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Brief communication

In situ synthesis of hybrid nanocomposite with highly order arranged amorphous metallic copper nanoparticle in poly(2-hydroxyethyl methacrylate) and its potential for blood-contact uses

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Abstract

A hybrid consisting of a highly ordered nanostructure of metallic Cu(0) nanoparticles embedded in a poly(2-hydroxyethyl methacrylate) (pHEMA) matrix was successfully synthesized by in situ photopolymerization, followed by in situ chemical reduction. The evolution of an ordered nanostructure of Cu(0) showing a nearly amorphous nature is discussed, and it is proposed that it is due to a coupling interaction between the Cu precursor and the unpaired O of the COOR group, associated with water molecules, among pHEMA molecules. The hybrids showed a negative surface charge and considerable improvement in blood compatibility compared to neat pHEMA and the widely used biomaterial polysulfonate.

Keywords: Copper nanoparticles; Cupric oxide; pHEMA; Hybrid; Surface charge

1. Introduction

Inorganic/organic hybrid materials have long attracted much attention because the beneficial properties from their individual components act synergistically. This unique character has been widely employed in a vast number of engineering and technical areas, especially in medical field, to producing biomedical devices with improved properties. With the advancement of nanomaterials technology, highly uniform hybrid composites with specific surface characters have become intrinsic for a number of medical uses in which, other than those requiring sufficient mechanical properties for load-bearing uses, contact between the surface of the medical device and tissues or blood in the human body is critical for the success of the device or implant. This has long been highlighted in the area of blood-contacting devices or implants, where the topological texture, electrical charge and hydrophilic nature of the hybrid surface are of prime importance [1,2]. Much attention has also been paid to the electrochemical behavior of the material's surface in order to inhibit the occurrence of thrombosis [3–5]. In general, although this has not yet been officially standardized, a material showing blood compatibility can be characterized in terms of, for instance, platelet adhesion, fibrinogen deposition and clotting time. However, it seems that blood-contacting devices currently on the market are not truly hemocompatible from the viewpoints of biology and biomaterials science [6]. The development of new biomaterials with improved blood compatibility has continuously attracted much attention. Recently, thromboresistant materials based on inorganic compounds, metals and metallo-organic compounds have received the most attention, as they have been shown to provide more advantages over conventional

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polymeric materials which are not truly thrombosis-free surface but are currently widely available on the market [7–9]. For instance, n-type oxides, which behave as semiconductors, provide a thromboresistant surface [10]. Organo-metallic compounds, such as lipophilic Cu complex [11] or metallic Cu particles [12] embedded in a polymeric matrix, where the catalytic generation of nitric oxide is generated from the composite surface, have shown promising outcomes in vitro, where both lipophilic Cu complex and copper particles were employed as catalysts to activate the redox chemistry by reduction of the nitrite or *S*-nitrosothiols into nitric oxide in the blood. These have successfully mimicked the nitric oxide forming activity of endothelial and other cells. However, the biocompatibility of the lipophilic Cu complex to the host is not yet fully understood, and its effect on the anti-tumor activity to DNA may increase the risk of biological complication [13]. Its lack of long-term activity has been another concern. With regard to the incorporation of copper particles, both 3 μm and 80 nm Cu particles (through mechanical mixing and dip coating) have been shown to produce blood clots, and it has been proposed that there are insufficient NO-generating species to effectively inhibit thrombus formation [12]. Increasing the surface roughness of a microcomposite with 3 μm Cu particles has also been suggested to cause a problem with regard to anti-clotting behavior, but this is less pronounced with 80 nm Cu nanoparticles.

The in situ synthesis of hybrid materials has long been technically attractive for their improved properties, such as blood compatibility, due to desirable electrochemical behavior, better physical uniformity and the greater chemical homogeneity of both the surface and bulk regions. Here, a novel in situ synthesis method is reported whereby a hybrid system based on the use of 2-hydroxyethyl methacrylate (HEMA) monomers that were photopolymerized in the presence of Cu^{2+} precursor was prepared by in situ synthesis, followed by in situ chemical reduction of the Cu^{2+} precursor to form a metallic Cu-containing hybrid. The hybrids were characterized by transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS) and streaming potential measurements. Interaction between blood and hybrids were examined in terms of the platelet adhesion test of whole human blood.

