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博　士　学　位　论　文

# 牡蛎壳双相磷酸钙/壳聚糖复合多孔骨修复支架材料的制备与表征

# The Preparation and Characterization of Oyster Shell Derived Biphasic Calcium Phosphate/Chitosan Porous Composite Scaffolds

杨　云

指导教师姓名：　张 其 清　教授

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# 中文摘要

骨组织工程支架材料一直是生物材料领域的研究热点之一，为骨缺损治疗提供了新方法。理想的骨组织工程支架材料不仅应具备良好的生物相容性和生物可降解性，还应具有三维多孔的贯通结构及易于成型等特点，并可为细胞和组织的生长提供结构支撑，起到引导组织再生作用。双相磷酸钙（BCP）是一类由羟基磷灰石（HA）和β-磷酸三钙（β-TCP）按不同比例混合而成的生物陶瓷材料，具有良好的生物相容性和骨传导性，可与自然骨发生生物化学反应从而形成牢固的骨性结合。具有适当孔径和孔隙率的多孔 BCP 支架可增大材料与组织液接触的表面积，为骨组织的生长提供有利通道和空间，促进新生骨组织长入材料内部。随着海洋资源的开发与应用，采用生物质碳酸钙制备磷酸钙陶瓷已引起人们的广泛重视。近年来的研究表明，牡蛎壳具有良好的生物相容性，已成为骨修复材料研究的热点。但由于牡蛎壳形状不规则，缺少适宜骨组织生长的天然多孔结构，极大限制了其在骨组织工程支架材料中的应用。

本论文首次尝试将废弃牡蛎壳原料应用到多孔磷酸钙陶瓷支架材料的制备中。首先通过水热法将牡蛎壳粉末转化为HA，然后采用有机模板复制法制备BCP多孔陶瓷支架，将其浸渍到壳聚糖（CS）溶液中，最后采用冷冻干燥技术制备BCP/CS复合多孔骨组织工程支架材料。通过对BCP/CS多孔支架进行理化及生物学性能表征，考察牡蛎壳原料在骨组织工程支架材料领域中的应用前景。主要研究进展及成果如下：

1. 僧帽牡蛎壳为方解石型碳酸钙，在水热过程中，牡蛎壳粉末通过表面的溶解重结晶（Dissolution-recrystallization）和内部的固态局域规整离子交换反应（Solid-state topotactic ion exchange reacion）转化为牡蛎壳羟基磷灰石（OHA）粉末。SEM、XRD和FTIR分析结果显示产物为片状的碳酸取代的缺钙型HA纳米颗粒。水热温度的升高、反应时间的延长及初始磷酸盐浓度的增加均可促进方解石向HA的转化。
2. 采用OHA粉末为原料通过有机模板复制法制备牡蛎壳双相磷酸钙陶瓷（O-HA）多孔支架。理化表征结果显示，该支架具有三维贯通的大孔结构，

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孔径为200~500 μm，孔壁表面具有均匀分布的微孔结构，孔隙率高达91.4 ± 1.2%，具有良好的渗透能力，在SBF浸泡实验中显示出良好的诱导类骨磷灰石生成能力，与商业购买HA粉末采用同样方法制备的多孔支架相比，O-HA多孔支架在细胞培养实验中显示出更加明显的促进MC3T3-E1细胞粘附与增殖作用，具有良好的细胞相容性。

3. 将O-HA多孔支架浸渍入CS溶液中，通过冷冻干燥法制成BCP/CS复合多孔支架材料。结果显示，BCP/CS复合支架具有三维贯通的多孔结构，孔径分布均匀，渗透力良好，可对血液迅速进行渗透，孔隙率为76.3~83.4%，抗压强度为0.46~0.77 MPa。
4. SBF浸泡实验表明，BCP/CS复合多孔支架可诱导类骨磷灰石形成，具有良好的生物活性。细胞培养表明，BCP/CS多孔支架具有良好的细胞相容性，有利于前成骨细胞的粘附与铺展，具有促进前成骨细胞的成骨分化作用，有利于新骨组织生成。动物植入实验表明，BCP/CS多孔支架植入兔体内表现为正常的炎症反应过程，无毒性，具有一定的生物可降解性和良好的组织相容性。

