Modified sol-gel coatings for biotechnological applications

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Abstract. The modified sol-gel derived silica coatings were prepared and characterized. The amino and methyl groups were introduced onto the colloidal silica. The silica coatings with different wettability properties: colloidal silica (water contact angle 17°), polysiloxane (61°), methyl-modified (158° and 46°) coatings samples were tested for CaCo-2 cells proliferation. Methyl-modified coating (46°) proved to be the best substrate for cell proliferation. CaCo-2 cell proliferation two days post seeding was significantly faster on almost laminine, fibronectin and collagen-1 coated samples compared to corresponding controls.

1. Introduction

Biological applications of sol–gel materials have been extensively studied during the past decade [1, 2]. Many reports have demonstrated that sol–gel materials can serve as host matrices for biological materials, from proteins to whole cells. A very large number of enzymes have been trapped within sol-gel glasses showing that they usually retain their catalytic activity and can even be protected against degradation [3-5]. The sol-gel derived inorganic matrices (films, micro-spheres or fibres) offer several advantages compared with organic polymers such as mechanical strength or chemical inertness. Bioreactors, biosensors and bio-chips can then be easily made via the sol-gel process as example sol-gel based bioreactor for enzymatic conversation of CO2 methanol [6, 7]. The sol-gel based immunosensor was also subjected to clinical samples [8]. Also, the sol-gel process was widely used for design of bioactive materials as glass, ceramic, cements or coatings [9-12]. Several authors reported sol-gel formation of artificial scaffolds for in vitro mammal cell cultivation using polymer silicate macroreticular composites [13, 14]. Hybrid sol-gel films were developed for tissue-derived cell growth [15]. And yet, taking into account, that the many applications of cell-cultures such as their use for production of various biochemicals, for growing and detecting of viruses and for production of antiviral vaccines, the potential of sol–gel materials to offer useful applications.

The surface properties of materials are important to biocompatibility of cell. It was shown [16-20] that various surface properties as type and the density of surface charge, balance between the hydrophilicity and the hydrophobicity on surface, the chemical structure and functional groups, surface topography and roughness, the interfacial free energy can affect cell attachment and growth.

This report presents investigations of the characterization and modification of colloidal silica coatings on glass substrates which could be useful for the adhesion and growth of cells. In further investigations, modified and protein coated sol-gel surface with different wettability properties have been tested for CaCo-2 cell proliferation.

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2. Experimental

**Sol-gel derived coatings.** The precursor of SiO$_2$ colloidal sol was prepared by the base catalyzed hydrolysis of tetraethylorthosilicate TEOS (Fluka, 99%) by the following method of preparation of Stöber silica [21]. The ammonia ethanol solution was added to the solution of TEOS in ethanol with continuous stirring at room temperature (20±2°C). The solutions with final silica concentration of 2% or 3% were prepared. The molar ratio of ammonium hydroxide to alkoxide was 0.2mol, to water – 0.4mol. The obtained reaction mixture was stored for 14 days at room temperature to allow hydrolysis as much as possible. The final product consisted of colloidal suspension of SiO$_2$ nanoparticles in an anhydrous solvent. The modification with (3-aminopropyl)triethoxysilane (APTES) was performed by immersing the silica coating for 12 h in toluene solution of APTES (2 g was dissolved in 50 ml toluene). Finally, the samples were dried for 2 h at 100 °C temperature and rinsed with toluene 2 times. Methyl-modified SiO$_2$ sols were prepared by adding different amount of hexamethyldisilazane (HMDS) or methytrimethoxysilane (MTMS) to the 2% colloidal silica suspension. The modified sols were aged 1 day (HMDS) or 7 days (MTMS) at room temperature. Polysiloxane 3% sol was obtained using acid hydrolysis of TEOS (TEOS:HCl:H$_2$O:EtOH molar ratio 1:0.01:4:37.4) in ethanol. Dip-coating method on both sides of the glass (Menzel-Glaser, 76x26mm) was employed to produce sol-gel coatings using apparatus KSV Instruments Ltd. KSV D™. The parameters of dipping were as following: immersion rate - 40 mm/min and dipping time - 20 s. IR spectra of the materials were recorded using ATR Perkin-Elmer Spectrum BX FT-IR spectrometer. The AFM images of the silica coatings on glass were performed on Multimode Scanning Probe Microscope (Digital Instruments).

For the characterization of surface properties, the measurements of water contact angle on KVS Instrument CAM 100 were recorded. The XPS-measurements have been performed with an Axis HS, Kratos Manchester, excitation: MgKα, 20 mA, 9 kV, 80 eV.

**Cell culture.** CaCo-2 cells from American Type Culture Collection (ATCC) were grown in advanced RPMI 1640 culture medium (Gibco, 12633-012) supplemented with 5% fetal bovine serum (FBS) (Gibco, 10106-169) and L-glutamine (2 mmol/l) without antibiotics, at 37°C in a CO$_2$ incubator (5% CO$_2$, 95% air, 95% relative humidity). FBS was heat inactivated prior to use (at 56°C for 30 min with gentle shaking). FlexiPERM micro 12 wells (Vivascience IV-50011436, growth area 0.3 cm$^2$) were stacked on microscope slides and VT079, VT104, VT111 and VT112 sample slides. The seeding number of cells was 1.5x10$^4$ cell/ cm$^2$.