2. Materials and methods

2.1. Materials

The hydrogel matrix was synthesized using HEMA (Sigma–Aldrich, Inc. USA, analytical grade) monomer, ethylene glycol dimethacrylate (EGDMA; Sigma–Aldrich, Inc. USA, analytical grade) as a cross-linker and benzoin methyl ether (BME; Tokyo Chemical Industry, analytic grade). For the in situ metal particle formation, polyvinylpyrrolidone (PVP, average mol. wt. 10,000; Sigma–Aldrich, Inc. USA, analytical grade) was used, together with $CuSO_4$ (Sigma–Aldrich, Inc. USA, analytic grade), employed as the precursor for metallic Cu formation, and hydrazine (Sigma–Aldrich, Inc. USA, analytical grade), used as the reducing agent. A nanosilica suspension with particle size ranging from 10 to 12 nm and 20% solid content (ChangChun group, Taiwan) was also used in the hybrid synthesis for controlling the porosity of poly(2-hydroxyethyl methacrylate) (pHEMA). Phosphate-buffered saline (PBS; Sigma–Aldrich, Inc. USA), glutaraldehyde (Fluka, Sigma–Aldrich, Inc. USA) and alcohol (99.5%, Sigma–Aldrich, Inc. USA) were used for the platelet adhesion test. All the reagents were used without further purification.

2.2. Cu–pHEMA hybrid synthesis

The Cu–pHEMA hybrid was prepared by applying ultraviolet (UV) irradiation to an aqueous solution containing 2.5 g of HEMA monomer, 0.1 ml of EDGMA as cross-linker, 0.03 g BME as initiator, 0.1 g SiO_2 suspension, 2 g of H_2O as solvent, and 0.05, 0.08 or 0.20 g of 1 M Cu^{2+} solution with 0.1 wt.% PVP for different hybrids, which were named 5C, 8C and 20C, respectively. The mixture was mixed vigorously during the course of reaction. To remove the dissolved oxygen from precursors, N_2 was purged for approximately 1 min. The mixture was then transferred to a sealed transparent plastic holder 50 mm × 50 mm × 0.5 mm in size and UV irradiated at 254 nm for 2 h to produce the Cu(II)–pHEMA hybrid. The Cu(II)–pHEMA hybrid was rinsed with distilled water to remove any unreacted species (e.g. cross-linker, monomer) and then subjected to in situ chemical reduction by immersing the as-synthesized Cu(II)–pHEMA hybrid into 50 ml of 0.5 M hydrazine solution at 40 °C for 24 h to form a final metallic Cu(0)–pHEMA hybrid.

2.3. Structural analysis

To investigate the structure of the Cu(0)–pHEMA hybrid, TEM (Philips/FEI Tecnai 20 G2 S-Twin transmission electron microscope) was employed. For TEM images, the particle size and particle distribution of the resulting Cu(0) in the hybrid can be examined. The resulting hybrid, 20C, was sliced using a Leica EM UC6 Ultramicrotome with an EMFCS cryoattachment at room temperature. Cross-sections of approximately 100 nm thickness were obtained by using a diamond knife. The ultrathin films of the hybrid were placed directly onto nickel grids. TEM micrographs were acquired at an operating voltage of 200 kV. The chemical state of the Cu(0)–pHEMA hybrid, 20C, was determined by XPS using a Thermo VG350 UK spectrometer equipped with Mg K_α at 300 W power at the anode. A survey scan of 0.1 eV steps for Cu $2p_{3/2}$ was taken.

