利用匍枝根黴菌(Rhizopus stolonifer)細胞壁組成RHIZOCHITIN作爲生物支架探討生物降解性及生物相容性

Biodegradability and Biocompatibility of the Scaffold Derived from Cell Wall Component of Rhizopus stolonifer

中文摘要

本研究利用延緩孢子產生的突變 Rhizopus stolonifer F6 菌株，經液體培養基培養得到海綿狀的菌絲墊。此菌絲利用 1N NaOH 在 121oC 處理 20 分鐘後凍乾得到的物質稱爲 RHIZOCHITIN (RC)。RC 再經 95% 酒精沖洗，以去除非皂化的酯類後得到的物質稱爲 alcohol-treated RHIZOCHITIN (ARC)。市售的正向控制組 BESCHITIN-W (BC)與 RC 和 ARC 作爲生物支架分別進行體外和體內的生物相容性和生物降解性測試。體外測試包括溶菌酶分解和纖維母細胞(L-929)培養，分別看生物支架前後重量改變與顯微鏡觀察(光學顯微鏡與電子顯微鏡)結構前後是否不同，得知溶菌酶在前三週 RC 和 ARC 材料重量減少明顯比 BC 還要少，表示 RC 和 ARC 不會很明顯的被溶菌酶分解，反之 BC 就明顯的被分解，在 SEM 中也可看到三者結構的改變。RC 和 ARC 不會影響纖維母細胞的存活，並且在細胞培養中也可以發現，RC 和 ARC 材料重量減少，可知 L-929 會對 RC 和 ARC 產生分解作用，但是 BC 不僅會降低 L-929 存活率外，而且只可以分解少許的材料。體內研究證明將 RC 和 ARC 植入 SD 大鼠皮下組織發現在兩週已完全被分解，然而 BC 在四週時還可被取出，表示 BC 在體內分解速度慢於 RC 和 ARC。實驗中 RC、ARC 和 BC 植入皮下組織均不會造成皮膚過敏和刺激性現象產生。最後，在植入後四週，利用直徑爲 8 mm 大小的鑽孔器，在植入的區域取出組織，進行 HE 和 PAS 染色，兩者結果均顯示 RC 和 ARC 在體內被分解的速度遠大於 BC。本研究得知從 R.stolonifer F6 細胞壁組成成分的物質，是具有生物相容性與生物降解性。

英文摘要

In the present study, the sporangia-formation-delayed mutant strain Rhizopus stolonifer F6 was used to obtain mycelium mattress by liquid culture. The mycelium mattress was treated with 1N NaOH at 121oC for 20 minutes and followed by lyophilization to have RHIZOCHITIN (RC). RC was then again washed with 95% alcohol to remove non-saponification lipid to get alcohol-treated RHIZOCHITIN (ARC). RC and ARC together with a commercial product BESCHITIN-W (BC) for positive control were employed as scaffold for the tests of biocompatibility and biodegradability in vitro and in vivo. In vitro tests included lysozyme degradation and co-culture to fibroblast cell line (L-929) with the weight change and microscopy (light

and scanning electron microscopy). Lysozyme did not digest RC and ARC at the first three weeks but significant degradation was observed in the group of BC by the decrease in weight and the observation under SEM. RC and ARC did not affect the survival of fibroblast cell line in culture condition with an obvious decrease in weight. However BC significantly decreased the survival rate of the fibroblast cell with only slightly degradation of the scaffold. In vivo study demonstrated that RC and ARC had totally degraded after 2 weeks imbedded in subcutaneous layer of SD rats and BC showed a pattern of slow degradation through a four-week observation. No allergic reaction or irritation on the skin of the rats was observed through the experiment for RC ARC and BC. Finally, the tissues of the embedded area were sampled by a punch of 8 mm in diameter after 4 weeks after imbedding of the scaffolds and section of the tissues revealed that RC and ARC were far more degradable than BC in both HE and PAS stains. The study suggested that the cell wall component derived from Rhizopus stolonifer F6 was biocompatible and biodegradable.