GROWTH OF VASCULAR CELLS ON SPUTTERED THIN LAYERS

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Abstract: Properties of gold films sputtered under different conditions onto borosilicate glass substrate were studied. Samples were examined by AFM, ARXPS, UV-Vis and ζ-potential. The samples were seeded with rat vascular smooth muscle cells and their adhesion and proliferation were studied. Gold depositions lead to changes in the surface morphology and roughness in comparison to pristine glass substrate. Gold deposition has a positive effect on the proliferation of vascular smooth muscle cells.

Keywords: glass; gold sputtering; surface properties; cell adhesion and proliferation

1. Introduction

Metal nanoparticles on oxide substrates have gained a markedly increasing consideration with regard to both scientific and technological purposes [1]. In particular, gold-silica nanosystems are among the most studied, thanks to their extensive applications in heterogeneous catalysis [2,3] and optics (non-linear devices, fiber optics chemical sensors) [1,4-6]. As a matter of fact, supported Au nanoparticles display size- and shape-dependent properties, which can further be tailored by varying their distribution on the substrate and interparticle spacing [4]. In particular, significant variations in the Au/SiO₂ optical response are induced by modifications of the system morphology from cluster-like systems, where gold nanoparticles are dispersed on the silica surface, to island-like structures, where Au aggregates are partially interconnected between each other, and ultimately, to continuous films [5,7]. The control of the Au particle distribution and concentration is therefore a keystone in order to develop nanosystems endowed with well-tailored optical properties. To this regard, in-situ and ex-situ optical methods for real-time growth monitoring and feedback-control of the deposition process have attracted a great deal of attention due to their non-destructive and non-invasive nature [8]. The use of a suitable modeling enables to obtain valuable information on the interrelations between structure and optical properties even for low-size nanosystems, whose detection and analysis is a hard task by means of conventional structural techniques alone [9]. Despite different SE studies on both Au island-like systems and continuous films on Si and SiO₂ have appeared in the literature [1,8,10], most investigations have not been completely exhaustive. In particular, the system evolution from clusters to continuous films as a function of the preparation conditions has never been thoroughly examined. The possibility of obtaining precise material features by modulating metal/substrate nanostructure and morphology has motivated the investigation of several preparation methodologies [1,10,11].

It is known that biocompatibility of a substrates is affected, besides of several other factors, by their electrical conductivity, chemical structure, surface morphology and roughness and wettability (polarity) [12]. In this work, we studied the surface morphology, ζ-potential, UV-Vis and ARXPS spectra and adhesion and proliferation of living muscle cells on gold structure sputtered on SiO₂ surface.

2. Experiments and Methods

The gold layers were sputtered on 1.8x1.8 cm² microscopic glass, supplied by Glassbel Ltd, CZ. Surface roughness of glass, measured over the area of 1x1 µm² and calculated as an average value from five different measuring positions, was Ra = 0.34 ± 0.06 nm [13]. The gold sputtering was accomplished on Balzers SCD 050 device from gold target (supplied by Goodfellow Ltd.). The deposition conditions were: DC Ar plasma, gas purity 99.995 %, sputtering time 10–400 s, current of 10–40 mA (discharge power 3–15 W), total Ar pressure about 5 Pa and the electrode distance of 50 mm. The power density of Ar plasma in our case was 0.13 W-cm⁻² and the average deposition rate was 0.15 nm s⁻¹. Glass substrate was cleaned with methanol (p.a.) and dried in a stream of N₂. Prepared samples were stored at laboratory conditions.

Mean thickness of gold films was measured by gravimetry using Mettler Toledo UMX2 microbalance. The thickness was calculated from the sample weights before and after sputtering using gold bulk density.

Ultraviolet-visible (UV-Vis) absorption spectra were recorded using a Varian Cary 25 SCAN UV-Vis spectrophotometer (PerkinElmer Inc., USA). UV-Vis spectra in the range from 300–900 nm were taken with 1 nm data step at the scan rate of 240 nm-min⁻¹. The results are presented as difference spectra (Δ absorbance) obtained by substraction of reference spectrum of pristine glass from the spectra of sputtered samples.

Concentration of silicon, carbon, oxygen and gold in glass surface layer was measured by X-ray photoelectron spectroscopy (XPS). Omicron Nanotechnology
ESCAProbe P spectrometer was used to measure Angle Resolved Photoelectron Spectra (ARXPS, error of 5%). Exposed and analyzed area had dimension 2x3 mm$^2$. X-ray source was monochromated at 1486.7 eV and the measurement at six different sample positions was performed using energy step size of 0.05 eV. The take off angles were 0°, resp. 81° with respect to the sample surface normal. The spectra evaluation was carried out using CasaXPS software. The element concentrations are given in at. %.