2.4. Zeta potential measurement

The surface charge (zeta potential) of the pHEMA and hybrid membranes were characterized using a streaming

potential apparatus, as detailed elsewhere [14]. In brief, an electrolyte solution was prepared with a 10^{-3} M KCl solution (dissolved in ultrapure water) at pH 7.4, where the pH value was adjusted by the addition of a 0.1 M NaOH or 0.1 M HCl solution. In each experiment, the solution flow through the membrane module was supplied by a peristaltic pump and the transmembrane pressure was controlled to between 0.2 and 1 bar, while the cross-flow velocity of the solution was fixed at 0.1 m s^{-1}. For each sample, four values of pressure difference (ΔP) were measured and the streaming potential (ΔE) data were recorded using a personal computer. The zeta potential (ζ) was calculated using the Helmholtz–Smoluchowski equation (Eq. (1)), where κ is the liquid conductivity, η is the liquid viscosity, ε_r is the liquid permittivity and ε_o is the permittivity of free space. The zeta potential was calculated from the slope of the $\Delta E/\Delta P$ curve, where $r^2 > 0.99$ for each sample

$$\zeta = \frac{\kappa\eta}{\varepsilon_r \varepsilon_0}\frac{\Delta E}{\Delta P} \quad (1)$$

2.5. Blood and platelet test

Fresh blood was drawn from a healthy adult volunteer. Care was taken to ensure that the volunteer was free of aspirin and other drugs, such as anticoagulant, that could bias the results. Fresh blood and platelet adhesion tests were performed in vitro to investigate the quantity of the adherent platelets on the Cu(0)–pHEMA hybrid. The Cu(0)–pHEMA hybrid, i.e. 20C, with dimensions of 1 cm × 1 cm, was immersed in 0.5 ml of platelet-rich plasma (PRP) solution in a 24-well plate and then incubated at 37 °C for 2 h. After terminating the adhesion test by adding 0.5 ml of PBS to each well, the samples were subsequently rinsed three times with a PBS buffer solution to remove any weakly adherent platelets. The firmly adhered blood components or platelets were fixed using 2.5% glutaraldehyde solution at 4 °C for 1 h. The samples with the adherent blood components or platelets were then dehydrated in graded ethanol solutions (30%, 50%, 70%, 95% and 100% for 15 min each at room temperature) and dried at 37 °C for 24 h. The samples were then characterized by field-emission scanning electron microscopy (JAM-6700 F) operated at 5 kV. Four SEM images were taken at a magnification of ×500 for each sample and five parallel measurements were carried out for each hybrid. The number of platelets on the membrane surface was counted for each image and the counting area could be estimated from the scale bar, thus quantifying both the numbers and the volume fraction of platelets on the membrane surface. The volume fraction of thrombosis coverage on the sample surface was counted on 20 fields chosen at random to obtain a good statistic analysis.

3. Results and discussion

Fig. 1a illustrates the nanostructure of the Cu(0)–pHEMA hybrid. The Cu(0) nanoparticles are relatively homogeneously distributed in the pHEMA matrix and show a relatively uniform size of about 2–5 nm in diameter, as can be seen in Fig. 1b, a high-resolution image of Cu(0) nanoparticles in pHEMA matrix. Fig. 1c is a selected area electron diffraction pattern, which confirmed that the Cu(0) nanoparticles are a poorly crystallized metallic phase, due to their relatively small crystallite size. The dimensional restriction of the primary Cu(0) nanoparticles, according to the work of Huang and Yang [15], should exhibit a quantum confinement effect, where the electrons may behave in a wave-like rather than a particle-like manner. Although no direct evidence supports this argument at present, it is reasonably believed that the Cu(0) nanoparticles in the pHEMA matrix should behave as a semiconductor, rather like bulk copper.

