Corrosion Behavior and Biocompatibility of Diamond-like Carbon-Coated Zinc: An In Vitro Study

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ABSTRACT: Owing to the desirable degradation rate and good biocompatibility, zinc (Zn) and Zn alloys are promising biodegradable implant metals in orthopedic and cardiovascular applications. Surface modification, such as deposition of coatings, is frequently implemented to further enhance their biological properties. In this study, diamond-like carbon (DLC) films are deposited on Zn by magnetron sputtering. The DLC films do not change the surface morphology of Zn but alter the hydrophobic properties with a contact angle of approximately 90°. Electrochemical and in vitro immersion tests reveal that the corrosion resistances of the DLC-coated Zn decrease unexpectedly, which is possibly due to galvanic corrosion between the DLC film and Zn substrate. Furthermore, the uncoated and coated Zn samples show hemolysis ratios less than 1%. The cells cultured in the Zn extract exhibit higher viability than those cultured in the extract of the DLC-coated Zn, suggesting that the DLC films decrease the cytocompatibility of Zn. The lower corrosion resistance has little influence on the hemolysis ratio, suggesting that hemolysis is not an obstacle for the design of Zn-based biomaterials. Our results show that the traditional concept of protection with DLC films may not be applicable universally and decreased corrosion resistance and cytocompatibility are actually observed in DLC-coated Zn.

1. INTRODUCTION

Biodegradable metals are considered to be next-generation biomaterials and magnesium, zinc, and iron are suitable candidates.1,2 With a standard electrode potential of −2.37 V versus standard hydrogen electrode (NHE), Mg degrades too fast naturally for many orthopedic applications (within 1−4 months), resulting in premature loss of mechanical strength and even failure of implants.3,4 Rapid degradation also produces a high-alkalinity microenvironment in the vicinity of the implant and accumulation of hydrogen, which may compromise tissue healing. On the other hand, Fe has a standard electrode potential of −0.44 V versus NHE and the degradation rate is too slow, meaning that the implant will remain in the body for a long time (over 2−3 years).5 Furthermore, corrosion products such as Fe(OH)3 and Fe3O4 cannot be excreted or metabolized easily.6,7 In comparison, the standard electrode potential of Zn is −0.73 V versus NHE and its moderate degradation rate may benefit wound healing.8 In addition to the more desirable degradation rate, Zn is a vital element essential to cellular transport in the forms of Zn fingers and Zn receptors.9 Zn can react with testosterone, thyroid hormones, and hormones in bone formation.9 The recommended daily intake of Zn is 8−20 mg/day for mature adults, and Zn deficiency impedes growth and development in infants and also causes decline in cognitive functions and behavioral problems.10

A cranial-maxillofacial internal fixation system made with Zn alloy was implanted into a patient in Air Force Medical University Stomatological Hospital (Xi’an, China), signaling large clinical potential. However, Zn also has some drawbacks. The strength and ductility of Zn are not sufficient for load-bearing implants, such as cardiovascular and orthopedic implants. In order to improve the biomechanical performance of Zn implants, researchers have developed various kinds of Zn
alloys such as Zn–Mg and Zn–Li.\textsuperscript{11,12} Fast release of Zn ions from the Zn substrate in the initial stage after implantation can adversely affect the cell viability because cells have a relatively low tolerance against Zn ions. Ma et al. used Zn-contained culture medium to culture endothelial cells and found that a concentration of approximately 6.5 ppm Zn ions would suppress the viability of the cells.\textsuperscript{13} Furthermore, in vitro cell cultures reveal that almost no cells can stay alive when directly cultured on Zn or Zn alloy surfaces.\textsuperscript{14,15} Owing to the inherent low electrode potential of Zn, alloying may not be suitable to avoid fast release of Zn ions from Zn-based implants, and therefore, surface modification to construct a physical barrier to suppress fast release of Zn ions appears to be a viable approach to improve the biocompatibility. However, these studies have mainly been limited to chemical conversion coatings, anodic oxidation, and plasma electrolytic oxidation,\textsuperscript{16–18} while other common techniques such as spray coating, atomic layer deposition, and magnetron sputtering are not very common.\textsuperscript{19–21} Magnetron sputtering is an industrial technique to deposit high-quality coatings for different applications, among which diamond-like carbon (DLC) films have both high corrosion resistance and biocompatibility.\textsuperscript{22–24} However, there is no report on the corrosion behavior and biological performance of DLC films on Zn. In this study, DLC films are deposited on Zn by magnetron sputtering and the corrosion and biological properties are investigated systematically.

