ZnO nanowires coated hydrophobic ~~and antibacterial~~ surfaces for various biomedical applications

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Abstract:

Recently, ~~the antibacterial~~ hydrophobic surfaces are finding many applications in the field of biomedical. This study is aimed to report the simple and facile method of hydrophobization of various surfaces like glass, semiconductor and polymer etc. used in biomedical field by using durable and water resistant ZnO nanowires coating. The change in contact angle of ethylenediaminetetraacetic acid (EDTA) anticoagulated whole blood (EDTA-WB) on various substrates like; glass, quartz, Si and polydimethylsiloxane (PDMS) before and after ZnO nanowires coating is reported. It was observed that the different type of substrates show great variation in contact angle of EDTA-WB, before and after ZnO nanowires coating. The substrates which are generally hydrophilic for EDTA-WB become hydrophobic after ZnO nanowires coating. This surface coating technique can be utilized in various biomedical applications for example in medical device and surgical equipments coating, orthopaedic dressings, in-vivo implants and corrosion resistance surfaces.

Key Words: Hydrophobization, Anticoagulated, EDTA, Whole Blood, Nanowires

1. Introduction

~~Antibacterial~~ H~~h~~ydrophobic surfaces are widely used in biomedical applications like orthopaedic dressings, in-vivo implants and corrosion resistance surfaces. Scientists and researchers from various disciplines are disseminating their knowledge for the rapid development of hydrophobic biomaterials with antibacterial activity. Various biomaterials like polyurethane, polydimethylsiloxane (PDMS), teflon, silicone rubber, poly(methyl methacrylate) (PMMA), stainless steel and titanium have been used in cochlear implants, heart valves, stents, dental implants, bone plates, joint replacement, skin repair, vascular grafts, catheters, tubing, drug delivery and wound dressing application [1]. Various nanomaterials used for hydrophobic coatings are manganese oxide polystyrene (MnO_2/PS), zinc oxide polystyrene (ZnO/PS), precipitated calcium carbonate [2] Carbon nano-tube structures etc. Recently, the semiconductor materials coating attract researcher's attention at the most. The biocompatibility, non-toxicity, anti-bacterial and antimicrobial properties, photo-catalytic activity and physiochemical stability of semiconductor material makes their coating most advantageous in biomedical application [3]. Among the semiconductor materials, ZnO is bio-safe and biocompatible material [4] and widely used in biomedical applications [5, 6]. Wang *et al.* showed that ZnO nanowires are completely bio-safe and biocompatibility when used in concentrations below 100µg/ml [7]. Due to the extreme repellence and bacterial resistance of ZnO nanostructures based hydrophobic coatings; there is more scope and wider potential for application uses in medical equipments and surgical tools. The primary purpose of hydrophobic coating is to repel water from the materials surface and act as sealant so that water can't penetrate through the surface. Nanostructures surface coating is an additional way to modify surfaces of both metals and polymers in an effort to increase hydrophobicity. This technique don't involve direct attachment of chemical groups or surface chemical alterations as the way conventional chemical modification techniques do, but still makes the surfaces more hydrophobic.

The coating technique must consider the materials properties as the response of the coated surface to the fluid may decide the performance of the finished device or material. The oxide nanostructures based coating is more durable than other gel-based coatings [7]. Carbon nanotubes coating is more expensive and the coating method is complex and difficult as compared to ZnO nanowires coating. Hence, the ZnO nanostructures based coating remains the most economic and efficient option of coating. If the ZnO nanostructures are hydrophobic in nature then it is very useful in avoiding the health risk by some of the bacteria's like Legionella which is typically found in water and can cause special type of pneumonia known as Legionnaries disease. Because, if the material surface is hydrophilic the bacteria's present in water may adhere to the biomaterial resulting in bio-film formation, which may cause pathogenesis [8-10]. For achieving antibacterial activity, many surface modifications techniques have been employed by the scientists and bioengineers for modification of surface physiochemical properties like silver coating and calcitite hydroxyapatite plasma sprayed coating, antibiotic impregnation into polymer matrix etc [11-13]. These techniques include harsh chemical treatment and are complex anti-bacterial coating methods. ZnO deposition by using drop coating method is very helpful to avoid the barrier of low heat resistance of many biomaterials mainly polymers. A smart approach is required to make the surface hydrophobic with antibacterial property in a simple and cost effective manner. If a hydrophobic semiconductor coating is available for biomaterials or bio-devices then it can serve dual purpose as it can make the biomaterial surface hydrophobic as well as bacteria resistant. These requirements stimulate an extensive research in the direction to prepare antibacterial hydrophobic semiconductor coating for biomaterials.

