Nano-Biointerface of Titania Nanotube Arrays Surface Influence Epithelial HT29 Cells Response

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ABSTRACT The unique structure of Titania Nanotube Arrays (TNA) provides larger surface area and energy to improve cellular interactions for nano-biomaterial implants and nanomedicine applications. TNA topography plays a critical role in cellular stability and cell survival. This nanostructure surface has been shown to modulate diverse cellular responses of cell adhesion, migration, proliferation and differentiation. The present study has found evidence which suggests that TNA nanoarchitecture structures may be beneficial for epithelial cells as a supply or storage route for nutrients and also for mediator growth signals. Thus, this nano-surface might act as a good modulator and communicator in cellular interaction because it could recruit and provide sufficient essential biological element for cell growth and its survival.

KEYWORDS: Titania Nanotube Arrays, Titanium Dioxide Nanosurface, Biological Interface, Bioengineered Nanomaterial

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INTRODUCTION

Titanium dioxide (TiO_2) nanotube arrays are also referred to as titania nanotube arrays (TNA) have garnered considerable interest as biomedical implant materials and nanomedicine applications (Xavier *et al.,* 2015; Swami *et al.,* 2011). TNA produced by anodization method inherent high quality and cost-effectiveness (Fadl-allah *et al.,* 2013; Guehennec *et al.,* 2007). Several works have reported TNA topology on various cellular responses such as cell adhesion, migration, proliferation and differentiation (Hazan *et al.,* 2016; Tan *et al.,* 2012; He et al. 2008; Webster & Ejiofor, 2004; Salata, 2004). The work indicates that the nanorough surfaces of implant materials could increase surface energy and specific cell adhesion factors resulting in a larger collection of proteins (such as vitronectin and fibronectin), which will subsequently promoting its cytocompatibility (Saharudin *et al.,* 2013; do Nascimento, 2010; Bigerelle & Anselme, 2005; Fujibayashi, 2004). In addition, this nanostructured surfaces have been shown to reduce inflammatory response more effectively compared to conventional materials (Taylor & Webster, 2011; Raimondo *et al.,* 2010). Furthermore nanosurface interaction with epithelial cells in spatial control are critical in producing improved biomaterial and implant geometry (Dhawan *et al.,* 2016; Nam *et al.,* 2016; Yashunsky *et al.,* 2010). This study will provide overview of nano-bio interface response of epithelial cell with TNA nanotopography.

METHODOLOGY

Cell Culture

Epithelial HT29 cells from the American Type Culture Collection (ATCC, USA) and TNA were prepared according to the method described by Mydin, R. B., et al. (2017). The dimension of the

TNA as follows: outer diameter: 100 nm, inner diameter: 60 nm, wall thickness: 15 nm, and length: 600 nm. TNA material is the test material while titanium foil (TiP-without any surface modification) and glass slide (Sigma-Aldrich, USA) were selected as control materials. All the test materials were standardized for 48 hours culture.

Field emission scanning electron microscope (FESEM)

Samples were fixed with 2.5 % glutaraldehyde (Sigma-Aldrich, USA) in 0.1 M PBS for 30 mins and followed by dehydrated series of 50 %, 75 %, 90 % and 99.5 % ethanol solutions for 10 mins interval before being air-dried at room temperature. The material surfaces were then subjected to SPI-MODULE Gold Coater before being examined under FESEM. The elemental analyses were performed via Energy dispersive X-ray spectroscopy (EDX).

Propidium iodide staining

The cultured growth medium in the plate wells (culture system) was removed and slowly doused three times with 1 X PBS before being fixed in 100 % cold methanol for 5 mins. Then, the solution was drained out and the samples were directly stained with 10 µg/mL propidium iodide (PI) from Sigma-Aldrich for 5 mins. Later, it was slowly rinsed three times with PBS and left in the laminar fume hood to dry. All the work should be done under minimum light exposure to avoid loss of fluorescent signal. Finally, the material surfaces containing cells were placed on glass slide, mounted with 90 % glycerol (Merck, USA), protected by coverslip and examined using fluorescent microscope with magnificent of (Olympus BX51, Japan) 200 X and 400 X. The microscope was equipped with a charge coupled device (CCD) camera at excitation wavelength of 450 nm band pass filter and 590 nm long pass filter for PI.