2. RESULTS AND DISCUSSION

2.1. Coating Characterization. The surface morphologies of the Zn and coated Zn samples are shown in Figure 1a, which reveal that sandpaper grinding and magnetron
sputtering do not alter the surface morphology appreciably. Figure 1b depicts the optical photographs, and Zn shows a silver-white appearance. After magnetron sputtering for 15 min (C-1#), the surface changes to light yellow and the color becomes darker with sputtering time. The color change provides evidence about successful deposition on C-1#, C-2#, and C-3#. The cross-sectional view of the C-3# sample suggested that the thickness of DLC coating was approximately 100 nm (Figure S1). Figure 1c shows the X-ray diffraction (XRD) patterns, and only Zn metal peaks are detected at 36.3, 38.9, 43.3, and 54.3° (JPCDS 04-0831) for all patterns, suggesting that the deposited films are thin or amorphous. Figure 1d shows the Raman spectra. For the hydrogen-free DLC film, the G peak at ∼1560 cm⁻¹ and D peak at ∼1380 cm⁻¹ represent the breathing mode of sp² bonded C atoms and bond stretching of all pairs of sp² atoms in chains and rings, respectively. Figure 1d shows a weak D peak and strong G peak from C-1#, C-2#, and C-3#, further corroborating successful deposition. Ultrasonic cleaning assay was applied to test the bonding force of DLC coating on Zn. No damaged area or color change was observed for the coating after ultrasonic cleaning (50 W, 40 kHz) in deionized water (Figure S2), suggesting the strong bonding force between the DLC coating and the Zn substrate.

The surface wettability is closely related to the corrosion resistance and cell adhesion. In general, a larger water contact angle indicates that it is more difficult for the liquid to permeate into the substrate and better corrosion resistance is expected. However, a smaller contact angle suggests stronger interactions between the substrate and liquid, bonding well for cell adhesion. The water contact angles of the samples are presented in Figure 2. The untreated Zn exhibits a contact angle of approximately 60°, and after coating with a layer of DLC, the contact angles of C-1#, C-2#, and C-3# increase to approximately 90°. The wettability is determined by the surface roughness and surface physical phase. In this study, the surface morphologies of the uncoated and DLC-coated Zn samples are similar, suggesting that the roughness is about the same. Therefore, the DLC films are responsible for the larger water contact angles. In fact, DLC films are widely used to fabricate a hydrophobic surface on various materials.

2.2. Corrosion Resistance. The corrosion resistance of the uncoated and DLC-coated Zn samples is investigated by electrochemical and immersion tests. Figure 3a shows the polarization curves, and the calculated values are displayed in Table 1. The anodic branches of the polarization curves of the coated samples are in principal identical to those of the bare sample, which might be because many nanometer holes exist among DLC coating and many areas of the Zn substrate can be in direct contact with water. Compared to the uncoated Zn, all the DLC-coated samples showed larger $E_{\text{corr}}$ and $j_{\text{corr}}$ values. Bigger $E_{\text{corr}}$ values suggest that the DLC-coated samples have better corrosion resistance, and a larger $j_{\text{corr}}$ value denotes the onset of corrosion for fast degradation. The $E_{\text{corr}}$ values of the three DLC-coated samples are about the same, but for a longer deposition time, the $j_{\text{corr}}$ value decreases slightly, indicating better corrosion resistance. The corrosion resistance follows the trend of Zn > C-3# > C-2# > C-1# and $R_p$ confirmed the tendency. Figure 3b shows the cumulative release of Zn ions from various samples after immersion in phosphate-buffered saline (PBS) for 7 days. The uncoated Zn sample shows the lowest Zn release followed by C-3#, C-2#, and C-1#, in line with the electrochemical results. That is, the DLC-coated Zn samples have worse corrosion resistance. Because Zn²⁺ would react with PO₄³⁻ to form the precipitate Zn₃(PO₄)₂ in PBS solution, the Zn²⁺ in the extract cannot precisely reflect the corrosion rate of bare Zn and coated Zn. Nevertheless, the release of accumulation concentration of Zn²⁺ in PBS can partially represent the corrosion resistance of various samples. Hence, we did not calculate the corrosion rates for the immersion test.

