Cells adhesion and growth on gold nanoparticle grafted glass

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A B S T R A C T

The surface of glass substrate was plasma treated, coated by gold nano-structures and subsequently grafted with nanoparticles. The samples were plasma treated, sputtered with Au nanostructures which was followed by grafting with biphenyl-4,4′-dithiol (BPD) and then gold nanoparticles. The wettability, optical and chemical properties and surface morphology were studied. The adhesion and proliferation of vascular smooth muscle cells (VSMCs) on the samples were investigated in-vitro as well. Grafting of gold nanoparticles with the dithiol increases the UV–vis absorbance, the surface becomes more hydrophobic, rougher and more rugged compared to pristine, sputtered and only dithiol treated surface. Gold nanoparticles bound over dithiol and Au nanostructures cause better cell proliferation than purely BPD treated or pristine glass.

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1. Introduction

Noble metal nanoparticles have unique electronic, optical, and catalytic properties. The integration of metal nanoparticles into thin films is particularly important for various applications, for example in tissue engineering, biological sensing and in the preparation of electronic nano-devices [1–4]. Gold nanoparticles (AuNPs) are very used metal nanoparticles. AuNPs have several distinctive physical and chemical attributes; their optical [5] and electrochemical properties [6] and catalytic activity are different from bulk gold. Other versatile features of AuNPs include chemical inertness, simple preparation, modification, and control of particle size. AuNPs colloidal gold particles of size ranging from ca 1 to ca 100 nm in size [7,8]. AuNPs are approximately four orders of magnitude smaller than human cells and as such they are of appropriate dimensions for applications in tissue engineering [9]. Particularly, nanoparticles may interact with biomolecules (e.g. enzymes, receptors, antibodies, DNA) and cells, but they are also very attractive candidates for use as carriers in medical diagnosis and treatment [10,11]. Moreover, AuNPs are known to possess very low or no cytotoxicity effect on cells making them useful for nano-medicine applications [12].

The use of AuNPs in biological environment requires the improvement their stability using ligands [13,14]. Noble metal nanoparticles were chiefly modified by thiols [13–15], disulfides [16], amines [5], phosphines [17], carboxylic acids and mercaptoalkanoic acid derivatives [14]. Sulfur possesses a huge affinity to metal surfaces and organosulfur compounds will therefore adsorb spontaneously [18]. The metal/sulfur interaction is strong enough to immobilize the thiol-groups on the surface of metal nanoparticles. Thiol stabilized AuNPs can exhibit desired reactivities due to the variety of functionalizations and the strong Au—S bond between the softly acidic Au and the soft thiolate base [19,20]. The formation of self-assembled mono- and multi-layer films of small-ligand-stabilized metal nanoparticles enables applications such as separative layers and chemical sensors [20–22].

In this work, we have treated the glass with argon plasma and Au was subsequently sputtered on the samples under different conditions. The AuNPs were then grafted on the biphenyl-4,4′-dithiol (BPD) activated glass/gold surface. Surface changes were studied with several analytical techniques (UV–vis and XPS spectroscopy, AFM microscopy, contact angles and electrokinetic analysis). The influence of the plasma treatment, Au deposition, grafting with BPD and then with AuNPs on adhesion and proliferation of living cells was evaluated in vitro method.

2. Materials and methods

The gold layers were sputtered on $1.8 \times 1.8\,\text{cm}^2$ borosilicate microscopic glass (supplied by Glassbel Ltd., CR).

The samples were treated in direct current (DC, glow, diode) Ar^+ plasma on Balzers SCD 050 device under the following conditions: gas purity 99.997%, flow rate $0.3 l s^{-1}$, pressure 10 Pa, electrode distance 50 mm and its area 48 cm^2, chamber volume approx. 1000 cm^3, and plasma volume 240 cm^3. Exposure time was 240 s, discharge power 8.3 W, and the treatment was accomplished at room temperature. The samples were cleaned by nitrogen flow.

The gold sputtering was accomplished on Balzers SCD 050 device from gold target (supplied by Goodfellow Ltd., England). The deposition conditions were DC Ar^+ plasma, gas purity of 99.995%, sputtering time was 20 and 150 s at deposition current of 20 and 40 mA (discharge power 6.6 and 15.2 W), Ar^+ pressure about 5 Pa, and electrode distance of 50 mm. Power density of Ar^+ plasma in was $0.13 W cm^{-2}$, the average deposition rate was $0.15 nm s^{-1}$. The glass substrate was cleaned with pure methanol and dried in a stream of N_2.

Immediately after sputtering, the samples were immersed in a methanol solution ($4 \times 10^{-3} mol l^{-1}$) of biphenyl-4,4′-dithiol (BPD) for 24 h [23]. Samples with grafted BPD (see Fig. 1) were cleaned with pure methanol and then immersed for 24 h into freshly prepared colloidal citrate stabilized solution of AuNPs (concentration ca $2.75 \times 10^{-9} mol l^{-1}$ [24,25]). The average diameter of the spherically shaped nanoparticles was ca 15 nm (as determined by TEM). Finally, the samples were again rinsed with methanol and dried in a stream of N_2.

UV–vis absorption spectra were measured using PerkinElmer's Lambda 25 UV–vis-NIR Spectrometer in the spectral range 300–900 nm at scanning rate of 240 nm min^{-1} and data collection interval of 1 nm. A pristine glass slide was used for background measurement. The typical data uncertainty obtained under this arrangement is below ±5%.

