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Influence of Hydrogen Discharged from Palladium Base Hydrogen Storage Alloys on Cancer Cells

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Abstract

The influence of discharged hydrogen from Pd-Ni based hydrogen storage alloys (HSAs) on cultured cells has been investigated. The susceptibility of cells to discharged hydrogen varied with the kind of cells. No influence was seen in the normal cells, while an effect of killing cancer cells was observed near the HAS and the region where the cell death was observed was limited to an extent of a few mm from the alloy surface. In order to examine the cause of the effects, the amount of gaseous hydrogen and hydrogen radicals released from the alloy surface and pH change of physiological saline aq. solution were measured. The amount of gaseous hydrogen and hydrogen radicals increased with time. The pH of physiological saline aq. solution decreased first and then recovered to the starting value after about 50h. The pH change behavior varied with alloy composition. It is inferred that the hydrogen radicals formed on alloy surface may bring a characteristic change in the cancer cells, leading to the effect of discharged hydrogen on cancer cell death.

Introduction

Recently, the influence of hydrogen on a human body attracts attention in the medical field. It has been reported that hydrogen gas, when inhaled in rodents, has an effect of reducing cytotoxic oxygen radicals and hence suppress the occurrence of diseases caused by oxygen radicals, such as arteriosclerosis, cataract, blood-flow injury in brain, liver and heart and so on[1-5].

In our earlier work on hydrogen storage alloys[6-8], it was revealed that Pd base HSAs have a good formability, a high pulverization resistance on absorption / desorption cycles and an easy activation characteristic and, moreover, the abs. /des. plateau pressures near atmospheric pressure can be obtained by adjusting alloy composition. In the process of the desorption, absorbed hydrogen in the metal lattice moves to the metal surface to become an active hydrogen, i.e., atomic hydrogen. This atomic hydrogen (called "hydrogen radical") has a very high reactivity. From this point of view, it was believed that hydrogen radicals might have notable influences on the occurrence of diseases caused by oxygen radicals. However, the existence of hydrogen radicals may be in a very short time because of its instability due to a high reactivity and gaseous hydrogen would be easily formed. Hence the effects of hydrogen radicals discharged from HSAs may be limited in a narrow area near the HSA. In our preliminary work it was found that discharged hydrogen from Pd-Ni based HSAs caused a cancer cell death.

In the present work to elucidate the influence of discharged hydrogen radicals on biological cells, Pd-3~11at% Ni alloys having plateau pressures near atmospheric pressure were used as hydrogen radical supplier and the influence of alloy composition and shape of HSA on hydrogen discharging behaviour and on biological cells were investigated.

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Experimental

Sample Preparation

Pd-3, 5, 8, 11at%Ni alloys were prepared by arc-melting pure materials (palladium > 99.9 wt% and nickel > 99.9 wt%) under argon gas atmosphere. The alloys were re-melted several times and annealed for 15 h at 1073K in vacuum for homogenization. After repeated rolling and annealing for stress relief, the alloys were rolled into a thin plate with 80μm thickness and cut into rectangular sheets of 2mm wide and 4mm long. To examine the effect of sample shape, Pd-5at%Ni alloys having various shapes were prepared from as cast samples, a cylindrical sample with dimensions of 3 mm in outer diameter(OD), 0.08mm thick(t) and 2 mm in height(h) ("standard" sample), a cylindrical sample with double outer diameter(OD=6mm, t=0.08mm and h=2 mm), a cylindrical sample with double volume(OD=3mm, t=0.165mm and h=2mm) and a hollow square sample with L=3mm, t=0.08mm and h=2 mm.

Measurement of Hydrogen Discharged from Pd-Ni Alloys

In order to investigate the behavior of discharged hydrogen, the measurements of hydrogen radical and pH change of physiological saline aq. solution and volumetric measurement of hydrogen gas evolved were performed.

The amount of hydrogen radicals considered to cause cell death was measured using radical trapping agent DBNBS (3, 5-dibromo-4-nitrosobenzenesulfonic acid sodium)[9]. The alloys after activation treatment were put into the solution of DBNBS (0.16ml) in ultrapure water (100ml) for 1~3h. After evaporation-drying of the solution by keeping at 333K, ultrapure water (5ml) was added and held at 333K for 1 h. Then centrifugal separation was carried out by 4000rpm for 12 minutes to obtain a supernatant. The spectrum of the supernatant was measured using the absorption spectrometer. The peak area was computed as an indication of the amount of hydrogen radicals.