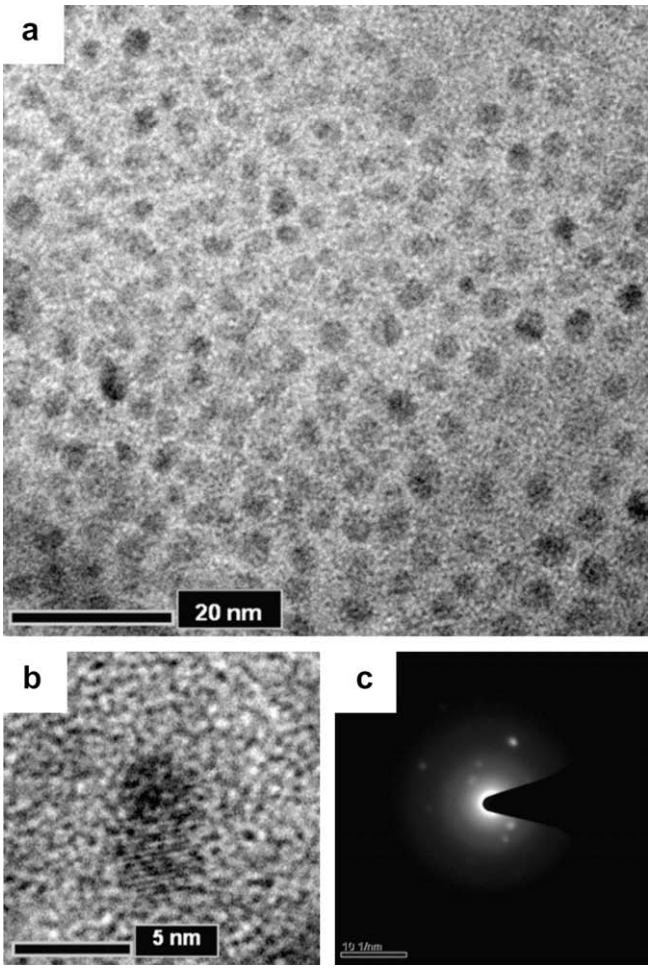


Fig. 1. TEM photographs of Cu(0)–pHEMA hybrid showing (a) an orderly packing configuration of Cu(0) nanoparticles distributed in the pHEMA matrix, (b) high-resolution image of a Cu(0) nanoparticle in pHEMA matrix where the nanoparticle has a size of about 3 nm in average, and (c) selected area electron diffraction pattern of a Cu(0) nanoparticle, indicated the Cu(0) a poorly crystalline (or amorphous) structure.

The highly order arrangement of the Cu(0) nanoparticles within the matrix, having a relatively constant particle-to-particle distance of 8–10 nm, suggests that it might results from a mechanism involved in the synthesis of the

Cu(II)–pHEMA hybrid. Upon hybrid formation, the Cu(II) ions may be anchored by the unpaired electron of O from either the COOR group of the HEMA monomers or from the H_2O in the reaction mixture to form coordination bonds, via, for instance, a coupling reaction, as schematically shown in Fig. 2b. The Cu(II) ions are immobilized in the network structure of the HEMA upon polymerization and remain in place while subjected to chemical reduction, resulting in the formation of amorphous metallic Cu(0) nanoparticles. This argument is also supported by the nanostructural development illustrated in Fig. 1a, where no fractal aggregation of the Cu(0) nanoparticles was observed in the resulting hybrid. In other words, the orderly packed configuration of the Cu(0) nanoparticles in the matrix suggests that the molecular network structure in the pHEMA matrix acts as a confined space for Cu(0) formation, as elucidated upon schematically in Fig. 2a. This also suggests an interaction between the Cu(II) ions and hydroxyl groups of pHEMA. Once the Cu(0) nanoparticles are nucleated by chemical reduction and grow, they develop in a confined nanometric space defined by the dimension of the pHEMA network (as a nanocage), rather than agglomerate to form fractal aggregates. This may then result in the regular arrangement of Cu(0) nanoparticles in a well-defined nanostructural network. Such highly uniformly distributed Cu(0) nanoparticles in the pHEMA ensures a nanometric-scale uniformity on the hybrid surface upon contact with blood-clotting proteins, such as fibrinogen, from undesirable adsorption.

