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Three-dimensional superhydrophobic copper 7,7,8,8-tetracyanoquinodimethane biointerfaces with the capability of high adhesion of osteoblasts†

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A three-dimensional superhydrophobic copper 7,7,8,8-tetracyanoquinodimethane (CuTCNQ) nanowire array with the capability of high adhesion of osteoblasts was demonstrated. The CuTCNQ nanowire array was constructed by using a combined vapor deposition technique. The superhydrophobic nanowire array exhibited very high adhesion of osteoblasts, indicating that the CuTCNQ nanowire array was a good biointerface.

The surface properties of biointerfaces are very critical to determine the interactive manner between the surfaces and cells.[1–3] In general, significant properties of the surfaces of biointerfaces mainly include chemistry, morphology and wettability.[4–9] The manipulation of these properties has been considered as an important project for the achievement of a set of properties that may be more appropriate for specific biological functions. In recent years, based on the synergic principle of molecular recognition and topographic interaction, a series of biointerfaces have been developed and demonstrated to be effective and advantageous in the diagnosis and monitoring of various diseases.[10–12] On these biointerfaces, the interactive manner between the surfaces and cells can be effectively regulated, and thereby highly efficient and precise detection can be achieved.[13,14] However, the wettability of these biointerfaces is often neglected. Most of the biointerfaces seem largely to be superhydrophilic–hydrophobic. The biological response (the interaction between surfaces and cells) on the nanostructured superhydrophobic surface is rarely considered. Therefore, the construction of a superhydrophobic biointerface to understand the interactive manner between the surfaces and cells is greatly desired.

Here, we demonstrated a combined physical and chemical vapor deposition method to fabricate a 3D superhydrophobic nanowire array biointerface based on copper 7,7,8,8-tetracyanoquinodimethane (CuTCNQ). The wettability of CuTCNQ nanowire array could be finely tuned from hydrophobicity to superhydrophobicity solely by controlling the reaction time. The cell adhesion results demonstrated that the superhydrophobic CuTCNQ biointerfaces exhibited much higher osteoblast adhesion than the hydrophobic surfaces (Scheme 1).

CuTCNQ is one of the most promising semiconductor materials, which has great potential in biosensors,[15–17] field-effect transistors[18] and electrochromic devices[19] due to its excellent conductivity. The great potential in biosensors stimulates us to find out whether this promising material can act as a good biointerface to interact with cells? In our study, CuTCNQ nanowire arrays were fabricated *via* a combined physical and chemical vapor deposition technique.[20] In a typical synthesis, TCNQ powder was firstly placed in a quartz boat. Then, the indium tin oxide (ITO) glass substrates covered with Cu were reversely placed in TCNQ powder and finally heated at 150 °C under vacuum. The process allows the TCNQ to vaporise and react with copper for the fabrication of CuTCNQ complexes. The synthesized CuTCNQ nanowire arrays are shown in Fig. 1. The nanowires were grown perpendicularly on substrates and they exhibited very high uniformity. The length of the nanowires could be well tuned from 0.81 ± 0.08 μm (short), 2.7 ± 0.28 μm (medium) to 7.9 ± 0.04 μm (long). Meanwhile, it is obvious that the density of the nanowires also increased with the increase of the heating time.

Fig. 2a shows the UV-Vis spectrum of the nanowires, the peaks at 392 nm were attributed to neutral CuTCNQ^0, while the peaks at 420, 744 and 842 nm were assigned to the TCNQ anion radical of CuTCNQ.[21] Fig. 2b shows the FTIR spectrum of the nanowires. The band at 2200 cm^{-1} was the typical absorption of $\mathrm{C{\equiv}N}$ stretching. The absorptions at 1351, 1577 and 1506 cm^{-1} were assigned to $\mathrm{C{=}C}$ stretching. The EDX revealed the existence of C, N and Cu elements on the nanowires (Fig. S1†). Fig. 2c shows the XPS results of the nanowires. The binding energy at 932.5 eV and 952.2 eV was assigned to Cu $2p_{3/2}$ and Cu $2p_{1/2}$, suggesting that the valence of Cu was

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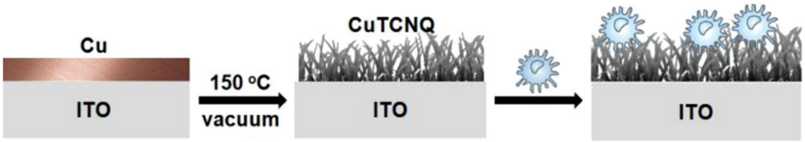

Scheme 1 Schematic illustration of the use of 3D CuTCNQ nanowire biointerfaces for highly efficient cell adhesion.

