聚乳酸/硫酸鈣複合材料於骨組織工程應用之評估－力學性質及細胞表現

Polylactic acid / calcium sulfate composite in bone tissue engineering – a biomechanics and cytocompatibility analysis

中文摘要

骨空腔缺損治療初期，缺損部位無法提供足夠支撐，需藉固定系統或骨移植維持力學穩定，傳統金屬材質剛性大，容易造成應力遮蔽，使鄰近部位骨質疏鬆，惰性高分子的發展可改善此缺點，但降解速率慢，阻礙新骨生長空間，影響骨融合的穩定。本研究以熔融混合方式將不同重量比例聚乳酸及硫酸鈣混合，製作可降解生物吸收性支架，複合材表面性質結果顯示隨著硫酸鈣比例的增加，複合材疏水性有顯著的降低，力學試驗結果中，聚乳酸與硫酸鈣以 7：3 比例混合的複合材兼具較高的降伏強度與楊氏係數，我們選擇此比例作爲複合材混合比例，將複合材以磷酸鹽緩衝溶液進行體外降解，結果複合材於三個月後降伏應力顯著下降，顯示複合材降解速率較聚乳酸材料快。離子釋放方面，去離子水與培養液浸泡結果都指出複合材的離子釋放速率則較硫酸鈣材料顯著下降($p<0.05$)。爲提供適合骨細胞生長貼附的孔洞表面，本研究以顆粒濾瀝方式將材料表面孔洞化，孔洞數目隨致孔劑比例增加而增多，高、低孔隙試片表面孔洞數爲 1：0.75，以偏光及電子顯微鏡分析孔洞複合材試片表面孔隙大小爲 250-400 μm，觀察降解對孔洞材料的影響，我們選擇高孔隙度複合材作爲實驗的孔洞試片進行降解實驗，經過三個月後孔洞性複合材的降伏強度較降解前顯著降低($p<0.05$)。使用初代培養的頭蓋骨細胞作爲骨母細胞的實驗模型，觀察骨母細胞在不同材料試片上的表現情形，在細胞貼附及增生的觀察中，細胞於複合材 4 小時的貼附率顯著較聚乳酸材料高($p<0.05$)，孔洞性複合材的貼附率又顯著較實體複合材試片高($p<0.05$)，細胞增生速率於各組試片間並無顯著差異。將細胞進行誘導培養三週，分析細胞生長情形，複合材試片上培養的細胞相較於聚乳酸組別有較高的鹼性磷酸酶活性($p<0.05$)、較高的 OPN 及 BSP 表現量($p<0.05$)，孔洞試片組別的細胞於第三週細胞內鹼性磷酸酶活性顯著較實體複合材組別高($p<0.05$)，而其 OPN 及 BSP 表現量也顯著較實體複合材組別高($p<0.05$)，茜素紅 S 染色結果，孔洞性試片組別鈣沉積量較實體試片高。由材料特性與材料降解力學評估及細胞實驗結果，我們認爲複合材有良好的初始穩定與較佳的親水性及良好的細胞親合性，符合支持新骨生成治療骨缺損要件滿足腰椎部分負載需求，並可支持缺損部位的力學穩定達三個月。材料的孔洞化造成初始力學強度的減弱與材料降解速度的加快，也同時促進骨母細胞貼附生長及表現，適合於頸椎等缺損及負載較小的部位應用。

英文摘要

In the early stage of the load-bearing bone defect treatment, the bone can not bearing the physical load as before. The surgeon needs to implant an additional fixation system or autograft to restore the stability in the defect area. The traditional implant may induce osteoporosis in the adjacent bone because the stainless steel with high stiffness easily lead to stress shielding. Although the inert polymer with lower stiffness can prevent the shortcoming, but the slowly degradation rate make the implant share space hinder the growth of new bone and affecting the stability of bone fusion. In this study, we used the melting method to prepare the biodegradable scaffold with mixing different weight ratio of polylactic acid and calcium sulfate. The surface properties of composite showed that the hydrophobicity of the composites can be significantly reduced with the increase in the ratio of calcium sulfate. The composite (PC) mixed polylactic acid with a 7:3 ratio of calcium sulfate has both higher yield strength and Young''s modulus in static compression test result. The mechanical properties of the composite also evaluate after 1 and 3 months immersion in phosphate buffer solution (PBS). The yield stress significantly decrease after 3 months immersion indicating the degradation rate of composite was faster than polylactic acid (P). The ion concentration of the degradation solution, deionized water and culture medium, in composite group was significantly lower than the calcium sulfate group. This study also use particulate-leaching method to make the composite surface porous. According to the light and electron microscopy analysis, the pore size is 250-400μm, and the pore number ratio of the high porous composite (pPC), composite with 200wt% pore-forming agents, and the low porous composite, with 100wt% pore-forming agents, is 1:0.75. The in vitro degradation test also performed to evaluate the mechanical properties of the pPC composite before and after immersion in phosphate buffer solution. After 3 months immersion, the yield stress of pPC composite was significantly lower than initial one.

In the research model, the primary cultured osteoblasts were harvested from the calvaria of fetal SD rat. In the cell adhesion and proliferation experiment, the 4 hours cell adhesion rate in the pPC group was significantly higher than the porous polylactic acid (pP), and also the pPC group was significantly higher than the solid composite group (PC). For the cell doubling assay result, there was no significance among the cell perliferation rates in various materials. To analysis the cell expression, we use the induction medium to culture the osteoblast for 3 weeks. The cell cultured in composite group has higher alkaline phosphatase (ALP) activity, osteopontin (OPN) and bone sialoprotein (BSP) mRNA expression than the polylactic acid group. The porous composite group has significantly higher ALP activity, OPN and BSP mRNA

expression than the solid composite group. The alizarin red s stain result shows that the porous composite group also had more calcium deposition. In conclusion, the composite has sufficient initial mechanical properties and maintain the defect area stability at last 3 months. The porous structure decrease the initial ultimate compression stress of the porous composites and increase the degradation rate of the material, but the rough surface also increase the cell adhesion rate and simulated the cell expression. This kind of porous composite may be suitable for the small loading area like cervical.