RESULT AND DISCUSSION

FESEM analysis of TNA and TiP surface after 72-hour incubation with the growth media, revealed that the surfaces was prone to adhesion of growth nutrients aggregates. The results suggest that most of growth nutrients aggregates may have channeled into the nanotubes cannel (small molecules that less than 60 nm size) and leaving some large growth nutrients aggregates (larger molecules that higher than 100 nm size) which formed like dried crystallites flakes structure on top the TNA surface (Figure 1b). It was noted that the inner diameter and outer diameter of TNA played an important role in the selection of nutrients that channeled into the nanotubular structure. Meanwhile, FESEM observations on TiP surface showed a thick-coating layer of growth nutrient aggregates (Figure 1a). EDX element analysis on TiP indicated the presence of higher carbon and oxygen elements compared to TNA surface. The source of carbon element could be possibly originated from growth nutrients media (the larger component such as albumin protein from fetal bovine serum). This finding confirmed that there were more growth nutrients settling on top of TiP surface compared to those on TNA surface. Therefore, there was a possibility that the growth nutrients might settle into and onto TNA nanotubular structure and thus contribute to the percentage of surface elemental distribution. More importantly, this surface condition mimics the characteristic of human bone surface structure that could help to minimize the interfacial stresses and delamination factors caused by limited access of body fluid (Jäger *et al.*, 2007; Oh *et al.*, 20011). Thus, the space present between TNA nanostructures exist as a nutrient storage area (in the cylindrical structure) and provide larger surface interaction for the cells on the material.