Here, our main focus is to develop a semiconductor material based hydrophobic coating for biomaterials. Our works include utilization of hydrophobic ZnO nanowires for hydrophobic coating on various substrates like; glass, quartz, Si and PDMS. After ZnO nanowires coating, the substrates become hydrophobic for EDTA-WB. The contact angle (CA) measurements before and after ZnO nanowires coating show a drastic change in surface wetting behaviour for EDTA-WB on various substrates. The observed results indicate that hydrophobization of various substrates is possible by ZnO nanowires coating which can be utilized for various biomedical applications.

2. Experimental section

2.1 *CA measurement setup*

The CA measurements were done by using commercially available image capturing setup by using sessile drop method on various substrates before and after ZnO nanowires coating. Figure 1(a) shows the schematic diagram of the experimental setup used for the measurement. A high definition camera is used for capturing the images. The sample volume of drop is about 5µl for each measurement. To minimize the error in measurements, five set of measurements were taken on a sample and then the average value was used for analysis. The available ZnO nanowires show hydrophilic nature. We have placed the nanowires in dark for 40 days to make them as superhydrophobic. The superhydrophobic ZnO nanowires coating on various substrates were done by using drop coating method.

2.2 *ZnO Nanowires coating process and hydrophobization*

The process of coating of the superhydrophobic ZnO nanowires on the substrates is shown in figure 1(b)~~2~~. ZnO nanowires coating was done by mixing the ZnO nanowires with alcohol (ethanol) and then ultrasonicating it for 15 min for better mixing. After mixing, the mixture is drop coated on various substrates. Different types of sample substrates are prepared by coating of ZnO nanowires like Si, PDMS, glass and quartz. CA is the measure of wettability and a useful gauge to determine the interactions of biomaterials with the surrounding biological environment having water like physiology [14]. The hydrophobization of various substrates after ZnO nanowires coating increases the CA of EDTA-WB on the substrates, thereby making the substrates hydrophobic for decreased blood wettability. Figure 1(c)~~3~~ shows the diagram of proposed method where the outer surface of the substrates are made hydrophobic by ZnO nanowires coating which increases the CA and hence reduces water wetting of the surface.

3. Results and discussion

3.1 *Sample Characterization*

The SEM image of the ZnO dispersed on Si substrate is shown in figure ~~4~~2a. The SEM image clearly reveals that the ZnO material has nanowires like morphology with average diameter of about 70 nm. The XRD spectra of the material illustrate that the ZnO has wurtzite phase (figure ~~4~~2b). The superhydrophobic ZnO nanowires dispersed on Si substrate shows water contact angle of about 156° as shown in figure ~~4~~2c.

3.2 *Blood cell counter*

The concentration of various blood cells in the fresh blood sample (used for CA) as measured by the automatic blood cell counter is as shown in table 1. For measurements of the concentration of various blood cells in the blood sample, Yark Abacus III automatic blood cell counter was used.

