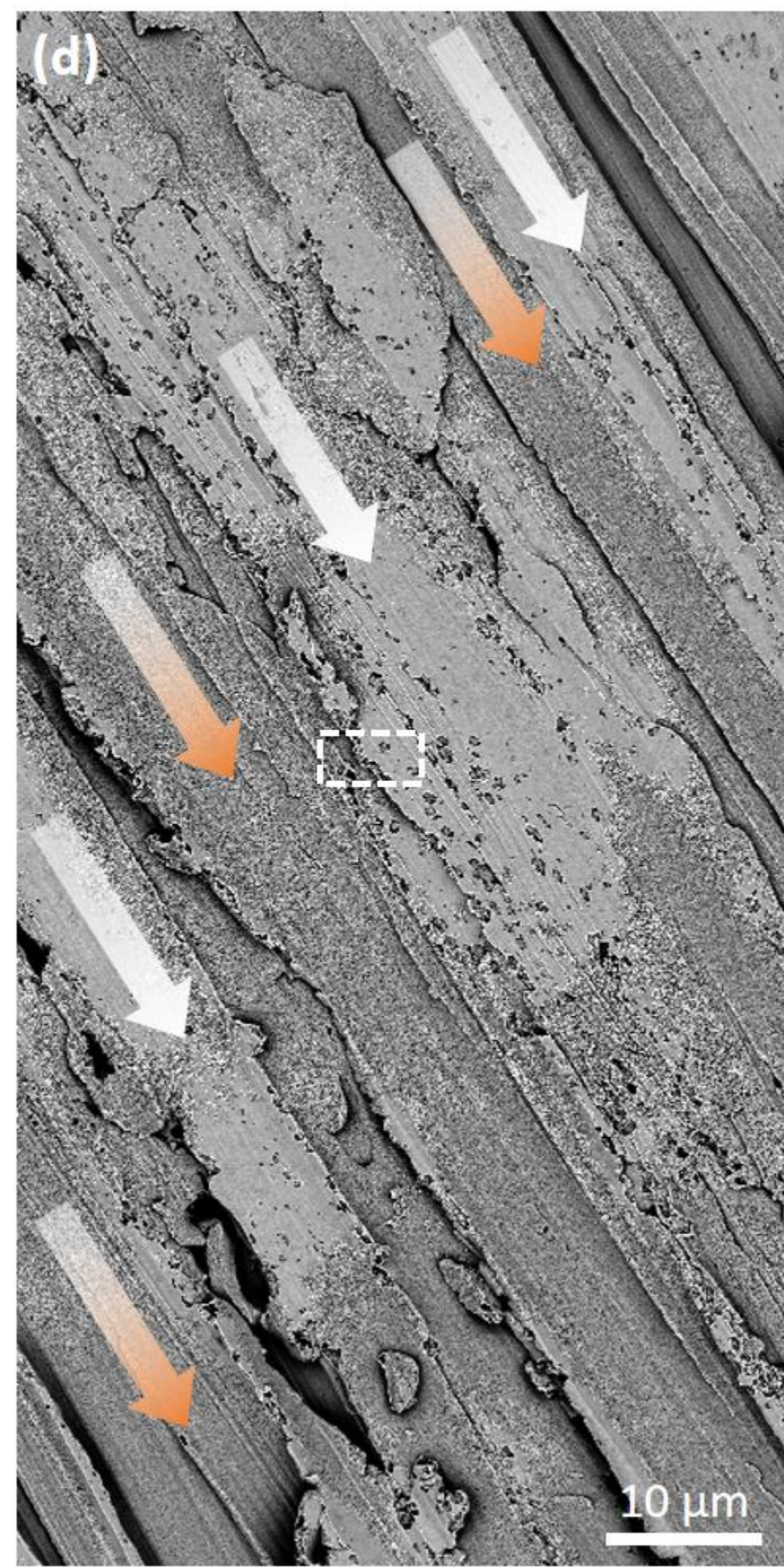
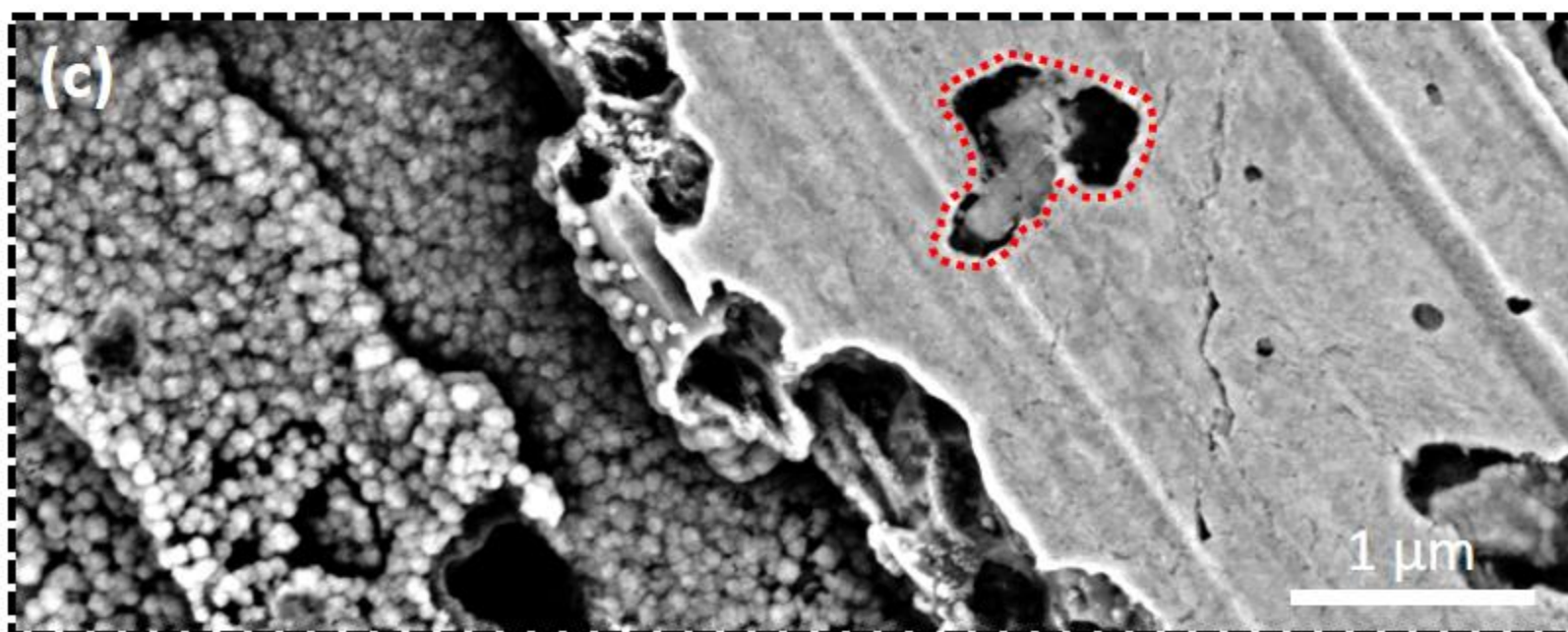
