

## Measurement of pH near Fibroblast Cells on Stainless Steel and Titanium

[S.Hiromoto](#)<sup>1</sup>, [T.Hanawa](#)<sup>1</sup>

<sup>1</sup> *Biomaterials Center, National Institute for Materials Science, Tsukuba, Japan.*

**INTRODUCTION:** The cells adhere to biomaterials through the points of contact with cell adhesive proteins. Around the cells, they generate extracellular matrix and various biomolecules and ions. Collagen and glycosaminoglycan in the extracellular matrix are long-chain molecules. They would raise the viscosity around the cells and mass transfer of molecules and ions around the cells would be prevented. The environmental change with cells affects the corrosion behavior of biometals. For example, when fibroblast cells are cultured on biometals, the precipitation of phosphate and calcium decreases and the electrochemical property of pure Ti changes [1-3]. Also, the redox potential at the interface is possibly modified because probably sulfate ion in the solution precipitates as sulfide/sulfite on stainless steel and pure Ti only when the fibroblast cells is cultured on the specimens [1,2].

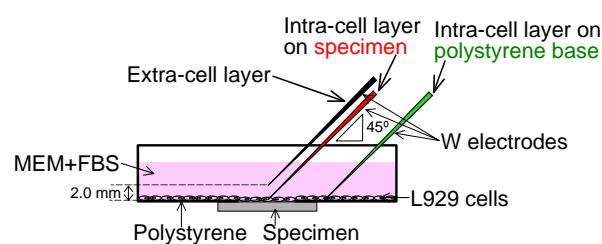
In this study, pH near murine fibroblast L929 cells on 316L stainless steel and pure Ti was measured using a tungsten microelectrode to investigate the effect of cells on the chemical environment of the biometals.

**METHODS:** The surface of 316L stainless steel and commercially pure Ti (99.5%) disks was polished with #600 grid SiC paper (Buehler Ltd.). After autoclaving sterilization, the specimens were fixed on the base of polystyrene cell culture dish. The dish with specimen was sterilized again. The dish without specimens was also prepared. Murine fibroblasts L929 cells were cultured in the dish for 605 ks (7days) with MEM+FBS (Eagle's minimum essential medium with the addition of 10vol% of fetal bovine serum) in a 5% CO<sub>2</sub> incubator. The cells covered the base of dish after 605 ks. For comparison, the dish with the specimens and MEM+FBS was placed in the incubator for 605 ks. When pH was measured, the dish was moved inside a thermostatic unit at 310 K and 5% CO<sub>2</sub> gas was flown over the dish.

A saturated calomel electrode (SCE) and a tungsten (W) microelectrode with the tip with a diameter of 3 μm and a length of 5 μm were used as reference and pH sensor electrodes, respectively. The tip of the W electrode was placed at the intra-L929 cell adhesive layer (a few

micrometer above the base) and 2-mm above the cell adhesive layer (*i.e.* extra-cell layer) on both specimen and polystyrene base as shown in *Fig. 1*. The tip was confirmed to be inside the cell layer under the observation by a stereoscopic microscope placed in the vertical line. In the case of the dish with only MEM+FBS, the tip of the W electrode was placed just over the base (a few micrometer above the base) and 2-mm above the base of the dish. The position just over the base is the same position of the intra-cell layer in the dish with cells. The measurement was performed on several dishes prepared in different experimental days and each dish was named alphabetically.

Open circuit potential ( $E_{open}$ ) of the W electrode was monitored using a potentiogalvanostat. The  $E_{open}$  was converted to pH using a pH- $E_{open}$  calibration curve measured in standard pH solutions.



*Fig. 1: Measurement position of the W electrode in presence of cells.*

**RESULTS:** *Figure 2* shows the pH of the intra-adhesive layer on the specimens and polystyrene base. The pH of the extra-cell layer is shown as a dotted line in *Fig. 2*. The pH values of the intra-cell layer on 316L and Ti specimens were lower than those on polystyrene base and of the extra-cell layer. The pH of the intra-cell layer on 316L specimen was lower than that on Ti specimen. The pH of the intra-cell layer on polystyrene base was as same as that of the extra-cell layer.