As mentioned earlier [11,12], copper, in either metallic form or chemical complex, should play a critical role in catalytic nitric oxide generation for improved blood compatibility. It is important to realize the chemical composition of the Cu nanoparticles prepared in situ and the possible oxidative state of the copper developed in the hybrids since both factors may also determine the electrochemical interaction involved in protein adsorption or platelet adhesion/aggregation. An XPS analysis on the pristine synthesis of Cu(0)–pHEMA hybrid before and after immersion in

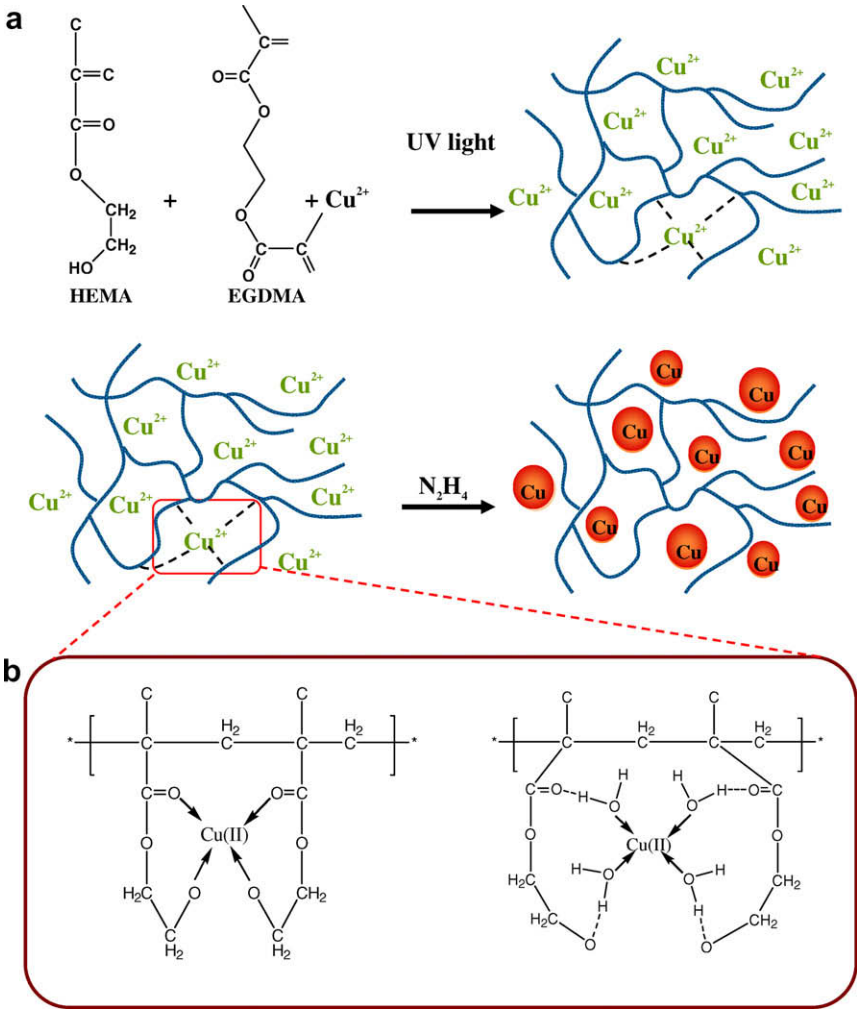
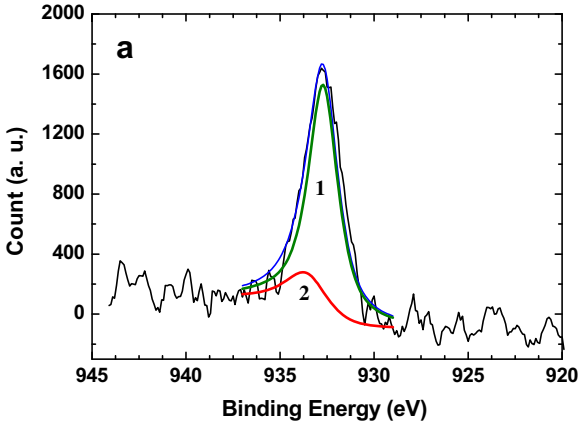
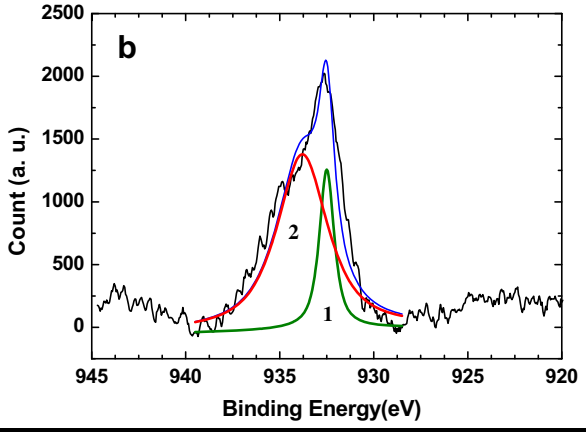