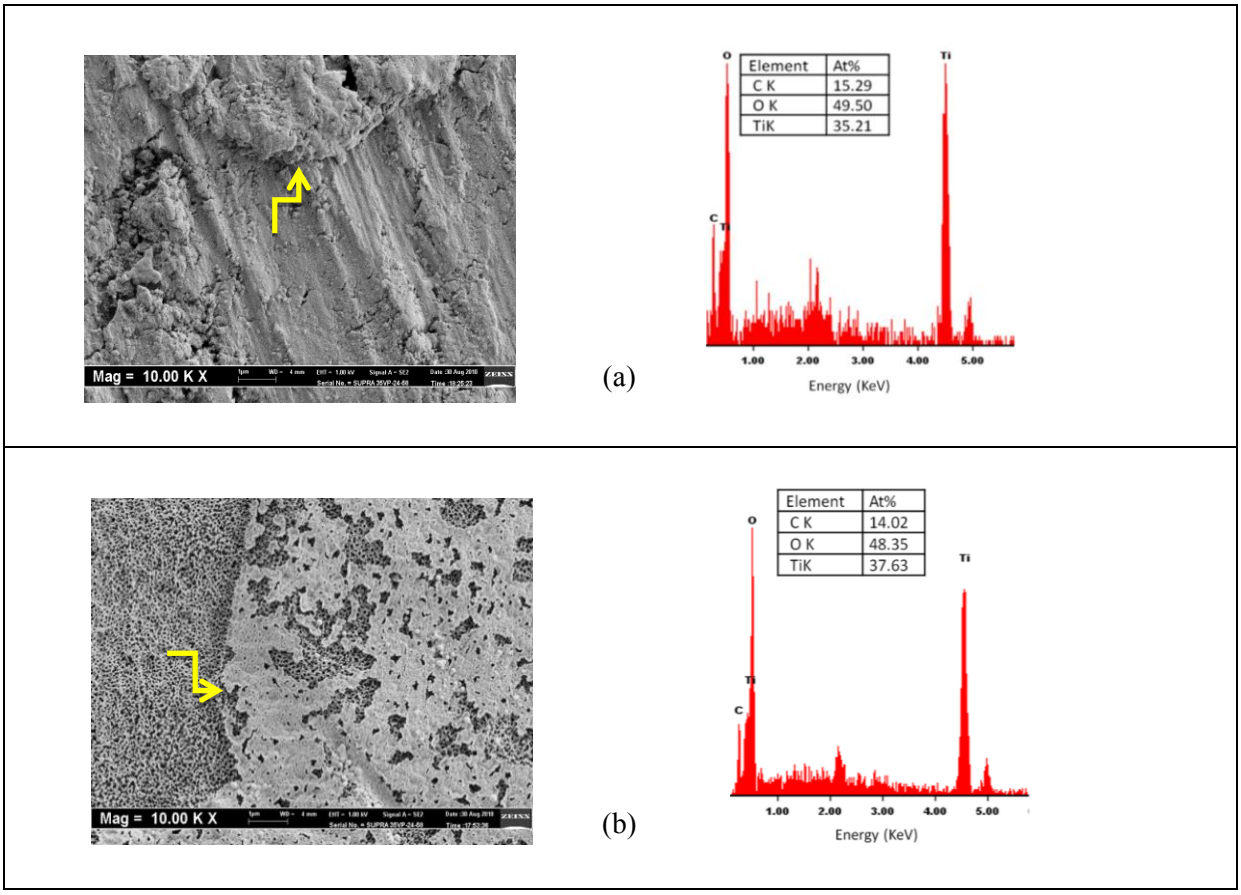

Figure 1. FESEM-EDX surface analysis of cell growth media on TiP and TNA. (a) Ti surface showed the growth media form a thick coating layer on the surface and (b) TNA showed the growth media forms a layer of dried crystallites flakes on the top the nanotube surface. From the EDX element analysis indicated the presence of main element, such as carbon (C), titanium (Ti) and oxide (O) on both material surfaces. TiP surface showed presence of higher carbon element in comparison to TNA surface, possibly contributes from the growth media component.

In this study, cell morphology assessment of epithelial HT-29 cells cultivated on TNA and glass surfaces was observed after 72-hour time point. Glass surface represented as control growth surface for the cells. Typical epithelial growth and spreading behavior were observed with cytoplasmic extension for the entire samples, as shown in Figure 2. Thus, the cells may exhibit cytocompatibility in the presence of colonized multi-layered cells which cover the TNA and glass surfaces. Cells morphology on TNA surface appeared to be flattened and spread well compared to its control counterparts. This indicates that the cell may be involved in proliferation and differentiation pattern (Hazan *et al.*, 2016; Bauer *et al.*, 2008). Thus, the nanocues advantages of TNA surface might recruit and provide sufficient essential biological component for cell growth; cell adhesion, migration, proliferation and differentiation activities. It is well known that cells respond the amount of proteins (growth factors) that are available on the surface interaction area. Detailed FESEM observation shows that, part of the cytoplasmic extension structure such as the cell's filipodium and lamellipodium (the end structure of the cytoplasmic extension) was not visible on TNA cells (Figure 2c). Thus, it was assumed that some part of the cell's cytoplasm might interact and remain in the inner part of the nanotube cylindrical structure. This phenomenon can be described based on the schematic diagram as illustrated in Figure 3. Interestingly, the distinctive topology of TNA nanosurface might also contribute to the interlocked configuration of cells, which results in rapid and strong adhesion of bone cells towards the implant surface.

