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Silicon Nitride Membrane for Cell Culturing

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Abstract. This paper presents a development in producing low residual stress PECVD SiN_x layers at high deposition rates and their application in cell culturing. The key factor in the novel process is the employment of up to 600 W high powers in high frequency (13.56 MHz). In conjunction with the adjustment of the reactant gases composition, the residual stress can be tuned to 4 MPa and high deposition rate up to 320 nm/min can be achieved meanwhile. Moreover, by using this optimized process, an 11 µm thick low stress SiN_x layer was produced, which will be used to fabricate large area windows for cell culture. Finally, a cell culture test by cultivating mouse stem cells onto porous membrane by the low stress PECVD SiN_x layers also indicated that these layers are biocompatible and are suitable for cell culturing.

Key words: Low stress, silicon nitride, PECVD, high power, cell culturing.

1. Introduction

Silicon nitride (SiN_x) is one of the widely used materials in a variety of biomedical implant applications, due to its good chemical inertness, high fracture toughness, high wear resistance and biocompatibility [1, 2]. For example, it can be used as conductive and dielectric layers, and selectively passivation layers against the *in vitro* and *in vivo* environments [3–5]; it also can be used in implantable microdevices and protein filtration applications as well-controlled, stable, uniform membranes [6], which have several advantages compared to polymeric membranes, such as no morphological change or degradability in biological fluids, adequate mechanical strength, and precisely

controlled pores features [7, 8]. All of these characteristics show that silicon nitride is a promising material for biomedical applications.

Currently, PECVD is one of the best methods to produce SiN_x layers, due to its lower process temperature (usually around 300°C), and more uniform and less stress variation through wafers. However, one drawback for PECVD SiN_x layers is the high residual stress generated by PECVD standard processes, normally around 120 Mpa. The high residual stress can greatly affect the membrane's performance; for example, the high intrinsic stress will lead to stress-induced failure via microvoid formation and structure [9, 10]; the wafers will break or be severely warped if the thickness is too large. On the other hand, for the biomedical applications, such as cell cultures, membrane with large areas and thick thickness is necessary, which means low stress is critical; otherwise, high stress inside the thick membrane will easily break the membrane, especially in the case of big exposed area.

Here, we report a novel process to produce low stress PECVD SiN_x layer and its biomedical applications. The developed process was able to produce near zero stress SiN_x layers with a high deposition in a PECVD reactor. The developed process was used to deposit thick SiN_x layer and produced 11 μm layers with around 2.5 MPa stress. These layers can be used to fabricate large area window which will be used in cell culture experiments. Lastly, nanoporous membrane made by low stress SiN_x was generated and used for the cell cultures. By cultivating D1-CDFA cells on the nanoporous membrane, it is shown that the cells can grow well on top of the porous SiN_x membrane and cells isolation results are satisfying.

2. Experimental procedures

The deposition of SiN_x layers were carried out using a plasma-enhanced chemical vapor deposition (PECVD) system (STS, Multiplex Pro-CVD). A schematic diagram of the equipment was presented by Chung et al in [11]. The unique characteristic of the system is that the plasma can be activated in two RF modes: at 380 kHz (LF) and/or at 13.56 MHz (HF). Another important characteristic of the equipment is that it offers the opportunity of selecting the power in a large range: between 0 and 600 W for HF mode and between 0 and 1 kW for LF mode. The depositions of the layers were performed using pure silane (SiH_4), ammonia (NH_3) and nitrogen (N_2). To avoid contamination of the deposited layer a predisposition of a SiN_x layer was performed each time after the plasma cleaning process (usually performed after a cumulative deposition thickness of 6 μm).

For characterization of the deposited layers 4″ silicon wafers, p-type, $< 100 >$ crystallographic orientation, 1–10 Ωcm were used. The wafer was initially cleaned in piranha (H_2SO_4: H_2O_2 in the ratio of 2:1) at 120°C for 20 minutes, rinsed in DI water and spun-dried. The native oxide of the layer was removed by dipping the wafer for one minute in a classical BOE solution. The stress characterization of the SiN_x films was performed with a stress measurement system (KLA Tencor FLX-2320). The thickness of the films was measured with a refractometer (Filmetrics F50).