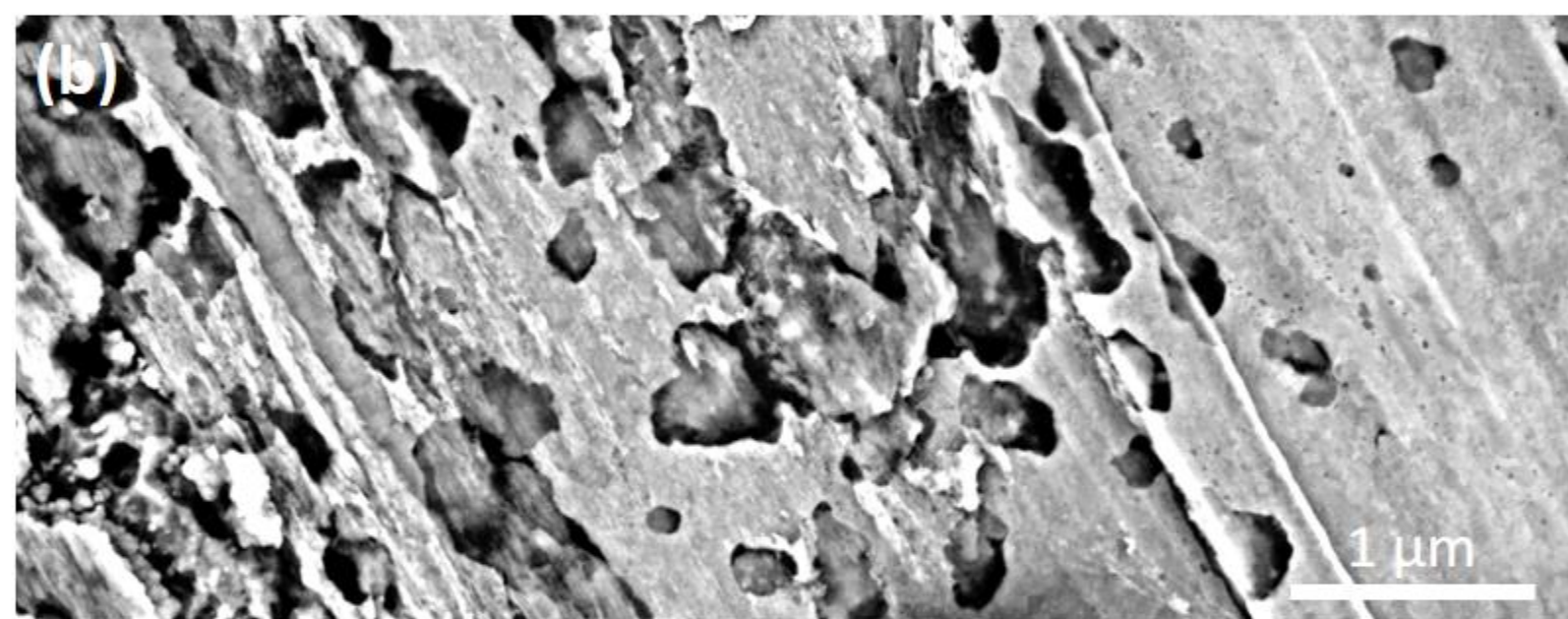
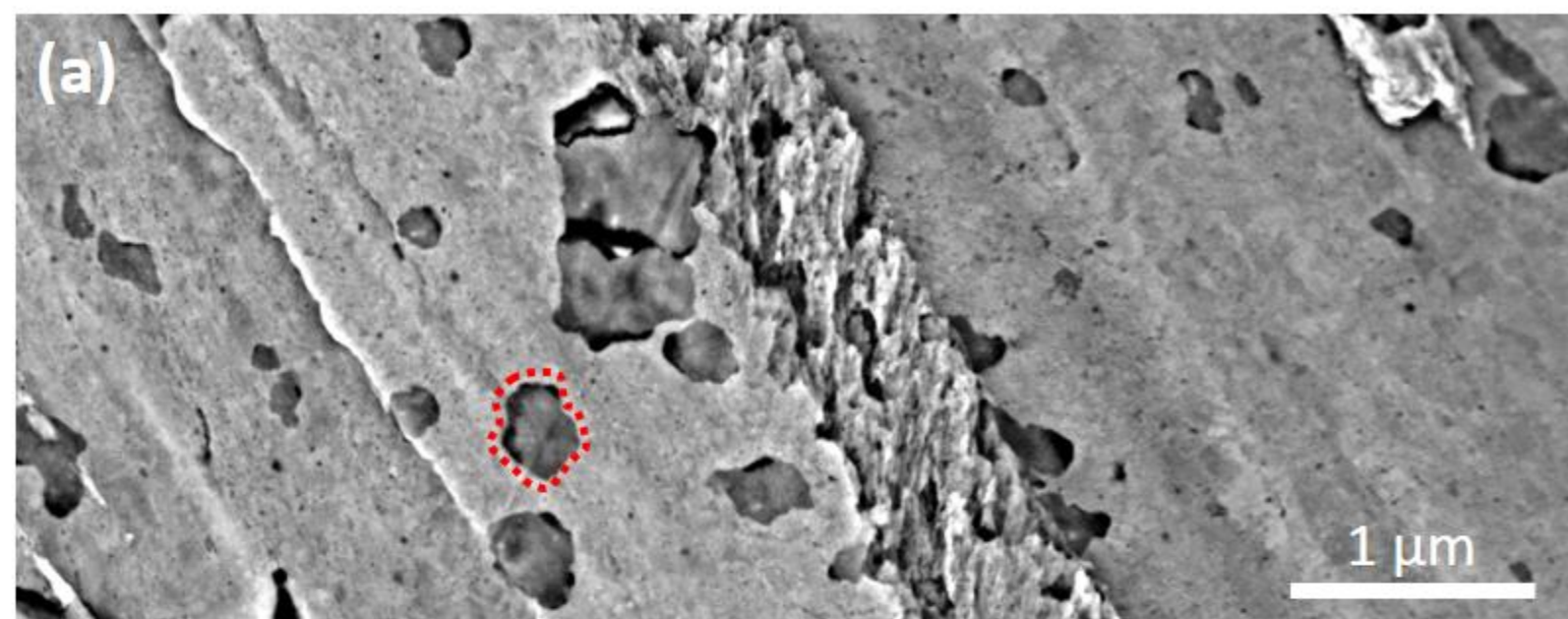
