

学校编码: 10384
学号: B200425021

分类号 _____ 密级 _____
UDC _____

厦 门 大 学

博 士 学 位 论 文

骨细胞与生物材料相互作用的生物芯片和
原位物理化学研究

**Investigation on bone cell-biomaterial interaction by
biochip techniques and in-situ physicochemical methods**

张 帆

指导教师姓名: 林昌健教授, 陈勇教授

专 业 名 称: 物理化学

论文提交日期: 2011 年 12 月

论文答辩时间: 2012 年 1 月

学位授予日期: 2012 年 月

答辩委员会主席: _____

评阅人: _____

2011 年 12 月

Investigation on bone cell-biomaterial interaction by biochip techniques
and in-situ physicochemical methods



A Dissertation Submitted to the Graduate School in Partial
Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

By

Fan Zhang

Directed by Prof. Changjian Lin and Prof. Yong Chen

Department of Chemistry, Xiamen University

December, 2011

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下,独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果,均在文中以适当方式明确标明,并符合法律规范和《厦门大学研究生学术活动规范(试行)》。

另外,该学位论文为()课题(组)的研究成果,获得()课题(组)经费或实验室的资助,在()实验室完成。(请在以上括号内填写课题或课题组负责人或实验室名称,未有此项声明内容的,可以不作特别声明。)

声明人(签名):

年 月 日

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

1. 经厦门大学保密委员会审查核定的保密学位论文，
于 年 月 日解密，解密后适用上述授权。

2. 不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年 月 日

目 录

中文摘要	I
英文摘要	III
第一章 绪 论	1
1.1 生物材料	1
1.1.1 常用的生物材料	3
1.1.2 生物材料涂层的构筑	5
1.1.2.1 羟基磷灰石涂层	5
1.1.2.2 二氧化钛涂层	8
1.2 细胞与生物材料的相互作用及其研究方法	11
1.2.1 细胞与生物材料的相互作用	11
1.2.2 细胞与生物材料相互作用的研究方法	13
1.3 生物微机电系统	15
1.3.1 生物芯片	15
1.3.2 生物传感器	16
1.3.3 微细加工技术	17
1.4 细胞和生物材料相互作用研究的原位物理化学技术	20
1.4.1 原子力显微镜	20
1.4.2 电化学阻抗谱	23
1.4.3 衰减全反射傅立叶红外光谱	28
1.5 本论文的研究目的和设想	31
参考文献	34
第二章 实验方法与仪器	56
2.1 电化学沉积制备方法	56
2.2 细胞和生物材料相互作用的原位物理化学研究方法	57
2.2.1 原子力显微镜	57

2.2.2 电化学阻抗谱	57
2.2.3 衰减全反射傅立叶红外光谱	57
2.3 细胞培养室与光刻室的搭建	58
2.3.1 细胞培养室	58
2.3.2 光刻室	58
参考文献	61
第三章 原代细胞培养方法及生物材料表面的体外细胞评价	62
3.1 引言	62
3.2 实验材料及试剂	65
3.2.1 实验试剂和材料	65
3.2.2 试剂配制	66
3.3 原代细胞的培养	66
3.3.1 原代成骨细胞的培养	66
3.3.2 MSCs 的培养	69
3.4 生物材料表面的体外细胞评价	70
3.4.1 MWCNTs/HA 复合膜层表面的体外细胞评价	71
3.4.2 OCP/胶原蛋白复合膜层表面的体外细胞评价	76
3.5 本章小结	79
参考文献	80
第四章 微流控系统 中的 MSCs 成骨分化研究	86
4.1 引言	86
4.2 芯片设计和细胞分化	87
4.2.1 芯片设计与制作	87
4.2.2 微流控芯片中细胞培养和分化	89
4.3 两种不同设计的微流控芯片的对比研究	90
4.4 微流控芯片中两种不同营养供应方式下的 hMSCs 成骨分化研究	94
4.4.1 微流控芯片中两种不同的营养供给方法	94
4.4.2 微流控芯片中 hMSCs 的增殖研究	95
4.4.3 微流控芯片中 hMSCs 的成骨分化研究	102