3. Fabrication process of low stress SiN_x layers

3.1. Deposition at high power in HF mode

The observation that in HF mode the tensile stress decreases with the RF power while the deposition rate is significantly increased was further investigated. A series of experiments have been completed to deposit SiN_xlayer in the power range of 100 W to 600 W. Figure 1 shows the variations of residual stress and deposition rate respectively with the power change. In these experiments, the pressure and temperature were set to be 900 mTorr and 300°C respectively while the reactant gas composition of SiH_4/ NH_3/ N_2 was 100/60/1500 sccm.

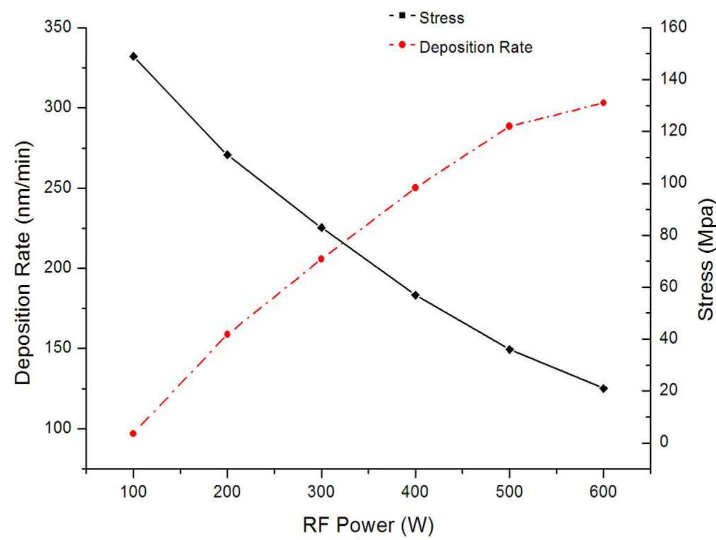

Fig. 1. Variation of residual stress and deposition rate with the power.

From the graphs, it can be observed that, the deposition rate increases strongly while the residual stress decreases with the increase of power. That is because; the increased energy of the electrons produced by higher power increases the dissociation of the main gases and as a result the deposition rate increases [12]. Moreover, the high dissociation rates of gases generated more N^+ species and consequently resulted in increased incorporation of N bonding in the SiN_x film. This results in compressive stress due to the volume expansion of the SiN_x film [13]. Therefore, higher power brings higher compressive stress, which compensates the tensile stress and leads to overall lower tensile stress. On the other hand, during the $SiH_4/NH_3/N_2$ process, the critical power to activate N_2 (9.8 eV) is much higher than that to activate NH_3 (4.6 eV for H–NH_2) [14]. Therefore, in the high power range (600 W), much more N_2 have been activated and dissociated, which means more N atom will react with SiH_4 and much more SiN_x will be generated. That is to say, the decreasing stress value

with the increase of power can be attributed to the high dissociation of N_2 which led to more N species and resulted in an increased of N–Si bonding in SiN_x film.

In a conclusion, the residual stress of SiN_x layer decreases and the deposition rate increases with the increase of power. Moreover, the residual stress of silicon nitride layer deposited under the ultra high power condition (600 W) can reach a low and high deposition rate were achieved simultaneously. Therefore, in the following investigations, all the powers were set to be constant to be 600 Watt.

3.2. Influence of N_2 flow rate

From above statement, it can be known the high RF power will "turn on" N_2 during the deposition process, which means N_2 is a critical factor effecting SiN_x residual stress.

Considering that N_2 supplies the N atom for reaction, the decrease of the N_2 flow rate will lead to "Si-rich" SiN_x layer which has lower residual stress. In this part, the flow rates of nitrogen have been changed from 1150 sccm to 2200 sccm with the power set to be constant at 600 W to investigate deposition rate and residual stress. The flow rates of SiH_4 and NH_3 were 120 sccm and 75 sccm, respectively, and the pressure was set to be 900 mTorr.