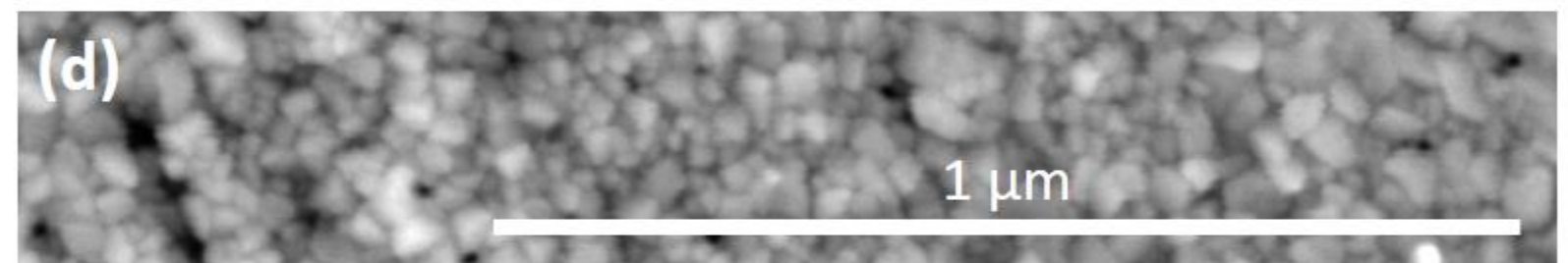