Hydrogen discharged from the HSA sample surface partly dissolves in physiological saline aq. solution in the form of hydrogen ion and the rest is released to air as gaseous hydrogen. Hydrogen absorbed sample of 1 g was put in 0.9% NaCl aq. solution at 313K in an incubator and pH change of the aqueous solution was measured using pH meter. The volume of gaseous hydrogen gas was collected using the collecting over water method.

Cell Experiment

Cancer cells* (HeLa, H1299, SW and DLD1) and normal cells* (MDCK, GP8 and NIH3T3) were employed in the cell experiment. Hydrogen absorbed sample (H+) and hydrogen free sample (H-) were put on laboratory dishes, 35mm in diameter, where the cells were cultured. The cell observation was performed to measure the range of cell death in the samples using the optical microscope. Then, the probability of survival cells were measured using Trypan blue reagent (dyeing for detection of cell death) and a cell counter after 24 h.

Results and discussion

Hydrogen Desorption Characteristics

The result of absorbance measurement to detect hydrogen radicals emitted from the sample surface is shown in Fig. 1. Two peaks were observed in the range of wavelength 400~460 nm and both peaks are considered to belong to the DBNBS azo compound. The area of peak1 in Fig.1, which may correspond to the amount of hydrogen radicals is shown for Pd-5 and 8at%Ni alloys in Fig.2. The discharged hydrogen radical increased with time and more hydrogen radicals were released in Pd-8at%Ni alloy, compared with Pd-5at%Ni alloy. This is due to the increase in the desorption plateau pressure with an increase in nickel content of the alloys.

The amount of gaseous hydrogen discharged from Pd-3~11at%Ni alloys in physiological saline aq. solution at 313K is shown in Fig. 3. Since the desorption plateau pressure was increased with nickel content of the alloys, approaching to the atmospheric pressure, the amount of released hydrogen gas increased with alloy composition. Evolution of gaseous hydrogen appears to be ceased in several hours. The amount of hydrogen gas released from the Pd-5at%Ni alloy showed a comparable value even after 10 times of absorption/desorption cycles.

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*HeLa: cervical cancer cell, H1299: lung cancer cell, SW and DLD1: bowel cancer cells, MDCK: renal epithelial cell, GP8: cerebrovascular endothelial cell, NIH3T3: fibroblast cell.

The pH change of physiological saline aq. solution is shown in Fig. 4. The pH value decreased up to 10~20 h, and then almost recovered to the original value after about 50 h. In the region where pH decreases, Tafel reaction ($2H_{lat} > 2H_{ads} > 2H^+ + e^-$ or H_2) may occur on the alloy surface. The pH reduction was considered to be caused by the hydrogen ion formation remaining an electron generated in the alloy. On the other hand, in the region where pH rises, the excess electron charged up in the alloy by the Tafel reaction may induce a change in the reaction from Tafel reaction to Volmer reaction ($2H^+ + e^- > 2\ H_{ads} > H_2$), where the hydrogen ion in the aqueous solution reacts with an electron in the alloy, resulting in a pH increase. As seen in Fig.4, the behavior of pH change differed with alloy composition and Pd-11at%Ni alloy showed the maximum change in pH.

The change in the amount of released gaseous hydrogen and pH, i.e., hydrogen ions, as well as the discharge of hydrogen radicals can be explained in terms of the change in plateau pressure with alloy composition and it is indicated that those are controllable with nickel content of the alloys.