**Protein coatings.** Laminin-1 from basement membrane of Engelbreth-Holm-Swarm mouse sarcoma (Sigma, L2020) was used. It was slowly thawed in the refrigerator and diluted in Hank's balanced salt solution (HBSS, Gibco, 14060-040). The surface was coated with a minimal volume (18 μl) of working solution (0.1 mg/ml) and left for 45 min. Excess fluid was sucked off and the surface was left to air dry before introducing the cell suspension. Fibronectin (Sigma F1141) solution in PBS (0.2 mg/ml) was used for coatings. A minimal amount (11 μl) of fibronectin solution was added to each well and left to dry for 45 min. Excess fluid was sucked off. The coated surface was rinsed with culture medium before the cell suspension was added. Collagen-1 (Sigma C7661) was dissolved in acetic acid (0.1 mg/ml). A minimal amount (18 μl) of collagen solution was placed in each well and the collagen was allowed to bind for two hours at 37 °C. Excess fluid was sucked off and the surface was left to air dry. The coated surface was rinsed with HBSS before adding the cell suspension.

**Assessment of cell proliferation.** Proliferation of cells was measured with the colorimetric cell proliferation BrdU (5-bromo-2-deoxyuridine) test (Kit No. 1 647 229, Roche) two days post-seeding on coated and non-coated VT samples and glass. The supernatant was replaced by 100 μl of fresh growth medium and 10 μl BrdU labelling solution was added. The cells were incubated for 90 min at 37°C. After removal of the supernatant, 150 μl of FixDent solution was added and the cells were left at room temperature for 30 min. The supernatant was sucked off and 75 μl of anti-BrdU-POD was added. The cells were then stored for 90 min at room temperature. Afterwards, the cells were washed three times with 150 μl washing buffer. 75 μl of substrate solution was added and left for some minutes protected from light for the colour to develop. 75 μl of the solution was transferred to 96-well microtiter plates and 20 μl of 1M sulphuric acid was added. The absorbance was measured at 450 nm.
Data were derived from three independent experiments and presented as means with standard deviations. The differences were analyzed using Student's t test on two populations and One-way ANOVA; p < 0.01 was considered significant.

3. Results and Discussion

For the characterization and biological tests modified nanostructured colloidal silica coatings on glass substrates were prepared. Surface modification by silanization reaction is used in numerous fields of biotechnology, where cellular adhesion and proliferation often need to be promoted for better biocompatibility. If silane used is APTES, the reaction is self-catalyzed by amine group of APTES. This reaction is attractive because it offers a chemical link between the silane and colloidal silica surface. The possible mechanism of the formation nanoparticles and of thin colloidal silica films by sol-gel method and the modification with APTES schematically are presented in Fig. 1.

![Figure 1. A schematic diagram of the steps involved in the sol-gel process used for the preparation of colloidal silica and amine-modified coatings.](image)

The prepared sol-gel derived colloidal silica coatings and modified coatings were characterized by wettability measurements using the contact angle measurements. One of the most frequently used methods of contact angle assessments is the sessile drop technique. The images of water drops on glass, colloidal silica and amino-modified coatings surface are shown in Fig. 2.

![Figure 2. Images of water drops on different surfaces: a) glass (contact angle 25°), b) colloidal SiO₂ (17°); amino-modified (angle 54°).](image)

The results showed that the colloidal silica surface (water contact angle 17°) is more hydrophilic compare to glass surface. The significance increase in the value obtained in the case of aminopropyl group introduction. The increasing depends on the chemical structure of the APTES. It is a short molecule with hydrophobic propyl residue and amine group at the end of the hydrocarbon tail.

The colloidal silica and amino-modified coatings were also characterized by IR and XPS spectroscopy. IR spectroscopy carried out on colloidal silica coatings and after modification with APTES indicates a significant decrease in the silanol bands (3740 cm⁻¹ and 3553-3670 cm⁻¹) and increase of new bands of aminopropyl groups. The bands were corresponding to both the symmetric
and asymmetric stretching of -H₂ (2930, 2870 cm⁻¹) and NH₂ (3360, 3280 cm⁻¹). The XPS analysis of amino-modified surface shows the changes amount of O, S, and C (Table 1.).

**Table 1. XPS data of coatings**

| Surface     | O 1s 533 eV | N 1s 400 eV | N 1s 401.5 eV | Si 2p 105 eV | C 1s 284 eV |
|-------------|-------------|-------------|---------------|--------------|-------------|
| colloidal SiO₂ | 55.3        | 0.5         | 0.0           | 29.01        | 0.2         |
| APTES       | 36.0        | 2.5         | 0.7           | 25.12        | 9.9         |
| -N-H₂ -NH₃⁺ | -NH₃⁺       | SiOₓ        |               |              |             |

The modification with aminopropyl groups results in a clear change of the N1s-Peak and C 1s (284 eV). Two components at ~ 400 eV with a concentration of ca. 2.5 At-% and at ~ 401.8 eV with a concentration of ca. 0.7 At-% can be found. The first one can be assigned to a NH₂ binding. The second peak can be related to a –NH₃⁺. Also, the Si/O-ratio has changed during this step.

