Growth of High-Quality Oxides on The Inner Surface of ECMO Circuit by Atomic Layer Deposition to Reduce Thrombus Formation

Outline

I. Background

A. ECMO, as an effective modality for life saving, however, with high risk of complications

Mechanical circulatory support has evolved markedly over recent years. ECMO (extra corporeal membrane oxygenation), essentially a variation of the cardiopulmonary bypass circuit used routinely in cardiac surgery, is instituted in an emergency or urgent situation for the management of life-threatening pulmonary or cardiac failure (or both) when no other form of treatment has been or is likely to be successful [1]. By removing blood from the patient and circulating it through an artificial lung with a pump, ECMO can provide pulmonary and cardiac support, depending on the cannula arrangement. Whereas cardiopulmonary bypass facilitates open heart surgery for a number of hours, ECMO maintains tissue perfusion and oxygenation for days to weeks. It is currently being used worldwide as temporary support, usually awaiting recovery of organs, or can be used as a bridge to a more permanent device or cardiac transplantation.

1) ECMO-related mortality and morbidity

Being invasive, complex, resource intensive, and serious related complications, ECMO is restricted its use in highly specialized centers and mostly reserved for patients with a high risk of death who have failed conventional management and where the underlying respiratory or cardiac disease is reversible [2]. Advances in ECMO have made it a more feasible rescue modality compared with early experiences. However, the mortality remains high with survival rate of 77% for neonatal respiratory failure, 53% for adult respiratory failure, 45% for pediatric cardiac failure, and 32% for adult heart failure. The principal causes of ECMO-related mortality and morbidity are bleeding (7-34%) and thrombosis (8-17%) [3].

2) Coagulopathy is the major device-related complications

ECMO is well known to cause coagulopathy. High extracorporeal blood flow (BF) along with the large artificial surfaces of ECMO circuit can trigger serial cascades of inflammatory response including coagulation, complement, cytokines and eicosanoid systems with crosstalk among them [4]. The continuous activation of the contact and fibrinolytic systems as well as consumption and dilution of factors occurs within minutes of initiation of ECMO [5]. Platelets adhere to surface fibrinogen and are activated and the resultant platelet aggregation and clumping causes numbers to drop [6]. Correction by platelet transfusion produces only a temporary increase in numbers. Prolonged duration of ECMO exacerbates these

negative effects rendering the critically ill patients at more potential risks of coagulopathy, either bleeding or thrombosis.

Bleeding can rapidly become life-threatening and appears to be out of proportion to the degree of coagulopathy and patient platelet count. Cannulation (7-20%) and surgical site bleeding (6-34%) are common. Intracranial bleeding (1-11%), especially in neonates, gastrointestinal haemorrhage (1-4%), and pulmonary haemorrhage (4-8%) may also occur [7]. Concurrently, extensive thrombosis will not only interferes the blood flow in the ECMO circuit leading to frequent tube changes, but also cause more devastating systemic complications, such as disseminated intravascular coagulopathy (DIC), cerebrovascular accident, pulmonary embolism, end organ failure, etc. Hemolysis is another well-recognized complication of ECMO with an incidence of between 5% and 8% and can reciprocally aggravate the coagulopathy [8]. The free Hb is monitored as the indicator for screening the microthrombosis in the circuit.

B. Current efforts to decrease the coagulopathy

To prevent clotting of the cannulae, tubing and particularly of the oxygenator, and, moreover, the serious systemic thromboembolic events, systemic infusion of unfractionated heparin and heparin-bonded circuits are usually needed [6,9]. The thrombus formation is reduced. However, bleeding risk then is increased. The balance between hemostasis and thrombosis is critical. To avoid the adverse effects from commonly-used pharmacological methods, and since blood-surface interaction is a crucial promoter of the subsequent inflammation leading to coagulopathy, it seems more fundamental to treat the major complications by modifying the material of ECMO circuit which now is mainly composed of polyvinylchloride (PVC) compounds. In most patients, the duration of support required is approximately 1 week or longer [10]. The biocompatibility is particularly an important issue regarding the extended period of ECMO use.

