Biocompatibility of atomic layer-deposited alumina thin films

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Abstract: Presented in this paper is a study of the biocompatibility of an atomic layer-deposited (ALD) alumina (Al_2O_3) thin film and an ALD hydrophobic coating on standard glass cover slips. The pure ALD alumina coating exhibited a water contact angle of 55° ± 5° attributed, in part, to a high concentration of —OH groups on the surface. In contrast, the hydrophobic coating (tridecafluoro-1,1,2,2-tetrahydro-octyl-methyl-bis(dimethylamino)silane) had a water contact angle of 108° ± 2°. Observations using differential interference contrast microscopy on human coronary artery smooth muscle cells showed normal cell proliferation on both the ALD alumina and hydrophobic coatings when compared to cells grown on control substrates. These observations suggested good biocompatibility over a period of 7 days *in vitro*. Using a colorimetric assay technique to assess cell viability, the cellular response between the three substrates can be differentiated to show that the ALD alumina coating is more biocompatible and that the hydrophobic coating is less biocompatible when compared to the control. These results suggest that patterning a substrate with hydrophilic and hydrophobic groups can control cell growth. This patterning can further enhance the known advantages of ALD alumina, such as conformality and excellent dielectric properties for biomicro electro mechanical systems (Bio-MEMS) in sensors, actuators, and microfluidics devices. © 2007 Wiley Periodicals, Inc. J Biomed Mater Res 87A: 100–106, 2008

Key words: ALD; atomic layer deposition; biocompatibility; bio-MEMS; conformal coatings; vascular smooth muscle cells

INTRODUCTION

The primary motivation for this paper is to present results obtained on the biocompatibility of atomic layer-deposited (ALD) alumina (Al_2O_3) and hydrophobic coatings. While these coatings are increasingly finding their way into micro electro mechanical systems (MEMS) as dielectric layers to prevent electrical shorting,[1] the work presented here represents the first study to determine the compatibility of ALD coatings for applications in Bio-MEMS. A combination of optical microscopy and an Alamar Blue (AB) assay is used to gain a quantitative measure of the biocompatibility of ALD alumina-coated glass cover slips with human vascular smooth muscle cells.

The number of biomedical applications for Bio-MEMS is increasing significantly. These include catheter-based blood pressure sensors,[2] thermal conductivity sensors,[3] microfluidic devices, and tools for neurosurgery.[4] All of these applications represent short-term use. An attractive opportunity is to implant sensors permanently for *in vivo* monitoring. However, this presents a significant set of challenges both in terms of an appropriate biological response and the reliability of Bio-MEMS. To this end, the need is evident for coatings that both protect the device from a fluidic environment and minimize the impact of the materials on the biological processes that they are measuring.

Many of the processes used to coat MEMS are line-of-sight, which ultimately limits their usefulness

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TABLE I
Summary of Known Properties of Atomic Layer-Deposited Alumina (Compiled From Previous Work[10,11])

Thickness (nm)	Deposition temperature (°C)	Resistivity (Ωm)	Leakage current (A/cm^2)	Electric field used for leakage current measurement (mV/cm)	Catastrophic breakdown (mV/cm)	Dielectric constant, *k*
115	177	1.00 E + 16	1.00 E − 10	1	–	7.5
12	177	1.00 E + 15	2.00 E − 09	3	5.3	5.9
Density (g/cm^3)	**Refractive index (*n*)**	**RMS roughness (nm)**	**FReS (hydrogen concentration)**	**RBS (oxygen/ aluminum ratio)**	**RBS (carbon)**	**RBS (nitrogen)**
3.0	1.6	0.4 ± 0.1	6.9%	1.56 ± 0.19	Undetected	Undetected

RBS = Rutherford backscattering; FReS = forward recoil spectrometry.

when considering raised or three-dimensional structures. A viable alternative is to use atomic layer deposition to produce coatings.[5] Such coatings are conformal, pinhole free, can be deposited with atomic layer precision at low temperatures, and adhere well to materials used in MEMS devices.[1]

Atomic layer-deposited films are available with a variety of compositions. In this work, we consider ALD alumina because of the previously demonstrated biocompatibility of bulk alumina.[6–9] In addition, the ability to modify the surface properties by modifying the hydroxyl group concentration (making the surface more hydrophilic or hydrophobic) is of scientific interest in terms of our basic understanding of biocompatibility.