Table 1. Concentrations of various blood components in the blood sample

S.No.	Component	Concentration
1	WBC	9.04×10^{9} /L
2	LYM	3.46×10^{9} /L
3	GRA	5.11×10^{9} /L
4	RBC	5.87×10^{12} /L
5	HGB	14.2 gm/dl
6	HCT	44.80%
7	PLT	2.48×10^{9} /L

3.3 *CA measurement*

Here, to analyze the blood interaction with different surfaces we have done the sessile droplet method based CA measurements on various surfaces with a 5µl blood droplet. The images of blood CA on various sample substrates before and after ZnO nanowires coating is shown in figure ~~5~~3(a-d). It is found that after ZnO nanowires coating, the blood CA on quartz substrate increases from 54.6° to 96.4°. For glass substrate, the blood CA value increases from 28.5° to 145.7°. For Si substrate it increases from 62° to 138.8° and for PDMS substrate the blood CA value increases from 81.4˚ to 131.6˚. The observed results revealed that the various types of substrates which are initially hydrophilic to blood become hydrophobic after ZnO nanowires coating. It indicates that the hydrophobization of various substrates is possible by ZnO nanowires coating. Hydrophobic surfaces are those on which the water drop makes a contact angle (CA) greater than 90°and if it is above 150° the surfaces are known to be superhydrophobic surfaces. Superhydrophobic surfaces are very difficult to wet and the surface-water interactions are very less [15]. Some of the naturally occurring superhydrophobic surfaces are lotus leaf, water strider, butterfly wings etc.

The wettability on ZnO surfaces can be explained by the Young's, Wenzel or Cassier-Baxter models as shown in figure 6. The Young's contact angle 'θ' for the liquid drop on a smooth solid surface, is determined by the surface free energies involved [16],

$$\cos \theta = (\gamma_{sv} - \gamma_{sl})\, \gamma_{lv} \qquad \text{(i)}$$

where γ_{sl}, γ_{sv}, and γ_{lv} are the solid/liquid, solid/vapor, and liquid/vapor tensions, respectively. The apparent contact angle 'θ_a' for a rough surface is given by the Wenzel model [17],

$$\cos \theta_a = r \cdot \cos \theta_b \qquad \text{(ii)}$$

where θ_b is a contact angle on a smooth surface and r is a surface roughness factor.

The Cassier – Baxter model is applicable once the contact is lost. It can be described by the Cassier equation [18],

$$\cos \theta_r = f_1 \cos \theta - f_2 \qquad \text{(iii)}$$

where θ and θ_r are the contact angles for smooth and rough surfaces, respectively; f_1 and f_2 are the fractional interfacial areas of ZnO and the air trapped between the surface and a water droplet, respectively.

The bar diagram representation of the variation in CA of undiluted EDTA-WB on different substrates before and after ZnO nanowires coating is shown in figure ~~7~~3(e).

3.4 *Variation in CA with blood dilution*

Figure ~~8~~5 shows the variation in the CA measured with different blood dilution ratios on ZnO coated samples of Quartz and PDMS respectively. The dilution of blood with distilled water is in the ratio of 1:25, 1:50 and 1:100 respectively. The CA measurement is one of the important methods for analyzing the surface properties in which the angle formed by the liquid drop on the surface of solid is measured. CA mainly depends upon the top molecular layers of solid and their properties. It is mostly used for characterizing the effect of adsorption of various films or other surface treatments on a solid surface in terms of surface energy change [19]. It also gives information related to the wetting properties of surface materials used for different devices/implants. The CA depends upon the adhesive forces between the liquid and solid formed by their molecular interactions. So, if the liquid interaction with solid surface increases, it will support lower CA and vice versa.