To investigate the corrosion mechanism of the uncoated and DLC-coated Zn samples, the morphologies of the corroded sample surfaces are examined after immersion in PBS. The result is shown in Figure 4. After corrosion for 1 day (Figure 4a), some particles appear on the surface of all the samples, but the marks created by the abrasive sandpaper are still visible on the uncoated sample. For the three DLC-coated samples, the structures caused by the abrasive sandpaper are almost covered by the corrosion products composed of nanoparticles. Notably, big particles of corrosion products on the surface of Zn and C-2# and C-3# were of the similar size. It is because C-1# exhibited the largest amount of released Zn ions (Figure 3b), and thus, more crystal nuclei formed, finally resulting in the smallest particles. Less Zn ions were released from C-2# and C-3# than from C-1#, so less crystal nuclei formed, and finally, some crystal nuclei form particles with larger size. However, at high magnification, the bare Zn substrate was observed for the Zn group, while particles with small size were deposited on all the DLC-coated samples. After corrosion for 7 days (Figure 4b), the surface of the uncoated Zn is covered by a compact corrosion layer. For the three DLC-coated samples, a large number of corroded particles are visible on the surface, and there is a loose corrosion product layer under the particles. The elemental compositions are listed in Table 2, and Zn, O, and P are detected in all the samples. Notably, larger O and P concentrations are detected on the DLC-coated samples than on the untreated Zn sample. The phase compositions detected using the XRD technology are presented in Figure 4c,d. Zn₃(PO₄)₂ feature peaks were detected for DLC-coated samples. Because the ion type surrounding the bare Zn substrate was the same as that surrounding DLC-coated Zn, the same corrosion product would be formed on the bare Zn surface. It might be because the layer of the corrosion product was too thin, so the Zn₃(PO₄)₂ precipitate on bare Zn was not detected by XRD. More intense peaks of Zn₃(PO₄)₂ are observed in the DLC-coated samples, disclosing more corrosion products, which is consistent with energy-dispersive X-ray spectroscopy (EDS) results.
Zn$_3$(PO$_4$)$_2$ particles are smaller than those on the bare Zn. More Zn ions are released from the substrate and the cathode. At the anode, Zn loses electrons to form Zn$^{2+}$, and at the cathode, H$^+$ from H$_2$PO$_4^-$ and HPO$_4^{2-}$ reacts with electrons to form H$_2$. The released Zn ions react with PO$_4^{3-}$ to form the Zn$_3$(PO$_4$)$_2$ precipitate. Figure 5c shows the formation of the corrosion layer on the surface of Zn. During initial corrosion, large corrosion particles are deposited on the Zn surface, and as time elapses, a compact Zn$_3$(PO$_4$)$_2$ layer forms. The faster corrosion of the DLC-coated samples means that corrosion particles are deposited on the DLC and Zn substrate.37 In this system, the Zn substrate is the anode and the DLC film is the cathode. At the anode, Zn loses electrons to form Zn$^{2+}$, and at the cathode, H$^+$ from H$_2$PO$_4^-$ and HPO$_4^{2-}$ reacts with electrons to form H$_2$. The released Zn ions react with PO$_4^{3-}$ to form the Zn$_3$(PO$_4$)$_2$ precipitate. Figure 5c shows the formation of the corrosion layer on the surface of Zn. During initial corrosion, large corrosion particles are deposited on the Zn surface, and as time elapses, a compact Zn$_3$(PO$_4$)$_2$ layer forms. The faster corrosion of the DLC-coated samples means that more Zn ions are released from the substrate and the Zn$_3$(PO$_4$)$_2$ particles are smaller than those on the bare Zn surface (Figure 5d). As corrosion progresses, more Zn$_3$(PO$_4$)$_2$ particles are deposited on the DLC films, but it is difficult for the corrosion products on the DLC-coated Zn to form compact layers, probably due to its chemical stability property.

