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Growth of Zircone on Nanoporous Alumina Using Molecular Layer Deposition

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Molecular layer deposition (MLD) is a sequential and self-limiting process that may be used to create hybrid organic/inorganic thin films from organometallic precursors and organic alcohol precursors. In this study, films of a zirconium-containing hybrid organic/inorganic polymer known as zircone were grown on nanoporous alumina using MLD. Scanning electron microscopy data showed obliteration of the pores in zircone-coated nanoporous alumina. An in vitro cell viability study indicated that the growth of human epidermal keratinocytes was the greatest on zircone-coated nanoporous alumina than on uncoated nanoporous alumina. Our results suggest that MLD may be used to create biocompatible coatings for use in many types of medical devices.

INTRODUCTION

Atomic layer deposition (ALD) is a sequential and self-limited thin film growth process that provides conformal films with excellent control over the film thickness.[1] ALD is used to create many types of ceramic or metal films, including films of alumina and tungsten.[2] It is widely used in the semiconductor industry but has only had limited use to this point in other emerging fields such as the biomedical device industry.

A subset of ALD is molecular layer deposition (MLD), which involves the use of bifunctional molecular precursors for "dry" growth of films via step growth polymerization.[3,4] Early MLD efforts involved growth of polyimide and polyamide films; growth of many other organic polymers, including polyurethanes, polyesters, and polyureas, has been subsequently demonstrated using this approach.[5–9]

ALD and MLD involve growth that occurs in a layer-by-layer manner. These processes typically involve binary reactions that are broken into discrete half cycles, in which the two precursors are temporally separated from each other. Purge steps commonly follow the introduction of each precursor and enable the removal of the excess precursor. Since a molecular layer is deposited for each pair of MLD half cycles, the MLD-grown film thickness is related to the number of reaction cycles.[10,11]

MLD exhibits several advantages over spin-coating and other "wet" processes for thin film growth.[12] Films with excellent control over composition and conformality may be obtained using this approach.[13] In addition, MLD may be used to coat high-aspect-ratio structures such as nanoparticles.[14] The growth rate in MLD is limited by the time needed move between precursors in the film growth chamber and the number of substrate surface sites.[4] Another benefit of the MLD approach is that it does not require catalysts or solvents.[11] The substrate temperature typically associated with MLD (60–175°C) is appropriate for film growth on many temperature-sensitive (e.g., polymeric) substrates.[4] As noted by Zhou and Bent,[12] MLD is compatible with many conventional vapor phase processes. In addition, efforts are underway to develop scalable, high-throughput MLD methods that are suitable for commercial use.[9]

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Many types of MLD films have been developed, including the metalcone (metal alkoxide polymer) films; examples of metalcones include alucones, zincones, titanicones, and zircones.[3,15]

In general, hybrid organic/inorganic thin films are created from an organometallic precursor and an organic alcohol precursor.[3,4] For example, alucone (aluminum alkoxide) hybrid organic–inorganic polymers can be created from aluminum alkyl precursors (e.g., trimethylaluminum) and organic precursors [e.g., ethylene glycol (EG)].[9,10,16] Hybrid organic–inorganic polymer films with well-controlled thicknesses have been demonstrated.[4,16,17] Films with porous oxide films have been created from MLD-grown films. For example, Liang et al.[14] created porous aluminum oxide films using a two-step process that involved (I) growth of a dense alucone film using MLD and (II) etching of the organic component of the film by either calcination in air or water etching.

Although the biomedical applications of MLD are potentially very interesting, only a few efforts have been undertaken to understand the biocompatibility of MLD-grown thin films to this point.[1,18] In this study, films of a zirconium-containing hybrid organic/inorganic polymer known as zircone (zirconium alkoxide) were grown on nanoporous alumina using MLD. Metalcones contain a metal center that is surrounded by an organic diol. In zircone, the metal center is zirconium and the organic diol is EG. Lee et al. showed that zircone films with different inorganic component:organic compontent ratios may be created by depositing a combination of zirconium oxide via ALD and zircone via MLD.[15] The substrate used in this study, nanoporous alumina, exhibits long-range ordering and high pore densities. It is being considered for use in a wide variety of biomedical applications, including immunoisolation (e.g., pancreatic islet cell encapsulation), drug delivery, and biofiltration.[19] Scanning electron microscopy was used to perform imaging of the pores, and an in vitro cell viability study was conducted to compare the growth of human epidermal keratinocytes on zircone-coated nanoporous alumina with that on uncoated nanoporous alumina.