*Figure 3* shows the pH just over the specimens and polystyrene base immersed in MEM+FBS without cells. The pH above the specimens and polystyrene base is shown as a dotted line in *Fig. 3*. The pH values just over 316L and Ti specimens were as same as those on polystyrene base, while the former was sometimes lower than

the latter. Also, the pH just over the specimens and polystyrene base were as same as that above the specimens and polystyrene base.

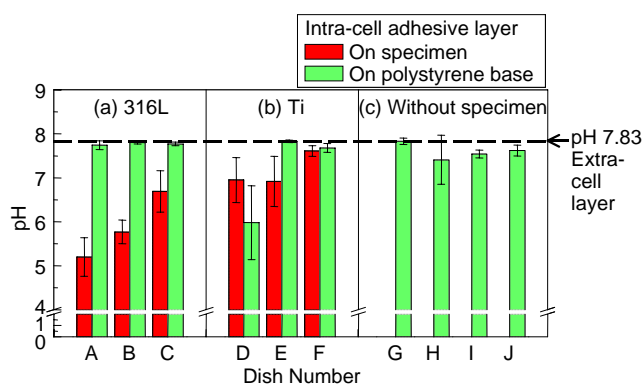


Fig. 2: pH of the intra-cell adhesive layer on the specimens and polystyrene base.

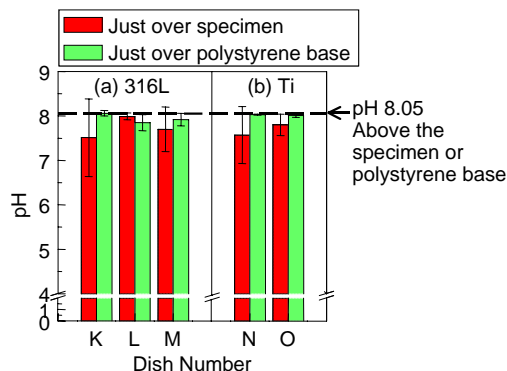


Fig. 3: pH just over and 2-mm above the specimens and polystyrene base without cells.

**DISCUSSION & CONCLUSIONS:** In presence of cells, only on the specimens, the pH of the intra-cell layer was lower than that of the extra-cell layer (Fig. 1). Without cells, the pH just over the specimen was also lower than that above the base (Fig. 2). These indicate that a certain interaction between the metallic materials and solution and cells causes the decrease of pH. The decrease of pH on 316L was larger than that on Ti, indicating that the corrosion reaction of the specimens is responsible for the decrease of pH because the corrosion resistance of 316L is generally lower than pure Ti. The decrease of pH at the intra-cell layer on the metallic materials is supposed to be caused by the acidifying effect of the dissolved metal ions. The dissolved metal ions are possibly accumulated at the interface between cells and materials due to the prevention of mass transfer by the extracellular matrix and cells.

The acidifying effect of metal ions depends on the concentration and the valence of the metal ions. The valence of the metal ions dissolved from 316L

and Ti was not known, on the other hand, the concentration of the metal ions from 316L specimen is estimated to be higher than that from Ti specimen. According to this, the acidifying effect of dissolved metal ions on 316L specimen was larger than on Ti specimen. On the other hand, the molecules and ions generated by the cells against the dissolved metal ions are supposed to be another cause of the decrease of pH. Further investigation is necessary.

**REFERENCES:** <sup>1</sup> T. Hanawa et al (2002) *Mater. Trans* **43**:3088-3092. <sup>2</sup> S. Hiromoto et al (2004) *Biomaterials* **25**:979-986. <sup>3</sup> S. Hiromoto et al (2002) *Electrochim Acta* **48**:387396.

**ACKNOWLEDGEMENTS:** This work was supported by Japan Society for the Promotion of Science (JSPS), Young Scientists (B), Grants-in Aid for Scientific Research (15760545, 2003).