### 2.3. Hemolysis Ratios and Cytocompatibility

The hemolysis ratio is a critical parameter for biomaterials, reflecting the damage to the surrounding erythrocyte. A hemolysis ratio larger than 5% is unacceptable in clinical applications. Figure 6a shows the hemolysis ratios of the samples. Because the corrosion resistance of the DLC-coated samples is poorer than that of the untreated Zn sample, the uncoated Zn and DLC-coated Zn samples exhibit ratios less than 5%, which are superior to Mg and Mg alloys, whose hemolysis ratios are larger than 50% caused by their poor corrosion resistance.38 The cytocompatibility is evaluated using the extracts. Figure 6b,c shows the cell viability of MC3T3-E1 after culturing in various extracts for 1 and 3 days, respectively. The cells cultured in the four 100% extracts exhibit very low viability (<20%) on day 1, and after culturing for 3 days, almost no living cells are detected in all the groups. When the extracts are diluted to 50%, the cell viabilities increase on day 1, especially for the bare Zn. However, on day 3, the cells in the DLC extracts show sharply decreased viability, but the cells cultured in the 50% Zn extract still show a viability of 85%. The viabilities of the cells cultured in the 25% extracts are similar to those cultured in the 50% extracts. After culturing for 3 days in the 25% extracts, the cell viability increases in the extracts of C-1#, C-2#, and C-3#. This trend is consistent with that of the corrosion resistance of the DLC-coated samples. In general, the bare Zn shows better cytocompatibility than the DLC-coated samples. Cells are sensitive to Zn ion concentration. It is reported that a Zn concentration of 6.5 ppm is high enough to inhibit the proliferation of endothelial cells and L929.13,39,40 However, as a comparison, when the concentration of Mg ions reaches approximately 790 ppm, it would damage the cell viability of rat bone marrow stem cells (rBMSCs).41 In this study, according to the immersion test, Zn ion concentration in the PBS extract was approximately 2 ppm. Considering that Zn ions would react with PO$_4^{3-}$ to form the precipitate Zn$_3$(PO$_4$)$_2$ in PBS solution and the extract obtained in the cell experiment was with an expose area of 4 mL/cm$^2$ (for the immersion test, the expose area was 10 mL/cm$^2$), the Zn ions in the culture medium were expected to be far higher than 2 ppm. Hence, the 100% extract was deadly for MC3T3-E1 cells, and after dilution, the cytotoxicity of the extract decreased along with the decrease in Zn ions. In our previous study, we also found that an excessive introduction of Zn ions to the surface of biomaterials exhibited cytotoxicity to rBMSCs.42

![Figure 3. (a) Potentiodynamic polarization curves and (b) cumulative release of Zn ions from different samples immersed in PBS for 7 days.](https://doi.org/10.1021/acsomega.1c00531)

**Table 1. Electrochemical Corrosion Parameters of Various Samples in PBS**

<table>
<thead>
<tr>
<th></th>
<th>$E_{corr}$ (V)</th>
<th>$j_{corr}$ (A/cm$^2$)</th>
<th>$R_{p}$ (kΩ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn#</td>
<td>−1.43</td>
<td>$3.17 \times 10^{-5}$</td>
<td>34.29</td>
</tr>
<tr>
<td>C-1#</td>
<td>−1.41</td>
<td>$1 \times 10^{-4}$</td>
<td>14.23</td>
</tr>
<tr>
<td>C-2#</td>
<td>−1.41</td>
<td>$8.34 \times 10^{-5}$</td>
<td>16.25</td>
</tr>
<tr>
<td>C-3#</td>
<td>−1.41</td>
<td>$6.18 \times 10^{-5}$</td>
<td>22.56</td>
</tr>
</tbody>
</table>
Although the corrosion resistance and cytocompatibility of the DLC-coated Zn samples decrease, there are several lessons we can learn from this study in order to guide and provide insights into the design and fabrication of coatings on Zn. First, the lower corrosion resistance has little influence on the hemolysis ratio, suggesting that hemolysis is not an obstacle for the design of Zn-based biomaterials. Second, considering the larger contact angle of the DLC-coated samples, a middle layer between the DLC coating and substrate (such as polydopamine) may suppress galvanic corrosion to improve the