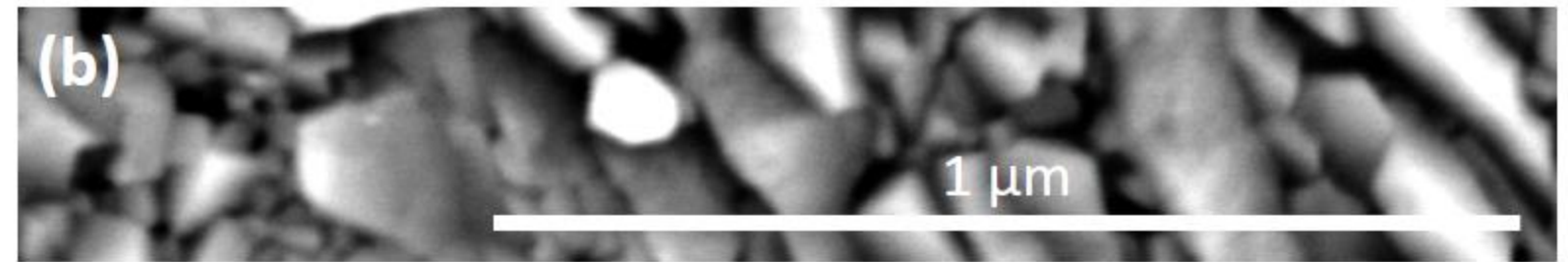
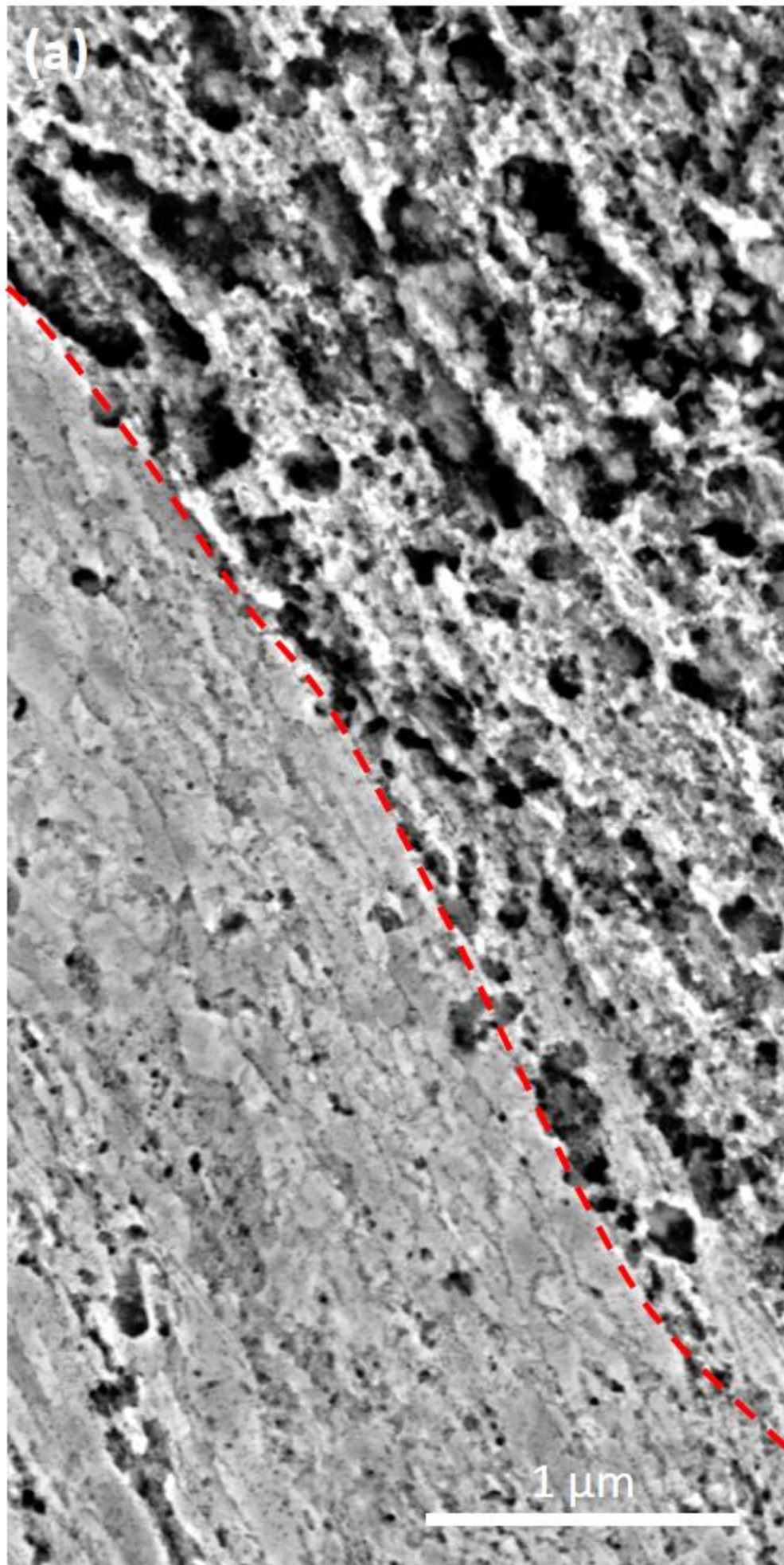