Cell experiment

The influence of hydrogen discharged from Pd-5 ,8 and 11at%Ni alloys on normal MDCK cell is shown in Fig.5. No distinct influence was observed in Pd-5at%Ni alloy (Fig.5a). On the other hand, exfoliation of cells was observed in the Pd-8at%Ni alloy (Fig.5b) and the Pd-11at%Ni alloy (Fig.5c). Since the Pd-8,11at%Ni alloys have higher desorption plateau pressures, compared with Pd-5at%Ni alloy, evolution rate of gaseous hydrogen was so high that normal cells were exfoliated from the base face of the cultivation dishes. The hydrogen free (H^-) and hydrogen absorbed (H^+) Pd-5at%Ni alloys were put in the 35 mm laboratory dishes where normal cells (MDCK, GP8, NIH3T3) and cancer cells (HeLa, H1299, SW, DLD1) were cultured. The probability-of-survival of normal and cancer cells after 24h is shown in Fig. 6 ((a) normal cells (b) cancer cell). As compared with H^- sample, the susceptibility to discharged hydrogen varies with the kind of cells in the H^+ samples. Cell death was observed in almost all of the cancer cells, especially in HeLa, H1299 and DLD1, while no influence was observed in cancer SW cell. The normal MDCK cell cultured together with the cancer HeLa cell was prepared. The appearance of the cells after an exposure of hydrogen discharged from Pd-5at%Ni alloy is shown in Fig.7. Exfoliation of cells was observed in only the cancer cells near the alloy. No influence was observed over the distance of 2~3 mm from the alloy for both of the cells. The optimal composition for selective influence, i.e., no influence in normal cells and cell death in cancer cells, is considered to be Pd-5at%Ni. Effect of sample shape on the range of cell death is shown in Fig.8. Pd-5at%Ni alloy samples having various shapes, “standard” cylindrical sample, cylindrical sample with double outer diameter, cylindrical sample with double volume and hollow square sample, were immersed into physiological saline aq. solution and the stereomicroscope photographs of cells dyed with Trypan-blue reagent were taken after 24h. The dark area in the photos shows the region of cell death. The range of cell death observed was summarized in Table 1. In all samples, cell death was seen inside the alloy sample and the range of cell death outside the sample is around 2 mm, regardless of sample shape.

It is well known that cytotoxic superoxide anion is generated in mitochondria[10]. Then, superoxide dismutase converts it into hydrogen peroxide[11] and glutathione peroxidise or catalase detoxified the hydrogen peroxide into H_2O in normal cells. Unless the latter reaction occurs, very harmful hydroxyl radicals may be produced, which react with nucleic acids and proteins. From the results of hydrogen discharging behaviour and its effect on biological cells, it is inferred that the hydrogen radicals emitted from the alloy surface may act on these reactions and bring a characteristic change in the cancer cells, leading to the effect of discharged hydrogen on cancer cell death.

Summary

To elucidate the discharged hydrogen radicals on biological cells, the influence of alloy composition and shape of Pd-Ni HSA on hydrogen discharging behaviour and on biological cells were investigated. From the cell experiment using normal and cancer cells, it was demonstrated that the hydrogen discharged from Pd-Ni HSAs selectively brings about cancer cell death. The results obtained are summarized as follows.

1) The amount of hydrogen radicals discharged from the Pd-5at%Ni alloy increased with time up to 48 h and Pd-8at%Ni alloy released more hydrogen radicals than Pd-5at%Ni alloy.
2) Hydrogen gas evolved from the HSAs increased within several hours in early stage and then the evolution was ceased. The amount of gaseous hydrogen evolved increased with an increase in the nickel content of the alloys. The pH value of physiological saline aq. solution where the Pd-Ni alloys were immersed, was once decreased up to 10-20h and was recovered to the original value after 50h. The amount of gaseous hydrogen and hydrogen ions as well as hydrogen radicals varied depending upon alloy composition. It is, therefore, possible to control the hydrogen discharging behavior by adjusting the nickel content of the alloys.
3) From the probability-of-survival measurement, reduction in the probability of survival was observed in HeLa, H1299, DLD1 (cancer cells) and GP8 (normal cell). Moreover, the influence of discharged hydrogen was observed even in the cells where the probability of survival did not decrease. It was suggested that the influence of discharge hydrogen depend on the kind of cells.
4) When using Pd-5at%Ni alloy as hydrogen supplier, cancer cell death and no influence on normal cells was observed, while both normal MDCK cell and cancer HeLa cell were affected by the discharged hydrogen from Pd- 8 and 11at%Ni alloys. It was, therefore, found that an optimal alloy composition for attaining the selective cancer cell death is Pd-5at%Ni. The cell death was observed in the whole region inside the samples having various shapes and the region of approximately 2~3 mm outside the samples.

Acknowledgement

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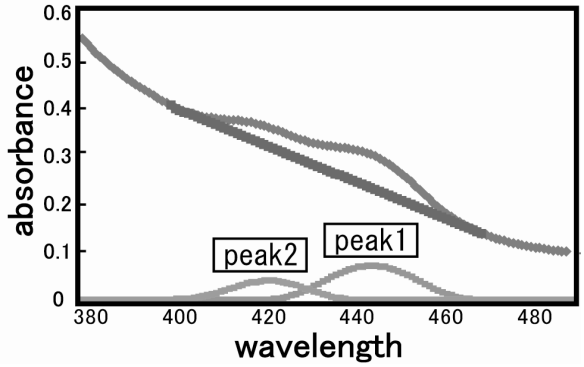

Fig.1 Absorbance of Pd-5at%Ni hydrogen storage alloys after 24h immersion in physiological saline aq. solution.