In various studies ZnO nanowires are used as coating material because ZnO exhibits higher antibacterial properties as compared to other metal oxides nanostructures [13]. The available reports show that ZnO is not only suitable to kill bacteria with irradiation of UV light, but it can also inhibits bacterial growth under normal visible light [20]. Wang *et al*. showed the UV photoactive property of ZnO, which is responsible for its antibacterial activity

$$\mathrm{ZnO} + h\nu \rightarrow e^{-} + h^{+} \qquad \text{(iv)}$$

$$h^{+} + \mathrm{OH}^{-} \rightarrow \bullet\mathrm{OH} \qquad \text{(v)}$$

$$e^{-} + \mathrm{O_2} \rightarrow \mathrm{O_2}\bullet^{-} \qquad \text{(vi)}$$

$$\mathrm{O_2}\bullet^{-} + 2\mathrm{H}^{+} + e^{-} \rightarrow \mathrm{H_2O_2} \qquad \text{(vii)}$$

When ZnO is irradiated with UV light having wavelength smaller than the band gap of ZnO, on the surface of semiconductor material i.e. ZnO, electron-hole generated as shown in equation (iv). The generated holes may react with surface hydroxyls or water (OH^-/H_2O) to produce hydroxyl radicals (•OH), equation (v) on the surface. Conduction band electrons react with molecular O_2 adsorbed on the surface to generate (O_2•) super-oxide radical, equation (vi). Finally, the radicals (•OH and O_2•) react with the bacterial cells to destroy and shrink it [21-23]. Along with antibacterial property, the ZnO water wetting properties can also be modified [24-26]. In few literature reports, we have found that it is possible to make ZnO as superhydrophobic without using any chemical treatment by just storing in dark for about 40 days. We have utilized the anti-bacterial hydrophobic property of ZnO nanowires and successfully prepared various hydrophobic substrates with the help of ZnO nanowires. Our work suggests, if we use superhydrophobic ZnO nanowires coating, then we can achieve a dual purpose. Along with the antibacterial activity of ZnO, we can achieve a hydrophobic property also. To use the hydrophobic and antibacterial property of ZnO, it is very easy to coat nanostructures on different material's surface like glass, quartz, silicon and PDMS, which are widely used for many biomedical applications. The ZnO deposition by using drop coating method (explained in experimental section) is very helpful to avoid the barrier of low heat resistance of many biomaterials mainly polymers.

4. Conclusions

With the advancement of nanotechnology at a rapid rate, nanomaterials are playing important role in development of biomaterials. For these advancements, the surfaces with special wettability properties have attracted the researchers for practical and fundamental research applications. In this study we focused on blood wettability including hydrophobic surfaces induced by biocompatible and safe nanoparticles of ZnO. Surface modification of various types of substrates by a biocompatible ZnO material is demonstrated. The nanowire like morphology of ZnO nanostructures is used for changing the CA on various types of substrates. The results reveal that ZnO nanowires coating is suitable for hydrophobization of various type of substrates which are showing hydrophilic nature for EDTA-WB. These studies help in evaluating the wetting behaviour of biomaterial for EDTA-WB for various applications in medical science like in the field of biosensors, transducers, corrosion resistance, liquid transportation, micro-fluidic systems, and bio-engineering.

Figure Captions

Figure 1. (a) Schematic diagram of the contact angle measurement setup, (b) Graphical representation of the steps followed in the coating of ZnO nanowires on various substrates, and (c) Schematic representation of the variation in contact angle on the outer surface of the

substrate (i) before ZnO nanowires coating and (ii) after ZnO nanowires coating for decreased blood wettability.

Figure 2. (a) SEM (b) XRD and (c) water contact angle images of the ZnO nanowires coated on Si substrate.

Figure 3. Contact angle on various substrates before and after ZnO nanowires coating: (a) quartz, (b) glass, (c) silicon, (d) PDMS samples, and (e) Contact angle of EDTA-WB on various substrates before and after ZnO nanowires coating.

Figure 4. Young's, Wenzel and Cassie-Baxter Models for hydrophobicity of solid surfaces.

Figure 5. Variation in contact angle with dilution of whole blood on quartz and PDMS samples.

