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利用形態　　：技術代行
支援課題名（日本語）　：Ti 上の Cu 微小構造物への細胞接着特性評価
Program　Title（in English）　：Evaluation of the cell adhesion on the Cu layer sputtered on the Ti substrate
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1. 概要（Summary）

The nanoscale roughness, wettability, and chemical bonding structures on the material surface can be modified by the plasma treatment. The Cu film was sputtered on the Ti to mimic the surface of the implant. The surface of the Cu was treated by the radicals generated from the microhollow-cathode discharge plasma. The adenocarcinomic human alveolar basal epithelial (A549) cell was seeded on the plasma-treated Cu surface to examine cell viability, cell morphology, and cytoskeleton.

2. 実験（Experimental）

~100 nm Cu layer was sputtered on the 10 mm x 10 mm Ti substrate by Professor Takeshi Kato at Nagoya University as a part of Nanotechnology Platform Program. This sample was washed with ethanol and DI water and placed in the 6-well dish.

Ar/O_2 non-equilibrium atmospheric-pressure micro hollow cathode discharge (HCD) radical source (FPC20-N2 Tough plasma, Fuji Machine Mfg. Co., Ltd.) shown in Fig. 1 was used in this study. The detail of this system was described elsewhere [M. Hori: Japanese patent JP 2012-14927, 2012.] but briefly, plasma was generated between the two comb-shape electrodes which were driven at 60 MHz with the applied voltage of 9 kV. The grounded electrode was placed at the downstream of the micro HCD to trap ions, electros, and UV/VUV. Ar at a flow rate of 5 slm was introduced from the upstream of the micro HCD radical source while adding 0~1.2% O_2. The distance from the exit of the radical source nozzle to the bottom of the 6-well dish was fixed at 15 mm. Treatment time was varied from 15 s to 120 s.

The absolute densities of the $O(^3P_j)$ and the $O_2(^1\Delta_g)$ were measured from our previous studies [H. Hashizume: 73rd fall meeting of JSAP. Session 8.6, 13a-E1-35, Sept. 2013, Ehime, Japan.] where the $O(^3P_j)$ density increased with the addition of O_2 up to 0.6% and decreased dramatically with the further addition of O_2. The $O_2(^1\Delta_g)$ densities increased linearly proportional to the addition of O_2.

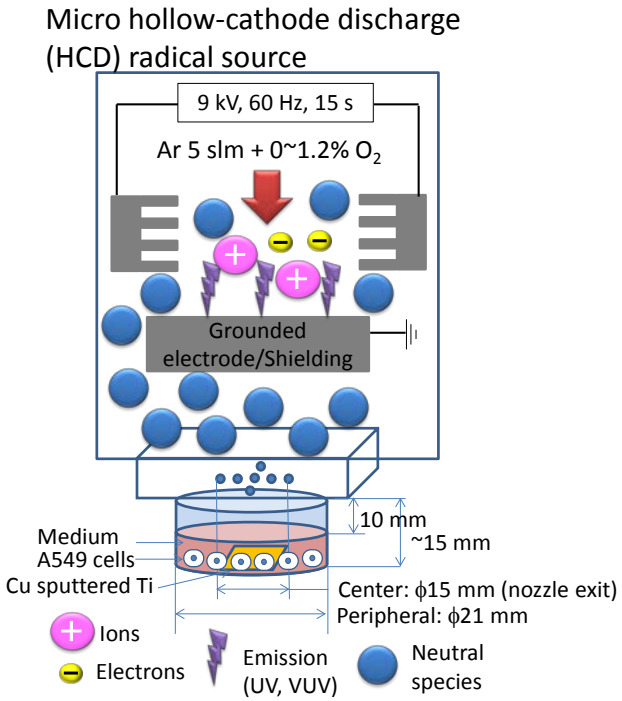

Fig.1 Experimental apparatus

After the radical source treatment of the Cu surface was completed, A549 cells were seeded and incubated for 24 h and 72 h. 50,000 A549 cells were seeded in the 6 well culture plates with 2 mL of complete medium which consisted of Dulbecco's modified eagle medium (DMEM) (044-29765, Wako Pure Chemical Industries, Ltd.) with 10 % of Fatal Bovine Serum (FBS). Cell viability was evaluated using the WST-8 assay and fluorescence activated cell sorting. Cell morphology, cytoskeleton, and the shape of the nucleus were observed by the inverted microscope after staining the fixed cells by Coomassie Brilliant Blue, Alexa Fluor® 488 phalloidin, and 4',6-Diamidino-2-Phenylindole (DAPI).

3. 結果と考察（Results and Discussion）

Preliminary results show that the cell viability seeded on the non-traeted Cu and radical-treated Cu while varying O_2 did not change significantly after 24 h and 72 h incubation. However, the shape of the cytoskeleton was significantly affected when the Cu surface was treated with O_2 radicals. The relationship between the presence of Cu_xO_y and the shape of the cytoskeleton is currently under investigation to elucidate the signal pathway that trigger the cell spreading.

4. その他・特記事項（Others）

<謝辞（Acknowledgement）>

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5. 論文・学会発表（Publication/Presentation）

<学会発表>

(1) S. Tajima, K. Ishikawa, K. Takeda, and M. Hori, Modification of A549 mitochondria activity, cell shape, and cell cytoskeleton by an atomic oxygen radical source, 2013 JSAP-MRS Joint Symposia Symposium O, 16a-M3-6, Sept 16, 2013, Doshisha University, Kyoto, Japan.

(2) S. Tajima, M. Sekine, H. Hashizume, M. Ito, T. Ohta, K. Takeda, K. Ishikawa, and M. Hori, Isolation of Neutral Species Generated from the Ar/O_2 Non-Equilibrium Atmospheric-Pressure Micro Hollow-Cathode Discharge for the Modification of the A549 Cells, 8th International Conference on Reactive Plasmas, 31st Symposium on Plasma Processing (ICRP-8/SPP-31), 6P-AM-SPD-P05, Feb 6, 2014, Fukuoka Convention Center, Fukuoka, Japan.

6. 関連特許（Others）

なし。