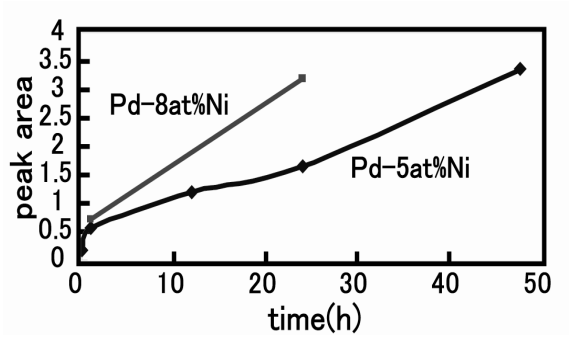

Fig.2 Peak area of the peak1 in Fig.1 for Pd-5 and 8 at%NI alloys.

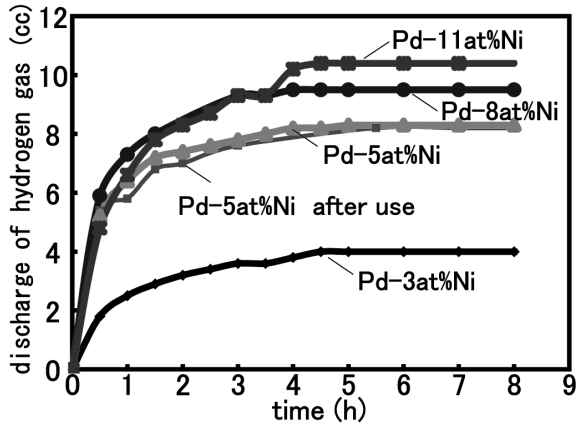

Fig.3 The hydrogen gas discharged from Pd-3~11at%Ni hydrogen storage

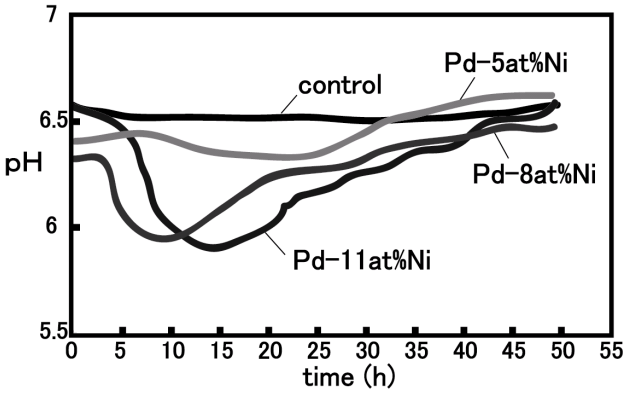

Fig.4 PH change in physiological saline aq. solution due to discharged hydrogen from Pd-Ni alloys.

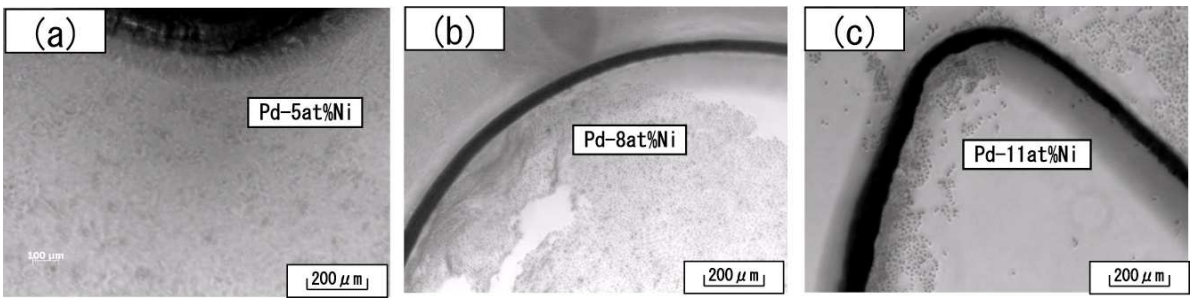

Fig.5 The appearance of normal MDCK cell near Pd-Ni alloys after 24h-immersion of (a) Pd-5at%Ni, (b) Pd-8at%Ni and (c) Pd-11at%Ni alloys.