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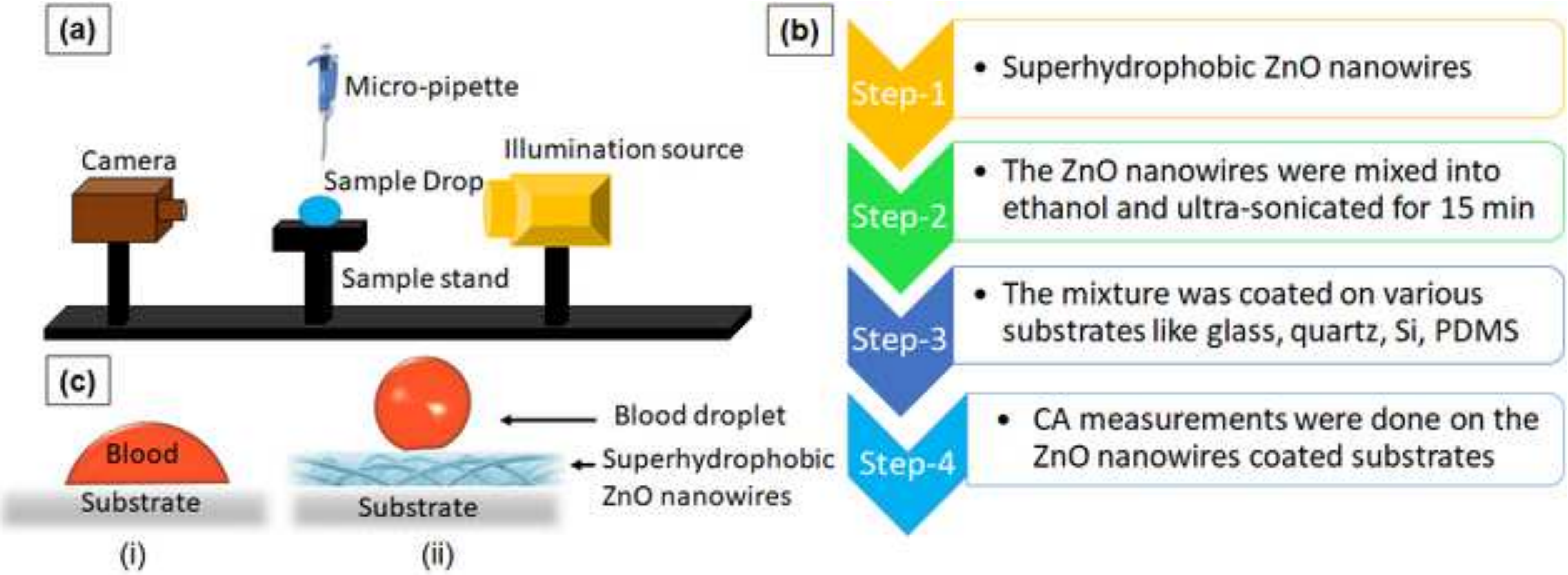
Figure 1



Step-1
- Superhydrophobic ZnO nanowires

Step-2
- The ZnO nanowires were mixed into ethanol and ultra-sonicated for 15 min

Step-3
- The mixture was coated on various substrates like glass, quartz, Si, PDMS