Table 2. EDS Results of the Various Samples after Immersion in PBS for 7 Days

<table>
<thead>
<tr>
<th></th>
<th>O (at. %)</th>
<th>P (at. %)</th>
<th>Zn (at. %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn#</td>
<td>27.7 ± 3</td>
<td>3.2 ± 0.3</td>
<td>69.1 ± 3.2</td>
</tr>
<tr>
<td>C-1#</td>
<td>47.2 ± 3.6</td>
<td>5.3 ± 0.1</td>
<td>47.4 ± 3.4</td>
</tr>
<tr>
<td>C-2#</td>
<td>56 ± 3.2</td>
<td>5.8 ± 0.19</td>
<td>38.2 ± 2.2</td>
</tr>
<tr>
<td>C-3#</td>
<td>54.9 ± 3.1</td>
<td>6.0 ± 0.4</td>
<td>39.1 ± 3.3</td>
</tr>
</tbody>
</table>

Although the corrosion resistance and cytocompatibility of the DLC-coated Zn samples decrease, there are several lessons we can learn from this study in order to guide and provide insights into the design and fabrication of coatings on Zn. First, the lower corrosion resistance has little influence on the hemolysis ratio, suggesting that hemolysis is not an obstacle for the design of Zn-based biomaterials. Second, considering the larger contact angle of the DLC-coated samples, a middle layer between the DLC coating and substrate (such as polydopamine) may suppress galvanic corrosion to improve the
corrosion resistance and biocompatibility. Third, the type of magnetron sputtering target is important. If the sputtered film is conductive and possesses a higher electrode potential than Zn, galvanic corrosion may occur. Hence, Mg, Mn, and Zr targets may be more suitable for magnetron-sputtered films on Zn.

3. CONCLUSIONS

DLC films are fabricated on Zn by magnetron sputtering. Although the DLC films show hydrophobic properties, the corrosion resistance of the DLC-coated samples is worse than that of the untreated Zn due to galvanic corrosion between the coatings and the metal substrate. A compact Zn₃(PO₄)₂

Figure 5. Schematic illustration of the corrosion reactions on (a) bare Zn and (b) DLC-coated Zn. Formation processes of the corrosion layers on (c) bare Zn and (d) DLC-coated Zn.

Figure 6. (a) Hemolysis ratios of different samples. Cell viabilities of MC3T3-E1 cells cultured in various extracts for (b) 1 day and (c) 3 days.
corrosion layer is formed on the bare Zn surface, but loose Zn₃(PO₄)₂ particles are deposited on the DLC-coated Zn samples. Nonetheless, both the uncoated and DLC-coated Zn samples show favorable hemolysis ratios. The lower corrosion resistance has little influence on the hemolysis ratio, suggesting that hemolysis is not an obstacle for the design of Zn-based biomaterials. Cells cannot sustain the viability when cultured in the 100% extracts. Nevertheless, with the increase in magnetron sputtering time, the DLC coating exhibited better corrosion resistance and cytocompatibility. Our results show that DLC films prepared in this study cannot improve the corrosion resistance and biocompatibility of zinc, but a more compact and thicker DLC film might work.

4. EXPERIMENTAL SECTION

4.1. Sample Preparation. Pure Zn (≥99.995%, Longshicheng Instrument Equipment Co., Ltd, China) was cut into pieces with a thickness of 2 mm and diameter of 8 mm. The Zn samples were ground with silicon carbide abrasive paper (1000#, Haofu Abrasive Company, China) and cleaned ultrasonically in ethanol (Sinopharm, China). They were then transferred to a reactive magnetron sputtering system (ATC-Orin SUHV with Load-Lock, AJA International, Inc., USA) with a high-purity C target. During deposition, the pressure of the chamber was kept at 8 mTorr, Ar flow rate was 20 sccm, and direct-current power was 90 W. Deposition was conducted for 15, 30, and 60 min to produce C-1#, C-2#, and C-3#, respectively.

4.2. Surface Characterization. The microstructures of Zn, C-1#, C-2#, and C-3# were observed by scanning electron microscopy (SEM; Hitachi-S3400 N, Hitachi, Japan), and optical images were captured using a digital camera. XRD (Rigaku, Japan) was used to evaluate the phase composition, and Raman scattering was conducted on the LabRAM (Horiba Jobin Yvon, France). A contact angle measurement system (SL200B, Solon, China) was used to evaluate the surface wettability of the samples.