1) **Systemic Heparinization and Heparin-Bounded Circuit to Prevent Thromboembolic Complications**

Serious coagulopathy has been the major complication during ECMO support, either bleeding or thrombosis, leading to high device-related mortality and morbidity. Several efforts have been made on this problem, mainly with the pharmacological interventions. To reduce the thromboemolic

complications, high doses of heparin is usually needed with its major anticoagulant effect by inactivating thrombin and activated factor X (factor Xa) through an antithrombin (AT)-dependent mechanism. However, bleeding risk then increases and attempts should be made to avoid or minimize all non-urgent invasive procedures. Operative interventions in patients on ECMO can be particularly hazardous because of the risk of bleeding and should be avoided if at all possible [11]. Heparin-bounded circuits were developed recently to reduce the use of systemic heparin and have been reported to reduce blood cell trauma [12], complement [13] and granulocyte [14] activation. Nevertheless, systemic heparinization is still advisable because of the risk of end organ damage from microthrombus and fibrin deposition, although the level of heparinization requito red is still under debate. Regular laboratory monitoring with activated partial thromboplastin time (APTT) is required to adjust the doses of heparin accordingly and hence makes resource intensive.

2) **Anti-Platelet Agents to Treat Coagulopathy**

Anti-platelet agents such as low-dose aspirin (acetylsalicylic acid) and short-acting GP IIb/IIIa inhibitors like tirofiban and eptifibatide have all been used to treat the thrombosis-bleeding complication by preventing platelets aggregation and possibly reducing inflammation. By using these platelet protecting techniques and adding an aprotinin infusion to block excessive fibrinolysis, one institution has reported a reduction in bleeding complications from 81% to 9% [15]. However, unlike clinical application of APTT to monitor heparin effect, there is no well-established method to guide the optimal use of anti-platelet agents in such a complex system.

C. Improving the biocompatibility of ECMO is a more fundamental approach

Whereas the pharmacological interventions would bring the critical imbalance between hemostasis and thrombosis, efforts to modify the ECMO system may be another essential method to approach the problem. Although ECMO remains a short-term support device, the use of such a circuit for extended periods (days to weeks) has required some modifications, among which the biocompatibility of the material of tubing and circuit system is an important consideration. Biocompatibility is a general term used to describe the suitability of a material for exposure to the body or bodily fluids. It is the ability of a material to perform with an appropriate response in a specific application. Biocompatibility is vital for medical devices. If a material used is not biocompatible, there may be complications such as extended chronic

inflammation at the contact point, generation of materials that are toxic to cells (cytotoxicity), cell disruption, restenosis after treatment, thrombosis, corrosion of an implant, etc. In the setting of ECMO support, using a biocompactible material is thought to minimize the blood-surface interaction, and hence give the control of the inflammatory response from the right beginning to prevent the following activation of serial cascades. Modification of the ECMO circuit with more biocompatible, especially hemocompatible material is reasonably a fundamental way to reduce the potential coagulopathy.

1) Concerns about PVC, the current material of ECMO circuit

PVC is the currently-used material in the ECMO blood tubing. Although it possesses unique physical and chemical properties, PVC was developed primarily for industrial use and later found their applications in biomedicine. Thus this synthetic material exhibits the incompatibility with blood and tissues. As in direct contact with blood, PVC is still prone to initiate the formation of clots, as the platelets and blood coagulation system are activated [16]. Besides, potential health hazards associated with the release of the plasticizer di-2(ethylhexyl)phthalate (DEHP) from the (PVC) tubing exist [17]. The lipid content of the liquid the PVC is in contact with, the temperature, and the duration of contact affects the leaching of DEHP with the greatest concern on the male reproductive system [18].

2) ZrO_2 and Al_2O_3 are well-known biocompatible and hemocompatible materials

In this subproject, ZrO_2 and Al_2O_3 are chosen as the thin-film materals deposited on the inner surface of ECMO circuit to reduce the thrombus formation because of its excellent biocompatiblility. ZrO_2 and Al_2O_3 are well-known biocompatible materials which have been widely utilized in artificial implants such as ears, teeth and joints, etc. They exhibit excellent material properties for biocompatible coating including good chemical resistance and dimensional stability, high wear resistant and hardness, zero water absorption, and noncorrosivity in saline environments. Recent researches have reported that ZrO_2 can be safely used as an excellent hemocompatible material in the right ventricular assist device [19]. Results of coagulation assays and platelet aggregation tests for the ZrO_2 implants showed no device-induced hemolysis or increase in platelet activation. In addition, biological depositions or wear were not observed on the ZrO_2 surface, indicating excellent biocompatibility and hemocompatibility. These results clearly demonstrate that ZrO_2 can be used safely and effectively as a blood-contacting tubing surface in ECMO circuit to improve the blood

compatibility. However, despite the broad applications of ZrO_2 and Al_2O_3 in artificial implants, traditional thin-film coating techniques are unable to deposit these hemocompatible materials on the inner surface of the PVC tubes in ECMO circuit.