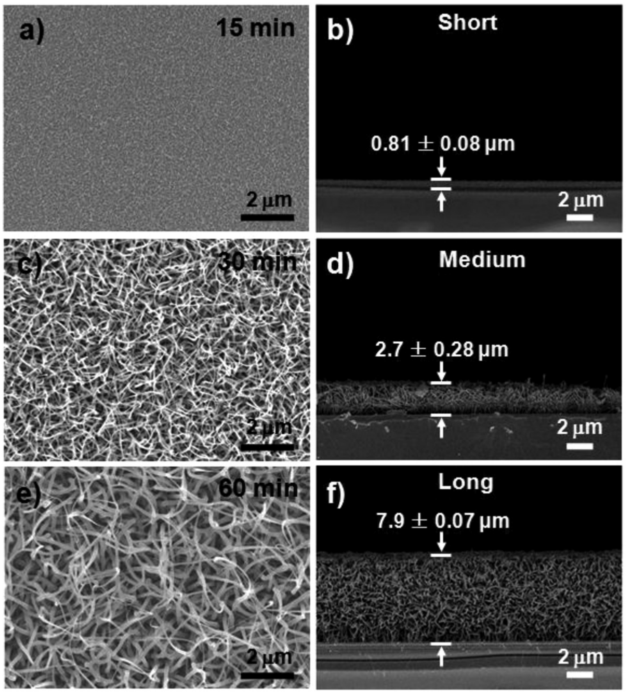

Fig. 1 SEM images of the synthesized CuTCNQ nanowire arrays with different growth times. (a, b) 15 min, (c, d) 30 min, and (e, f) 60 min. The length of nanowires increased with the increase of the reaction time.

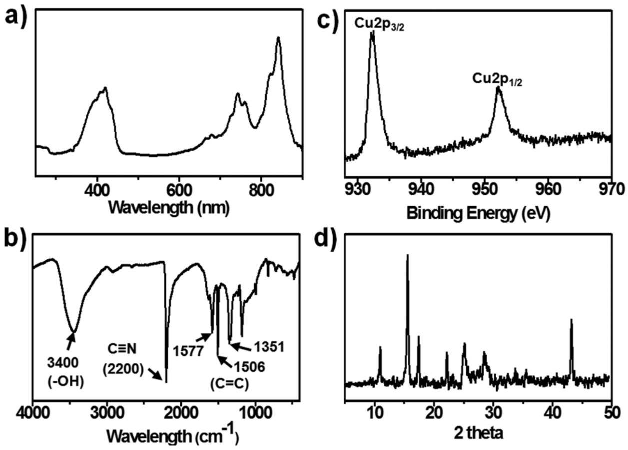

Fig. 2 Characterization of CuTCNQ nanowires. (a) UV-Vis, (b) FTIR, (c) XPS and (d) XRD patterns of nanowires. These results confirmed that the obtained products were CuTCNQ.

essentially Cu^+. Meanwhile, the N 1s orbitals appeared as a single feature at 398.6 eV, which was indicative of one type of TCNQ.[22] The XRD and the selected-area electron diffraction (SAED) pattern of the nanowires identified that nanowires belong to phase I in accordance with the previous literature (Fig. 2d and S2†).[22,23] These results confirmed that the well-defined CuTCNQ nanowires could be successfully fabricated.

The wettability of the as-prepared CuTCNQ nanowire arrays was characterized comprehensively. In general, the micro–nanostructured roughness can significantly amplify surface wetting behaviors. According to the assumption of Wenzel model for a nanowire arrays, the surface roughness is proportional to the length and density of nanowires. And therefore, the contact angle is usually increased with the increase of the roughness.[24] As shown in Fig. 3, the contact angles for the short, medium and long nanowires were 123.3 ± 1.8°, 144.8 ± 3.6° and 159.2 ± 2.6°, respectively. This result showed that the contact angle on the surface of CuTCNQ nanowires increased with the surface density of the nanowires as well as their length. Thus, the increase of the length and density of nanowires would directly result in the change of surface wettability of CuTCNQ nanowires from hydrophobicity to superhydrophobicity. The adhesion force of water droplets on the superhydrophobic CuTCNQ surface was recorded by using a high-sensitivity microelectromechanical balance system. The results suggested that water droplets on the superhydrophobic CuTCNQ surface exhibited a very high adhesion force (69.42 ± 5.73 μN) (Fig. S3†), implying that the superhydrophobic CuTCNQ surface was a Wenzel state.[25]

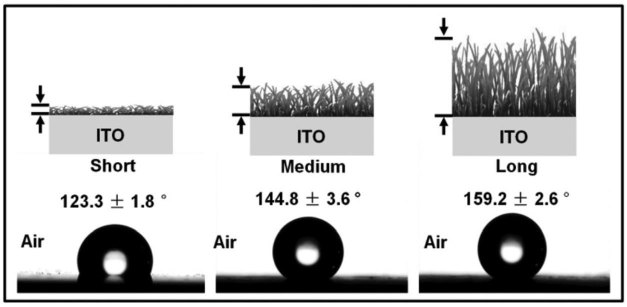

Fig. 3 The wettability of CuTCNQ nanowire arrays with different nanowire lengths. The contact angle increased with the increase of the length of nanowires. The wettability of CuTCNQ nanowire arrays could be finely tuned from hydrophobicity to superhydrophobicity.