AFM images of colloidal silica and amino-modified coatings obtained are shown in Fig. 3.

*Figure 3. AFM images of modified surface of a) colloidal silica coatings b) modified with APTES. Image size 2x2 μm*

AFM images shows that colloidal silica coating is composed of ≈ 25-35 nm silica particles. The surface of coating is rather smooth, the roughness is \( R_{MS} = 4.3 \) nm, and the particles show tendency to form agglomerates. It is evident from images, that the nanosilica surface is modified by amine groups. The surface roughness is \( R_{MS} = 12.54 \) nm and particles agglomeration occurs.

With the aim of finding the optimal hydrophobicity for cells growth, the methyl-modified silica coating were prepared. The surface of silica nanoparticles was modified by adding different ration of reagents hexamethyldisilazane (HMDS) or methyltrimethoxysilane (MTMS) to colloidal silica sol. The water contact angles of surface coatings are shown in Fig. 4.

*Figure 4. Water contact angles of surface coatings obtained from methyl-modified sols*

The colloidal silica particles are covered by hydroxyl groups, after HMDS or MTMS addition, some of the hydroxyl groups are replaced by methyl groups. The contact angle of water increased with increasing amount of HMDS or MTMS, but HMDS modified coating has higher contact angle than
MTMS compound. In case HMDS, each monomer of HMDS consists of two trialkylsilane groups, which gets attached to the surface. Hence HMDS modified coating shows the highest contact angle (158°) due to its better hydrophobic covering.

The CaCo-2 cells from American Type Culture Collection (ATCC) were grown on sol-gel derived coatings with different wettability properties. The coatings obtained from colloidal silica (VT104), polysiloxane sol (VT111), methyl-modified sols (VT079 and VT112) were selected for proliferation test. The sol composition and data of water contact angles of coatings are shown in Table 2.

| Sample   | Sol composition          | Water contact angle (°) |
|----------|--------------------------|-------------------------|
| VT079    | methyl-modified SiO₂     | 158                     |
| VT112    | methyl-modified SiO₂     | 46                      |
| VT104    | 3% colloidal SiO₂        | 17                      |
| VT111    | 3% polysiloxane          | 61                      |

Cell behaviour on biomaterial surface is modulated by the concentration, composition and conformation of absorbed extracellular matrix (ECM) proteins [22]. The sol-gel coatings were coated with a minimal volume of different proteins as laminin, fibronectin or collagen-1 solutions. Results of proliferation on coatings surfaces are presented in Figs. 5 and 6.

**Figure 5.** Proliferation of CaCo-2 cells on VT079, VT104, VT111, VT112 samples and glass as control surface. ★: marks those cases where there is significant difference in values (p < 0.01, Student's t test on two populations) between VT samples and glass control.

**Figure 6.** Proliferation of CaCo-2 cells on laminin-1, fibronectin and collagen-1 coated VT079, VT104, VT111 and VT112 samples and corresponding controls. ★: marks those cases where there is significant difference in values (p < 0.01) between protein coatings and corresponding controls.

When only simple samples (Fig. 5) are compared the means within all samples are significantly different (p < 0.01, One-way ANOVA). Proliferation on VT079 was significantly slower and on VT112 significantly faster (p<0.01) then on glass control (Fig. 5). This two substrates were both
methyl-modified, however contend of HMDS was 7.17 % in VT079 and 1.25 % in VT112. The means within the surfaces for all protein coated VT samples and controls are significantly different (p < 0.01, One-way ANOVA) and almost all protein coated samples stimulated proliferation compared to the corresponding controls (Fig. 6). Laminin-1 coated VT104 sample was the only protein coated surface where proliferation was significantly slower (p<0.01) then on control.

4. Conclusions

The modified sol-gel derived silica coatings were prepared and characterized. The coatings of colloidal silica (VT104, water contact angle 17°), polysiloxane sol (VT111, 61°) methyl-modified sols (VT079, 158° and VT112, 46°) with various wettability properties were tested for CaCo-2 cells proliferation. Methyl-modified coating VT112 proved to be the best substrate for cell proliferation. Cell proliferation two days post seeding was significantly faster on almost all proteins coated samples compared to corresponding controls. The difference in drop contact angle between VT079 (158°) and VT104 (17°) is the biggest. From our results we can assume, that other surface properties are more significant for protein adsorption and consequent for cell growth and proliferation. The advent of hybrid sol–gel materials opens new possibilities for tailoring efficient substrates for cell adhesion and growth. A major advantage of these materials is that the quantity of preparation methods and chemicals with which one can design almost any desired surface property is practically unlimited.

Acknowledgements

The financial support from the Integrated Project CellPROM (No. NMP4-CT-2004-500039) under the 6th Framework Programme for Research and Technological Development in the thematic area of “Nanotechnologies and nano-sciences, knowledge-based multifunctional materials and new production processes and devices” is gratefully acknowledged.

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