Typical electrical and chemical properties of ALD alumina are shown in Table I.[10,11] The electrical measurements for ALD alumina were performed on n-type Si(100).[10] The presence of an approximately 1.2-nm-thick interfacial SiO_2 layer between the ALD alumina and the Si(100) lowers the dielectric constant (*k*) for the 12 nm-thick ALD alumina film from 7.5 to 5.9.[10]

Atomic layer deposition

Atomic layer deposition (ALD) utilizes sequential, self-limiting surface reactions of chemical species to deposit thin films.[5,12] The deposition of alumina (Al_2O_3) on silicon is achieved by using sequential exposures of trimethylaluminum (TMA, $Al(CH_3)_3$) and water.[13,14] A schematic drawing of a typical ALD reaction sequence is shown in Figure 1.

In the first reaction, labeled (A), $Al(CH_3)_3$ reacts with the hydroxyl (OH*) group (the asterisk hereinafter representing the surface-active species), and deposits a monolayer of methyl-terminated aluminum. This reaction stops after all the hydroxyl species have reacted with the TMA. In the second half of the process, labeled (B), water reacts with the $AlCH_3$* species and deposits a monolayer of hydroxyl groups. The second reaction stops after all the methyl species have reacted with the H_2O. The surface is now ready for another exposure of TMA. These reactions are repeated in a cyclic manner to achieve the desired film thickness.

Because each reaction is self-limiting, the Al_2O_3 deposition occurs with atomic layer precision. The number of reaction cycles determines the thickness of the coating. A linear growth rate of 0.12 nm per cycle was observed during the experiment. This growth rate is the normal growth rate for ALD alumina at 177°C.[14] Atomic force microscopy (AFM) images revealed that the Al_2O_3 films on Si(100) were extremely flat and uniform (see Table I). From Rutherford backscattering spectroscopy (RBS), the levels of carbon and nitrogen in the alumina films were below the limits of detectability.[11]

After completing the H_2O reaction, the Al_2O_3 film is left with a very high coverage of AlOH* groups on the surface. At room temperature, this hydroxyl coverage may be almost twice as large as the coverage of hydroxyl groups on SiO_2 surfaces, generally accepted to be approximately 4.6×10^{14} OH

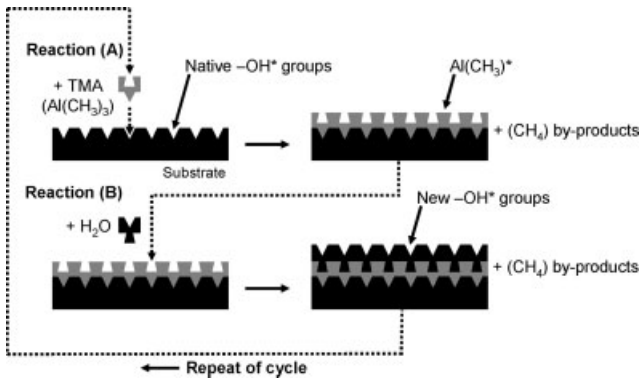

Figure 1. ALD reaction scheme showing two self-limiting half reactions, (A) and (B), which combine to give Al_2O_3 coatings of well-defined chemistry and thickness, in which the $Al(CH_3)$* and the —OH* groups are the surface reactive species and CH_4 is the reaction by-product.

groups/cm^2.[15–17] This population of AlOH* groups was utilized during a gas phase technique to functionalize the surface and render it highly hydrophobic. This technique, described in detail elsewhere,[18] comprises an ALD alumina seed layer and the subsequent adsorption of a functional hydrophobic monolayer using a non-chlorinated precursor. The precursor was synthesized from chlorosilane available commercially (Gelest, Morrisville, PA).

MATERIALS AND EXPERIMENTAL METHODS

Materials

Commercially available borosilicate microscope glass cover slips were used to compare uncoated surfaces with those coated with ALD alumina and a hydrophobic coating. Glass cover slips were chosen primarily because of size limitations in the ALD reactor and because of their widespread use in cell biology.