4.3. Electrochemical Evaluation. Potentiodynamic polarization was performed in the PBS using an electrochemical analyzer (CHI760C, Shanghai, China). A three-electrode cell with a graphite rod as the counter electrode, the saturated calomel electrode as the reference electrode, and the sample as the working electrode with an exposed area of 0.5 cm² was adopted. Before the assessment, the sample was stabilized in PBS for 400 s to obtain a stable open-circuit potential, and the test was carried out at a scanning rate of 10 mV/s. The corrosion potential (Ecorr) and current density (icorr) were derived by Tafel extrapolation. The corrosion resistance (Rp) was calculated according to the Stern–Geary formula

\[ j_{\text{corr}} = \frac{b_i b_k}{2.3(b_i + b_k)} \times \frac{1}{R_p} \]

where \( b_i \) and \( j_{\text{corr}} \) were obtained according to Tafel extrapolation.

4.4. Immersion Test. Immersion tests were conducted to evaluate release of Zn ions and morphology of the corroded sample surface. The backside of the sample without C deposition was sealed with silicone rubber to prevent the influence from the untreated side. For each group, four parallel samples were placed in a flat plastic bottle and 20 mL of PBS was added according to ASTM G31-71. After every 24 h, the PBS in the bottle was collected and 20 mL of fresh PBS was introduced. The Zn concentration in the collected PBS was determined by inductively coupled plasma atomic emission spectroscopy (Varian Liberty 150, USA). To examine the morphology of the corroded sample surface, the samples were immersed in PBS as aforementioned, and on days 1 and 7, the samples were obtained and rinsed with ultrapure water. After air drying, the corroded samples were examined by SEM, EDS, and XRD.

4.5. Hemolysis Ratio. The backside of the samples was sealed as described in Section 4.4, and the human blood was obtained from healthy adults. The hemolysis ratio assay was conducted according to the literature.\(^{44,45}\) In detail, 0.8 mL of the whole blood was diluted with 1 mL of 0.9 wt % NaCl. In the experiment, 0.9 wt % NaCl and ultrapure water were used as controls, respectively. The samples were transferred to a 24-well plate, to which 1.5 mL of 0.9 wt % NaCl was added. After incubation at 37 °C for 30 min, 30 μL of the diluted blood was added to each well and incubated at 37 °C for another 60 min. Afterward, the medium was collected and centrifuged at 3000 rpm for 5 min. A total of 100 μL of the medium of each sample was transferred to a 96-well plate, and the optical density (OD) was measured at 545 nm on the Cytation 5 multi-mode reader (Biotek, USA). The hemolysis ratio was calculated by the equation

\[ \text{Hemolysis ratio} = \left( \frac{\text{OD}_{545}\text{ of sample} {\text{positive control}}}{{\text{AP}_{545} - \text{AN}_{545}}} \times 100\% \right) \]

where \( \text{AS}_{545} = \text{AN}_{545} \), \( \text{AP}_{545} - \text{AN}_{545} \times 100\% \), where \( \text{AS}_{545} \), \( \text{AN}_{545} \), and \( \text{AP}_{545} \) are the OD values of the sample, negative control, and positive control, respectively.

4.6. Cell Viability. The backside of the samples was sealed as described in Section 4.4. The sterilized samples were placed on a 24-well plate, to which 2 mL of α-MEM culture medium was added. After incubation for 24 h, the extract was collected (denoted as 100% extract). The 100% extract was diluted with α-MEM culture medium two and four times and denoted as 50% extract and 25% extract, respectively. The MC3T3-E1 cells (5 × 10⁵ cell/well) were used for a 72-well plate. After culturing for 24 h, the culture medium was replaced with various extracts for each sample. After 1 and 3 days, the cell viability was assessed using AlamarBlue assay (Invitrogen, USA).

4.7. Data Analysis. All the measurements were conducted in triplicate. The results were presented as mean ± standard deviations (SD) and analyzed using GraphPad Instant software (GraphPad Prism software, Inc., USA). The significance of the differences among various groups was analyzed by one-way analysis of variance followed by Tukey–Kramer multiple comparison post-test for multiple comparisons. \( P < 0.05 \) was considered to be statistically significant.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c00531.

Cross-sectional view of the C-3# sample and surface view and optical view of the C-2# sample after ultrasonic cleaning for 1 min (PDF)

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Notes

The authors declare no competing financial interest.

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