牙齦纖維母細胞對骨移植材料的反應

Response of human gingival fibroblasts to bone grafting

中文摘要

自骨移植手術被應用於治療骨缺損以來，各式各樣的天然與人造骨移植材料，被提出或研發出來，至於生物體對這些材料是如何反應，則是臨床骨移植手術時必須考慮的。結締組織裡的纖維母細胞(fibroblast)，經常是骨移植材在植入體內後，早期到達移植材表面的細胞。因此，如果纖維母細胞和移植材有良好的相容性，並能在新生骨質形成階段裡擔任正面色，則骨移植手術後，纖維母細胞的早期包圍將有益於骨缺損的復原再生。研究選擇氫氧磷灰石(Hydroxyapatite；HA)、脫鈣冷凍乾燥骨(Demineralized Freeze-Dried Bone Allograft；DFDBA)、磷酸三鈣 (Beta-Tricalcium Phosphate； b-TCP)、和非陶瓷型氫氧磷灰石 (Non-ceramic hydroxyapatite；NCHA)等四種臨床常用的骨移植材，以觀察人類纖維母細胞(human gingival fibroblast； HGF)對這些骨移植材的反應。 首先以MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenylterazolium bromide) Assay 來分析骨移植材對人類纖維母細胞的毒性。同時將牙齦纖維母細胞與上述種骨移植材共同培養，在第 1、3、7、14 天固定、並於試料處理後，分別以倒立式相位差顯微鏡、掃苗式與穿透式電子顯微鏡、光學顯微鏡等形態學研究方法，評估纖維母細胞對骨移植材的反應。另以纖維母細胞單獨培養作爲對照組。研究結果發現，經 MTT Assay 的檢測，四種骨移植材料均不具有細胞毒性。由形態學觀察發現，在第１、３天時，除了HA 群之外可以觀察到明顯細胞聚集及附著的現象，特別在 b—TCP 群組中，可見在第１天就有細胞聚積與附著現象，並有顆粒被吞噬、以及細胞變大與數量變多的現象。.第７天之後， 所有群組都可觀察到細胞朝同一方向規則排列的情形，但仍以 HA 群組較不明顯。HA 群到第７天之後可觀察到細胞漸有聚集的現象。由結果了解上述四種骨移植材均具有良好的細胞相容性，而 HA 的細胞聚集與附著現象均較它種骨移植材爲慢。b—TCP 周圍的纖維母細胞有較它種骨移植材早期聚集與附著的現象，有助於手術後傷口的癒合及移植材的早期固定 ，另外b—TCP 群的細胞吞噬現象可能有助於骨移植材的吸收，與爾後骨形成的鈣化，對新生骨的再生將有正面的影響。

英文摘要

Lots of natural and artificial bone grafting materials have developed after bone transplantation treatments being used clinically .The evaluation of tissue responses to the materials becomes important when transplantat- ion surgery being considered. The fibroblasts in connective tissues contact the bone grafting materials at the early stage of wound healing after the bone transplanta-tion surgery done. Therefore ,well

biocompatibility between the grafted materials and the surrounding fibroblasts are always important for theresults of surgery . Furthermore , if the fibroblasts ,which contact with the grafting materials being constructive to the osteogenesis in healing of bonydefects , the cells will accerlate new bone formation and lead to excellent bone healing . Four grafting materials : hydroxyapatite (HA) , Beta-tricalcium phosphate (b- TCP) ,Demineralized Freeze-dried Bone Allograft , and Non-Ceramic hydroxyapatite(NCHA) was evaluated for cytotoxicity with MTT assay .And the four materials was also co-culutured with human gingival fibroblasts. At the 1st,3rd,7th and 14th day , these specimen will be treated and observed with light microscope , inverted phase contrast microscope , scanning and transmission electron microscope .Fibroblasts were cultured alone and observed as the control.The results showed no cytotoxicity to the bone grafting materials in MTT(3 - (4, 5-dimethylthiazol - 2 - yl)-2 ,5 -diphenyl terazolium bromide) assay. In the morphological observation , the cell aggregation was found at the 1st and 3rd day in b-TCP , NCHA , and DFDBA groups ,but not in case of HA group. Especially , the cell aggregated and adhesed to the graft particles at the 1st day in b-TCP group.The observation also showed cell size and cell amount increasing , and phagocytosis effect in b-TCP groups. After the 7th day , the fibroblasts were arranged in order , but not significant in the HA group .At the 7th day, the cell aggregation was observed in HA group. According to the result , the four bone grafting materials have well biocompatability ,but the fibroblast aggregation and adhesion are slower in HA group than in the other groups. The earlier aggregation and adhesion on the fibroblasts around the b-TCP particle will enhance the wound healing and materials fixation after the bone transplantation surgery .The phagocytosis effect of b-TCP can the rapid resorption of b-TCP , the calcification and ossification , which will enhance the new bone formation process.