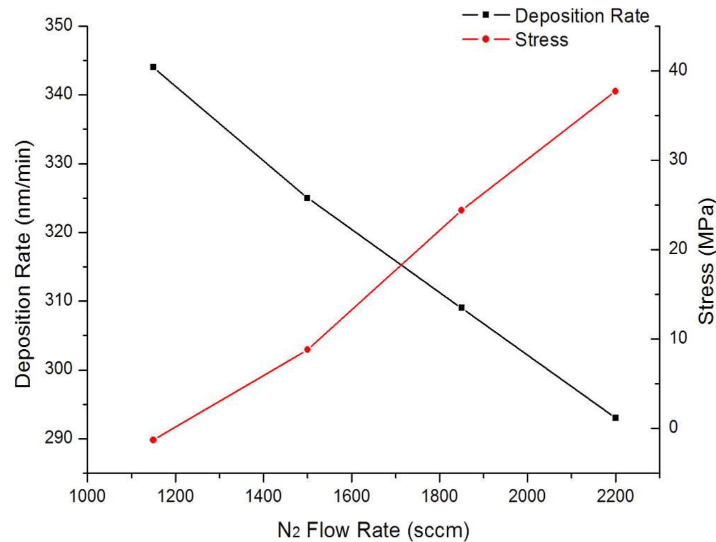

Fig. 2. Variation of residual stress and deposition rate under different N_2 flow rates.

From the Fig. 2 it can be observed that the deposition rate increases and the residual stress decreases as the N_2 flow rate decreases from 2200 sccm to 1150 sccm. The drastically decreasing N_2 flow (which is much higher than the NH_3 and SiH_4 flows) has a huge impact decreasing the total gas flow rate (from 2395 to 1345 sccm). With the pressure constant, the residence time of the gas in the chamber increases.

A longer residence time results in a higher dissociated rate of ammonia in the plasma with the effect of increasing of the deposition rate. Moreover, from the figure, the stress of the lowest N_2 flow rate was compressive (negative value).

3.3. Influence of NH_3 flow rate

In order to investigate the relationship between residual stress / deposition rates of SiN_x layer with the NH_3 flow rate, the NH_3 flow rate was varied from 45 sccm to 100 sccm with the SiH_4 and N_2 flow rate to be constant at 120 sccm and 2200 sccm respectively. The pressure was 900 mTorr and the power was 600 W. The results were shown in Fig. 3.

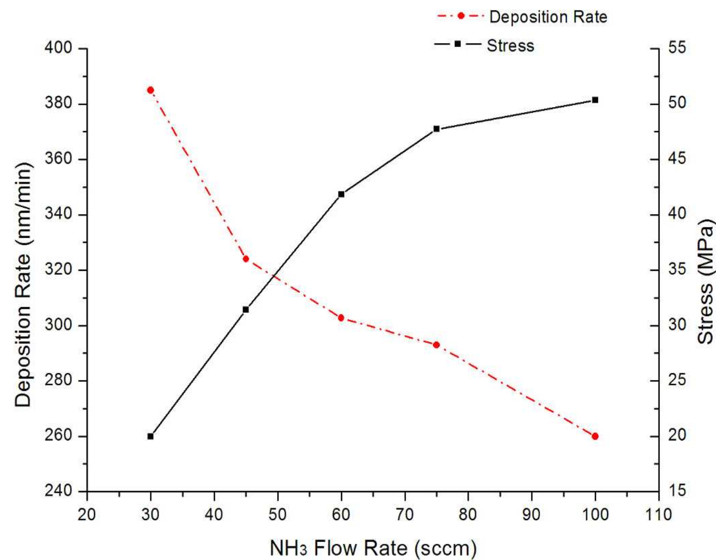

Fig. 3. Variation of residual stress and deposition rate under different NH3 flow rates.

It can be seen that the deposition rate increased and residual stress decreased when the NH_3 flow rate decreases. This is because in ultra high power range, both SiH_4 and NH_3 become activated, and when there is also sufficient excess NH_3, almost all of the SiH_4 react with it to form tetra-aminosilane, $Si(NH_2)_4$, and the triaminosilane radical, $Si(NH_2)_3$. The latter is the dominant precursor species for film growth. It decomposes on the surface and in a "condensation zone" beneath the surface in a process whereby an NH_2 and an H from neighboring precursor radicals combine to form an NH3 molecule, which is evolved into the plasma [15]. The resulting Si and N dangling bonds pull together to propagate the Si-N network, and this generate the tensile stress. On the other hand, low NH_3 flow rate will result in "Si-rich" SiNx layer and the high silicon content yields lower stress level [16]. Therefore, higher NH_3 flow rate leads to higher tensile stress. The decreasing of the deposition rate with the increasing of NH_3 flow rate can be explained by the cumulative effect of decreasing

the number of Si species in the plasma that generates also a decreasing of $Si(NH_2)_3$ – the film growth precursor. Similar results were achieved when the deposition power was changed. In addition Lee et al reported in [17] the decrease of the deposition rate with the NH_3/SiH_4 ratio.

