Assessment of GaN chips for culturing neuron cells

1. Introduction

GaN-based materials are emerging as the important materials for the optical devices in the blue and green wavelengths. These devices are essential for full-color display and pickup head in high-definition digital-video-disk~HD-DVD systems. The wide direct band gap and excellent thermal conductivity make these materials good candidate for the high-temperature field-effect transistors. In addition, because of the useful properties of GaN such as chemical stability, high hardness, excellent heat-resistant properties and electrical properties, GaN films have a wide range of applications.

Now most of the electronics for intracerebrum implant are silicon-based devices, but the biocompatibility of silicon is poor [1,2]. Thus, whether the favorable electrical characteristics of GaN films can make them a possible use in a cellular chip is an interesting topic. For example, electrode-neuron implants could be used as neural prostheses, as have been used clinically in pain relief, functional electrode stimulation [3] and Parkson's disease [4]. This study investigated the biocompatibility of GaN films using primary neuronal cells and assessed the effect of u-type, n-type and p-type of GaN films on neuronal network formation. Neuron cell morphology was observed by metallography microscope and LDH assay was used to count cell survived on the GaN films. The long-term objective of this study is to develop a GaN-based field-effect transistor arrays (FET).

2. Materials and Methodes

2.1. Preparation of GaN films

GaN films were grown in a separate-flow horizontal reactor by atmosphere-pressure MOCVD. The details of separate-flow horizontal reactor have been described in [5]. The main reactant source was separated by a quartz plate, where we introduced the flow rate of 60 mmol/min in trimethylgallium~TMGa and 5000 cc/min in NH3 flowing into the upper and the bottom streams, respectively. In this work, the 4 mm GaN epitaxial layers were grown on the sapphire substrates through structures of buffer layer. Buffer layer is the conventional nucleation layer. The substrate was first precleaned at 525 °C to grow the GaN nucleation layer with a thickness of 0–1000Å, followed by raising to 1000 °C to grow the; 4 μm GaN epitaxial layer. The temperature was then raised to 1130 °C to grow the 4 μm *p*-type Mg-doped GaN layer. SiH4 was used forSi dopant with various Si mole flow rates ranging from 0.43 to 0.65 nM/min form n-type GaN.

2.2. Cell culture

Cerebellar granule neurons were prepared from 7-day-old Wistar rats according to Levi et al. [6] with slight modifications. Briefly, neurons were dissociated from freshly dissected cerebelli by mechanical disruption in the presence of trypsin and DNase. Subsequently, neurons were added to the culture wells at a density of 10×10^5 cells/well [7] in basal Eagle medium (BME; Gibco) supplemented with 10% fetal calf serum (FCS; Gibco), 25mm KCl, penicillin G (100 IU/ml) and streptomycin (100 mg/ml). Cultures were maintained at 37℃ in a humidified atmosphere of 95% air/5% CO_2.Cytosine arabinoside (10 mm) was added to the culture medium 1 day after plating to prevent replication of non-neuronal cells.

2.3. Cell morphology

For morphological observation, the cells adhering to the membrane were washed with PBS and then .fixed with 2.5% glutaraldehyde in PBS for 1 h at 4℃. Subsequently, the cells were postfixed for 1 h in 1% osmium tetroxide at 25℃. After thorough washing with PBS, the specimens were dehydrated by graded ethanol changes, critical point dried and examined by metallography microscopy.

2.4. Assessment of neuronal survive

Neuronal survival was evaluated by NAD^+ reduced to $NADH/H^+$ by the LDH-catalyzed conversion of lactate . For the LDH assay [8], the culture medium was removed after a predetermined culturing time , and then cells were incubated with 0.2ml of Triton X-100 for 30 min at 37℃. After incubation, add 0.1ml/well assay medium containing cells and add 0.1ml/well LDH kit shake to triplicate wells and shaken for 30 min. The optical density of the formazan solution was read on an ELISA plate reader at 490 nm and reference wavelength at 630nm.

3.Results

3.1. Characterisation of GaN films

Film roughness estimated from the AFM data was found to be 4.6, 7.1, 8.5 and 13.7 nm for the u-type, n-type, p-type GaN films and silicon film, respectively. Since the difference of roughness among them was small [9], the effect of roughness on the behavior of neurons were neglected in this work.

The contact angle formed between a water drop and the surface of U-type, N-type, P-type GaN film and silicon film and TCPS were 45±2.3°, 47±2.1°, 63±1.7°, 65±1.7° and 65±2.5°. Clearly, the U-type and N-type GaN films are more hydrophilic, but the hydrophobic property of P-type GaN film is very close to those of silicon film and TCPS.

3.2. Primary cerebellar granule neurons attachment and morphology

After incubation of 3 days, cerebellar granule neurons cultured on the u-type and n-type GaN films have formed neurite networks, exhibiting almost normal adhesion and neurite formation (Fig.1 (a) and (b)). For the case of neurons cultured on the p-type GaN film, less neuritis but more obvious cell aggregation were observed (Fig.1 (c)). A similar cell behavior was observed for neurons cultured on the silicon films (Fig.1 (d)). It is noted that neurons cultured on the p-type GaN film and silicon film formed clusters of neurones with long neurites protruding up to 150 μm from the cell bodies. For neurons cultured on the TCPS, cell aggregate had a more round shape than the p-type GaN film and the silicon film. However, p-type GaN film and silicon film did not exhibit the dense network of fibers that could be visualized on the u-type and n-type GaN films.