4.5 本章小结	106
参考文献	108
第五章 生物材料微图案和微电极阵列及其与细胞的相互作用	113
5.1 引言	113
5.2 生物材料微图案的构筑及其表面成骨细胞的 AFM 表征	115
5.2.1 硅片表面微图案的构筑及其表面成骨细胞的 AFM 表征	115
5.2.2 玻璃表面钛微图案的构筑及成骨细胞的 AFM 表征	128
5.3 生物材料微图案薄膜的构筑及其与 MSCs 的相互作用	131
5.3.1 银和羟基磷灰石微图案的构筑及其与 MSCs 的相互作用	131
5.3.2 高分子聚合物微图案的构筑及其与 MSCs 的相互作用	136
5.4 微电极阵列的设计、制作及其与细胞相互作用的阻抗研究	139
5.5 本章小结	157
参考文献	159
第六章 活细胞的实时衰减全反射傅立叶红外光谱检测研究	169
6.1 引言	169
6.2 DMEM 培养基及其成骨细胞悬液薄膜层衰减全反射傅立叶红外光谱研究	171
6.3 室温下 MG63 活细胞的实时衰减全反射傅立叶红外光谱研究	173
6.4 MG63 活细胞与材料相互作用过程的实时衰减全反射傅立叶红外光谱研究	180
6.5 本章小结	191
参考文献	192
第七章 结论与展望	197
7.1 主要结论	197
7.2 研究工作展望	199
作者攻读博士学位期间发表与交流论文	201
致 谢	203

厦门大学博硕士学位论文摘要库

Contents

Abstract in Chinese	I
Abstract in English	III
Chapter 1 Introduction	1
1.1 Biomaterial	1
1.1.1 Biomaterials in Common Use.....	3
1.1.2 Construction of Biomaterial Coating.....	5
1.1.2.1 Hydroxyapatite Coating.....	5
1.1.2.2 TiO ₂ Coating.....	8
1.2 Interaction between Cell and Biomaterial and Its Research Methods	11
1.2.1 Interaction between Cell and Biomaterial.....	11
1.2.2 Research Methods of Interaction between Cell and Biomaterial.....	13
1.3 Bio Micro Electro Mechanical System	15
1.3.1 Biochip.....	15
1.3.2 Biosensor.....	16
1.3.3 Micro-Fabrication Technology.....	17
1.4 In situ Physical and Chemical Characterization Technique for the Study on Interaction between Cell and Biomaterial	20
1.4.1 Atomic Force Microscopy.....	20
1.4.2 Electrochemical Impedance Spectroscopy.....	23
1.4.3 Attenuated Total Reflection Fourier Transform Infrared.....	28
1.5 Objectives and Contents of the Dissertation	31
References	34
Chapter 2 Experimental and Instruments	56
2.1 Preparation Method of Electrochemical Deposition	56
2.2 In situ Physical and Chemical Characterization Technique for the Study	