MATERIALS AND METHODS

The nanoporous alumina was fabricated using a two-step anodization-based approach.[20] A 99.999% pure aluminum sheet (thick = 1 mm) was electropolished in a mixture of perchloric acid and ethanol (1:4 volumetric ratio) for elimination of surface irregularities; a temperature of 7°C and a constant voltage of 20 V were used for the electropolishing process (duration = 3 min). Nanoporous alumina was obtained from two-step anodization in 0.3-M sulfuric acid solution (temperature = 0°C, voltage = 25 V); the first anodization step (duration = 12 h) and the second anodization step (duration = 20 h) were separated by an alumina etching process that involved a chromium (IV) oxide (CrO_2)-based solution (temperature = 65°C, duration = 5 h). The aluminum layer was removed in a copper(II) chloride ($CuCl_2$)-based solution at room temperature for about 20 min. The alumina barrier layer was etched in 0.1-M phosphoric acid at 30°C for 80 min. The nanoporous membrane was detached using 0.1-M phosphoric acid at 30°C for 30 min. The resulting membrane exhibited a nanohole diameter of 20 nm, a nanofilter diameter of 1 cm, and a nanofilter thickness of 100 μm (Fig. 1).

The zircone film was deposited using zirconium tert-butyloxide (ZTB), ($Zr[O(CH_3)_4]$; 99%, Sigma Aldrich, St. Louis, MO, USA) and EG ($HO(-CH_2)_2OH$; Reagent Plus >99%, Sigma Aldrich, St. Louis, MO, USA).[20] Figure 2 shows the possible mechanism for zircone film growth via MLD.[15] The precursors were carried into a viscous flow reactor as described elsewhere using ultra-high-purity nitrogen (Airgas, Radnor Township, PA, USA).[20] The nitrogen was also used to separate the precursors and to purge the system between cycles. The optimal dosing and purging conditions for one cycle in the viscous flow reactor are given as (Dose A (s)/Purge A (s)/Dose B (s)/Purge B (s)). The optimal

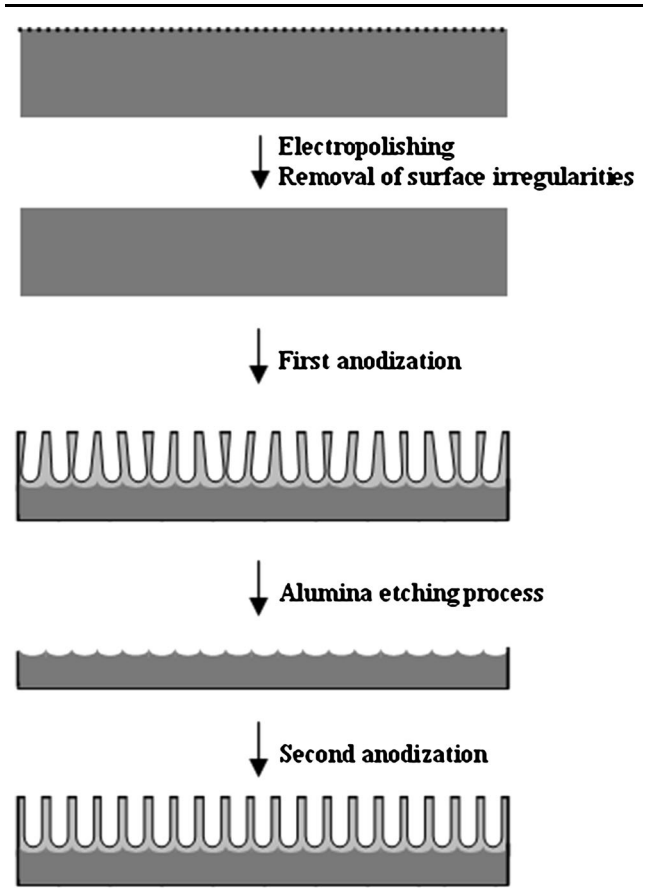

Fig. 1. Preparation of highly ordered nanoporous alumina. Reproduced from Ref. 19 with permission of The Royal Society of Chemistry.