Atomic layer deposition

ALD was performed in a viscous flow reactor.[19] Although there are no theoretical limitations to the size of the reactor that can be used, the flow tube or reaction zone used for these experiments was a stainless steel tube 50.8 cm long with a 3.5-cm inner diameter, heated externally with ceramic heaters. The tube had a reaction region 2.5 cm wide by 18 cm long in which seven glass cover slips were placed and subsequently heated by radiation and convection from the hot walls.

The system utilized a viscous flow of nitrogen carrier gas to transport the reactants to the substrate and to purge the reaction products from the flow tube. A mass flow controller was used to regulate the nitrogen flow to a total of ~200 sccm. These conditions yielded a ~2.5 m/s flow velocity in the reaction chamber. The reactants were alternately pulsed into the gas flow using pneumatic valves controlled by a computer program. The glass substrates were coated with 500 AB cycles (see Fig. 1) at 177°C. These 500 AB cycles correspond to ~60 nm thickness of ALD alumina. The choice of temperature is based on the optimum deposition rate for the process. In practice, the process can be applied at close to room temperature with a resultant increase in cycle time due to decreased reactivity and longer purge times.[11]

To produce the hydrophobic variation, an ALD alumina seed layer was deposited at 140°C using the approach described above. This Al_2O_3 layer provides a dense and uniform concentration of surface —OH groups. A hydrophobic precursor, tridecafluoro-1,1,2,2-tetrahydrooctyl-methyl-bis(dimethylamino)silane ($C_8F_{13}H_4(CH_3)Si(N(CH_3)_2)_2$), was then chemically bonded to the surface hydroxyl groups on the ALD seed layer. On flat substrates, this procedure produces a film that is highly hydrophobic with a water contact angle of 108°.[18] The typical thickness of the outermost hydrophobic layer is ~1 nm, based on the knowledge of the chemical structure of the precursor as well as high resolution TEM studies.[18]

Cell culture

Human coronary artery smooth muscle cells (CASMC) were obtained commercially (Cambrex Bio Science, Wakersville, MD). The cells were cultured in smooth muscle cell media (SMCM) and supplemented with 5.5 m*M* glucose, 5% fetal bovine serum (FBS), 50 μg/mL gentamicin, 50 ng/mL amphotericin-B, 5 μg/mL insulin, 2 ng/mL human recombinant fibroblast growth factor (hFGF), and 0.5 ng/mL human recombinant epidermal growth factor (hEGF). The cells were then incubated in a humidified environment at 37°C in 5% CO_2 and 95% air.

At 80% confluency, the cells were rinsed in HEPES, an organic chemical buffer, and then detached from the surface of T75 cell culture flasks using a 0.025% trypsin in ethylene diamine tetra acetic acid (EDTA) solution for 2 min. The CASMCs were resuspended in a trypsin neutralizing solution, centrifuged into pellet form and resuspended in smooth muscle cell media as a cell suspension for the experiments. Cells that were available at passage numbers 7 and 8 were used in the experiments. Cell passaging or splitting is a technique that keeps cells alive and growing under cultured conditions for extended periods of time.[20]

Cell proliferation and measurement of cell activity

The effects of proliferation of coronary artery smooth muscle cells on ALD alumina-coated substrates were compared to the same cell batch grown on glass control substrates. This comparison assessed whether biologic function was retained on the ALD alumina-coated substrates. An Alamar Blue (AB) (Alamar Blue™, Biosource International, Camarillo, CA; Alamar Blue assay, US patent 5,501,959, Trek Diagnostic Systems, Cleveland, OH) assay was used to determine cell proliferation viability/activity over 7 days of growth on the various substrates. The AB assay, which incorporates a redox indicator that changes color in response to metabolic activity, is commonly used to assess quantitatively the viability and/or proliferation of mammalian cells and micro-organisms.[21] As cells grow, the chemical reaction of the growth media causes the Alamar Blue to change from a blue (oxidized) to a red (reduced) form. The color change is due to the uptake of oxygen by the cells in the media and its replacement by hydrogen.

