MORPHOLOGY OF BIOMEDICAL THIN FILMS PREPARED BY MAPLE

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Abstract: Biomedical thin films of fibrinogen were deposited by a novel laser processing technique MAPLE - Matrix Assisted Pulsed Laser Evaporation. The influence of deposition condition on thin film morphology and roughness was studied using confocal laser scanning microscopy and scanning electron microscopy. Results of experiments and our experiences with a new confocal laser scanning microscope LEXT are presented.

Introduction

Biomedical fibrinogen thin films have been developed for medical and pharmaceutical applications.

Fibrinogen (also called Factor I) is a 340 kD glycoprotein encoded on human chromosome 4 and produced by the liver. The usual concentration of fibrinogen in blood is 2 – 4 mg/l. The conversion of fibrinogen to fibrin is the final stage in blood clotting. During normal blood clotting, fibrinogen is broken down by an enzyme called thrombin into short fragments of fibrin. Thrombin also activates a substance called Factor XIII. Factor XIII helps weave the fibrin fragments into a complex lattice, closing off injured blood-vessel walls. Blood platelets attach to the fibrin fragments, clumping together to form blood clots and stop bleeding. When the level of fibrinogen is decreased during some disease man can bloody.

Materials and Methods

MAPLE (Matrix Assisted Pulsed Laser Evaporation[1]) is vacuum based physical vapour deposition technique. The laser beam of a KrF excimer laser (λ = 248 nm, $\tau \geq$ 20 ns, f =10 Hz) was focused on the double polished Si (111) substrate, placed in the interaction vacuum chamber. The substrate was optically polished and cleaned before deposition. The vacuum chamber was pumped out to a pressure of 10^{-4} Pa by a turbomolecular pump and subsequently filled with nitrogen (see Table 1). The target material is cooled to temperatures between -40°C and -190°C. Low concentration of biomaterial in solvent is used. The laser beam is absorbed by matrix, material (fibrinogen dissolved in physiological serum) is ejected from target and carried towards substrate. The target-substrate distance was set 3 cm.

Morphological properties of the films were studied using confocal microscopy and scanning electron microscopy.

Table 1: Summary of deposition conditions and fibrinogen thin film thickness (laser repetition rate 10Hz).

Sample	Vacuum pressure [10^{-4} Pa]	N_2 pressure [Pa]	Energy/pulse [mJ]	Spot area [mm^2]	Fluence [mJ/cm^2]	Thickness (nm)	Deposition rate (Å/pulse)	Roughness [μm]
N 1	5	15	109	36	302	191	0.0382	0.0470
N 2	7	15	39	30	100	7	0.0013	0.0027
N 3	3	15	76	30	200	81-256	0.0426	0.0131
N 4	3	15	121	30	400	72-545	0.109	0.0074
N 5	3	15	95	30	300	154-476	0.0952	0.0335
N 6	2	15	150	30	500	433-771	1.75	0.0280
N 7	2	5	95	30	300	71-158	0.0395	0.0119

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Confocal laser scanning microscopy (CLSM)[2] is a non-destructive technique with major advantages over conventional optical microscopic methods. The principle for this special kind of microscopy was developed by M. Minsky in 1953. Integrating a light microscope, a scanning laser and a computer, confocal microscopy allows the generation of three-dimensional images.

For 2D and fine 3D surface profile measurement we used a new confocal laser scanning microscope for ultra-precise measurement LEXT (Olympus, 408 nm LD Laser, resolution 0,01µm, total magnification 120x – 14 400x). No vacuum pumpdown or sample preparation were required, samples were studied directly on the microscope stage.

Results

Thin films morphology was measured by confocal laser scanning microscopy in real time. The thickness of deposited thin films was in the range 7 nm – 771 nm. Surface morphology of biomedical thin films created under the similar deposition conditions was the same (see Fig.) No remarkable changes in films morphology were observed for sample N3, N4, N5 (see Fig. 6 and Fig. 9). The deposition rate for sample N3, N4, N5 is approximately the same.

