

誌謝

時光飛逝，很快的就進入了我碩士階段的尾聲，回想當年由大學畢業進入研究所就讀，使我的生活轉變很大，我很榮幸的跟隨一位研究奈米生物方面的老師 黃國華教授，讓我不僅學習到研究生物方面的知識也學習到做人處事的道理，雖然在這塊領域上，我只是一個剛涉略其中的人，對於生物方面的研究還不算專精，但這兩年中跟隨著老師的研究方向邁進，讓我對生物領域的研究越來越感興趣。現在的社會上，已不再需要只會單一方向專精的人，而是可以跨領域的吸收並且應用的人，專一的研究固然重要，但是跨領域的結合將可以帶給學生更多的創造力與思考方向，在本實驗室中就是有這樣的好處。轉眼間，兩年的時光過去了，在這兩年裡不只有老師陪伴著我們不分日夜的細心指導，還有師母 洪孟燕 女士、洪耀欽 醫師，都給予我相當的關懷及照顧，我永遠不會忘記師母每一頓豐盛的大餐，以及辛苦的帶領我們到山上抓蜘蛛，教導我學習應該有的態度，在此實驗室中我也學習到了管理帳務的相關知識，非常感謝從我進入本實驗室就一直帶領我的敘安學長，也感謝大勳學長不厭其煩的幫我借儀器，以及同屆的這些值得信賴的好夥伴：小馬、小孟、阿書，當然不可或缺的還有這些可愛的學弟妹們，讓我在這兩年裡面不僅有研究上的同儕，也添加了不少生活上的樂趣。在我的求學階段走了好長的一段路，不僅要感謝培育我的父母還要感謝一位最重要的人—林佩蓉，作為我的女朋友的她陪伴著我走過一關關的困難，雖然常常因為研究而忽略了她，但她都能體諒我讓我能夠順利完成學業。即將要離開實驗室，心中有萬般的不捨，祝福實驗室日益壯大，學弟妹們研究順利。

奈米表面應用於造骨細胞的生長調控和人工植入物的設計

研究生：戴世明

指導教授：黃國華 教授

共同指導：洪耀欽 教授

國立交通大學奈米科技研究所

中文摘要

仿造細胞外基質[extracellular matrix(ECM)]作成的微米基材促進細胞生長跟分化，但是對於奈米結構是否促進細胞生長分化卻少有人說明，為了指出奈米結構表面是否控制細胞的生長情形，我們將 MG63 成骨細胞培養在奈米點陣列上，而這些奈米點的分布範圍由 10-nm 到 200-nm。奈米表面對於細胞的生長、型態、貼附、細胞骨架以及礦化能力將被明確的探討，在所有的實驗數據中，50-nm 的表面最適合 MG63 細胞生長。我們將四種不同型態的牙釘：Uncoated、TPS、HA、Nano Tite，以電子顯微鏡 (electron microscope) 作細部結構的探討，並且應用於生物體外以及臨床實驗上的研究。此篇研究中指出約 50-nm 的奈米結構的確可以有效的在生物體外調控成骨細胞的生長，而在臨床研究上擁有與 50-nm 的奈米點結構類似的 HA 牙釘也可使成骨細胞生長得比同樣擁有奈米結構的 Uncoated 和 TPS 牙釘以及結構定義不明確的 Nano Tite 牙釘好。

接著我們製作出一個包含有九片奈米點表面結構片段的裝置，此表面結構有 Flat、10-nm、50-nm、100-nm 和 200-nm。接著將 HELA、C33A、ES2、PA-1、TOV-112D、TOV-21G、MG63 和 NIH-3T3 細胞培養於我們做出的裝置上三天，計算細胞的個數來當作其細胞成長的程度，而 SEM 可觀察出細胞型態變化的程度，再利用 vinculin 以及 F-actin 螢光染色來觀察細胞在不同表面上的貼附能力以及細胞骨架分布情形，並且將以上四種關於細胞的不同特性以數據的方式呈現。我們可以利用此裝置來分辨出 HELA 和 C33A 兩種癌細胞之間向外侵略能力的差異，不僅如此，我們的裝置對於分處於不同階段的 ES2、PA-1、TOV-112D、TOV-21G 四種癌細胞其生長也有不同的表現，我們還更進一步的探討特定奈米點表面對於 MG63 細胞的生長影響。