Figure 5



(e)

Oxide growth	Corrosion sites	0.1 X <i>E. coli</i>		1 X <i>E. coli</i>		5 X <i>E. coli</i>	
0.45% NaCl		+++	-	X	--	X	---
0.9% NaCl		++	-	X	--	X	---
1.8% NaCl		+	-	++	--	X	---

Figure 6

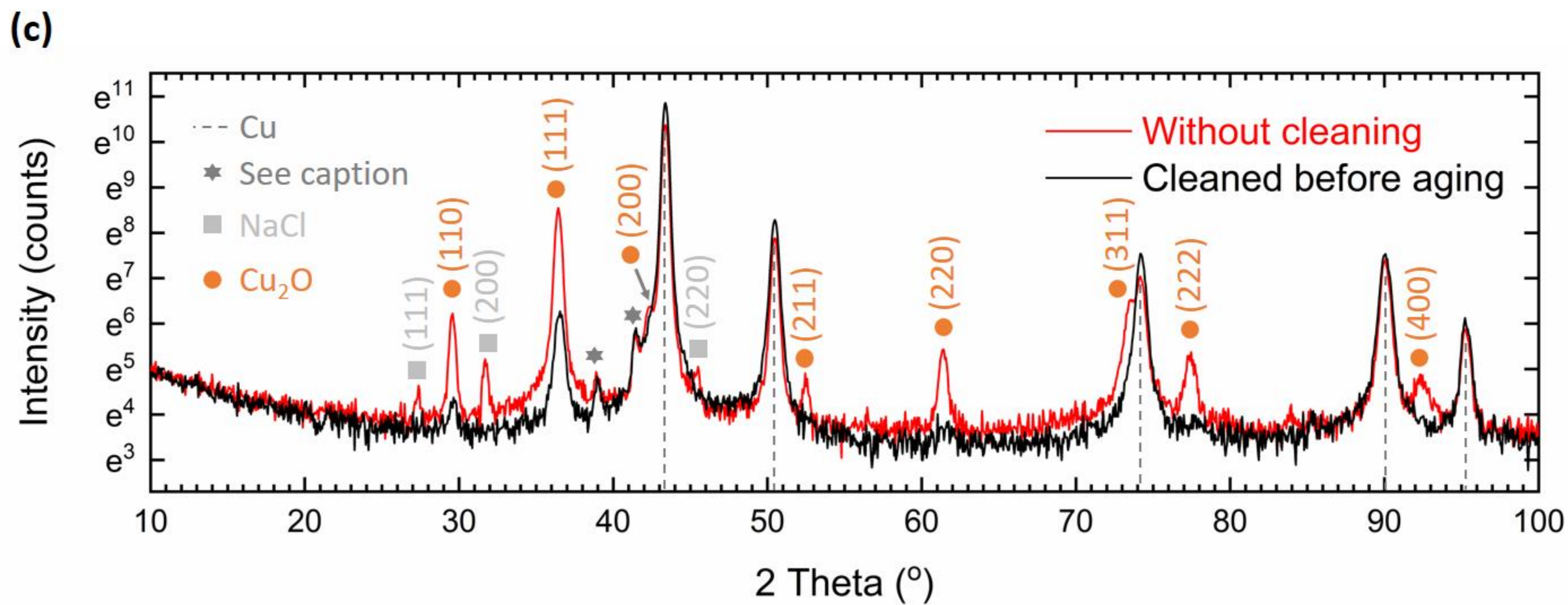
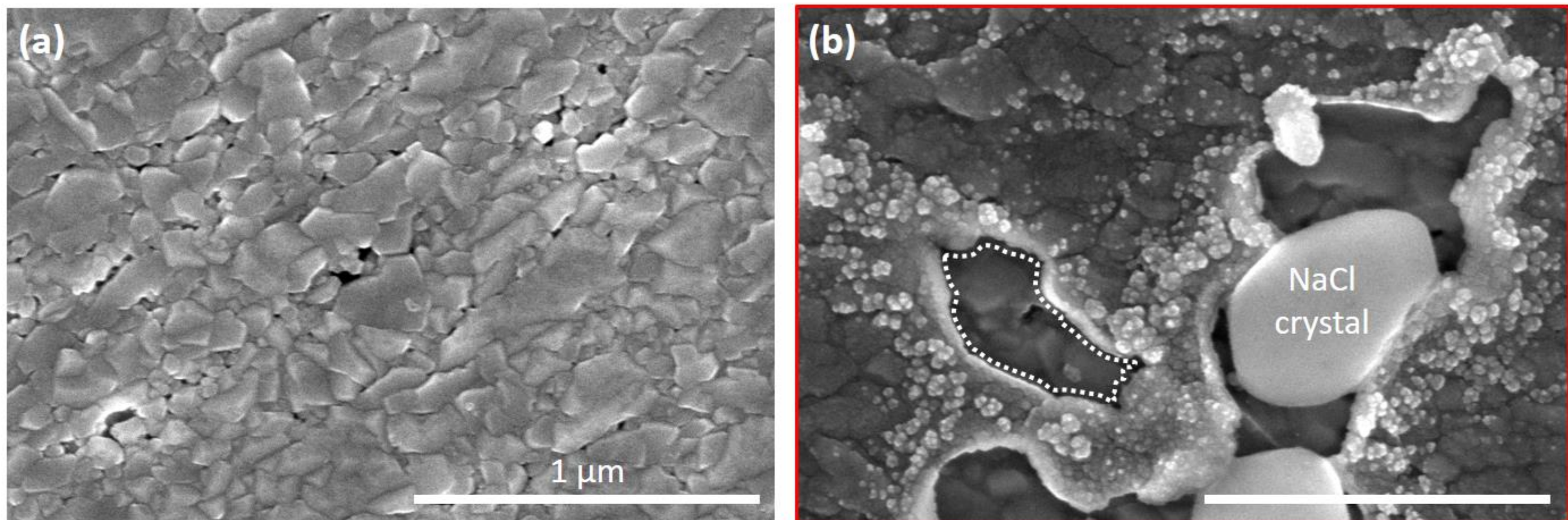
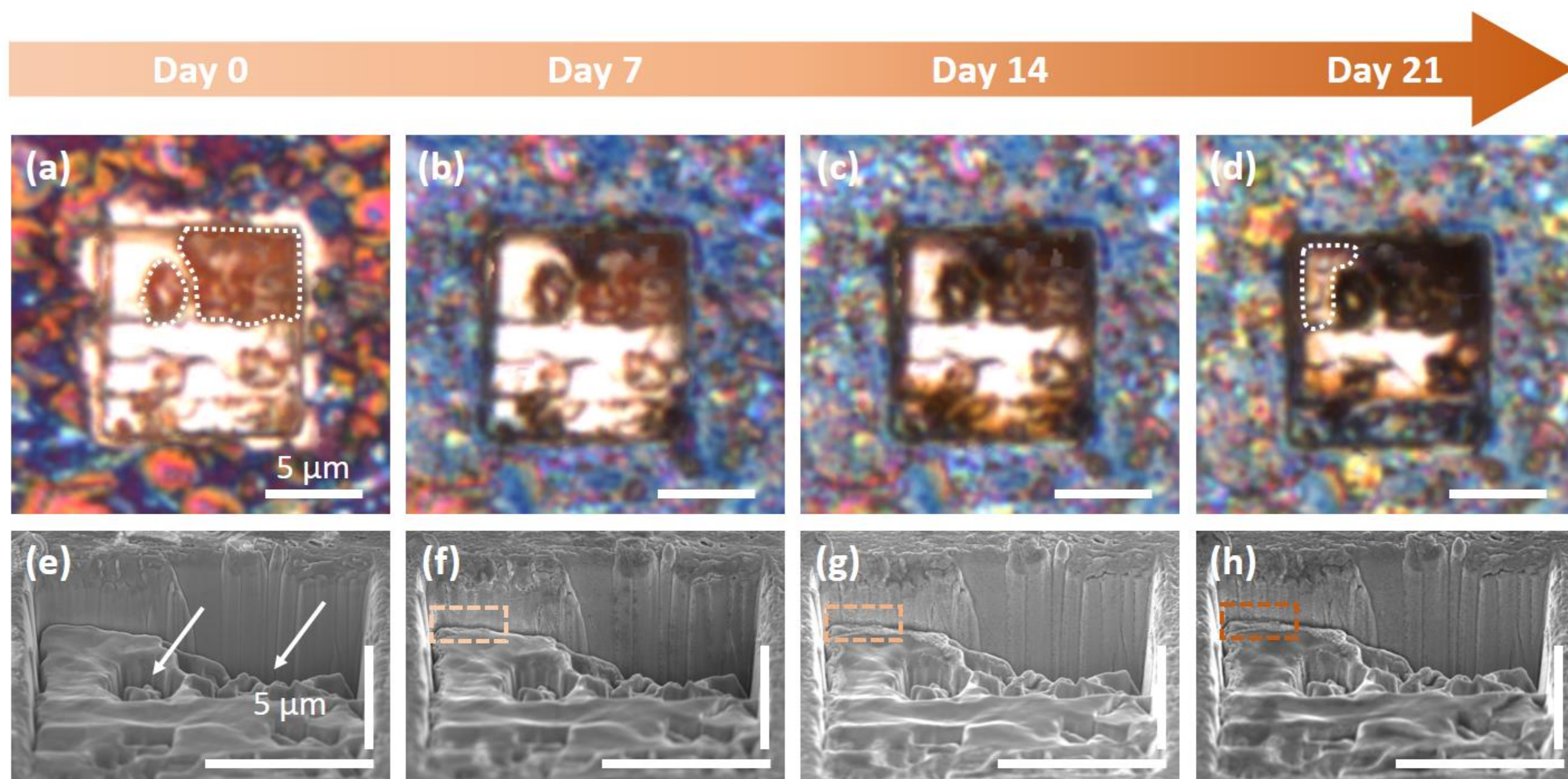
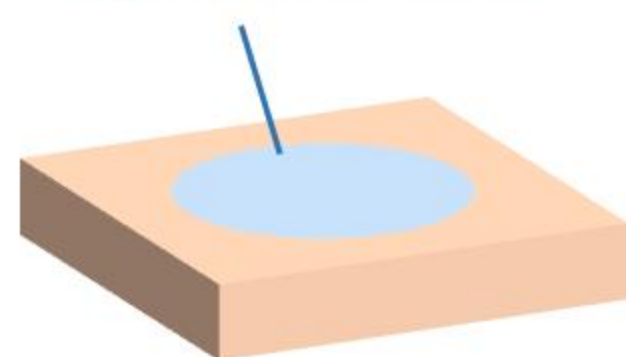