**关键词：**牡蛎壳；羟基磷灰石；β-磷酸三钙；壳聚糖；多孔支架。

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# Abstact

Bone tissue engineering scaffold is one of the hotspots in biomaterial research fields, which provides a new way to treat bony defects. An ideal bone tissue engineering scaffold should possess good biocompatibility, controllable biodegrade rate and good machinability for rapid shaping. A three-dimensionally (3D) interconnected macroporous structure is crucial to bone tissue engineering materials. The scaffolds could provide good conditions for cell growth, differentitaion and proliferation and guide tissue regeneration. Hydroxyapatite/β-tricalcium phosphate (HA/β –TCP) biphasic calcium phosphate (BCP) are most widely used as bone tissue engineering materials due to their excellent biocompatibility and osteoconductive properties. When implanted in vivo, the BCP ceramics can form chemical bond with bone tissue. BCP porous scaffolds with suitalbe pore sizes and porosity could provide a favorable environment for bone ingrowth, increase the contact area between material and body fluids. With the development of marine resource, the preparation of calcium phosphate ceramics from biogenetic calcium carbonate ($CaCO_3$) has become a new researching topic on bone repair materials. In recent years, oyster shells have been proven to be biocompatible and bioactive bone grafting materials. However, the application of oyster shells in bone tissue engineering scaffold materials is limited by their irregular shapes and a less porous structure.

In the present work, we have newly developed a macroporous calcium phosphate bone tissue scaffold from oyster shell waste. HA nano-particles were synthesized via hydrothermal conversion of oyster shell powders. BCP porous scaffolds were fabricated using polymer replication technique from as-prepared HA nano-particles. Finally, BCP/CS porous scaffolds were obtained by freezing and lyophilized the porous ceramics scaffolds impregnated with CS solution. We investigated the physicochemical and biological properties of the BCP/CS porous scaffolds, and assessed its potential application in bone tissue engineering scaffold materials. The

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main progresses and achievements of this dissertation are outlined as following:

1. The *Crassostrea angulata* shell powder was composed of a calcite polymorph. HA nano-powders were prepared by the hydrothermal conversion of oyster shell powders. The exterior calcite converts to HA through dissolution-recrystallization, the interior calcite converts to HA via a solid-state topotactic ion-exchange reaction. The physicochemical properties of products were studied using SEM, XRD and FTIR analysis. The results showed that the obtained powders were plate-like, carbonated and calcium defected HA nano-powders. Higher reaction temperature, longer reaction time and higher concentration could promote the hydrothermal conversion of calcite into HA.
2. BCP scaffolds were fabricated using polymer replication technique from as-prepared HA nano-particles. The obtained scaffolds had a porosity of 91.4 ± 1.2% and showed an excellent permeability due to the open macropores (200~500 μm) and interconnected micropores in macropore walls. The BCP scaffolds showed a good ability to form apatite during the simulated body fluid (SBF) immersion process. The synthetic scaffolds were found to be non-cytotoxic and displayed better biocompatibility than pure HA scaffolds when seeded with pre-osteoblasts cells.
3. BCP/CS porous scaffolds were fabricated through freezing and lyophilized BCP porous scaffolds impregnated with CS solution. The results indicated that the BCP/CS porous scaffolds had a 3D interconnected macroporous structure and an excellent permeability. The porosity and compressive strength of composite scaffolds was 76.3~83.4% and 0.46~0.77 MPa, respectively.
4. The BCP/CS porous scaffolds showed a good ability to form apatite in the simulated body fluid (SBF) immersion test. From the *in vitro* cell culture, BCP/CS porous scaffolds showed good cytocompatibility and could promote the proliferation and differentionation of the pre-osteoblast cells. In the animal implantation experiment, BCP/CS scaffolds showed some signs of normal inflammatory reaction. The composite scaffolds were nontoxic and showed a certain degree of biodegradation and good tissue compatibility.

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**Keywords**：Oyster shell；Hydroxyapatite；β-tricalcium phosphate；Chitosan；Porous scaffold

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