All substrates were degreased by soaking in 100% ethanol for 10 min each, dried under a laminar flow hood and sterilized under a UV lamp for 20 min on both sides before exposure to cells. Experiments were performed using untreated six-well cell culture plates. Each well was considered an individual data point. The number of cells plated in each experiment was determined using a standard commercially available hemocytometer. Initially, 125 μL of cell suspension was added to each substrate surface in the six-well cell culture plates and incubated for

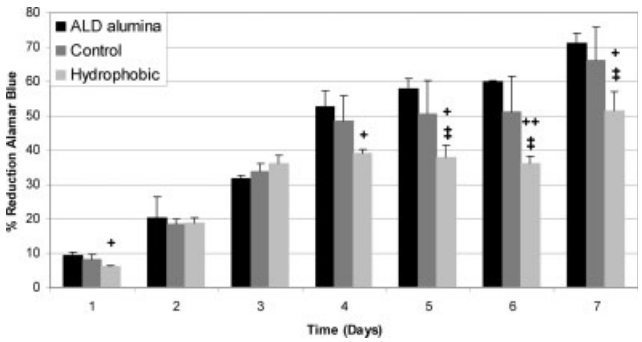

Figure 2. Reduction in Alamar Blue (%) over 7 days, comparing ALD alumina-coated cover slips with hydrophobic-coated cover slips and uncoated cover slips (control). Data is represented as a bar for each time point and reported with the associated mean ± standard deviation (SD). Data is reported as mean ± SD, where ‡ shows a significance test for cases versus control, (‡ $p < 0.05$) and + shows a significance test for ALD alumina versus hydrophobic (+ $p < 0.05$ and ++ $p < 0.01$).

fifteen minutes prior to the addition of 2 mL of SMCM and supplements.

The optimum cell-seeding density was determined to be 6×10^4 cells per well from a standard calibration curve obtained over 48 h using concentrations ranging from 0.5 to 36×10^4 cells/mL. Alamar Blue was added to the cell culture wells at 1:10 (v/v) ratio and incubated over the 5 days. Aliquots of 200 μL of the Alamar Blue medium were transferred at each time point to a 96-well cell culture plate and absorbance read at 570 and 600 nm using a commercially available automated plate reader. Absorbance was recorded every 24 h over a period of 7 days.

Statistical analysis

All experiments were performed for 7 days in triplicate and repeated at least twice. Cell proliferation data were analyzed using standard analysis of variance (ANOVA) techniques (Graph Pad In Stat, GraphPad Software, San Diego, CA) and Students Newman-Keuls post-hoc comparisons, with a value showing $p < 0.05$ considered significant. The mean ± standard deviation and ± standard error of the means are presented in the graphs.

Optical microscopy

Optical micrographs of the proliferating vascular smooth muscle cells were recorded using a differential interference contrast (DIC) microscope equipped with a digital camera. Cells were imaged directly on the cover slip. These were prepared by removing the cover slip with cells attached from the media and gently rinsing them to minimize cell detachment in distilled deionized water. This was immediately followed by recording optical micrographs.

RESULTS AND DISCUSSION

Alamar Blue assay

The main objective of using the Alamar Blue assay is to quantify the proliferation of the human vascular smooth muscle cells on the ALD alumina- and hydrophobic-coated glass cover slips. The uncoated borosilicate glass cover slips served as a control. As stated previously, a cell plating density of 6×10^4 cells/mL was chosen from the standard curve experiment. The cell suspensions were placed on the coated and uncoated cover slips (control samples) as per the procedure described above under Cell Proliferation and Measurement of Cell Activity.

The experiments were performed in triplicate to take into account the variability within a particular run. The results are presented as a percent reduction AB of the two coated substrates and the control shown in Figure 2. The difference in AB reduction between the control and the two coated substrates (ALD alumina and hydrophobic) as a function of time is shown in Figure 3. These results clearly demonstrate the different responses of the two substrates versus the control.