Figure 7



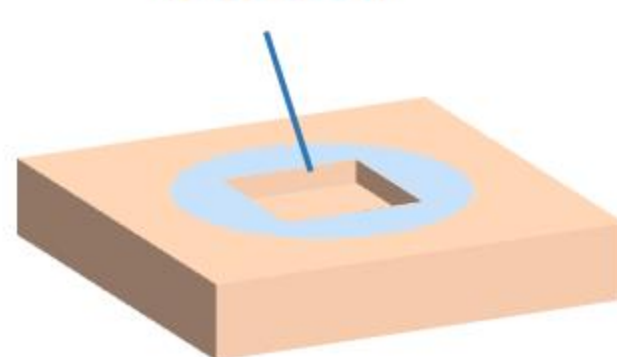
Oxidised copper after exposure to saline



Hole fabrication by FIB



"Fresh" area revealed



Atmospheric aging



"Fresh" area also got oxidised

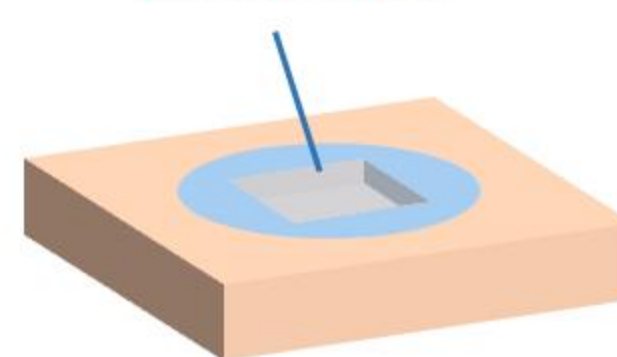
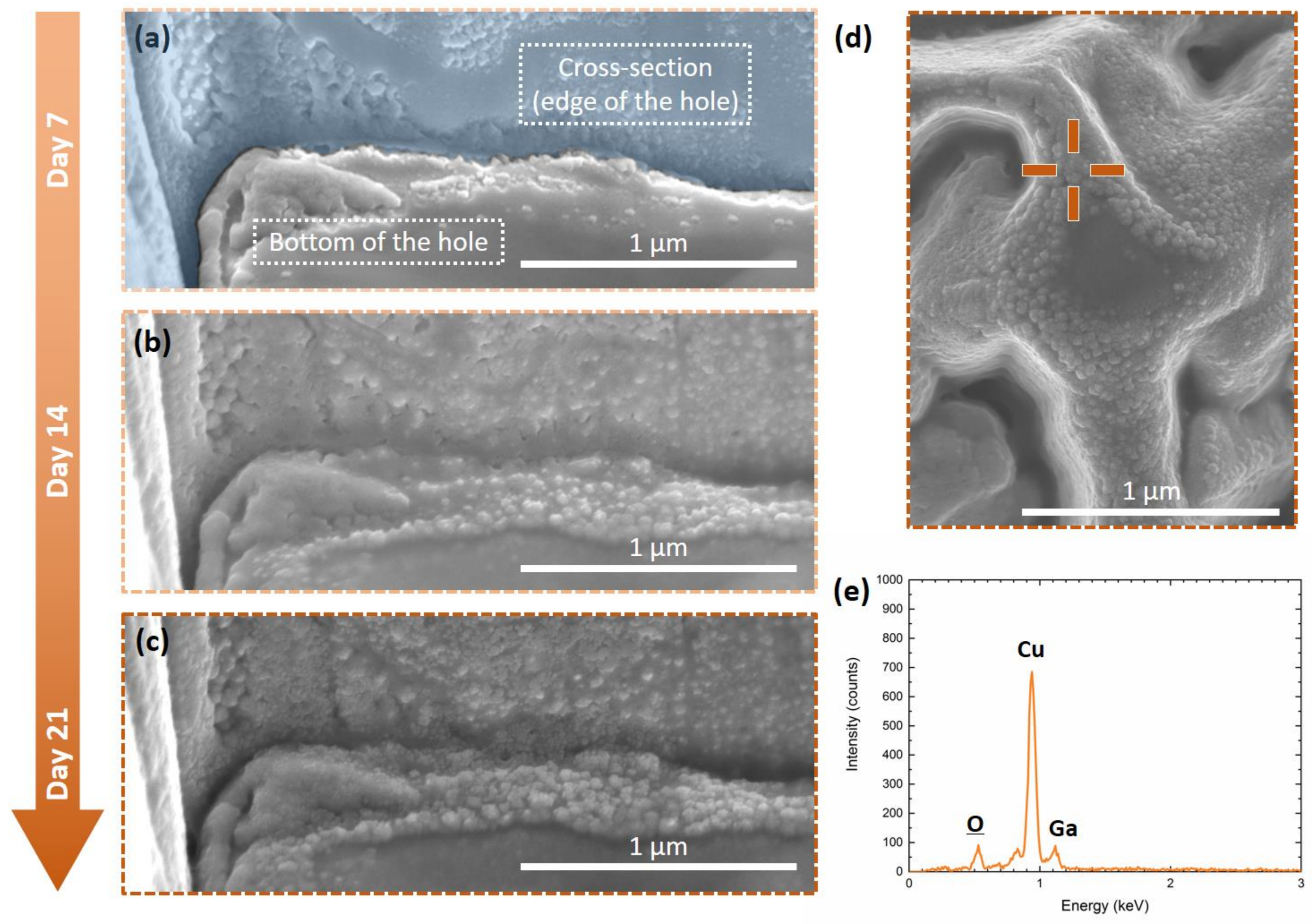
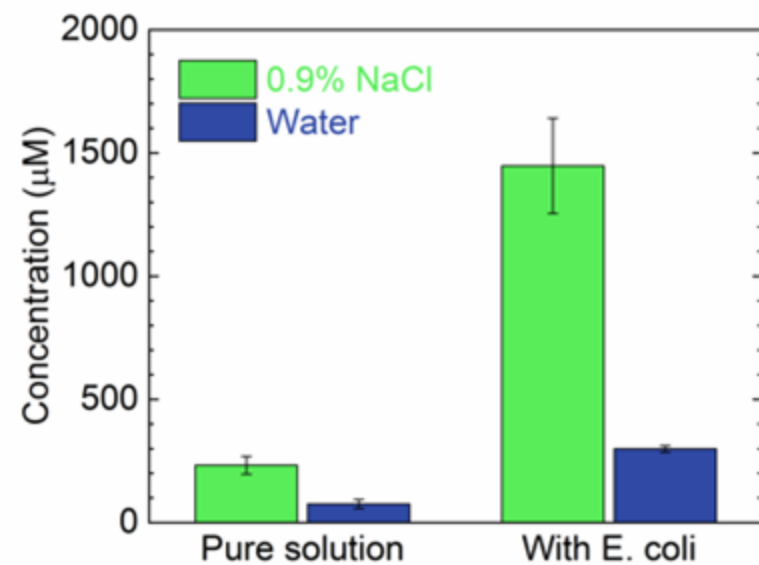