On the 5th day, neurons cultured on the u-type, n-type and p-type GaN films exhibited a dense fiber network with a random distribution of neuritic processes and increased the percentage area of the surface covered by cells (Fig.2 (a)-(c)). Conversely, Fig. 2 (d) shows the very low amount of cell coverage on the silicon film after incubation of 5 days. At time points of 3rd and 5th day, neurons cultured on the TCPS showed a similar result.

Figure 3 shows the relative cell number 3 and 5 days after cell seeding, which was quantitatively determined from the ratio of the LDH released from survival cells attached to the films to that of cells attached to TCPS. The LDH assay relies on the ability of the viable cells, thus the LDH value obtained is directly proportional to the cell number on each film. Cells cultured on all GaN films show near to or more than 200% survival of cells relative to TCPS. However, only 74±15 % and 33±0.7 % cell survival were observed on the silicon film relative to TCPS after 3 and 5 days in culture. LDH assay confirmed qualitative observation of neuron morphologies (Figs. 1 and 2). Thus, the effect of GaN and silicon films on the cultured neurons was noted, indicating that the GaN films may be used to culture neurons.

4. Discussion

Surface topography and chemistry of substrate have been show to be important in determining cell-substrate interactions and influencing survival and differentiation of cultured neurons [9-12]. Fan et al. have explored the different reaction of cells to varying nanotopographies on the same surface chemistry [9]. The roughness of the substrate may influence the contact area of the cell membrane with the substrate. In this study, the cell density of adherent cells and cell differentiation are independent of the types of the GaN films. This may be the difference of roughness of the GaN films

is too small to affect the behavior of cultured neurons, which is consistent with the results of Fan et al [9]. On the other hand, the U-type and N-type GaN films have more hydrophilic surface, but neuronal behaviors on them are similar to on P-type GaN film. The correlation between the contact angle and the cell adhesion can be considered with respect to the interface energy. However, the good compatibility of GaN films for neuron culture is not related to the hydrophilic property of their surface because silicon and P-type GaN films have similar water contact angles. Therefore, the surface physical and chemical of the GaN film itself may significantly affect neural adhesion and the morphology of adherent cells. Of course, it needs further study to develop the applications of GaN films in the biomedical field.

References

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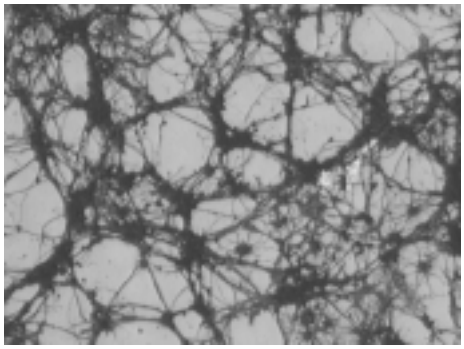
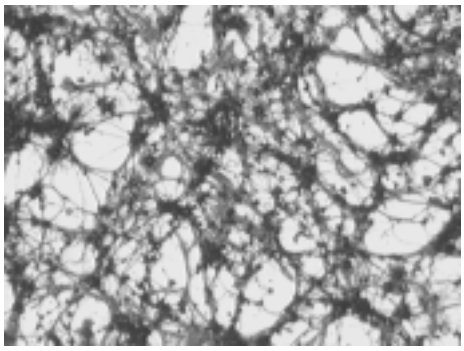


(a)



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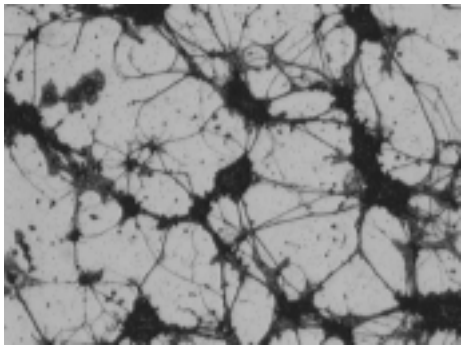

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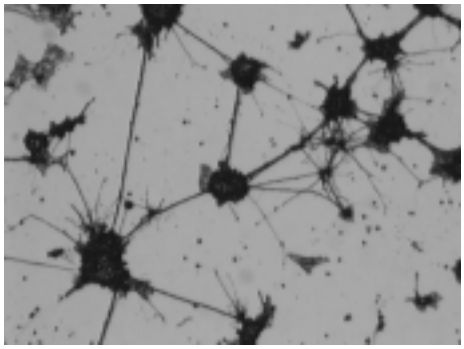