Electrokinetic analysis (zeta potential) of all samples was accomplished on SurPASS Instrument (Anton Paar GmbH, Austria). Samples were studied inside the adjustable gap cell with an electrolyte of 0.001 mol l$^{-1}$ KCl, all samples were measured eight times at constant $\varphi$H = 6.0 and room temperature (error of 5 %). For the data evaluation Helmholtz-Smoluchowski (HS) equation was used.

The surface morphology of glass and gold sputtered glass was examined by Atomic force microscopy (AFM) using VEEOCP II setup, surface roughness (Ra) was measured in tapping mode (Bruker Corp., USA). Si probe RTESP-CP with the spring constant 0.9 N m$^{-1}$ was used. By the repeated measurements of the same region (1x1 µm$^2$ in area), we prove that the surface morphology did not change after three consecutive scans.

Cell culture, adhesion and proliferation

For the study of cell adhesion and proliferation of six samples, gold coated under different conditions, were used. The glass samples were sterilized for 1 hour in ethanol (75%), air-dried, inserted into polystyrene 12-well plates (TPP, Switzerland; well diameter 20 mm) and seeded with vascular smooth muscle cells (VSMCs) derived from the rat aorta using an explantation method [14]. VSMCs were seeded on the samples with the density of 16 000 cells·cm$^{-2}$ into 3 ml of Dulbecco’s modified Eagle’s Minimum Essential Medium (DMEM; Sigma, USA, Cat. No. D5648), containing 10% fetal bovine serum (FBS; Sebak GmbH, Aidenbach, Germany). Cells were cultivated at 37°C in a humidified air atmosphere containing 5% of CO$_2$. The number and the morphology of initially adhered cells was evaluated 24 h after seeding. The cell proliferation activity was estimated from the increase in the cell numbers achieved on the 3$^{rd}$ and 6$^{th}$ days after seeding [15]. The number and the morphology of the cells on the sample surface were then evaluated on microphotographs taken under an Olympus IX 51 microscope (objective 20x; visualized area of 0.136 mm$^2$), equipped with an Olympus DP 70 digital camera. The number of the cells was determined using the image analysis software NIS-Elements. For each sample type, 20 independent measurements were performed. The number of adhered and proliferated cells was determined from the 6 samples.

3. Results and discussion

Properties of gold films sputtered under different conditions onto borosilicate glass substrate were studied. Thin Au films exhibit structure-dependent UV-Vis optical spectra [16]. The delta absorption UV-Vis spectra of the samples which are gold sputtered for the sputtering times 20 and 150 s at the discharge currents from 10 to 40 mA is shown in Fig. 1. The absorbance of gold structures increase with increasing sputtering time and discharge current and film thickness as could be expected. Discontinuous and inhomogeneous layers are composed of nanometer-sized gold particles. It is well known that the optical absorption of the structures composed of gold islands is a function of island size and density [17]. On the UV-Vis spectra broad band of plasmon resonance, situated at about 500 nm, is clearly visible. The band is more pronounced on the samples sputtered for longer times and at higher discharge currents. As could be expected, the film thickness is an increasing function of the sputtering time and discharge current as well.

![Fig. 1 UV–Vis spectra of gold films deposited on glass. Sputtering times 20 and 150 s and discharge currents 10, 20, 30, and 40 mA. The numbers in right side are thicknesses of Au layer in nm.](image)

Surface chemistry of gold films deposited on glass was analyzed by ARXPS and electrokinetic analysis. Concentration of silicon, carbon, oxygen and gold in glass surface layer was measured by ARXPS technique. Element concentrations (in at. %) of pristine glass and gold coated glass is summarized in Table 1. By XPS analysis traces of titanium, potassium and sodium were found in glass substrate (SiO$_2$). The concentration of silicon in the sample surface layer is highest for the glass substrate and it decreases with increasing thickness of the deposited gold layer as could be expected. For thicker layers the silicon is not seen in XPS spectra at all. On the samples sputtered at 20 mA the surface concentration of gold increases with increasing deposition time from 22.5 % to 51.9 %. For the
deposition at 40 mA concentrations increase from 34.7% to 42.8% is observed.