Figure 8

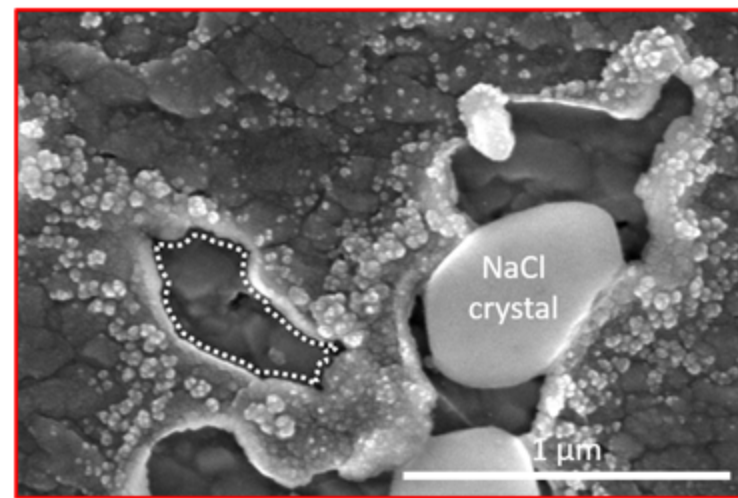
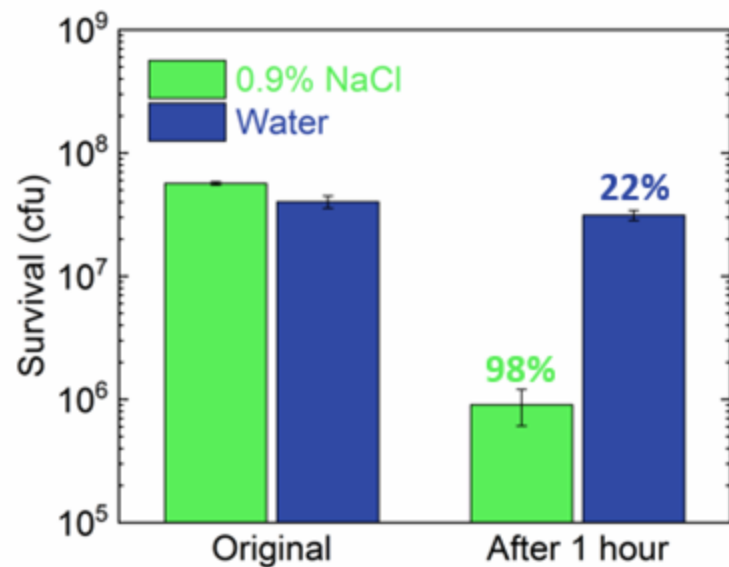


# Sodium chloride (NaCl) for copper surface

## 1. Assists copper release



## 3. Introduces atmospheric corrosion



## 2. Enhances antibacterial efficiency