D. Atomic layer deposition (ALD)

ALD is a thin-film deposition process for preparing high-quality oxides with atomic-layer accuracy. It offers many benefits including accurate and facile thickness control, excellent step coverage and conformality, high uniformity over a large area, low defect density, good reproducibility, and low deposition temperature. These features clearly account for the feasibility to deposit high-quality hemocompatible thin films on the whole surface of complex 3-D structures, such as the inner surface of PVC tubing in ECMO circuit, using the ALD technique.

II. Specific Aims

Surface modification of circuit material to improve the hemocompatibility

In this project, we will aim at improvement of the biocompatibility of the material used in ECMO circuit to reduce the device-induced coagulopathy. A new technique of atomic layer deposition (ALD) will be applied to coat biocompatible and hemocompatible zirconium dioxide (ZrO_2) and aluminum oxide (Al_2O_3) nanolaminate thin films on the inner (luminal) surface of ECMO circuit. The ALD technique is known to be capable of coating high-quality oxide thin films on a variety of complex surfaces. The advantages of ALD lie in its capability to produce high-quality and pinhole-free films on the surface of complicated 3-D structures with excellent uniformity and conformality as well as accurate thickness and composition control at a single atomic layer. Besides the preparation of the hemocompatible thin films on the ECMO tubing surface using ALD, surface enhanced Raman spectroscopy (SERS) is another crucial technique utilized in this subproject to optically probe the biomedical molecular characterization associated with blood coagulation and platelet activation. Moreover, the ALD technique will also be used to provide further enhancement of the functionalization, stability and reproducibility of the SERS substrates, so as to push the SERS technique into a more practical diagnostic tool in clinical monitoring. The safety and effectiveness of the newly-prepared ECMO circuit will be evaluated in the following in vitro and in vivo studies.

III. Research Design and Methods

A. Atomic Layer Deposition (ALD)

ALD is a thin-film deposition technique for preparing high-quality oxides with atomic-layer accuracy. The most distinctive feature of ALD is that each precursor is alternately pulsed into the reaction chamber, as shown schematically in Fig.5. When the first precursor is introduced into the chamber, the precursor molecules absorb upon the substrate surface by chemical absorption, resulting in the saturative absorption of one monolayer of precursor on the surface. Between the precursor pulses, the chamber is purged with an inert gas to remove all the excess precursors and by-products. As the next precursor is dosed in, it reacts with the precursor previously absorbed on the surface, producing one monolayer of solid product and gaseous by-products. Contrary to the conventional chemical vapor deposition (CVD), the chemical reactions in ALD proceed only at the substrate surface, resulting in self-limiting and layer-by-layer (or "digital") growth. The self-limiting or self-terminating reaction also suggests that precise control of precursor homogeneity is not necessary. The only requirement is that sufficient precursor molecules are needed to cover the adsorption sites on the surface. In addition, ALD can provide conformal coatings on complex 3-D structures due to the diffusion of vapor-phase precursors into porous structures as well as the surface-controlled self-limiting growth.

The advantages of ALD are summarized as follows [20]:

1. Precise and easy thickness control with one monolayer accuracy.
2. Accurate composition control and facile doping for achieving high doping concentrations.
3. Stoichiometric films with low defect densitiy.
4. High uniformity over a large area, leading to large-area and large-batch capacity.
5. Excellent conformality and good step coverage.
6. Low deposition temperature.
7. Good reproducibility.

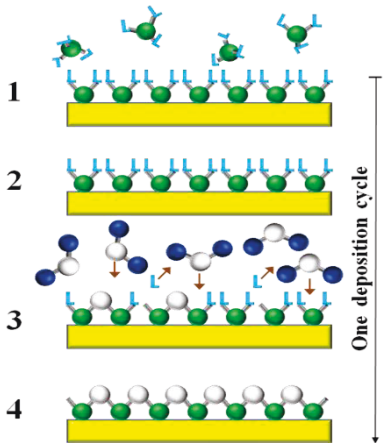

Figure 5 Schematic diagram of an ALD cycle (1–4) for depositing one monolayer of oxide. The green, white and blue balls, and symbol L refer to the metal, oxygen and hydrogen atoms, and the ligand of precursor, respectively.