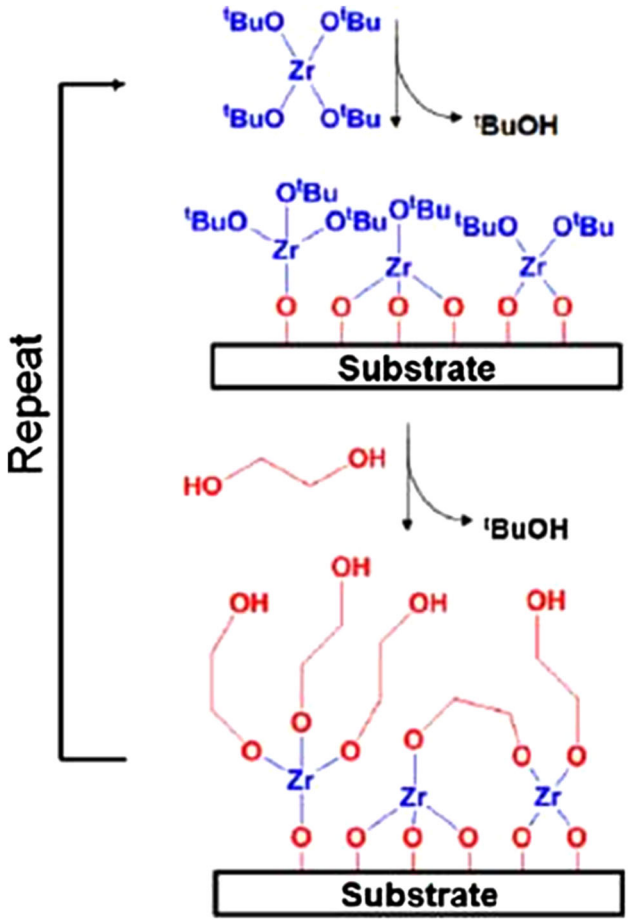

Fig. 2. Schematic depicting the growth of zircone MLD films using zirconium tert-butoxide and EG. Reproduced from Ref. 15 with permission of John Wiley & Sons, Inc.

dosing conditions for the zircone film were (0.5/75/1/50). The deposition temperature was set at 150°C for all films. The growth rate for the zircone film was 0.78 Å per cycle. The substrates contained circular openings (diameter = 10 mm), which contained nanoporous alumina. Imaging of uncoated and zircone-coated nanoporous alumina was performed using a JSM-7401F field emission scanning electron microscope (JEOL, Tokyo, Japan).

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used to compare the viability of human epidermal keratinocytes on zircone-coated nanoporous alumina and uncoated nanoporous alumina. Nanoporous alumina, with and without zircone coatings, were sterilized and prepared for cell culture studies. The substrates were sterilized with ultraviolet (UV) B light in a cell culture hood. Both top and bottom surfaces were exposed to the UVB light; the substrates were rotated 90° after 1 h of light exposure to ensure that all of the surfaces were exposed. To isolate the biocompatibility of the nanoporous material and not the surrounding substrate, sterilized stainless steel washers of 12.7 mm inside diameter were used to occlude the surrounding substrate and to prevent cellular growth everywhere except the nanoporous material. Prior to their use, stainless steel washers