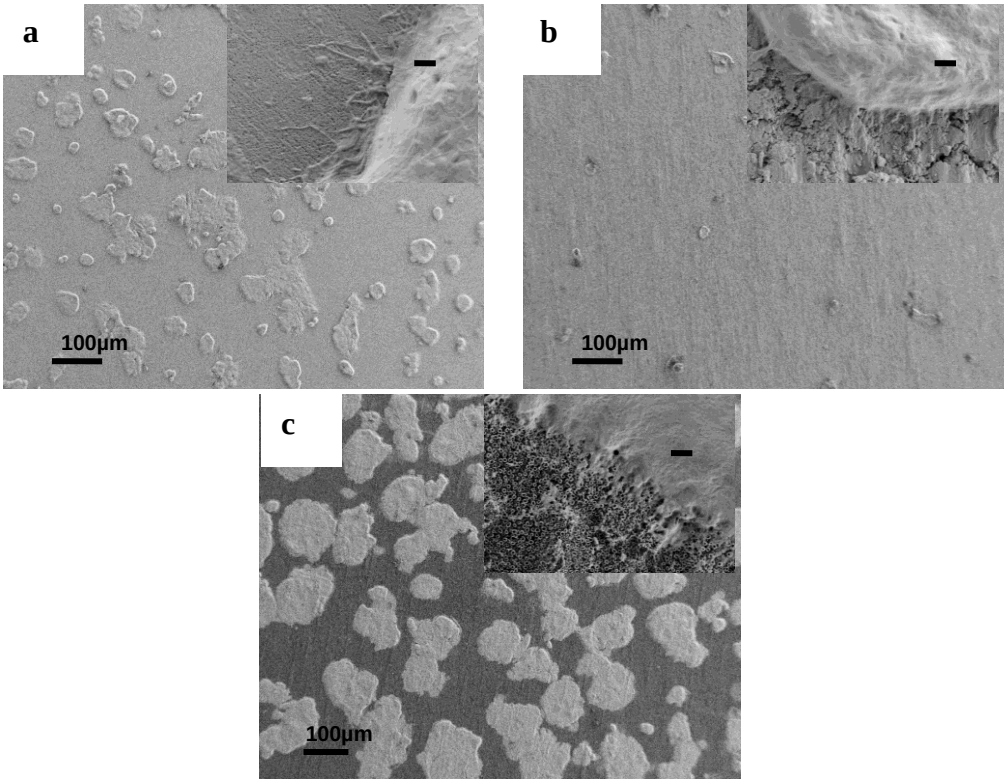

Figure 2. TNA induced morphological changes on the cells. The epithelial HT-29 cells were cultured on (a) Glass Slide, (b) TiP and (c) TNA. FESEM images show cells grown on the TNA indicating elevated cell numbers and a wider spread of cell cytoplasmic extension compared to reference. Three morphological features were observed: (a) flat spread out with filipodium and projecting cytoplasmic extensions; (b) flat hemispheroidal with a lamellipodium; (c) flat extensive cytoplasmic extensions but the filipodium and lamellipodium cannot be visualized (hyphothesis: some of the cytoplasmic may go inside the nanotube holes).

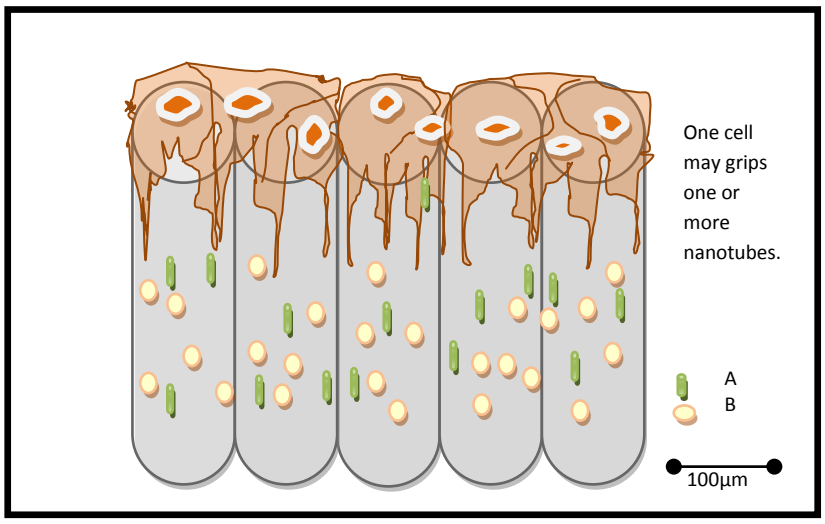

Figure 3. The schematic diagram shows the hypothesis of some part of the cell structure such as the filopodia (end structure of cytoplasmic extension) might interact with the inner structure of TNA. (A) Protein absorptions; (B) cells mediators signal and nutrients. The hole like structure is supposed to support early protein adsorption and plays an important role as a good modulator and communicator between the implant material and tissue.

CONCLUSION

This study have revealed the potential cell-TNA interactions at cellular and molecular levels. Noted that the TNA nanoarchitecture structures is beneficial for cells as a supply or storage route for nutrients and also for mediator growth signals. Based on these findings, the intricate molecular mechanism behind cell-TNA interactions are crucial for positive cell growth regulation and nanosurface mechanosensitivity activities which could contribute for better cellular responses

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