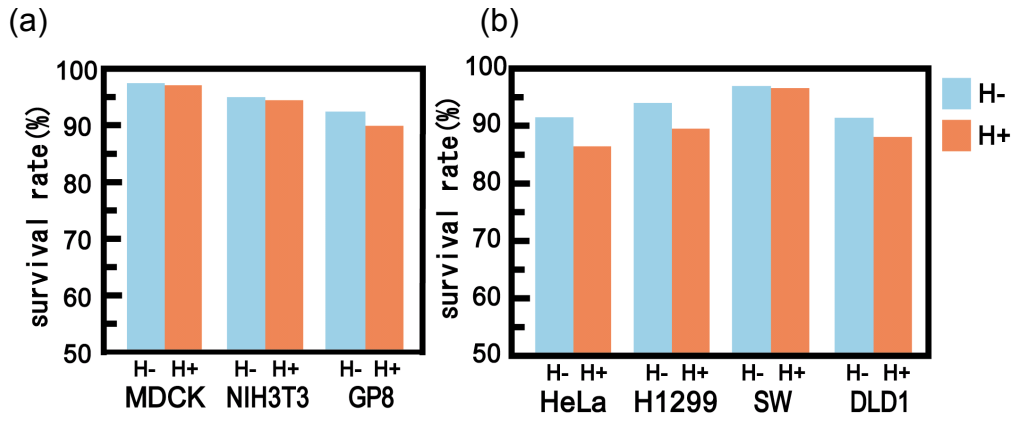

Fig.6 The probability-of-survival of (a) normal cells and (b)cancer cells.

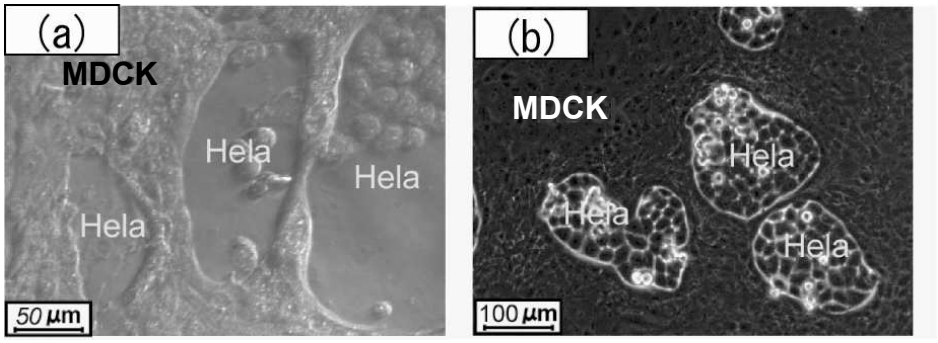

Fig.7 The appearance of cultured cells after 24h-immersion of Pd-5at%Ni alloy (a) MDCK + HeLa cells near the alloy and (b) MDCK + HeLa cells apart from the alloy.

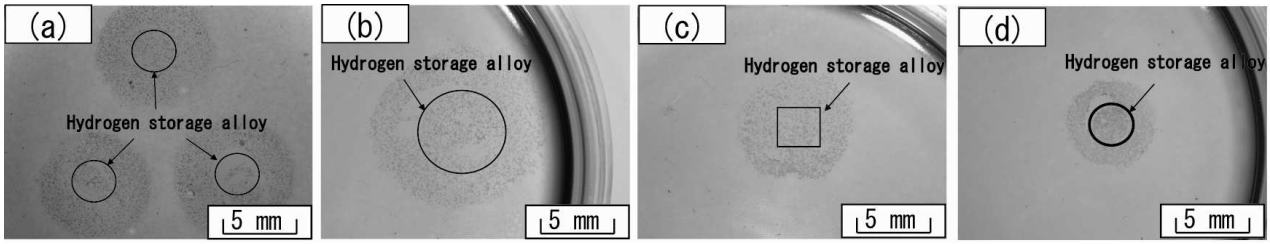

Fig.8 Influence of sample shape on the range of cancer HeLa cell death. (a) “standard” cylindrical sample, (b) cylindrical sample with double outer diameter, (c) square shape sample and (d) cylindrical sample with double volume.

Table 1 The range of cancer HeLa cell death for various sample shape.

Sample form	Cell death range inside	Cell death range outside(mm)
Standard sample	whole area	2.0～2.5
Cylindrical with double diameter	whole area	2.0～2.5
Square pipe	whole area	2.0～2.5
Cylindrical with double volume	whole area	1.5～2.0

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