on Interaction between Cell and Biomaterial	57
2.2.1 Atomic Force Microscopy.....	57
2.2.2 Electrochemical Impedance Spectroscopy.....	57
2.2.3 Attenuated Total Reflection Fourier Transform Infrared.....	57
2.3 Construction of Cell Culture Room and Photolithography Room	58
2.3.1 Cell Culture Room.....	58
2.3.2 Photolithography Room.....	58
References	61
Chapter 3 Primary Cell Culture and Cell Culture Experiments in vitro on Biomaterials	62
3.1 Introduction	62
3.2 Experimental Materials and Reagents Preparation	65
3.2.1 Experimental Reagents and Materials.....	65
3.2.2 Reagents Preparation.....	66
3.3 Primary Cell Culture	66
3.3.1 Primary Osteoblast Culture.....	66
3.3.2 Culture of MSCs.....	69
3.4 Cell Culture Experiments in vitro on Biomaterials	70
3.4.1 Cell Culture Experiments in vitro on MWCNTs/HA Composite Film.....	71
3.4.2 Cell Culture Experiments in vitro on OCP/Collagen Composite Film.....	76
3.5 Summary	79
References	80
Chapter 4 Differentiation of MSCs on Microfluidic Chip	86
4.1 Introduction	86
4.2 Chip Design and Cell Differentiation	87
4.2.1 Design and Preparation of Chip.....	87
4.2.2 Cell Culture and Differentiation on Microfluidic Chip.....	89
4.3 Comparison of Two Different Microfluidic Chips	90
4.4 Osteogenic Differentiation of hMSCs on Microfluidic Chip by Using Two	

Types of Methods of Nutrient Feeding	94
4.4.1 Two Types of Methods of Nutrient Feeding on Microfluidic Chip.....	94
4.4.2 Proliferation of hMSCs on Microfluidic Chip.....	95
4.4.3 Differentiation of hMSCs on Microfluidic Chip.....	102
4.5 Summary	106
References	108
Chapter 5 Fabrication of Micro Patterned Biomaterials and Micro Electrode Array and the Interaction between Them and Cells	113
5.1 Introduction	113
5.2 Fabrication of Micro Patterned Biomaterials and AFM Characterization of Osteoblast on Its Surface	115
5.2.1 Fabrication of Micro Pattern on Si Surface and AFM Characterization of Osteoblast on Its Surface.....	115
5.2.2 Fabrication of Micro Patterned Ti on Glass Surface and AFM Characterization of Osteoblast on Its Surface.....	128
5.3 Fabrication of Micro Patterned Biomaterial Films and the Interaction between them and MSCs	131
5.3.1 Fabrication of Micro Patterned Ag and Hydroxyapatite and the Interaction between Them and MSCs.....	131
5.3.2 Fabrication of Micro Patterned Macromolecule Polymers and the Interaction between Them and MSCs.....	136
5.4 Design and Fabrication of Micro Electrode Array and Impedance Research on Its Interaction with Cells	139
5.5 Summary	157
References	159
Chapter 6 Real-time ATR-FTIR Monitoring of Living Cells at Room Temperature	169
6.1 Introduction	169

6.2 ATR-FTIR Research on DMEM Medium and Osteoblasts Suspension Thin Films	171
6.3 Real-time ATR-FTIR Monitoring of Living MG63 Cells at Room Temperature	173
6.4 Real-time ATR-FTIR Research on the Interaction between Living MG63 Cells and Biomaterials	180
6.5 Summary	191
References	192
Chapter 7 Conclusions and Future Work	197
7.1 Conclusion	197
7.2 Future Work	199
Selected Publications and Conference Presentations	201
Acknowledgement	203

厦门大学博硕士学位论文摘要

中文摘要

现代微系统技术及各种原位物理化学方法,为生物表面/界面的研究提供了前所未有的机遇和全新的研究层次。本工作致力于应用发展的微流控和微电极阵列(MEA)生物芯片、原子力显微镜(AFM)、电化学阻抗谱(EIS)和衰减全反射傅立叶红外光谱(ATR-FTIR)等先进技术,从不同的侧面和层次研究骨细胞与生物材料相互作用过程及机制。

主要研究内容有:(a)建立昆明小鼠的原代颅骨成骨细胞和长骨骨髓基质干细胞(rMSCs)的培养,并应用于新型复合生物材料的研究;(b)考察微流控生物芯片中流体状态(静止和流动)对人类骨髓基质干细胞(hMSCs)成骨分化的影响;(c)应用微加工技术制备微图案化的表面薄膜,应用AFM原位表征微图案化表面的活成骨细胞三维形态,控制不同表面细胞的生长、黏附和形态;(d)设计并制作钛微电极阵列芯片及其细胞培养的原位EIS监控电解池,并进一步高通量地研究复合生物材料表面与细胞的相互作用;(e)提出一种MG63活细胞的实时ATR-FTIR监控的新方法,并用来研究细胞与TiO₂及羟基磷灰石(HA)材料表面的相互作用。主要研究结果如下:

1. 细胞培养实验结果显示:羟基磷灰石/碳纳米管(HA/MWCNTs)复合材料具有良好的生物相容性;电化学制备的磷酸八钙/蛋白质(OCP/protein)复合膜层可显著增强成骨细胞的黏附与生长。
2. 在两种微流体状态中,细胞可存活数周,但显示不同的增殖和分化行为。在液压控制的流动培养室中,细胞快速生长,但液压产生的液体流动不利于钙节结的形成。在初始细胞密度($2 \times 10^4 \sim 4 \times 10^4$ cells/cm²)下,液槽控制的静态培养室中细胞形态和茜素红染色图显示了最佳的成骨分化效果。
3. 厚度为100~200 nm的微图案表面可对活细胞局部三维形态进行选择性地控制:当细胞厚度和微图案高度比值小于3.2时,细胞表面能根据基底显示相应的微图案形态;当二者比值在3.9~7.8之间时,细胞表面不会显示基底的微图案形貌;有效控制金属薄膜厚度为20~30 nm,可产生具有良好透光性和导电性的医用金属表面,便于进一步开展实时的电化学和光学显微联合监控工作;单独的Ag薄膜对细胞具有毒性并且无法在其表面进行HA

的电化学沉积；应用电化学沉积和光刻技术，可制作微图案化的 Cr-Ag-HA 薄层，rMSCs 可在 Cr-Ag 和 Cr-Ag-HA 薄膜表面生长；应用微接触印刷术 (μCP) 在钛膜层表面制备了高分子图案钛-聚赖氨酸-聚乙二醇 (Ti-PLL-PEG)，可控制 rMSCs 的黏附和生长。

4. 采用多通道电流控制的选择性电化学沉积方法，可将 Ag、HA 和 Ag-HA 涂层集成于同一钛 MEA 芯片上；对比钛微电极阵列表面的不同涂层与活细胞相互作用的 EIS，并应用等效电路 $R_{\text{med}}(R_{\text{ox}}Q_{\text{ox}})(R_{\text{pro}}Q_{\text{pro}})(R_{\text{cell}}Q_{\text{cell}})$ 进行拟合；涂层和细胞增殖对 Ti 和溶液的界面的电化学行为具有一定的影响； R_{cell} 元件数值与涂层的生物相容性和细胞增殖具有一定的关联；根据 R_{cell} 元件数值判断生物相容性的顺序如下：Ti-Ag-HA>Ti-HA>Ti-Ag>Ti。
5. 由于水和培养基的强红外吸收谱峰会干扰活细胞全谱的获得，本论文通过原位光谱背景扣除法，获得活细胞的红外吸收光谱变化信息，在 1370~2000 和 3500~3940 cm^{-1} 出现一系列重现性良好的向下红外谱峰；相比于 Ge 和 Ge-TiO₂，Ge-HA 与细胞有强的相互作用，产生一个归属于 P-O 不对称伸缩振动的位于 1016~1079 cm^{-1} 的红外吸收峰，在分子水平说明 HA 具有更好的生物活性。

关键词：成骨细胞；干细胞；生物材料；生物芯片；原位技术

Abstract

Microsystems and in-situ physicochemical methods are providing new opportunity and different sides in understanding of biological interface. The emphases of this thesis are to study bone cell-biomaterial interaction, by developed microfluidic chip, microelectrode array (MEA) chip, atomic force microscopy (AFM), electrochemical impedance spectroscopy (EIS) and attenuated total reflectance fourier transform infrared spectroscopy (ATR-FTIR).