To investigate how the wettability of CuTCNQ nanowire arrays affect the interactive manner between the surfaces and cells, the adhesive capacity of cells on the CuTCNQ nanowire surfaces was demonstrated. Osteoblasts, an important cellular component of bone, were selected as the candidates. As we know, osteoblasts is a key functional cell for the formation, secretion and mineralization of the bone matrix. It plays an important role in the regeneration of the bone tissue and osseointegration of implant materials.[26,27] MC3T3-E1 as one of the osteoblasts has been widely used as a model cell in bone biology. Thus, it has a profound significance to investigate the interactive manner of MC3T3-E1 on the CuTCNQ nanowire arrays, such as the adhesive ability and cell viability on the 3D nanostructured surface. The MC3T3-E1 was cultured on the CuTCNQ nanowire arrays for 30 min. As shown in Fig. 4a, when the length of the nanowires was short, a small number of MC3T3-E1 are adhered on the surface of nanowire array. Only a few MC3T3-E1 cells were observed under the whole view. However, with the increase of the length of the nanowires, the number of adhered cells increased obviously (Fig. 4b–d). The variance analysis was used for the analysis of the statistical results of cell experiments. The results showed a significant difference among the three groups ($P < 0.05$). Except MC3T3-E1, other cells, such as 3T3 and T24, were also employed for cell adhesion experiments. The results demonstrated that the number of adhered cells also increased with the increase of the length and density of the nanowires (Fig. S4†). These results suggested that with the change of wettability of CuTCNQ surfaces from hydrophobicity to superhydrophobicity, the number of adhered cells were significantly increased. Fig. 4e shows the SEM images of the adhered cells on the superhydrophobic CuTCNQ nanowire surface. It was observed that many cellular pseudopods with diameters of about 100–200 nm were stuck to the surface of CuTCNQ nanowire arrays, demonstrating that this superhydrophobic surface with a Wenzel state is more beneficial for cell adhesion. Cells always tend to prefer hydrophobic surfaces. The superhydrophobic Wenzel state that is a wet-contact mode,[28–31] provides enhanced topographic interactions between surfaces and cells. Therefore, when the cell suspension is added onto the superhydrophobic surface of CuTCNQ nanowire array, more cells would be trapped into the rough matrix of the surface.

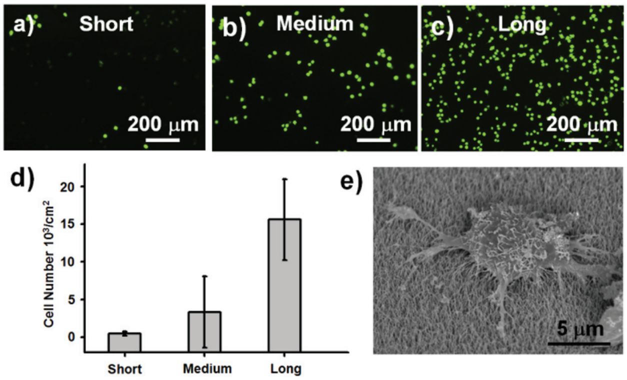

Fig. 4 (a–c) Comparison of cell-adhesion performance of CuTCNQ biointerfaces with different wettabilities. (d) The influence of the length of nanowires on the cell number. (e) SEM image to visualize the topographic interaction between the captured cells and CuTCNQ nanowires.

The cell viability of MC3T3-E1 on superhydrophobic CuTCNQ surfaces was also considered. After cell adhesion, the cell viability of MC3T3-E1 on the superhydrophobic CuTCNQ surfaces at different incubation times is calculated (Fig. S5†). The adhered cells on the CuTCNQ surfaces showed very high viability at different incubation times (>91%), indicating that the CuTCNQ nanowire array is a promising biointerface for the adhesion of osteoblasts.

Conclusions

We have developed a superhydrophobic CuTCNQ biointerface which exhibited very high adhesive ability for osteoblasts (MC3T3-E1). CuTCNQ nanowire arrays were fabricated *via* a combined physical and chemical vapor deposition technique. The wettability of CuTCNQ can be finely tuned from hydrophobicity to superhydrophobicity solely by controlling the reaction time. Consequently, the cell adhesion results demonstrated that the superhydrophobic CuTCNQ nanowire arrays exhibited very high adhesion capacity for osteoblasts.

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