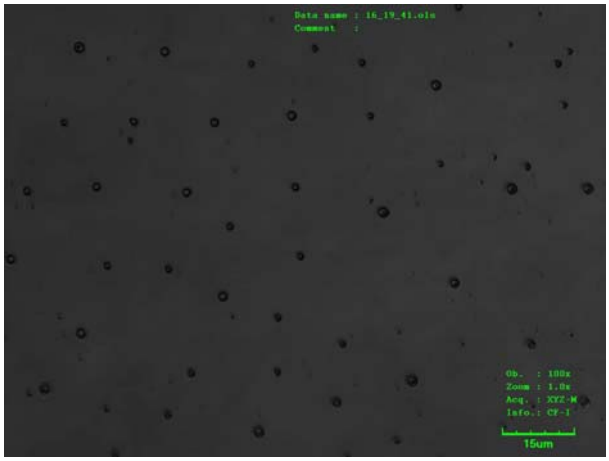

Figure 1: Confocal laser scanning microscopy - 2D analysis fibrinogen thin film sample N2.

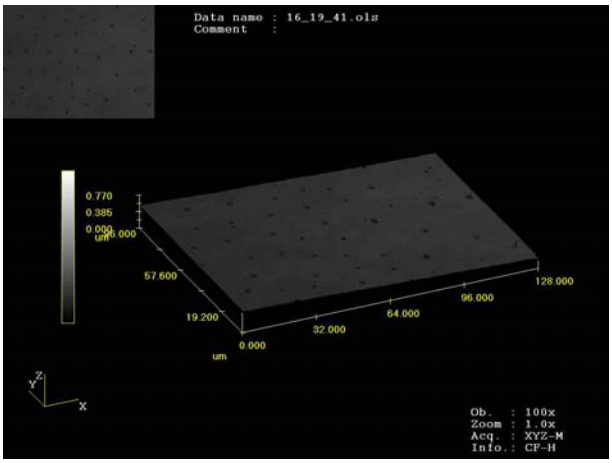

Figure 2: Confocal laser scanning microscopy - 3D analysis fibrinogen thin film sample N2.

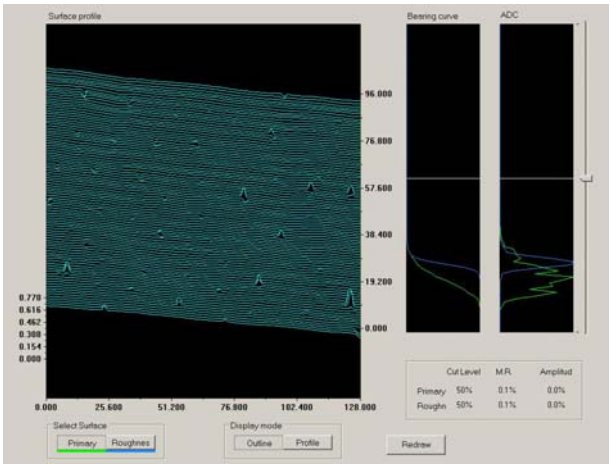

Figure 3: Confocal laser scanning microscopy. Roughness analysis fibrinogen thin film sample N2.

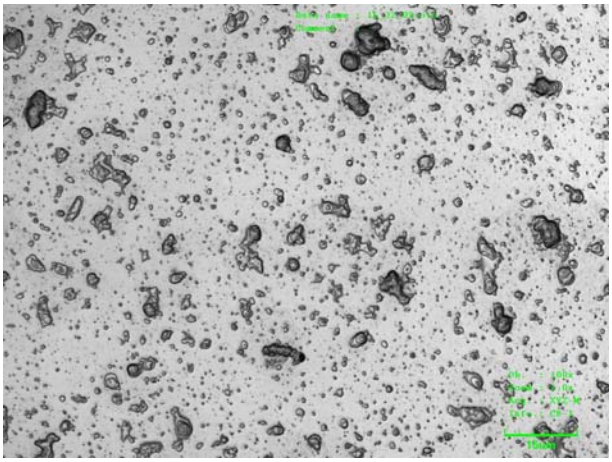

Figure 4: Confocal laser scanning microscopy - 2D analysis fibrinogen thin film sample N3.