我們架設了一個簡單且可分析表面結構對於細胞特性影響的平台，這簡單的製造流程可以大量的生產並且降低生產成本，更甚的，這個裝置可以用來分別出癌細胞的級別與侵蝕能力，並且帶給現今人工植入物質一個參考的指標，此裝置提供組織工程以及癌症治療一個方便且快速的檢測平台。



Application of nanosurface to modulate growth of osteoblasts and designing of artificial implants

Student: Shih-Ming Tai

Advisor : Dr. Guewha Steven Huang

Co-advisor : Yao-Ching Hung

Institute of Nanotechnology National Chiao Tung University

Abstract

Microstructure that mimics extracellular substratum promotes cell growth and differentiation while cellular reaction to nanostructure is poorly defined. To evaluate modulating ability of nano-scaled surface, MG63 osteoblasts were grown onto nanodot arrays with dot diameters ranging from 10 to 200 nm. Cell proliferation, morphology, adhesion, cytoskeleton, and mineralization were evaluated. Nanodot with 50-nm in diameter behaved the best in all evaluation. Four different types of dental implants, Uncoated, TPS, HA, and Nano Tite, were characterized by electron microscope and subjected to in vitro and clinical test. Here we show that nanostructure is capable of modulating the in vitro growth of osteoblasts at approximately 50-nm in diameter. Best clinical outcome for dental implants with nanostructure of similar dimension (HA) behaves the best compared to nanoscaled structure (Uncoated and TPS) and undefined structure (Nano Tite).

We have fabricated a nanodevice composed of a matrix of 9 nanodot arrays with various

dot sizes ranging from flat surface, 10-nm, 50-nm, 100-nm, and 200-nm. HELA, C33A, ES2, PA-1, TOV-112D, TOV-21G, MG63, and NIH-3T3 cells were seeded on the device and cultured for 3 days. Cell density was counted to examine the proliferation of cells. Scanning electron microscopy (SEM) was performed to assess the morphological change of cells. To evaluate cell adhesion and cytoskeleton reorganization, immunostaining specific to vinculin and actin filaments was performed. The scores of cell proliferation, morphology, distribution of focal adhesions, and cytoskeleton organization were obtained. We were able to distinguish the invasive ability of HELA versus later-staged C33A. Ovarian cancer cell lines (ES2, PA-1, TOV-112D, and TOV-21G) also exhibited differential growth parameters which are associated with the cell type, grade, and stage. Modulation for the growth of MG63 was also achieved.



We have established a platform which can assess basic parameters for cell growth. The simplified fabrication process ensured mass production and cost down. Apparently, the device was capable of distinguishing cancer cell line of various stages and also provided basic designing parameters for artificial implants. Our device will serve as a convenient and fast tool for tissue engineering and cancer treatment.

Contents

| | |
|----------------------------|------|
| Acknowledgment..... | i |
| Chinese abstract..... | ii |
| Abstract..... | iv |
| Contents..... | vi |
| Chapter I..... | vi |
| Tables of Chapter I..... | vii |
| Figures of Chapter I..... | vii |
| Chapter II..... | viii |
| Tables of Chapter II..... | ix |
| Figures of Chapter II..... | ix |