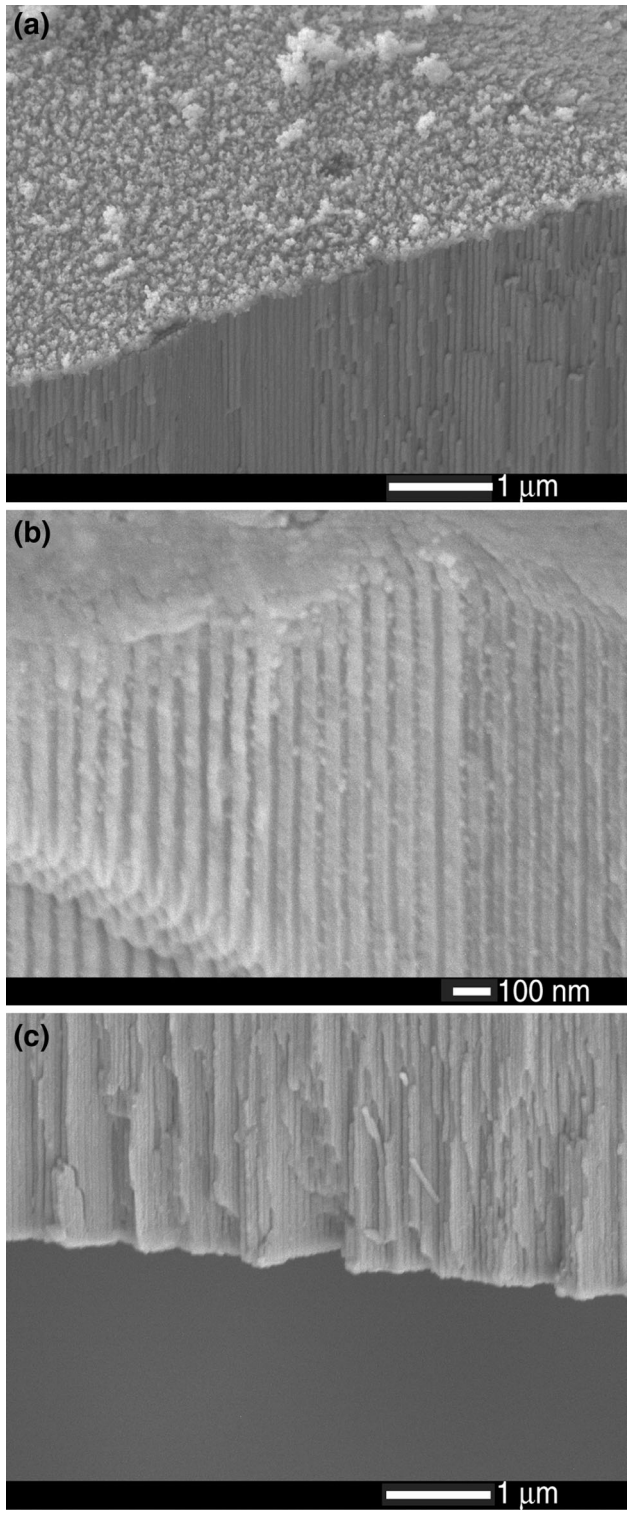

Fig. 3. Scanning electron micrographs of zircone-coated nanoporous alumina. (a) Cross section of the front side; pore features were not readily noted. (b) Cross section of the front side; some nanoparticles were noted within the pores. (c) Cross section of the back side; open pores were noted.