Fig. 2. A schematic drawing for the proposed synthesis scheme of Cu(0)–pHEMA hybrid, where (a) the Cu(II) were localized and chemically reduced by N_2H_4 in situ to metallic Cu(0) nanoparticles and (b) the Cu(II) ions were assumed to be coupled with the unpaired O of COOR groups along the pHEMA chain network, forming a regularly arranged nanostructure, as illustrated in Fig. 1a.



#	Position (eV)	Intensity	FWHM (eV)	Area	Area(%)	Component
1	932.7	1528.1	1.9	3809.8	83.5%	Cu
2	933.8	279.5	3.9	754.5	16.5%	CuO



#	Position (eV)	Intensity	FWHM (eV)	Area	Area(%)	Component
1	932.5	1257.565	0.95	1433.8	21.1%	Cu
2	933.8	1378.599	3.2	5361.6	78.9%	CuO

Fig. 3. The Cu $2p_{3/2}$ peaks of XPS spectra of (a) as-synthesized Cu(0)–pHEMA hybrid and (b) after immersion in PBS for 24 h. Showing that a metallic Cu(0) was mainly characterized for the as-synthesized hybrid, however, this turned into Cu(II) after PBS immersion, suggesting the Cu(0) nanoparticles are oxidized to copper oxide, Cu(II).

PBS is shown in Fig. 3a and b, respectively. In Fig. 3a, the Cu $2p_{3/2}$ NPs spectrum of pristinely synthesized Cu(0)–pHEMA hybrid shows a peak at 933.79 ± 0.2 eV, which is mainly attributed to Cu(0), while a much weaker feature, at 934.3 ± 0.2 eV, is attributed to a small amount of CuO. A corresponding fraction of the copper species at a similar concentration can be correlated with the area under the spectrum, and this determines that the metallic form of the Cu(0) accounts for 83.5% and CuO 16.5% of the resulting Cu(0)–pHEMA hybrid. On other words, the copper developed in the Cu(0)–pHEMA hybrid is a mixture of mainly metallic Cu(0) and a small fraction of CuO, after in situ chemical reduction. The presence of a small amount of divalent CuO suggests an incomplete reduction reaction of Cu(II) ions in the matrix. This Cu(II), i.e. CuO, may be located in the inner region of the metallic Cu(0) nanoparticles since the reduction reaction is considered to be a diffusion-controlled process. However, after immersion in PBS for 24 h, the resulting spectra, shown in Fig. 3b, indicate that most of the metallic Cu(0) is turned into ionic CuO, i.e. Cu(0) has a concentration of 21.1% and CuO, 78.9%, as a result of oxidation. This finding is indicative of the tendency of an electron-giving characteristic of the amorphous metallic Cu nanoparticles in the matrix. The presence of metallic copper nanoparticles, according to a recent report [12], should provide the redox capability necessary for thromboresistance.