Chapter I

| | |
|--|---|
| 1.1 Introduction..... | 1 |
| 1.2 Experimental Methods..... | 3 |
| 1.2.1 Chemicals..... | 3 |
| 1.2.2 Fabrication of nanodot arrays..... | 3 |
| 1.2.3 Cell culture..... | 4 |
| 1.2.4 Scanning electron microscopy..... | 4 |
| 1.2.5 Immunostaining of vinculin and actin filament..... | 4 |
| 1.2.6 Von Kossa staining..... | 4 |
| 1.2.7 Alizarin Red S staining..... | 5 |
| 1.3 Results and Discussions..... | 6 |
| 1.3.1 Fabrication of nanodot arrays for the growth of MG63..... | 6 |
| 1.3.2 Nanostructure modulated proliferation, cell adhesion, and cytoskeleton | |

| | |
|---|----|
| organization of MG63..... | 7 |
| 1.3.3 Nanostructure modulated mineralization of MG63..... | 10 |
| 1.3.4 Structural analysis for the clinical implants..... | 13 |
| 1.3.5 Evaluation of MG63 proliferation and growth on clinical implant.... | 15 |
| 1.3.6 Clinical outcomes..... | 17 |
| 1.4 Conclusions..... | 19 |
| Reference..... | 20 |

Tables of Chapter I

| | |
|---|----|
| Table 1.1 | 14 |
| Structural characterization for nanostructures on the dental implants | |
| Table 1.2 | 17 |
| Clinical outcome for dental implants | |
| Table 1.3 | 18 |
| Clinical outcome for multiple types of implant in the same patient | |

Figures of Chapter I

| | |
|--|----|
| Figure 1.1 | 7 |
| Fabrication of tantalum-based nanodot arrays using AAO processing | |
| Figure 1.2 | 9 |
| Modulation for the growth, proliferation, and cytoskeleton of MG63 | |
| Figure 1.3 | 10 |
| Mineralization of cultured MG63 by the von Kossa staining process | |
| Figure 1.4 | 11 |
| Correlation between mineralization versus size of nanodot arrays | |

| | |
|---|----|
| Figure 1.5 | 12 |
| Mineralization of cultured MG63 by Alizarin Red S staining | |
| Figure 1.6 | 12 |
| Correlation between mineralization versus size of nanodot arrays | |
| Figure 1.7 | 13 |
| SEM image showing the detail micro- and nano-structure of dental implants | |
| Figure 1.8 | 15 |
| DAPI and actin filament staining of MG63 onto implants | |
| Figure 1.9 | 16 |
| SEM image for MG63 grown onto implants | |

Chapter II

| | |
|---|----|
| 2.1 Introduction..... | 22 |
| 2.2 Experimental Methods..... | 24 |
| 2.2.1 Chemicals..... | 24 |
| 2.2.2 Fabrication of the nanodevice, a matrix of nanodot arrays..... | 24 |
| 2.2.3 Cell culture..... | 25 |
| 2.2.4 Scanning electron microscopy..... | 25 |
| 2.2.5 Immunostaining of vinculin and actin filament..... | 26 |
| 2.2.6 Definition for the scores of proliferation, apoptosis, focal adhesion, and cytoskeleton organization..... | 26 |
| 2.3 Results and Discussions..... | 27 |
| 2.3.1 The fabrication of an integrated nanodot array device..... | 27 |
| 2.3.2 Assessment of proliferation, apoptosis, cell adhesion, and cytoskeleton reorganization for cultured cells..... | 29 |

| | |
|----------------------|----|
| 2.4 Conclusions..... | 39 |
| Reference..... | 40 |

Tables of Chapter II

| | |
|--|----|
| Table 2.1 | 30 |
| Cell lines were tested by the nanotopography | |
| Table 2.2 | 38 |
| Scores of cells were grown on the nanodevice | |

Figures of Chapter II

| | |
|---|----|
| Figure 2.1 | 28 |
| Fabrication of integrated nanodevice for the screening of cellular response | |
| Figure 2.2 | 31 |
| SEM images of cells seeded on nanodot arrays | |
| Figure 2.3 | 32 |
| Immunostaining to show distribution of vinculin in cell cultured | |
| Figure 2.4 | 33 |
| Immunostaining to show distribution of actin filament in cell cultured | |