Table 1. Element concentration of gold films deposited on glass (SiO₂). Sputtering times 20 and 150 s and discharge currents 20 and 40 mA.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Si (2s)</th>
<th>C (1s)</th>
<th>O (1s)</th>
<th>Au (4f)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0°</td>
<td>81°</td>
<td>0°</td>
<td>81°</td>
</tr>
<tr>
<td>SiO₂</td>
<td>17.7</td>
<td>10.8</td>
<td>23.4</td>
<td>68.7</td>
</tr>
<tr>
<td>20 mA</td>
<td>11.9</td>
<td>1.8</td>
<td>34.1</td>
<td>58.3</td>
</tr>
<tr>
<td>20 s</td>
<td>-</td>
<td>-</td>
<td>55.5</td>
<td>68.8</td>
</tr>
<tr>
<td>150 s</td>
<td>3.0</td>
<td>0.8</td>
<td>39.2</td>
<td>64.0</td>
</tr>
<tr>
<td>40 mA</td>
<td>48.9</td>
<td>65.5</td>
<td>8.3</td>
<td>6.3</td>
</tr>
</tbody>
</table>

Electrokinetic potential (ζ-potential) was measured on the samples gold sputtered at 10–40 mA for 20 and 150 s. The measured values of ζ-potential are summarized in Fig. 2. The measured values of ζ-potential are summarized in Fig. 2. No pronounced dependence of the ζ-potential on the deposition time and current is seen. Significant variations observed on particular samples reflect more likely surface heterogeneity of the glass substrate. One can see from Fig. 2 that the ζ-potentials of pure materials, glass and gold, are close each other, so that the electrokinetic analysis is not suitable for examination of glass-gold system. Differences in the ζ-potential on the samples sputtered at 20 mA could be due to the dramatic increase in the surface roughness. Pronounced increase of the ζ-potential on the sample sputtered at 30 mA for 150 s. All surface properties (composition, polarity, roughness and morphology) of the foils are expected to affect significantly the measurement of zeta potential [18].

The interaction of vascular smooth muscle cells (VSMCs) with gold sputtered glass substrate was studied by using a microscope. The number of adhered and proliferated cells just after seeding is shown in Fig. 4. For comparison in a control experiment the cells were also seeded, under the same conditions, on standard tissue polystyrene (TCPS). On the 1st and 3rd day after the seeding the number of adhered cells on the pristine glass and glass/gold substrates was minimal especially in comparison with TCPS. On the 6th day (cells proliferation) after the seeding the number of cells on pristine and gold coated glass increases dramatically. The cell growth on pristine glass is slower than on TCPS. Gold coating results in dramatic increase in VSMCs proliferation, which is higher than that on the TCPS. Analogous increase in cell proliferation was ob-
served also on substrates which are chemically grafted with gold nanoparticles [15]. From in vitro experiments the viability of VSMC cells was determined to be 60% and 95% after 1 and 6 days after seeding, respectively. Maximum number of cells is observed on the sample sputtered for 20 s at 40 mA. Surface chemistry, wettability and morphology are known to facilitate VSMCs proliferation [12, 15, 20].

Fig. 4 The number of VSMCs after different cultivation times (1\textsuperscript{st}, 3\textsuperscript{rd}, and 6\textsuperscript{th} day). On pristine glass, gold-coated glass (20 and 150 s sputtering times and 20 mA discharge current), and gold-coated glass (20 and 150 s sputtering times and 40 mA discharge current). The number of VSMCs on TCPS was used as a standard.

4. Conclusions
Glass substrates sputtered with gold for different sputtering times and at different discharge currents were studied. The thickness of the deposited gold film is an increasing function of the sputtering time and the discharge current. The UV-Vis absorbance of gold films increase with increasing sputtering time and discharge current and the film thickness. Gold deposition leads to dramatic changes in the surface morphology and roughness in comparison to pristine glass substrate. AFM images prove the creation of separated gold islands in initial deposition stage and a continuous gold coverage for longer deposition times. Gold deposition has a positive effect on the proliferation of vascular smooth muscle cells. The largest number of cells was observed on sample sputtered with gold for 20 s and at the discharge current of 40 mA. This sample exhibits low relative roughness. Under the present experimental conditions, the specific contribution of individual factors to cell interaction with the substrate cannot be classified separately. The gold/glass structures studied in this work could find an application as biosensors.

Acknowledgements
This work was supported by MSMT No. 12/2013 and by GA CR under project P108/12/G108.

5. References
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