白蛋白披覆鈦金屬表面的體外實驗

Albumin grafted titanium surfaces in vitro study

中文摘要

植入性生物材料的表面不僅與宿主組織直接接觸，且於生物相容性扮演關鍵性角色，爲改善植體與組織的整合性進而控制植體界面反應，減少非特定蛋白質的吸附，本研究利用低溫電漿(glow discharge)來活化鈦金屬表面以連接生物活化性白蛋白（albumin），發展一植體表面處理技術。鈦金屬表面先以氬氣電漿清潔，接著以丙烯胺(allylamine)電漿處理，使經氬氣電漿清潔後的表面附著上胺基(-NH2)，再以交鏈劑戊二醛（glutaraldehyde）連接固定白蛋白於低溫電漿處理後的鈦金屬植體。經氬氣電漿處理後的鈦金屬植體表面分別以掃描式電子顯微鏡(SEM)及原子力顯微鏡(AFM)進行植體表面粗糙度分析;以 X 光光電子能譜術(XPS)分析白蛋白吸附於鈦金屬後其化學鍵結的改變;爲探討經低溫電漿處理的鈦金屬植體表面對固定白蛋白的影響，故以低掠角 X 光繞射儀(GIXRD)進行電漿與無電漿處理鈦金屬的結構分析;XPS 分析白蛋白吸附於鈦金屬後化學鍵結的改變；利用 Protein A Gold 方法間接定量白蛋白接著情形，再以 AFM 觀察奈米金粒子的分佈情形，並將金屬植體接著一系列的反應以 SEM 進行表面觀察。此外，鈦金屬植體完成一系列的反應後進行離體實驗，分別將披覆白蛋白之鈦金屬植體以類骨母細胞(osteoblast-like cell, MG 63)及(MC3T3-E1)兩種細胞分別以 5 x104 cell/ml 濃度培養，經 1 小時、8 小時、24 小時及 48 小時後以 MTT assay 計算細胞存活率及細胞活性，並以 SEM 觀察此細胞形態的變化。經 SEM 及 AFM 觀察得知，氬氣電漿處理的時間與表面潔淨度及表面粗糙度有正相關，顯示經電漿解離的氬離子與原子具清潔及離子轟擊(ion bombardment)鈦金屬植體表面的功能。以 GIXRD 得知，經電漿處理後的鈦金屬植體繞射角度及相對繞射強度並無顯著移位及遞減，亦即具(002)優選繞射晶面的鈦金屬植體經電漿處理後其表面並無任何氬氣損傷(argon damage)及氮化效應(nitridation effect)產生，取而代之經電漿離子化的丙烯胺以胺基(-NH2)型態成功地附著於鈦金屬植體，此結果將有益於對白蛋白的固定。經 XPS 分析白蛋白吸附於丙烯胺電漿處理後的鈦金屬植體化學鍵結能的改變得證，經丙烯胺電漿處理後比無電漿處理的鈦金屬植體較易於披覆白蛋白。經 SEM 觀察類骨母細胞披覆於白蛋白鈦金屬圓片得知，實驗組的鈦金屬上有類似圓形球狀體披覆，且培養 8 小時後已開始附著於鈦金屬片上， 24 小時之附著情形更爲良好，且細胞觸角已開始向外伸長，相對未經電漿處理的鈦金屬植體具較高的細胞活性。以上顯示使用低溫電漿處理技術可使鈦金屬植體胺基化，加上交鏈劑戊二醛的處理，可使鈦金屬植體表面交連上白蛋白。因此以低溫電漿處理進行植體表面處理的方法可提供一個將已知生物活性分子鍵結到鈦金屬植體，使得植體植入後能引導並促進組織復原之應用參考。

英文摘要

The surface of implantable biomaterials is in direct contact with the host tissue and plays a critical role in determining biocompatibility. In order to improve the integration of implants, it is desirable to control interfacial reactions such that nonspecific adsorption of proteins is minimized and tissue-healing phenomena can be controlled. In this regard, our goal is to develop a glow-discharge method to functionalize titanium surfaces by the covalent immobilization of bioactive organic molecules and evaluate the bioactivity in vitro. Titanium plate first was cleaned by glow discharge using argon plasma to eliminate surface contaminants and to produce a consistent and reproducible titanium oxide surface layer. Then an intermediary allylamine deposition was covalently linked to the oxide layer by glow discharge, followed by the covalent binding of albumin to the free terminal NH2 groups using glutaraldehyde as a coupling agent. Surface morphology observation and surface roughness were performed by scanning electron microscopy (SEM), and atomic force microscopy (AFM). Variation of chemical properties on titanium surfaces was analyzed by X-ray photoelectron spectroscopy (XPS). In addition, XRD was performed to realize of plasma-treatment effect. Composition variations of titanium surface with and without plasma treatments were analyzed by energy dispersive spectrometry (EDS). Surface coverage by bound albumin was evaluated by SEM visualization The results implied that the albumin was successful grafted on the titanium plates by glow discharge technology and this method could offer the possibility of covalently linking selected molecules in order to guide and promote the tissue healing that occurs during implant integration in bone and soft tissue. According to SEM and AFM investigation, argon plasma-treated time is proportional to surface roughness and cleaning. It revealed the bombardments of energetic radicals and ions during plasma treatment. Based on XRD results, the density and shape of reflections indicate changes in the phase of titanium plate. The XRD peak of (002) preferred-orientation titanium plate is not shifting. It implied the plasma treatment didn’t lead to argon damage and nitridation effect. Allylamine with terminal NH2 groups was successfully linked with plasma-treated titanium plate. Ionized allylamine was deposited on titanium plate by XPS analyses. The binding energy shifting of N1s peak is obvious. It revealed the allylamine was ionized by plasma treatments, and dangling bond was acted as medium to linking albumin. The variation of cell cultures on titanium surface with and without plasma treatment were observed by SEM. Osteoblast-like cells (MG 63 and MG3T3) were cultured on the plasma-treated titanium plates from 8 to 48 hours. The result showed the osteoblast-like cells spread radically after 8 hours, and better adhesion were observed after 24 hours. It implied that the plasma-treated titanium surface possesses the better bioactivity and

biocompatibility than that without plasma modification. The plasma treatment process plays an important role in helping tissue-healing phenomena. It can be not only provided clean titanium surface, but also make dangling bond NH2 on plasma-treated titanium surface. It was better for linking glutaraldehyde and albumin absorption on titanium surface. It is evident that plasma treatment will promote the tissue-healing.