The surface charge of the Cu(0)–pHEMA hybrids was determined at pH 7.4 in 10^{-3} M KCl solution. Fig. 4 shows the zeta potential of the hybrids with different weight con-

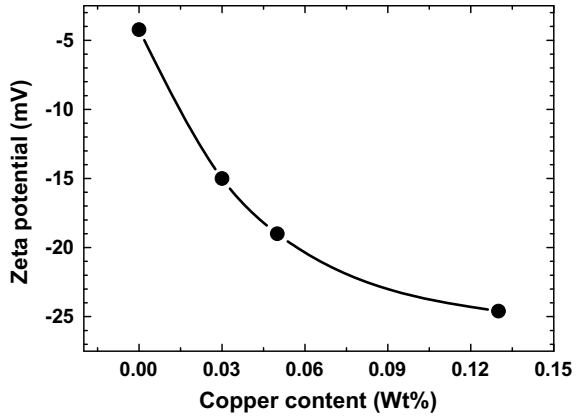


Fig. 4. Effect of the nano copper particles on zeta potential of Cu(0)–pHEMA hybrid with different copper particle content (weight %). Concentration of KCl = 1×10^{-3} M at pH 7.4.

tents of the copper nanoparticles (0%, 0.03%, 0.05% and 0.13%), corresponding to samples of pure pHEMA and hybrids 5C, 8C and 20C, respectively. It can be seen that the surface charge of the neat pHEMA and Cu(0)–pHEMA hybrids showed negatively charged at pH 7.4 and became more negative with increasing incorporation of the Cu(0) nanoparticle, to a value as high as −24.6 mV in this study. The increase in the negative charge of the hybrid surface with the incorporation of relatively small amount of metallic Cu nanoparticles, i.e. from 300 to 1300 ppm, suggests that it is the result of the highly uniform distribution of the small Cu nanoparticles, i.e. a few nanometers, in the hybrid, whereby a higher surface potential of the total Cu nanoparticles, due to relatively large total surface area, can be cumulatively achieved. Copper nanoparticles of Cu(0)–pHEMA can be oxidized to CuO in aqueous solution, as proved by XPS, and it was demonstrated that CuO particles show a negative surface charge at pH 7.4 [16], which may be due to the formation of hydroxide ions (OH^-) on the surface of the CuO–pHEMA hybrid [17]. However, the increase in the surface charge of the hybrids is gradually reduce as the Cu concentration continues to increase, reaching a constant level at pH 7.4. In the case of neutral pH, a limited number of hydroxide ions (OH^-) can be adsorbed on the surface of Cu(0)–pHEMA hybrid, causing the surface charge ζ to approach a constant value. Accordingly, a surface with an increased negative charge should exhibit improved anti-blood-clotting behavior [5], together with the redox ability of Cu nanoparticles reported earlier, further reinforcing the argument that the hybrids currently prepared should have an improved thromboresistant property.