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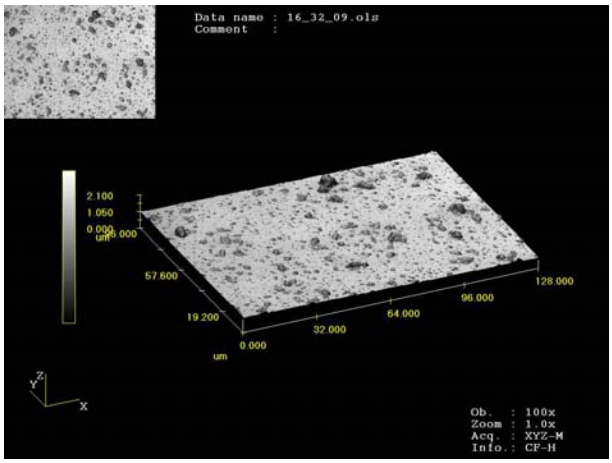

Figure 5: Confocal laser scanning microscopy - 2D analysis fibrinogen thin film sample N3.

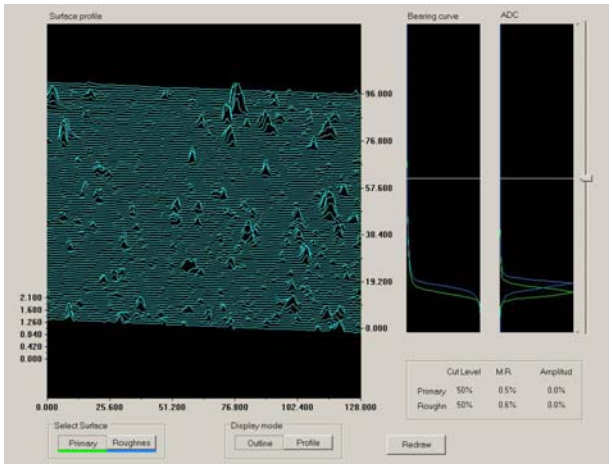

Figure 6: Confocal laser scanning microscopy. Roughness analysis fibrinogen thin film sample N3.

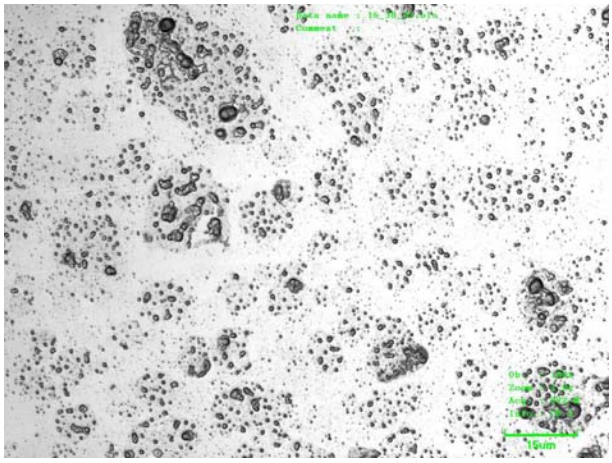

Figure 7: Confocal laser scanning microscopy - 2D analysis fibrinogen thin film sample N4.

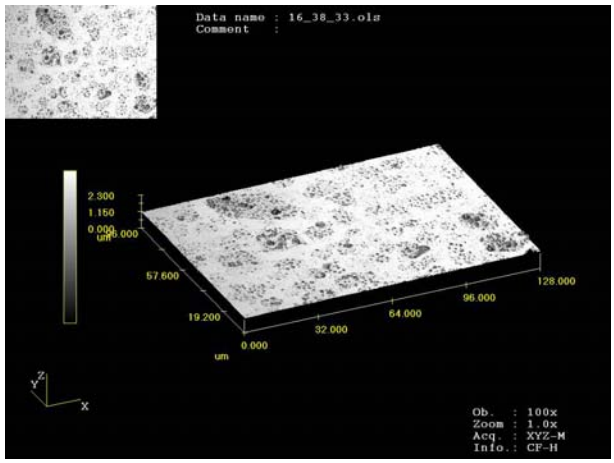

Figure 8: Confocal laser scanning microscopy - 3D analysis fibrinogen thin film sample N4.

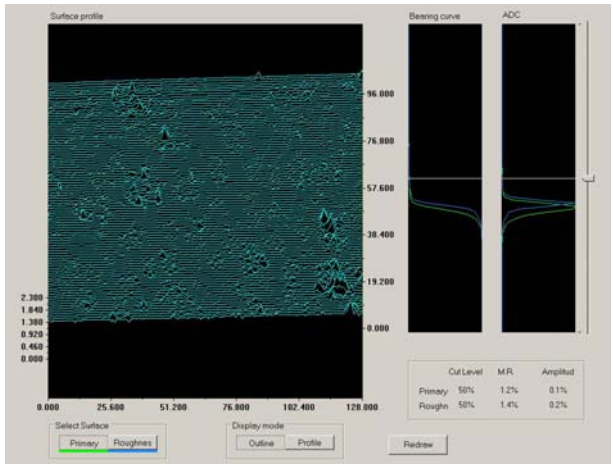

Figure 9: Confocal laser scanning microscopy. Roughness analysis fibrinogen thin film sample N4.