3.4. Influence of SiH_4 flow rate

The influence of SiH_4 flow rate is presented in Fig. 4. For this test, the SiH_4 flow rate was altered between 70 and 120 sccm, while NH_3 and N_2 were kept constant at 60 sccm and 1500 sccm. By increasing the SiH_4 flow rate, an increase number of Si-based radials are generated in the plasma with the effect of increasing of the deposition rate. In the mean time, the deposited SiN_x layer will be "Si rich" and as a result the stress decreases with the increasing of SiH_4 flow rate. It can be noted that with the increase of SiH_4 at 120sccm, the resultant stress in the layer is around 13 MPa tensile, while the deposition rate increased up to 340 nm/min.

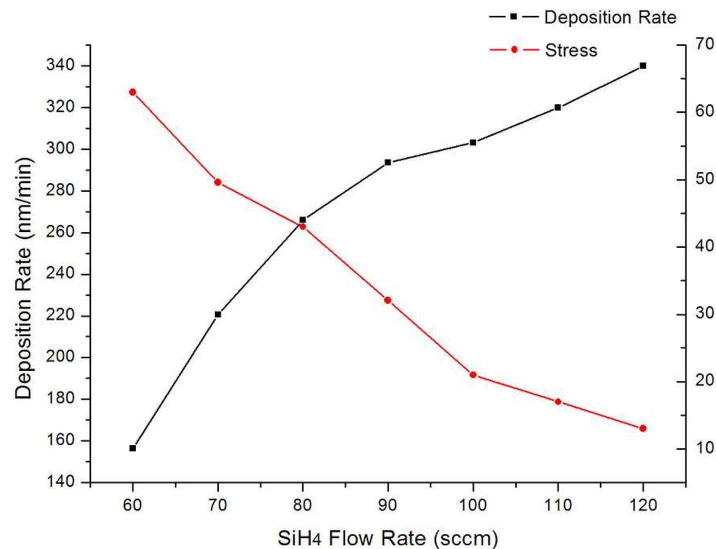

Fig. 4. Variation of residual stress and deposition rate under different SiH_4 flow rates.

3.6. Fine tuning of SiN_x residual stress

According to the influences of reaction parameters to the SiN_x residual stress, an optimized recipe to produce low stress and high deposition rate has been generated. In this optimal recipe, a gas mixture $SiH_4/NH_3/N_2$ of 120/75/1200 sccm was used and other conditions are: power 600 W, temperature 300°C and pressure 900 mTorr. The resultant stress was 4 MPa tensile and the deposition rate was 320 nm/min. In the following deposition process, this optimal recipe was employed.

4. Biomedical application of low stress silicon nitride membranes

4.1. Thick SiN_x layer with low stress

For biomedical applications, a thick membrane is necessary for big surface and easy for manipulation to avoid diaphragm breakage during the cleaning and dicing process. Therefore, a thick PECVD SiN_x layer with low stress is promising for biomedical applications. By using the optimal recipe generated, an 11μm thick SiN_x layer with around 2.5MPa tensile stress was generated and the SEM picture of the SiN_x layer is presented in the Fig. 5.

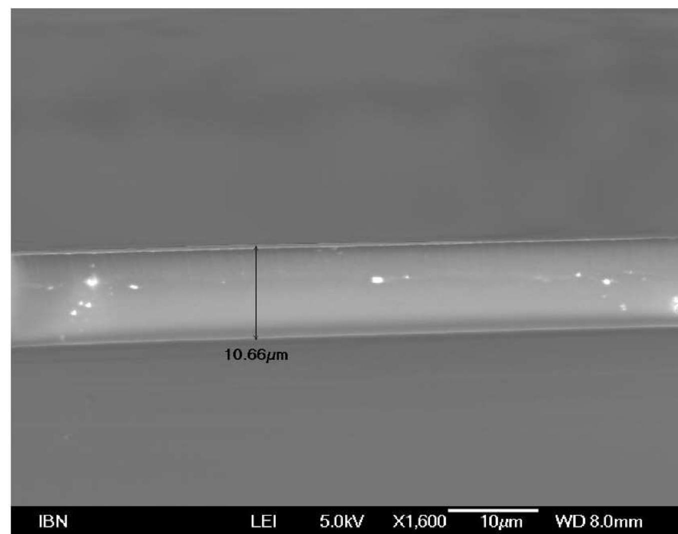

Fig. 5. The SEM picture of thick SiN_x layer.