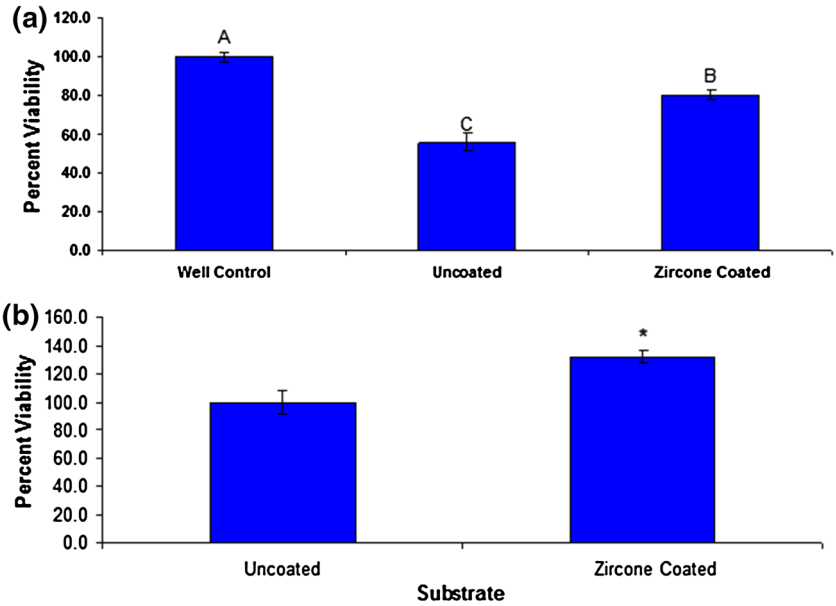

Fig. 4. (a) MTT viability of human epidermal keratinocytes grown on the nanoporous alumina with or without zircone coating; viability was normalized to the well control. Different letters denote statistical difference ($p < 0.05$) in viability between the substrates. (b) MTT viability of human epidermal keratinocytes grown on the nanoporous alumina with or without zircone coating. The viability was normalized to the uncoated nanoporous alumina. Asterisk denotes statistical difference ($p < 0.05$) in viability between the substrates.

were sterilized with two 1-h changes of 70% ethanol in sterile petri plates, followed by two additional rinses in Hank's Balanced Salt Solution (HBSS). The test materials were then transferred to sterile 6-well culture plates with stainless steel washers over the nanoporous regions, rinsed in sterile HBSS and in cell culture medium, and then equilibrated in cell culture medium in the incubator until seeded with human epidermal keratinocytes.

Cryopreserved first pass neonatal human epidermal keratinocytes (Lonza, Walkersville, MD, USA) were seeded in 75-cm^2 flasks and grown to 75% confluency, harvested, and seeded on each membrane (n = 3 uncoated; n = 2 coated) in 6-well plates at 100,000 cells per well. Cells were also seeded in the wells alone, which served as the control (n = 3 per plate), to monitor cell growth. Once the human epidermal keratinocytes in the well controls reached ~70% confluency, the media were changed and the cells were grown for an additional 24 h. To assess cell viability, the stainless steel washers were removed, the media were replaced with the MTT solution, and the human epidermal keratinocytes were incubated for 3 h. The media were aspirated, the cells were rinsed in HBSS, and 1 mL of isopropyl alcohol was added to the human epidermal keratinocytes in each well to solubilize the formazan crystals within the cells. Then 100 μL of isopropyl alcohol was added to a new 96-well plate and the absorbance was quantitated at 550 nm in a Multiskan RC plate reader (Thermo Fisher Scientific, Waltham, MA, USA).

The mean viability data were calculated by normalizing the uncoated and coated nanoporous alumina to the well controls and by normalizing the coated nanoporous alumina to the uncoated nanoporous alumina. Significant differences ($p < 0.05$) were analyzed using the PROC GLM Procedure (SAS 9.2 for Windows). When significant differences were noted, comparisons were performed using Dunnett's t test at $p < 0.05$ level of significance.

RESULTS AND DISCUSSION

Figure 3 contains scanning electron micrographs depicting zircone-coated nanoporous alumina. Figure 3a shows the cross section of the front side of zircone-coated nanoporous alumina. Pore features were not readily noted in this image. Figure 3b shows a cross section of the front side of the zircone-coated nanoporous alumina. This figure confirms closure of the pores and shows nanoparticles within the pores. Figure 3c shows a cross section of the back side of zircone-coated nanoporous alumina. Open pores were noted.

The use of washers controlled the growth of human epidermal keratinocytes and confined them to the nanoporous material over the underlying substrate circular opening. This approach ensured that human epidermal keratinocyte growth was restricted to a specific area so that data were consistent. Human epidermal keratinocytes grew on both the coated and uncoated nanoporous alumina. However, human epidermal keratinocyte viability

showed a significant decrease ($p < 0.05$) on both uncoated nanoporous alumina and zircone-coated nanoporous alumina compared with the controls, with viability being 80% for the coated nanoporous alumina and 56% for the uncoated nanoporous alumina compared with the controls (Fig. 4a). Human epidermal keratinocyte viability showed a significant increase ($p < 0.05$) on the zircone-coated nanoporous alumina normalized to the uncoated nanoporous alumina (Fig. 4b). Overall, the zircone-coated nanoporous alumina did not show a decrease in human epidermal keratinocyte viability compared with the uncoated nanoporous alumina.

