GRAPHENE FOREST DEVICES AS CELL SCAFFOLDS FOR STEM CELLS

Yukihiro Okamoto[1], Hitoshi Watanabe[2], Kazutoshi Kubo[2], Hiroki Kondo[2], Noritada Kaji[1,2], Manabu Tokeshi[3], Masaru Hori[2], Yoshinobu Baba[1,2,4]

[1]FIRST Research Center for Innovative Nanobiodevices, Nagoya University, JAPAN, [2]Nagoya University, Japan, [3]Hokkaido University, Japan, [4]Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), JAPAN

ABSTRACT

We developed carbon nanowalls devices (CNWs), on which graphenes vertically stand in the nanometer spacing like "graphene forest", with different wettability. CNWs permitted cell adhesion and proliferation, and especially super hydrophobic CNWs enabled easy and less invasive cell collection. Furthermore, collagen coated CNWs successfully enhanced the differential ability of the human mesenchymal stem cells (hMSC) to osteoblast cells compared to collagen coated polystyrene culture dishes. Thus, CNWs have superior many properties as cells scaffolds and are expected to be useful for regenerative medicine

KEYWORDS

Carbon nanowall, Cell scaffold, Nanostructure, Stem cells

INTRODUCTION

In regenerative medicine, cell scaffolds are significantly important as well as cells and growth factors [1]. One of the most promising scaffolds would be CNWs developed by our group [2] because CNWs have large surface area and high aspect ratio, which would promote cell adhesion and proliferation. In addition, the size, wettability, and the space between CNWs could be easily controlled. Despite of these superior properties, there has been no report about the application of CNWs for cell scaffolds. Therefore, in this paper, we investigated CNWs' performance as cell scaffolds in detail.

EXPERIMENT

CNWs with different wettability were fabricated according to our previous paper with our own apparatus as shown in Figure 1 [3]. Briefly, a radical injection plasma-enhanced chemical vapor deposition (RI-PECVD) system, which consists of two plasma sources, was employed to prepare CNWs. One of plasma source is the surface wave plasma (SWP). It generates the high density proton radical, which is injected to under plasma region. The other is very high frequency (VHF) capacitively coupled plasma (CCP). Operating conditions for CNWs preparation were as follow: The power of SWP and CCP were 400 and 300 W, respectively. The pressure was kept at 1 Pa with methane and hydrogen gas. The flow rate of each gas is 50 and 100 sccm for methane and hydrogen gas, respectively. After growth of CNWs, for the control of the CNWs' wettability, the atmospheric pressure plasma (APP) and CCP in the vacuum were irradiated on the CNWs' surface. For the preparation of super hydrophilic surface from hydrophilic surface, APP with argon and oxygen gas was employed according to the paper [4], which experimental conditions were as follow: The power of the atmospheric pressure plasma source is 9 kV. The flow rate of gas is 2 and 50 sccm for argon and oxygen, respectively. On the other hand, for the preparation of super-hydrophobic surface from hydrophilic surface, RI-PECVD with tetrafluoromethane and argon gas was employed, which experimental conditions were as follow: The power of CCP is 200 W. The pressure was kept at 160 Pa with tetrafluoromethane and argon gas. The flow rate of each gas is 36 and 10 sccm for tetrafluoromethane and argon, respectively.

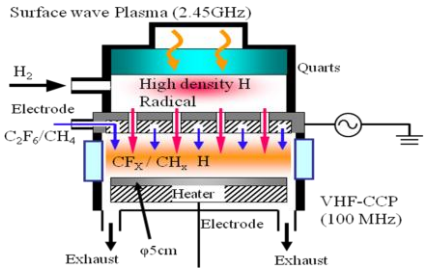

Figure 1. Schematic for carbon nanowall fabrication device

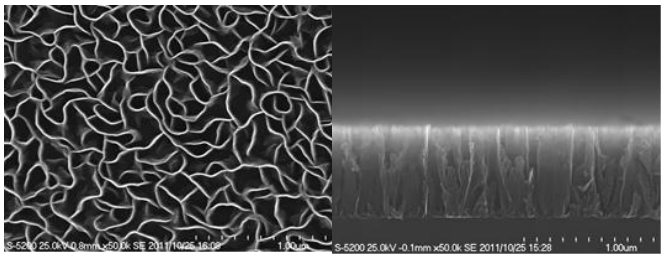

Figure 2. SEM images of Carbon nanowalls

SEM images of prepared CNWs show that we can successfully prepared CNWs, which height, space and thickness are 600 nm, 200 nm and 10 nm, respectively as shown in Figure 2. In addition, we could control CNWs' wettability and obtain super hydrophilic and super hydrophobic CNWs as shown in Figure 3.

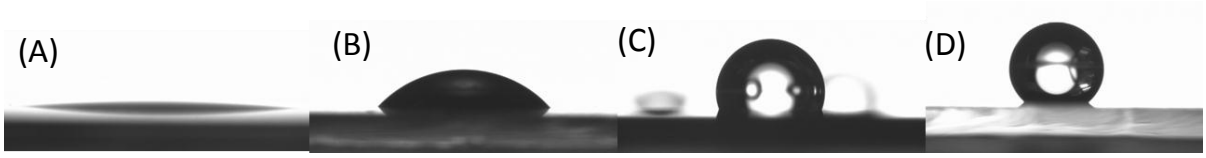

Figure 3. Contact angle measurements of each carbon nanowalls prepared by (a) atmospheric plasma followed by methane gas treatment, (b) only methane gas treatment, (c) hexafluoroethane gas treatment and (d) fluorine treatment followed by hexafluoroetahne gas treatement