Step-4
- CA measurements were done on the ZnO nanowires coated substrates

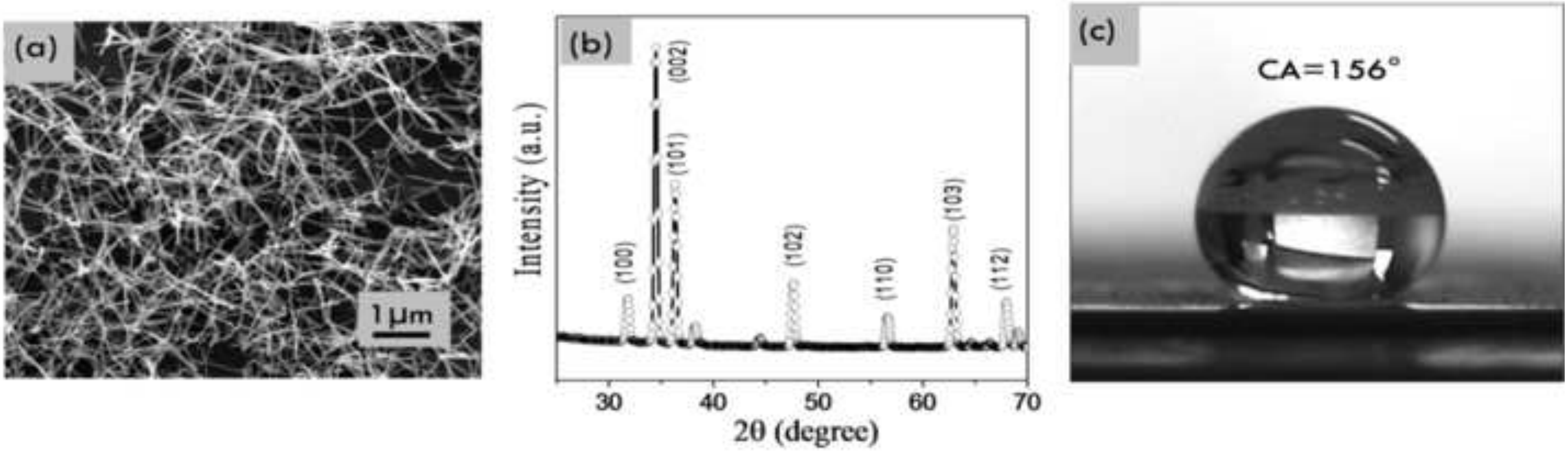

(a)
1 μm
(b)
Intensity (a.u.)
(100)
(002)
(101)
(102)
(110)
(103)
(112)
30
40
50
60
70
2θ (degree)
(c)
CA=156°