Figures 2 and 3 show that the percentage of Alamar Blue reduction for the ALD alumina-coated cover slips is slightly higher than that of the uncoated glass cover slips. However, the difference is not statistically significant. In the case of the hydrophobic coating, the AB reduction was found to be statistically lower ($p < 0.05$), showing a difference from the control glass cover slip and the ALD alumina-coated substrates after 4 days. With reference to Figure 3, the ALD alumina-coated substrate shows a slight decrease at day 7. This decrease is most likely associated with contact inhibited cell growth and is indicative of a higher cell proliferation rate on ALD alumina.

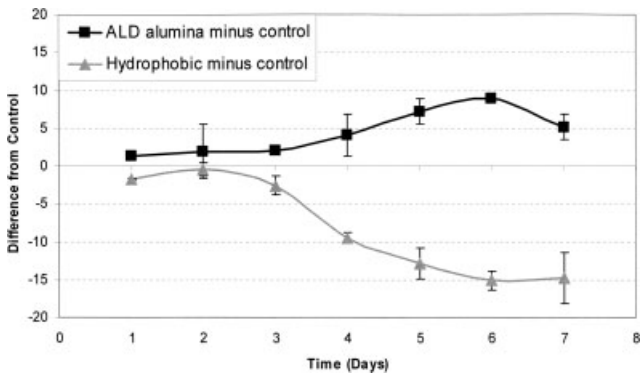

Figure 3. Difference (mean ± standard error %) from control for ALD alumina and hydrophobic coating versus time (days).