To assess CNWs' performance as cell scaffolds, at first, serum protein adsorption on CNWs surface was estimated by BCA method. The results indicate that adsorbed amount on CNWs was twice as much as on the glass surface with similar wettability. Therefore, we can expect cell adhesion and proliferation on CNWS. Subsequently, to investigate cell adhesion and proliferation on CNWs, tumor cells were cultured on CNWs with different wettability. SEM images in Figure 4 show that cells extended and adhered on CNWs with moderate wettability, while showed round shape on super hydrophobic CNWs. The reason of this phenomenon has still been unclear but seems to be related to the amount and structure of adsorbed protein. In addition, the cell numbers after 4 days cultures were estimated and the results showed that CNWs with moderate wettability had highest cell proliferation, while super hydrophobic CNWs seemed to have no proliferation as shown in Figure 5. However, detail observation made clear that cells detached from super hydrophobic CNWs surface when cells migrated or proliferated, and revealed that detached cells were alive and could proliferate. Therefore, super hydrophobic CNWs enable us to retrieve cells with less invasive manner and are useful for regenerative medicine.

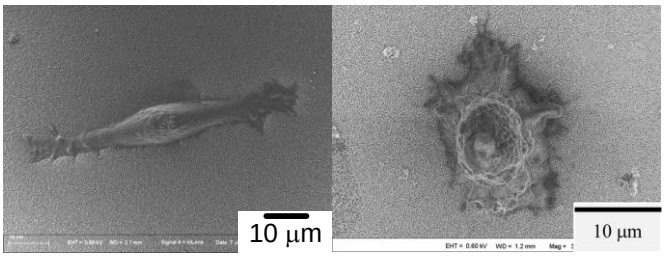

Figure 4. SEM images of tumor cells on CNW with moderate wettability (left) and with super hydrophobic (right)

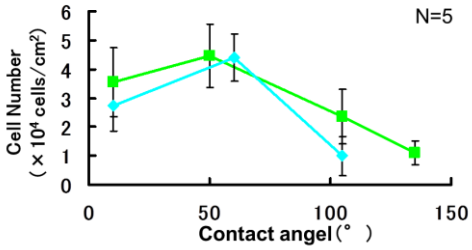

Figure 5. Cell numbers on CNWs(■)and glass surfaces (◆) after 4 days culture. Cells: HeLa cells (1 ×10 ^{4} cells /cm^2)

Finally, the effect of CNWs on the differential ability of hMSCs was also investigated. Figure 5 shows the results of alkaline phosphatase staining after culture on CNWs surface and glass surface, and that hMSCs on CNWs can differentiate to osteoblast as well as on the glass surfaces. In addition, quantification of alkaline phosphatase activities was demonstrated to assess the differentiation to osteoblasts. Figure 6 indicates that collagen coated CNWs enhanced the differentiation ability of hMSCs to osteoblast compared to the polystyrene dishes and glass surfaces. The reason of this enhancement has not been made clear, but would be due to the stiffness and electric property of CNWs.

Thus, CNWs have superior properties as cell scaffolds and would be expected to be useful in regenerative medicine.

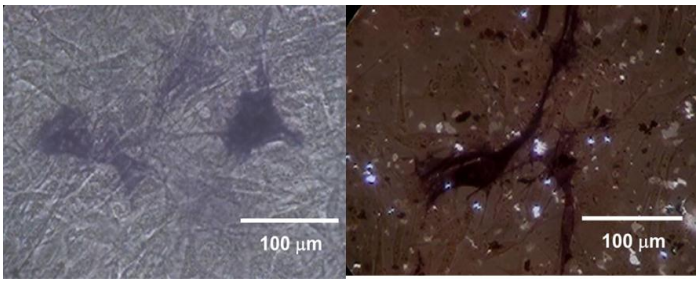

Figure 5. Optical micrographs of hMSCs stained with alkaline phosphatase kits on glass surface (left) and CNWs (right)

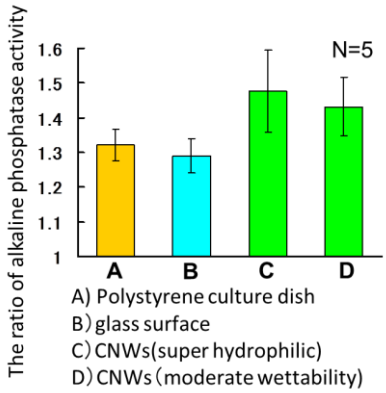

Figure 6. Comparison of alkaline phosphatase activity between collagen coated each substrate

CONCLUSION

Carbon nanowalls with hydrophobic or hydrophilic surfaces were prepared by our developed method. These carbon nanowalls permitted cells to adhere and proliferate. Interestingly, on super hydrophobic carbon nanowalls, cells easily detach from the surface in moving and proliferation. Therefore, with super hydrophobic carbon nanowalls, we can retrieve cells in less invasive manner. In addition, carbon nanowalls can promote hMSCs to higher differentiation into osteoblasts compared to other surfaces. Thus, carbon nanowalls have superior properties as cell scaffolds and are expected to contribute to regenerative medicine.

ACKNOWLEDGEMENTS

This research is supported by TOYOAKI SCHOLARSHIP FOUNDATION.

REFERENCES

[1] R. Langer, J. P. Vacanti. *Science*, **1993**, *260*, 920.

[2]M. Hiramatsu *et al*. *Appl. Phys. Lett.*, **2004**, *84*, 4708.

[3]H. Watanabe, *et al*., *Jpn. J. Appl. Phys.*, **2012**, *51* 01AJ07.

[4]Inui, *et al*., *Applied Physics Express*, **2010,** *3*, 126101.

CONTACT

Y.Okamoto E-mail: okamoto@nanobio.nagoya-u.ac.jp