Figure 3

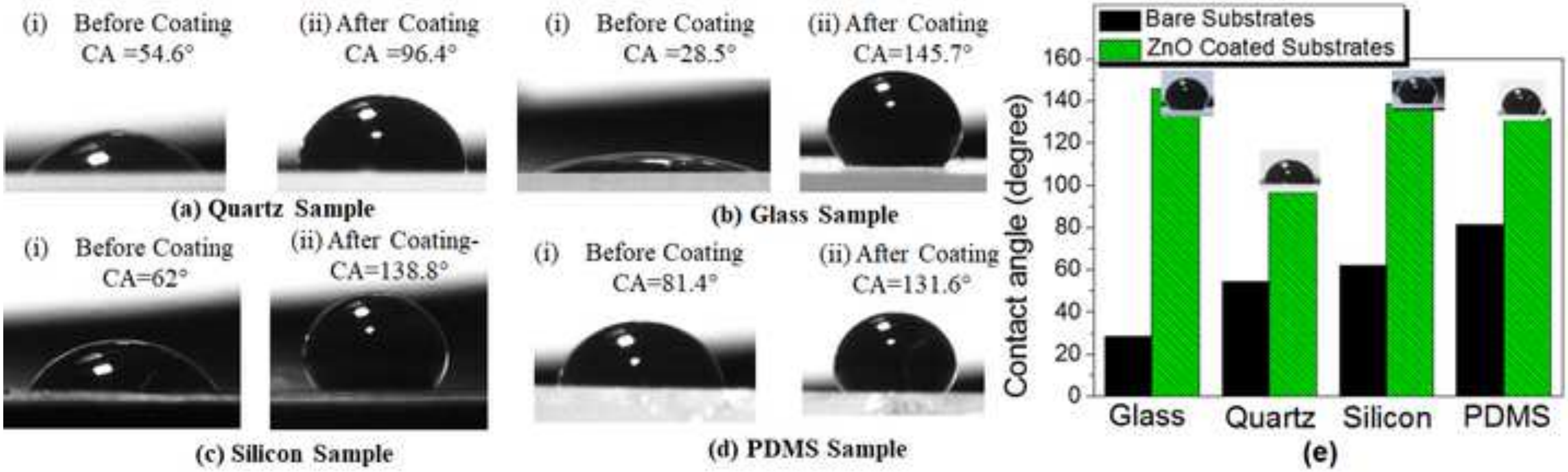
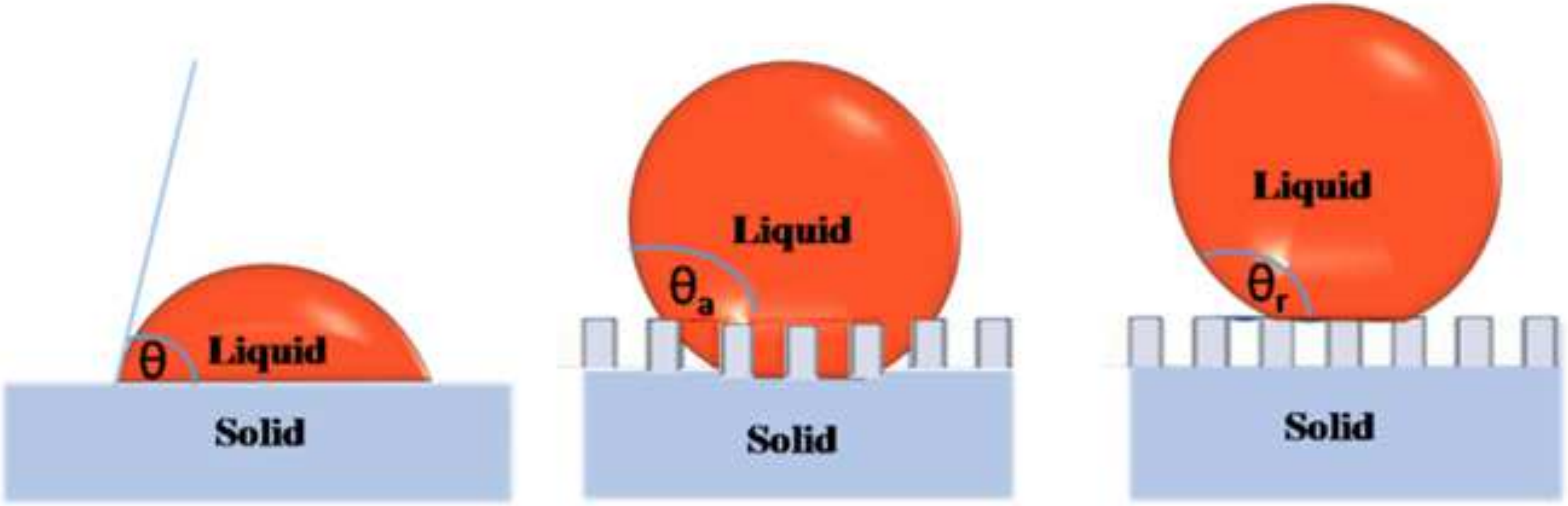

(i) Before Coating
CA =54.6°
(ii) After Coating
CA =96.4°
(a) Quartz Sample
(i) Before Coating
CA =28.5°
(ii) After Coating
CA=145.7°
(b) Glass Sample
(i) Before Coating
CA=62°
(ii) After Coating-
CA=138.8°
(c) Silicon Sample
(i) Before Coating
CA=81.4°
(ii) After Coating
CA=131.6°
(d) PDMS Sample
Bare Substrates
ZnO Coated Substrates
Contact angle (degree)
0
20
40
60
80
100
120
140
160
Glass
Quartz
Silicon
PDMS
(e)