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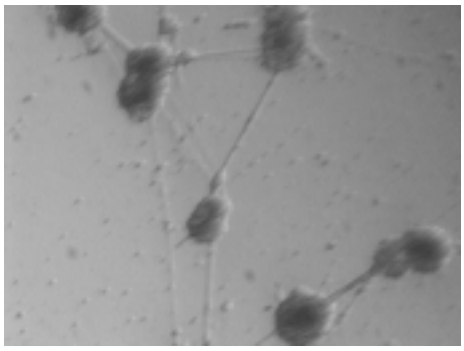
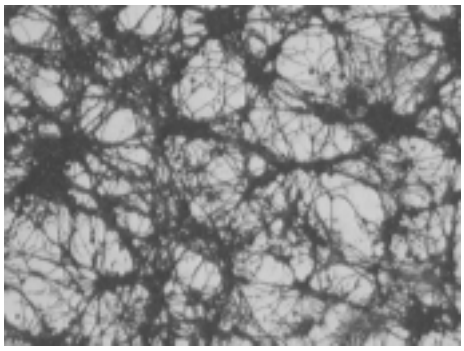


(e)

Fig.1. Primary cerebellar granule neurons cultured on (a) U-type GaN film, (b) N-type GaN film, (c) P-type GaN film, (d) silicon film and (e) TCPS for 3 div (100 ㅧ).



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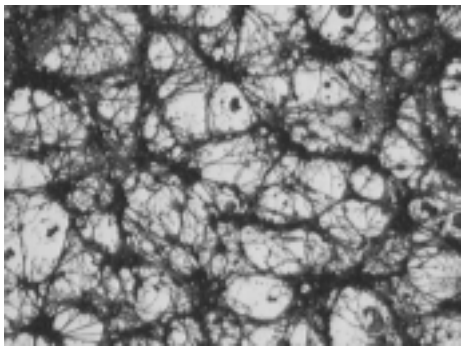

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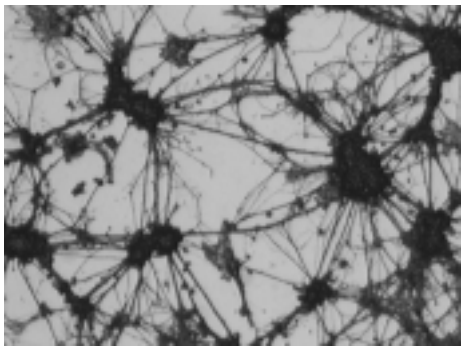

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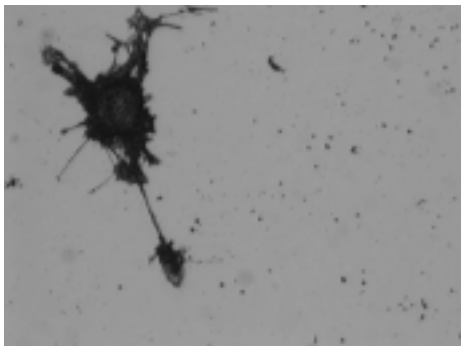

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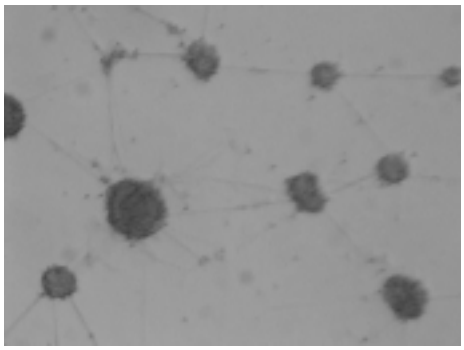

(e)

Fig.2. Primary cerebellar granule neurons cultured on (a) U-type GaN film, (b) N-type GaN film, (c) P-type GaN film, (d) silicon film and (e) TCPS for 6 div (100×).

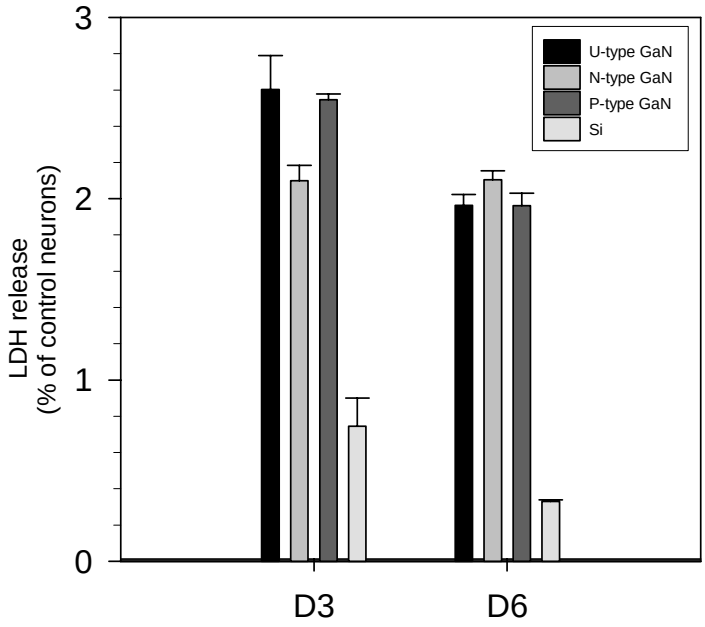

Fig.3. Relative LDH activity of neurons cultured on U-type GaN, N-type GaN, P-type GaN and silicon films.