By using the optimized recipe, nanoporous membrane with 400 nm pores size by low stress SiN_x layer was fabricated by the process shown in Fig. 6 and the SEM picture of the porous membrane is shown as Fig. 7.

4.2. Nanoporous membrane and cell culture test

Moreover, the experiment to test the biocompatibility of the nanoporous SiN_x membrane in an in vitro cell attachment assay with the mouse D1 mesenchymal stem cells as the model cell line was completed. Cells were stained with the cell tracker dye CFDA SE (Molecular Probes, Invitrogen) for easy visualization. Twenty-four hours after cell seeding, cells were found to adhere strongly onto the nanoporous SiNx membrane, which can be seen from Fig. 8. The picture shows the mouse D1 mesenchymal stem cells adopting a normal spread out morphology on the porous support.

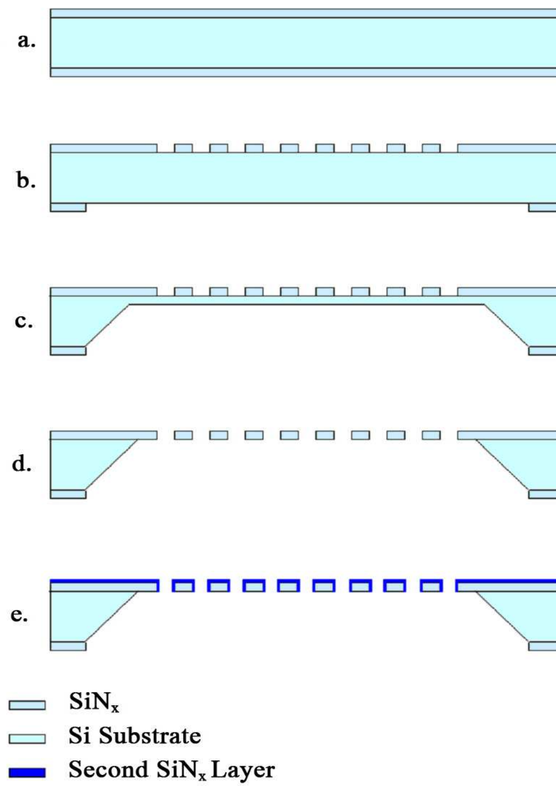

Fig. 6. Fabrication process for the nanoporous membrane.
(a. SiN_x Deposition on the both sides; b. Feature patterned on the both sides; c. Single side KOH etching; d. Both side KOH etching; e. Adjust the holes)

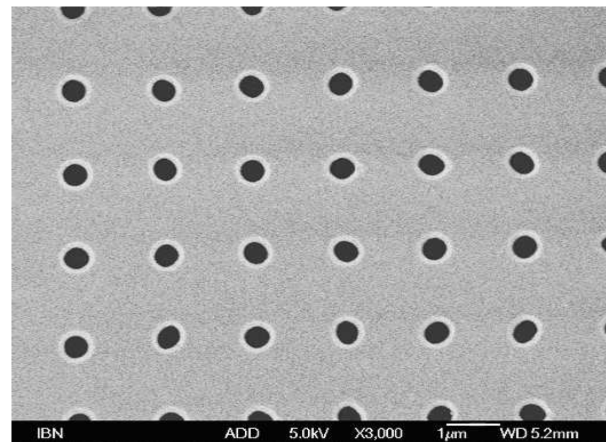

Fig. 7. The SEM picture of the nanoporous membrane with 400 nm pore size.