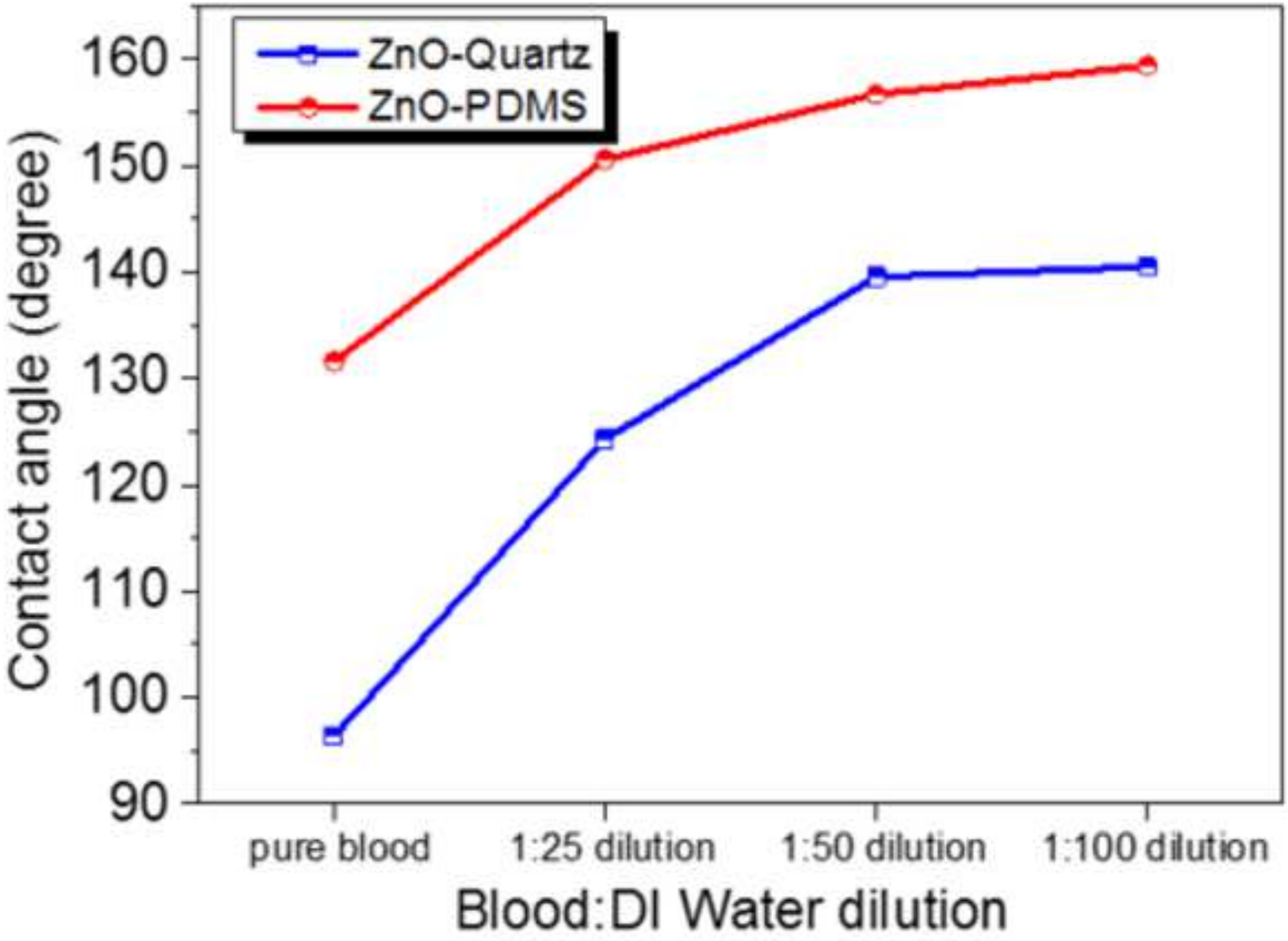
Figure 4



(a) Young's Model
$\cos\theta = (\gamma sv - \gamma sl)/ \gamma lv$

(b) Wenzel Model
$\cos\theta_a = r\cdot\cos\theta b$

(c) Cassie Model
$\cos\theta_r = f_1 \cos\theta - f_2$


ZnO-Quartz
ZnO-PDMS
Contact angle (degree)
160
150
140
130
120
110
100
90
pure blood
1:25 dilution
1:50 dilution
1:100 dilution
Blood:DI Water dilution