CONCLUSION

In this study, zircone films were grown on nanoporous alumina using MLD. Scanning electron microscopy data showed obliteration of the pores in the nanoporous alumina by the zircone film. The in vitro cell viability study did not show a decrease in human epidermal keratinocyte viability on the zircone-coated nanoporous alumina when compared with the uncoated nanoporous alumina. These results suggest that MLD may be used to create biocompatible coatings for use in many types of medical devices. For example, MLD-grown films may have use in drug delivery and in other medical device applications.[12]Additional efforts are needed to optimize the coating thickness to eliminate obliteration of pores. As noted by Zhou and Bent,[12] a better understanding of MLD growth behavior would facilitate additional use of MLD. In addition, an increase in the number of linking reactions used in MLD would facilitate utilization of this process.[12]

REFERENCES

1. S.M. George, *Chem. Rev.* 110, 111 (2010).
2. R.L. Puurunen, *J. Appl. Phys.* 97, 121301 (2005).
3. B.H. Lee, B. Yoon, A.I. Abdulagatov, R.A. Hall, and S.M. George, *Adv. Funct. Mater.* 23, 532 (2013).
4. M.E. Alf, A. Asatekin, M.C. Barr, S.H. Baxamusa, H. Chelawat, G. Ozaydin-Ince, C.D. Petruczok, R. Sreenivasan, W.E. Tenhaeff, N.J. Trujillo, S. Vaddiraju, J. Xu, and K.K. Gleason, *Adv. Mater.* 22, 1993 (2010).
5. H.I. Shao, S. Umemoto, T. Kikutani, and N. Okui, *Polymer* 38, 459 (1997).
6. A. Kim, M.A. Filler, S. Kim, and S.F. Bent, *J. Am. Chem. Soc.* 127, 6123 (2005).
7. J.S. Lee, Y.J. Lee, E.L. Tae, Y.S. Park, and K.B. Yoon, *Science* 301, 818 (2003).
8. T.V. Ivanova, P.S. Maydannik, and D.C. Cameron, *J. Vac. Sci. Technol. A* 30, 01A121 (2012).
9. J.S. Park, H. Chae, H.K. Chung, and S.I. Lee, *Semicond. Sci. Technol.* 26, 034001 (2011).
10. S.M. George, B. Yoon, and A.A. Dameron, *Acc. Chem. Res.* 42, 498 (2009).
11. D.M. King, X. Liang, and A.W. Weimer, *Powder Technol.* 221, 13 (2012).
12. H. Zhou and S.F. Bent, *J. Vac. Sci. Technol. A* 31, 040801 (2013).
13. G. Ozaydin-Ince, A.M. Coclite, and K.K. Gleason, *Rep. Prog. Phys.* 75, 016501 (2012).
14. X. Liang, M. Yu, J. Li, Y.B. Jiang, and A.W. Weimer, *Chem. Commun.* 46, 7140 (2009).
15. B.H. Lee, V.R. Anderson, and S.M. George, *Chem. Vap. Depos.* 19, 204 (2013).
16. A.A. Dameron, D. Saghete, B.B. Burton, S.D. Davison, A.S. Cananagh, J.A. Bertand, and S.M. George, *Chem. Mater.* 20, 3315 (2008).
17. X. Liang, D.M. King, P. Li, S.M. George, and A.W. Weimer, *AIChE J.* 55, 1030 (2009).
18. A.I. Abdulagatov, R.A. Hall, J.L. Sutherland, B.H. Lee, A.S. Cavanagh, and S.M. George, *Chem. Mater.* 24, 2854 (2012).
19. S. Lee, M. Park, H.S. Park, Y. Kim, S. Cho, J.H. Cho, J. Park, and W. Hwang, *Lab Chip* 11, 1049 (2011).
20. J.W. Elam, M.D. Groner, and S.M. George, *Rev. Sci. Instrum.* 73, 2981 (2002).