By using the nanoporous membrane fabricated by stress controlled silicon nitride membrane, a micromachined membrane-based biocapsule with pore size precisely controlled can be expected, which allows free exchange of nutrients, waste products, and secreted therapeutic proteins between the host and encapsulated cells, but excludes lymphocytes and antibodies that may attack foreign cell. Microfabricated inorganic encapsulation devices by silicon nitride membrane may provide biocompatibility, in vitro chemical and mechanical stability, tailored pore geometries, and superior immunoisolation for encapsulated cells over conventional encapsulation approaches. By using microfabrication techniques, structures can be fabricated with spatial features from the sub-micro range up to several millimeters. These multi-scale structures correspond well with hierarchical biological structure, from proteins and sub-cellular organelles to the tissue and organ levels.

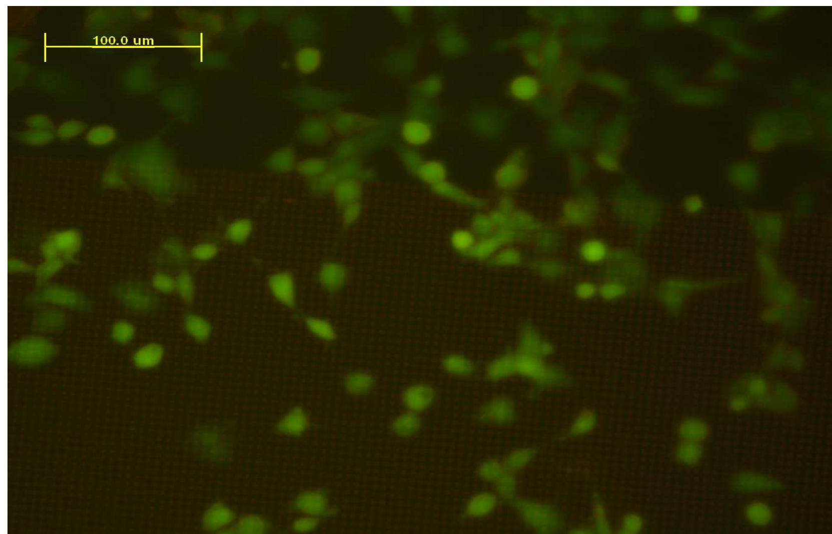

Fig. 8. Mouse D1 mesenchymal stem cells with the cell tracker dye CFDA SE.

5. Conclusions

This paper presented a new method for the fabrication of low stress SiN_x layer in PECVD systems and its applications at fabrication of thick SiN_x membranes, nanoporous membrane and cell culturing.

References

[1] NEUMANN A., RESKE T., HELD M., JAHNKE K., J. Mater. Sci.: Mater. Med., **15**, p. 1135, 2004.

[2] CIARLO D. R., Biomed. Microdevices, **4(1)**, p. 63, 2000.

[3] SANTINI JR. J. T., CIMA M. J., LANGER R., Nature, **397**, p. 335, 1999.

[4] HÄMMERLE H., KOBUCH K., KOHLER K., NISCH W., SACHS H., STELZLE M., Biomaterials, **23**, p. 797, 2002.

[5] LI Y., et al., Controlled Rel., **100**, p. 211, 2004.

[6] DESAI T. A., et al., Biosensor & Bioelectronics, **15**, p. 453, 2000.

[7] LEONI L., DESAI T. A., Adv. Drug Del. Rev., **56**, p. 221, 2004.

[8] PRAKASH R. S., I. PhD Thesis, (UMI Dissertation Services), p. 80, 1994.

[9] WINCHESTER K. J., DELL J. M., J. Micromech. Microeng., **11(5)**, p. 589, 2001.

[10] MACHENZIE K. D., REELFS B., DEVRE M. W., WESTERMAN R., JOHNSON D. J., Characterization &, Unaxis USA, Inc, 2004.

[11] CHUNG C. K., TSAI M. Q., TSAI P. H., LEE C., J. Micromech. Microeng., **15(1)**, p. 136, 2005.

[12] SCHMID P., ORFERT M., VOGT M., Surf. Coating Technol., **98**, p. 1510, 1998.

[13] SMITH D. L., ALIMONDA A. S., VON PREISSIG F. J., J. Vac. Sci. Tech. B: Microel., **8**, p. 551, 1990.

[14] VOSSEN J. L., J. Electrochemical Society, **126**, p. 319, 1979.

[15] LEE J. Y. M., SOORIAKUMAR K., DANGE M. M., Thin Solid Films, **203**, p. 275, 1991.