The main work covers: (a) culture of primary skull osteoblast and long bones Mesenchymal Stem Cells (rMSCs) from Kunming mouse and their application in biomaterial research; (b) investigation on effects of fluid states (static and flowing states) on osteogenic differentiation of human Mesenchymal Stem Cells (hMSCs) on microfluidic chip; (c) fabrication of micro-patterned thin films with physicochemical methods and micromachining technologies, to control cells adhesion and morphology characterized by in-situ AFM; (d) design and preparation of home-made electrolytic cells integrated with Ti MEA chip for high-throughput investigation on real-time EIS monitoring of interaction between composite biomaterials and cells, and (e) a novel approach of real-time ATR-FTIR monitoring developed to study the interaction between cells and biomaterials including TiO₂ and hydroxyapatite (HA). The main results and progresses are outlined as follows:

1. The cell culture experiment shows that the HA/MWCNTs composite has good biocompatibility and the electrochemically prepared OCP/protein coating exhibits an excellent cell attachment and growth.
2. In both fluid states, cells survived for several weeks but showed different proliferation and differentiation. In the flowing state, cells grew fast but the formation of calcium nodes was less efficient. Under quasi static conditions with an initial cell density between 2×10^4 and 4×10^4 cells/cm², both morphology micrographs and Alizarin Red staining images showed the best osteogenic differentiation.
3. Micro patterns with thickness of 100~200 nm could selectively control the local

3-D cells morphology: when the height proportion between cells and micro pattern was less than 3.2, the cells showed micropatterned morphology according to substrates, and the proportion from 3.9 to 7.8 can not be used to control cells morphology. The thin metallic films in the thickness of 20~30 nm were deposited in order to be used as a substrate with both good transparency and electrical conductivity for synchronous microscopical and electrochemical monitoring. A thin film of Ag alone was harmful for rMSCs and could not be used as a substrate for electrochemical deposition of HA. Micropatterned Cr-Ag-HA was fabricated by electrochemical deposition and photolithography. The rMSCs can be cultured on both Cr-Ag and Cr-Ag-HA thin films. Micro-contact printing (μ CP) was used to fabricate Ti-PLL-PEG patterns which could control adhesion of rMSCs.

4. Selective electrochemical deposition based on a novel procedure of multichannel current control was used to fabricated Ti MEA chip integrated with Ag, HA and Ag-HA. The EIS of MG63 cells on different surfaces on the MEA chip was monitored. The equivalent circuit $R_{\text{med}}(R_{\text{ox}}Q_{\text{ox}})(R_{\text{pro}}Q_{\text{pro}})(R_{\text{cell}}Q_{\text{cell}})$ was used to obtain satisfactory fitting results. The existence of coatings and proliferation of cells influenced the interface phenomenon between Ti and solution. The element R_{cell} can be related to the biocompatibility of coatings and proliferation of cells. Better biocompatibility was evaluated according to the R_{cell} values in the following order: Ti-Ag-HA>Ti-HA>Ti-Ag>Ti.
5. The strong IR absorption of water and medium greatly interfered with the complete spectrum obtained from live cells. The IR absorption variation of adherent cells at room temperature was monitored. Many reproducible downward peaks at 1370~2000 and 3500~3940 cm^{-1} were observed. Compared to Ge and Ge-TiO₂, a new IR absorption band at 1016~1079 cm^{-1} in the spectra of cells on Ge-HA indicated the stronger interaction between Ge-HA and cells.

Keywords: Osteoblast; Stem Cell; Biomaterial; Biochip; In-situ Technology.

Degree papers are in the "[Xiamen University Electronic Theses and Dissertations Database](#)". Full texts are available in the following ways:

1. If your library is a CALIS member libraries, please log on <http://etd.calis.edu.cn/> and submit requests online, or consult the interlibrary loan department in your library.
2. For users of non-CALIS member libraries, please mail to etd@xmu.edu.cn for delivery details.

厦门大学博硕士论文摘要库