For example, Al_2O_3 thin films can be deposited by ALD using alternating pulses of $Al(CH_3)_3$ (TMA, the precursor of Al) and H_2O vapor (the oxygen precursor) in an N_2 or Ar carrier gas flow:

$$\text{Surface-OH} + Al(CH_3)_3 \rightarrow \text{Surface-O-Al-}(CH_3)_2 + CH_4 \quad \text{(A)}$$

$$\text{Surface-O-Al-}(CH_3)_2 + 2H_2O \rightarrow \text{Surface-O-Al-OH} + 2\,CH_4 \quad \text{(B)}$$

Repeating these reactions in an ABAB... sequence (AB is called one ALD cycle), Al_2O_3 thin films can be prepared with one monolayer accuracy. The thickness of the Al_2O_3 thin films can be controlled accurately by the number of ALD cycles. These distinguished features of ALD have gained considerable attention in recent years to overcome many technical problems, especially in the semiconductor industry where the complex geometries and extremely small device size down to the nanometer scale strongly call for the demand of ALD.

In addition to the advantages of the thermal-mode ALD mentioned above, plasma-enhanced ALD (PEALD) yields many benefits such as

1. Lower deposition temperature.
2. Wider choice of precursors and materials.
3. Improvement in material quality due to a higher film density and a lower impurity level.
4. Ability to control stoichiometry of the films.

A remote plasma configuration in PEALD was constructed to reduce the plasma damage on the deposited films. Oxygen radicals generated by remote plasma will be used as the oxidants in the PEALD process to enhance the reactivity of reactant gas and prevent the use of water vapor as the precursor. In

addition, the deposition temperature in the PEALD process can be further decreased to be lower than 100°C, which is very substantial for the thermolabile substrates like the PVC tubing in ECMO circuit.

One may wonder if ALD is a too slow and expensive process for the biomedical applications. Actually, ALD has become a cost-effective thin-film deposition technique due to its large-area and large-batch capacity. The scale-up of ALD chambers to large batch capacity is facile and straightforward due to the self-limiting and layer-by-layer growth, high uniformity, excellent conformality, and good reproducibility of ALD. Large batch-type ALD reactors up to 150 wafers are commercially available now [21]. For instance, large batch ALD processing has been used to improve the throughput and applied in the massive production of solar cells. Another example of commercial application of ALD is the protective coating on jewellery. A robust and massive batch processing up to 2000 work pieces (please see Fig.6) has made ALD a cost-effective technique.

B. Conformal biocompatible coating by ALD at low temperature

The distinguished benefits of ALD as mentioned above can be further applied in the biomedical industry. For example, the ALD technique enables the coating of artificial implants with high-quality biocompatible thin films to minimize the impact between the implants and the biological processes inside the human body. The benefits of ALD for biocompatible coatings mainly come from the excellent conformality and uniformity together with the pinhole-free structure. Biocompatible ALD films can be used to reduce diffusion as well as improve corrosion and wear resistance. An ALD thin film only a few nanometers thick is flexible enough and thus prevent cracking, formation and spreading of detrimental

Figure 6 A batch ALD reactor with 2000 work pieces being coated simultaneously (Copyright Beneq Oy, Finland).

particles. Furthermore, ALD can be used to prepare the surface which resist bacterial growth and

accumulation of biological materials. Another advantage of ALD is that the deposition can be performed at relatively low temperatures (<150°C), thereby limiting thermal damage to the thermally fragile and temperature-sensitive substrates.

In this subproject, biocompatible and hemocompatible ZrO_2/Al_2O_3 nanolaminate thin films will be coated on the PVC tubing surface of ECMO circuit using ALD to reduce the thrombus formation. It is particularly appropriate to prepare high-quality thin films on the whole surface of complicated 3-D structures, such as the inner surface of PVC tubes, using ALD due to its high conformality. It may be noted that traditional thin-film coating techniques, including physical vapor deposition (PVD) and CVD, are unable to deposit these oxides on the inner surface of PVC tubes. The introduction of Al_2O_3 into the thin films will prevent the aging of ZrO_2 [22] and improve the adhesion to the PVC tubing surface. Other biocompatible materials, such as silicon dioxide (SiO_2), titanium dioxide (TiO_2), and titanium nitride (TiN), will be also prepared and tested to evaluate their hemocompatibility in ECMO circuit in this subproject.