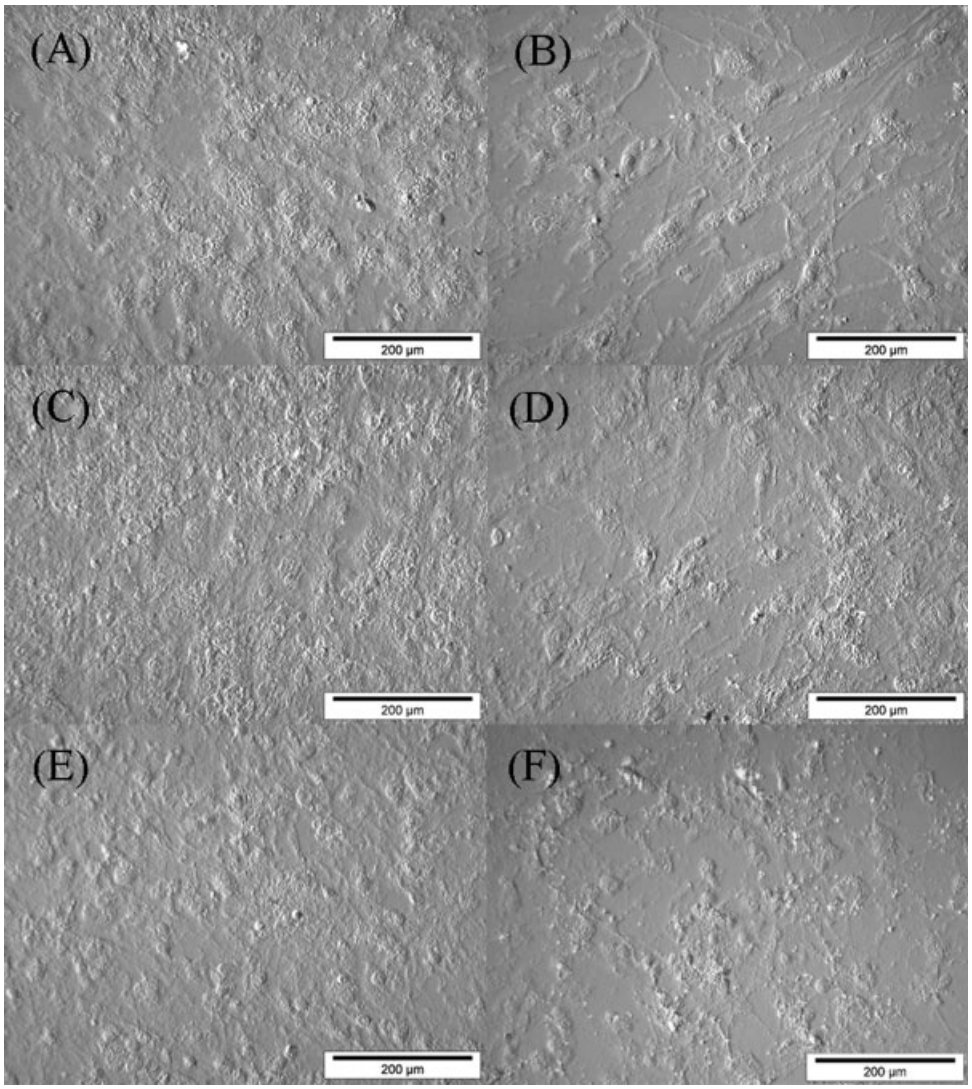

Figure 4. Differential interference contrast micrographs of human vascular smooth muscle cells, comparing cell proliferation on ALD alumina-coated glass cover slips with uncoated (control) glass cover slips. (A) ALD alumina coating after one day, (B) ALD alumina coating after 5 days, (C) hydrophobic coating after one day, (D) hydrophobic coating after 5 days, (E) control after one day, and (F) control after 7 days.

Microscopy

The images of the cells on the substrates in Figure 4, recorded after 1 and 5 days of exposure, show that the human coronary artery smooth muscle cells have adhered to the coated substrates and have formed a coherent layer. Proliferation rates were typical for *in vitro* conditions, whereby cells spread randomly with time on the surface without any organized structure when compared to the elongated morphology that would be observed *in vivo*.

The cells consistently appeared to proliferate equally or more rapidly during the first 24 h on the ALD alumina-coated glass cover slips than on the uncoated control as indicated by the higher cell density. This result is in contrast with the Alamar Blue results that showed no statistical difference between the two samples after 24 h. At day 7, some visual differences were apparent between the samples, with the cells attached to the ALD alumina surface appearing less densely populated than the control, suggesting an increase in cell spreading and average cell size.

As mentioned under the section titled Atomic layer deposition of the Introduction, there is a large concentration of hydroxyl groups present on the surface of the ALD alumina, which contributes to its hydrophilicity. The measured contact angle for ALD alumina is $55^\circ \pm 5^\circ$. This contact angle is very similar to the contact angle for the glass cover slips of $61^\circ \pm 2^\circ$. The hydrophilic nature of the ALD coating, typically desirable for cell adhesion and growth,[22,23] can explain the observed results. Increasing the contact angle to 108° by incorporating tridecafluoro-1,1,2,2-tet-

rahydro-octyl-methyl-bis(dimethylamino) silane on the surface of the ALD alumina had a statistically significant impact on the AB reduction.

These results show that modifying the hydrophilicity of the surface coating impacts cell viability. ALD alumina was shown to be biocompatible. The advantage of using ALD, aside from ALD being a conformal coating method, is that the surface properties can be tailored to a given application. Further work is underway to determine quantitatively the impact of changing surface chemistry and electrical properties on cell proliferation and growth.

CONCLUSIONS

Cell adhesion and growth have been successfully demonstrated on atomic layer-deposited alumina thin films on glass cover slips. The use of an Alamar Blue assay as a measure of biocompatibility suggests that coating glass cover slips with 60 nm of ALD alumina results in very similar levels of biocompatibility when compared with the uncoated glass samples. Modifying the ALD coating via the addition of hydrophobic groups leads to a statistically significant reduction in Alamar Blue, which is interpreted as a reduction in biocompatibility of the coating. These observations pave the way for the adoption of ALD alumina coatings for Bio-MEMS and other devices coming into contact with biological media.

Further work is required to expand this study using a variety of other materials relevant to Bio-MEMS, such as polysilicon. The possibility also remains of whether or not ALD alumina can be applied to gold structures that are commonly used in MEMS devices, and whether or not these ALD alumina-coated structures are biocompatible. In addition, no evaluations have been made to determine the long-term performance of these coatings. Questions also remain as to why the ALD alumina-coated samples did not show a more significant improvement over the uncoated glass cover slips, and whether or not there is a direct relationship between contact angle and biocompatibility. Consequently, there is a need for further investigation in terms of optimizing the ALD coating for the best chemical and surface properties.

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