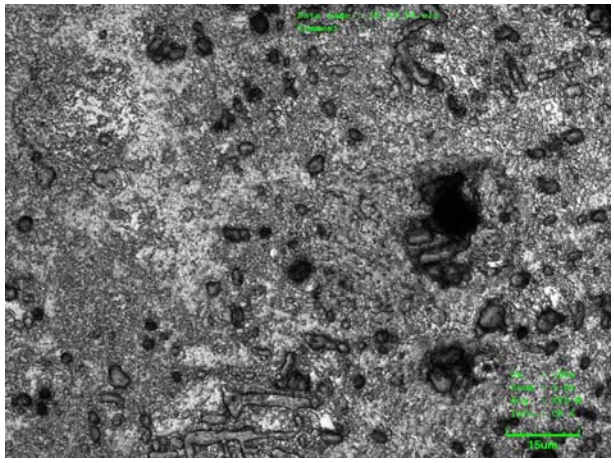

Figure 10: Confocal laser scanning microscopy - 2D analysis fibrinogen thin film sample N1.

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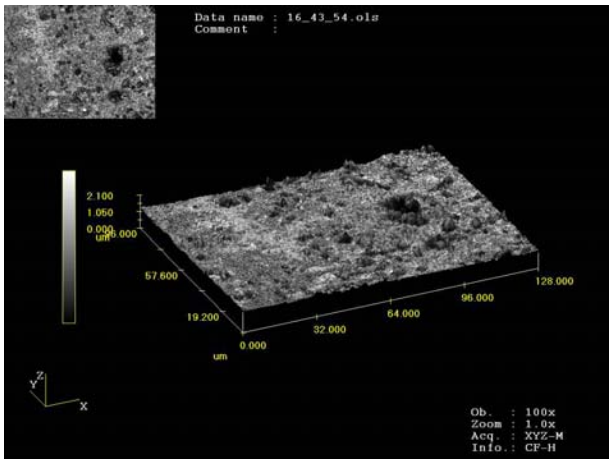

Figure 11: Confocal laser scanning microscopy - 3D analysis fibrinogen thin film sample N1.

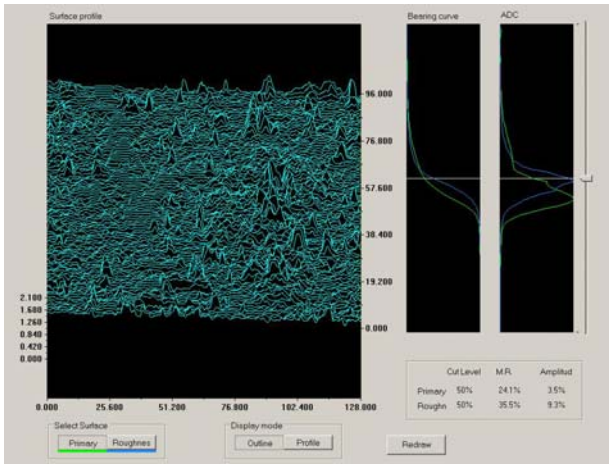

Figure 12: Confocal laser scanning microscopy. Roughness analysis fibrinogen thin film sample N1.

Discussion

The measurements of thin films roughness were done. No significant differences were obtained between similar deposition conditions. Confocal laser scanning microscopy is suitable used for ultra-fine observation and measurements.

Conclusions

MAPLE technology was used for deposition of thin biomedical films. The fibrinogen thin films surface and morphology were studied using new confocal laser scanning microscope LEXT. We have reported fibrinogen thin films morphology.

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[1] CHRISEY D. B., HUBBLER G. K. (1994): 'Pulsed Laser Deposition of Thin Films' (J. Wiley and Sons, New York)

[2] VO-DINH T. (2003): 'Biomedical Photonics - Handbook' (CRC Press LLC, Boca Raton)

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