C. Testing of new material

1) Physical characterization

The thickness and dielectric constant of the ZrO_2/Al_2O_3 nanolaminate thin films will be measured using spectroscopic ellipsometry (SE). The structure of ZrO_2/Al_2O_3 nanolaminate thin films will be characterized by transmission electron microscopy (TEM), scanning electron microscopy (SEM), and X-ray photoelectron spectroscopy (XPS). Atomic force microscopy (AFM) will be used for the analysis of surface morphology and roughness.

2) Biological characterization

Basic biological evaluations following ISO 10993/EN 30993 including chemical, mechanical, thermal and aging testing of the ZrO_2/Al_2O_3 nanolaminate thin films are arranged.

3) Hemocompatibility test

Healthy non–smoking volunteers are included to donate fresh bloods via venipuncture for in vitro hemocompatibility test, while those who are pregnant, with bleeding disorders or taking anticoagulants, such as aspirin, NOAC, are excluded. A series of relevant assessments is done as the followings:

a、 Serum levels of blood platelet-activating-factors

The blank PVC or PVC with T-ALD/PE-ALD Al_2O_3 films are planned to be immersed in whole blood for 1hr. The bloods are then collected and the sera were separated to determine the levels of blood platelet-activating-factors including prothrombin, sP-selectin and PAF acetylhydrolase.

b、 Adhesion and morphology of blood cells

The blank PVC and Al_2O_3-coated PVC (T-ALD and PE-ALD) samples are planned to be immersed in whole blood and incubated at 37°C for 1 hr under the static condition to check blood-surfaces adhesion,. The samples are then washed gently with phosphate buffered saline (PBS), fixed with 2.5% glutaraldehyde solution for 2 hr at room temperature, and dehydrated in a gradient of ethanol/distilled water mixtures. Finally, the samples are undergone critical-point drying, and subsequently coated with gold ions and then inspected using a scanning electron microscopy.

c、 Inflammatory response

After 1 hr immersion in whole blood, the inflammatory responses induced by blank PVC or surface-modified PVC samples are determined. The blood cells are collected, washed, and incubated with CD4/CD8, CD42b, CD45, or CD61/CD62P antibodies for 15 minutes on ice, and sorted using a fluorescence-activated cell sorter to evaluate the inflammatory responses.

IV. Projected Timeline

Year / Development Stage	1st Year				2nd Year				3rd Year			
	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4	Q1	Q2	Q3	Q4
Synthesis & improvement of potential targets Design & modification of new medical devices	→	→	→	→	→	→	→	→				
Optimization of leads & device models Prototype development Pilot-scale manufacturing						→	→	→	→	→		
Validation with clinical samples In vitro efficacy evaluation						→	→	→	→	→	→	→

VIII. Anticipated Outcomes

A. Clinical improvement

(1) Improvement of the device: The coagulopathy which are the major causes of device-related morbidity and mortality can be reduced, supporting the ECMO as a safer and more feasible modality for life saving. The clinical application of ECMO may be extended.

(2) Improvement of patients' outcome: Morbidity and mortality of the critically ill patients on ECMO will decrease because of not only avoiding devastating consequences from coagulopathy, but also less inflammatory response from the blood-surface interaction. The imbalance of inflammatory system is believed to be one of the crucial pathophysiological processes leading to organ failure.

(3) Improvement of the critical care for patients on ECMO: This approach from the fundamental way of material modification instead of pharmacological intervention makes the current complex critical care simpler and easier with less complications and treatments. Besides, regular laboratory monitoring to guide the dose adjustment of medications is not required.

(4) Cost & labor reduction: The cost and labor to treat the critically ill patients on ECMO will be reduced since the intensity of both treatments and monitoring is reduced for a smoother course. In addition, frequent changes of tubing system and oxygenator (around NTD 2,000,000 for one exchange) are avoided since there is lower incidence of hemolysis and thromboembolic problems which may lead to tube obstruction and plasma leakage.

B. Beneficial impacts on biomedical industry

(1) The distinguished benefits of ALD have attracted much attention to overcome a variety of technical problems, such as in the semiconductor industry in which the complex device structure with nanometer scale strongly call for the demand of ALD. However, the ALD applications in the biomedical industry still need to be developed. Based on the excellent performance and film quality of the ALD technique, this project seeks to explore and discover the potential applications and commercialization of ALD in the biomedical industry, including the biocompatible and hemocompatible coatings as well as the functionalization and stabilization of SERS substrates. The successful implementation of ALD for producing high-quality coatings on biomedical devices is expected as a key manufacturing technique in the future biomedical industry.

X. References

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