The coagulation of blood consists of a cascade of reactions that can be divided into two pathways – the extrinsic and intrinsic pathways – and platelets can accelerate thrombosis through the secretion of bulk phase agonists, fibrinogen-mediated platelet–platelet aggregation and the acceleration of thrombin production [18]. Therefore, the extent of platelet adhesion to biomaterials is often used as an index of blood compatibility [19]. In this study, platelet adhesion and platelet activation on the surface of the Cu(0)–pHEMA hybrid was one of the main focuses in order to evaluate the hemocompatible properties of the hybrid. Fig. 5 shows that a trace amount of platelets adhered to the surface of the Cu(0)–pHEMA hybrid after 2 h immersion with human whole blood, and compares this group with PSF (polysulfonate, a reference material) and pure pHEMA (the control group). The PSF showed a considerable amount of platelet adhesion and pHEMA showed some degree of platelet adhesion, which agree with the observations reported in the literature [20,21]. The amorphous metallic Cu(0)–pHEMA hybrid showed much improved anti-platelet adhesion behavior over these two well-known groups, with as little as 30% of the adhesion amount of platelet compared to that of neat pHEMA being observed for the hybrids, and improving the anti-platelet adhesion over PSF by about 92%. Although the exchange of electrons from blood proteins to the surface of medical devices has been considered to induce blood coagulation [5], the actual mechanism(s) of adhesion of platelets from whole blood to the hybrid surface is not clearly understood. In spite of the ambiguity, it can be reasonably assumed that the Cu nanoparticles in the pHEMA trigger an electron-transferring process from the amorphous Cu(0) nanoparticles to the blood-clotting proteins. At the same time, the negatively charged hybrid surface is expected to inhibit adsorption of the blood-clot-inducing protein (fibrinogen, negatively charged), which should minimize or eliminate protein adsorption/denaturation as a result of electrostatic repulsion and prevent electrons from being transferred from the protein to the negatively charged hybrid surface. Either the former or the latter mechanism, or a combination of both, could effectively inhibit undesirable platelet adhesion.

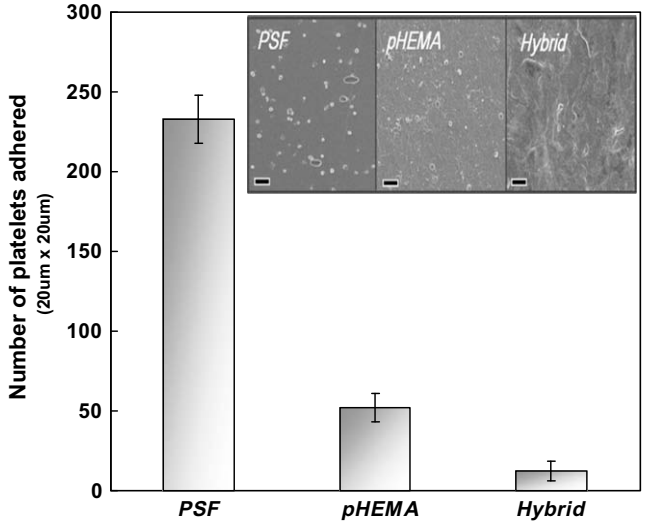


Fig. 5. The blood platelet adhesion test showed that the Cu(0)–pHEMA hybrid demonstrated an improved anti-platelet adhesion behavior by ~70% and 92% compared to that of the neat pHEMA and PSF, respectively. (The scale bar of SEM image is 10 μm.)

We can conclude that the metallic Cu nanoparticle-pHEMA hybrids reported in this communication exhibited outstanding anti-platelet adhesion property compared to neat pHEMA and a widely used biocompatible material, PSF. The highly ordered nanostructure of the hybrids offers unprecedented physical and chemical uniformity which, together with their highly negatively charged property, makes them outstanding anti-blood-clotting materials. The electron-giving potential of the hybrids, as revealed in an earlier electrochemical test [5], together with the XPS spectral variation in Fig. 2, also reinforce their thromboresistant character. A further study of blood compatibility in terms of Cu-catalyzed nitric oxide generation by the hybrids is under electrochemical investigation and will be reported upon shortly.

4. Conclusion

A well-defined hybrid structure has been successfully synthesized comprising amorphous metallic Cu nanoparticles with an orderly packing configuration within a pHEMA matrix. The oxidation of the Cu nanoparticles was confirmed by XPS analysis. Relatively uniform copper nanoparticles, about 2–5 nm in diameter, and a relatively constant particle-to-particle distance of 8–10 nm were observed in the Cu(0)–pHEMA hybrid structure, suggesting that an unprecedented chemical and physical homogeneity can be achieved. The negative surface charge of the Cu(0)–pHEMA hybrid was confirmed by its surface streaming potential and is expected to play an important role in reducing the adsorption of platelets. Thus, the experimental results demonstrate that the Cu(0)–pHEMA hybrid is a promising candidate for thromboresistant applications.

Acknowledgement

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