

Effect of Polyaniline-coated Surface on the Formation of Multicellular Spheroids of Hepatocytes

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Polyaniline was formed on indium tin oxide coated glass by electrochemical method. On the polyaniline surface, hepatocytes from adult rat rapidly formed multicellular spheroids for 2 days, and showed high amount production of albumin. It is shown that polyaniline is suitable for hepatocytes culture.

Many attempts have been made to develop an artificial liver support system to treat patients with fulminant hepatic failure. Recently, research effort has been focused on bioartificial liver using liver cells^{1,2} because of its synthetic function. Especially, the properties of surface are very important factor in the investigation of novel functional support materials for the animal cell culture. Therefore, modification of surface has been performed by using the many chemicals, such as lactose.³ In these studies, multicellular spheroids formation on the material surfaces was employed as the critical factor of materials, because high activities of hepatocytes are expressed by multicellular spheroids formation and the spheroids are known to maintain the ability to secrete albumin for a long period.⁴

In this study, we attempted to exploit the novel suitable material for animal cell culture, which can be synthesized more easily than lactose-modified materials and to accomplish the rapid formation of multicellular spheroids. We modified the surface of indium tin oxide coated glass (ITO glass) by several chemicals to get the information about the effect of materials surface on the spheroid formation. The results obtained here suggested that the introduction of amino group and coating with polyaniline (PA), one of conducting polymers, to the ITO glass surface were very effective for the spheroid formation. Therefore, we focused on the investigation of the effect of PA-coated surface.

Polyaniline was electrochemically formed on ITO glass surface from aniline aqueous solution (0.2 M)⁵ at constant voltage (5 V) for 5 s by using sulfonic acid (0.1M) as electrolyte. Amount of albumin secretion from the cells was measured by enzyme immunoassay.⁶ Cell, cultivation and other methods were reported previously.⁶ As polyaniline has two chemical state, that is doped state (oxidation state) and neutral state (reduction state), the effect on spheroid formation was examined in both state. Doped-PA has plus charge and sulfonate ion as counter ion in its structure. Neutral-PA has no charge and no counter ion. Non-treated ITO was used as control run.

Figure 1 shows microphotographs of hepatocytes cultivated for 2 days. On the doped-PA surface, multicellular spheroids were formed only for 2 days cultivation (Fig 1a). However, hepatocytes did not form spheroids but still spreaded over surfaces of neutral-PA-coated (Fig 1b) and non-treated surface (Fig 1c) for 2 days cultivation. Hepatocytes formed spheroids on these three surfaces for 4 days cultivation. These results suggest that doped-PA-coated surface is very effective for rapid formation of multicellular spheroids. The neutral-PA-coated

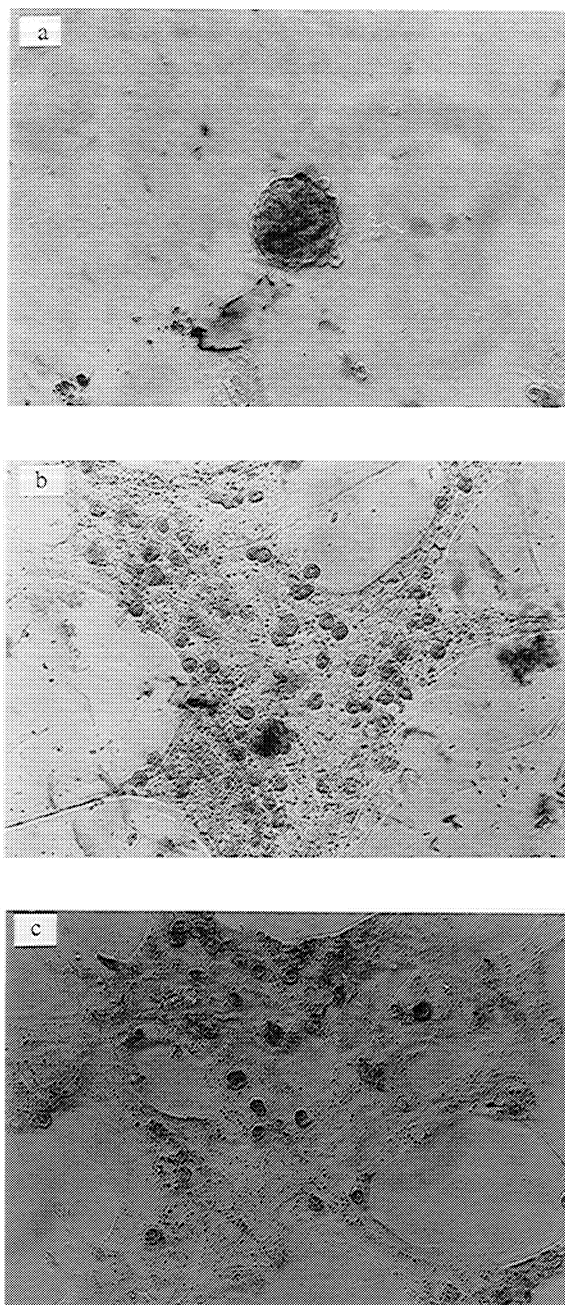


Figure 1. Micrographs (x250) of hepatocytes cultured on doped- (a) and neutral-PA (b) -coated and none-treated (c) ITO glasses for 2 days cultivation.

surface, however, is not effective in our object. It could be also predictable that the amount of albumin secretion from the cells on doped-PA-coated surface would be the greatest in these three cases.

Figure 2 shows the amount of albumin secretion from cultures using doped-and neutral-PA-coated surface and control

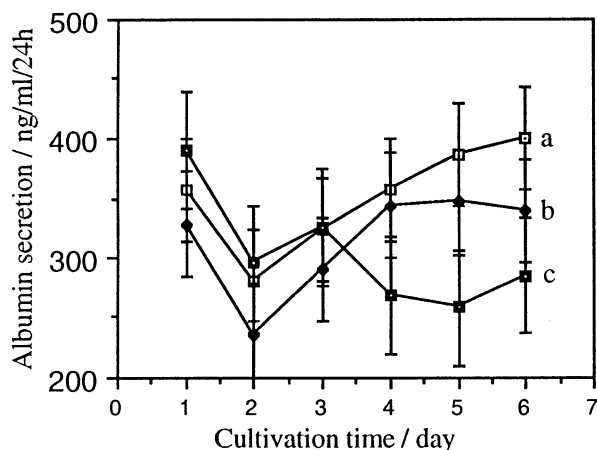


Figure 2. The amount of albumin secreted for 24 h from hepatocytes cultured on doped- (a) and neutral-polyaniline (b)-coated and none-treated (c) ITO glasses. Standard deviation is shown by bar in the figure.

run. Cells cultivated on doped-PA-coated surface gave the fastest speed of albumin secretion in these experiment (Fig 2). This result suggested that the cells on the doped-PA-coated surface maintained high activity for long period, and agreed with the prediction derived from the results of spheroid formation. We have also examined other conducting polymer, such as polypyrrole (Py), however, we have not found effective material like PA. Doped-PA has ionized structure as described above. It is considered that such ionic structure is an important factor for rapid spheroid formation. Moreover, we also have been carrying out the experiment using other conducting polymers.

Throughout this study, we could clarify the effectiveness of polyaniline for hepatocytes culture.

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