Static contact angles (CA) of distilled water (pH = 6.0), characterizing structural and compositional changes caused by the gold deposition and by grafting process, were measured at room temperature on two samples and in seven positions using a Surface Energy Evolution System (SEES, Masaryk University, Czech Republic). Drops of $8.0 \pm 0.2 \mu l$ volume were deposited using automatic pipette (Transferpette Electronic Brand, Germany) and their images were taken after a 5 s delay. Contact angles were then evaluated using SEES code.
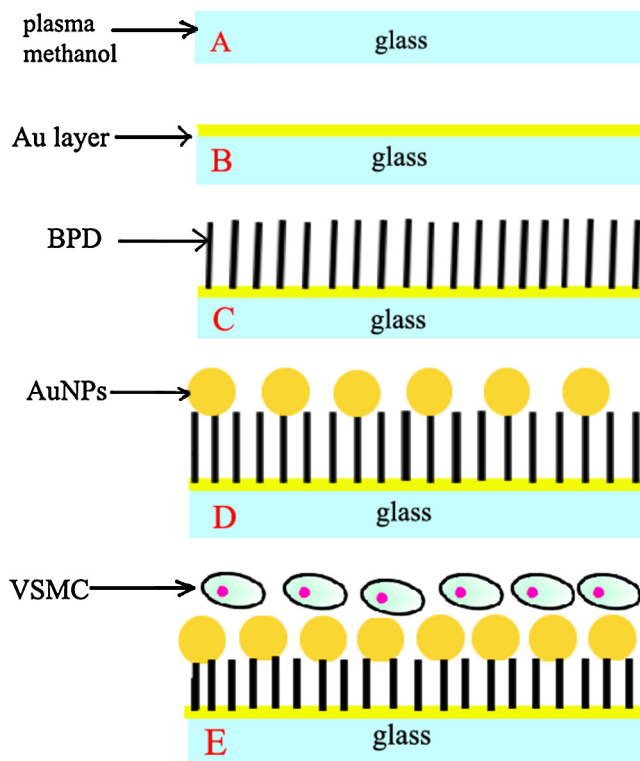

Fig. 1. Schema of sample preparation: (A)—plasma modification, (B)—sputtering of Au, (C)—BPD treatment, (D)—grafting with AuNPs and (E)—cells adhesion and proliferation.

The chemical composition of prepared structures was determined from X-ray photoelectron spectra (ARXPS), measured by Omicron Nanotechnology ESCAProbeP spectrometer. X-ray source was monochromated at 1486.7 eV and area of $2 \times 3 mm^2$ was exposed and analyzed. Spectra were measured stepwise with a binding energy step of 0.05 eV, the take off angles were 0° and 81° according to surface normal. The spectra evaluation was carried out
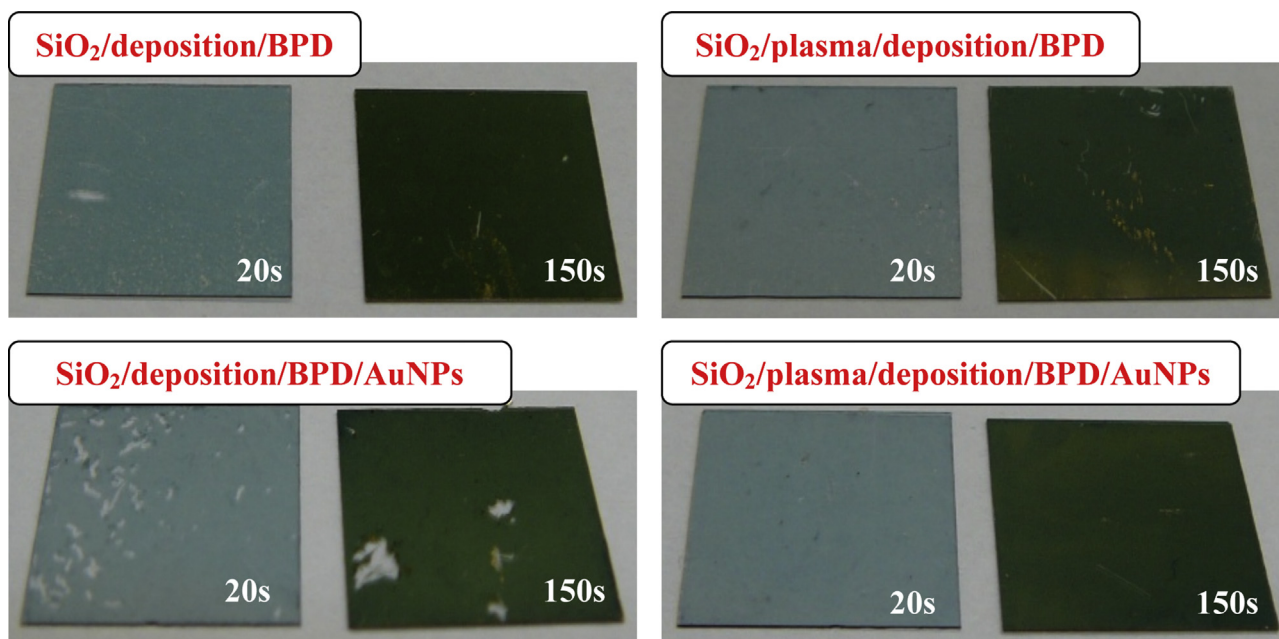

Fig. 2. Photographs of samples with sputtered Au (time 20 and 150 s, current 20 mA) and subsequently grafted with BPD and AuNPs: (i) deposition/BPD, (ii) plasma/deposition/BPD, (iii) deposition/BPD/AuNPs, and (iv) plasma/deposition/BPD/AuNPs.