論文名稱:	注射型生醫材料與間葉幹細胞於硬骨組織工程之應用
	Applications of Injectable Biomaterials and Mesenchymal Stem Cell in Bone Tissue Engineering
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摘要:	背景: 外傷後或腫瘤切除後骨頭缺損的修補對於骨科及顱顏面外科是一個常見的問題. 目前的標準治療方法還是使用自體骨移植. 但是取自體骨時的併發症,不可預期的被吸收率等限制了此項手術的應用.而組織工程結合細胞,材料及生長因子是目前修補顱顏骨缺損最先進的方法.為了縮短恢復期減低手術傷害,使用小傷口的內視鏡手術為未來的趨勢.而使用可注射式的生物材料正可附合這樣的需求. 可注射式材料也可與生長因子做均勻的混合以促進骨頭生長.可注射式材料也可用於修補不規則的缺損. 本研究主要使用幾種不同的注射型生醫材料結合骨髓幹細胞或脂肪幹細胞來做顱顏骨缺損的修補. 材料與方法:首先,我們先要熟悉各種動物脂肪幹細胞及骨髓幹細胞的擷取,及純化方法.並且熟悉幹細胞的骨誘導所需的染色,生化分析及聚合酵素連鎖反應. 第一實驗我們使用一個新合成的溫敏感性水膠(Hyaluronic-acid-g-chitosan-g-NIPAM hydrogel (HACPN))當細胞支架,將其與骨髓幹細胞混合及骨誘導後.分別測試其體外骨誘導的能力及體內異位骨增生及豬顱顏骨缺損重建的能力.因為合成的水膠的缺點是無法完全被吸收及可能產生有毒代謝物,所以第二個實驗使用自體組織膠來當細胞支架.我們混合脂肪幹細胞或骨髓幹細胞與自體組織膠及磷酸鈣的載體來測試其體外骨誘導及體內骨生成的能力.因為自體組織膠的機械強度較弱及生長因子較少.第三個實驗我們使用雷射燒結的聚己內酯骨支架混合富含血小板的組織膠當載體結合脂肪幹細胞來修復豬之下顎骨缺損. 結果:首先我們成功的從動物體分離出脂肪幹細胞及骨髓幹細胞且成功的在體外進行骨誘導.第一個實驗我們發現 HACPN 是一個好的細胞載體,可結合骨髓幹細胞成功的在體外進行骨誘導,可在裸鼠背部成功誘發異位骨增生.也可成功的修復豬的顱顏骨缺損.第二個實驗發現自體組織膠的細胞攜帶能力相當好,也可使幹細胞在體外達到很好的骨誘導能力.由豬顱顏骨修復的實驗中可發現,結合自體組織膠及磷酸鈣的載體不管使用脂肪幹細胞或骨髓幹細胞都可達到不錯的骨生成.而結合自體組織膠及骨髓幹細胞則次之,最差的則是自體組織膠及脂肪幹細胞.第三個實驗則發現結合富含血小板膠的組織膠及雷射燒結的支架可使脂肪幹細胞在下顎骨的修復產生緻密的骨生成.體外實驗也發現脂肪幹細胞在這一組可以有較好的貼附,生長及骨分化. 結論: 可注射型的水膠可以成為一個好的細胞載體來修復骨缺損.當結合磷酸鈣或者雷射燒結的支架可增強他的機械強度及骨修補能力.

英文摘要:

Background: The regeneration of bone defect after trauma or tumor ablation is a common problem in orthopedic and craniomaxillofacial surgeons. Traditionally, autogenous bone grafts are the gold standard to induced bony regeneration. However, there are some disadvantages that limit their use such as donor site morbidity, variable degree of resorption and the need for a secondary surgery exposure. Tissue engineering is a growing, advancing method for management of the craniofacial defect by combination of cells, scaffolds and signal molecules. To minimize surgical trauma and shorten the recovery time, minimal incision by endoscope-assisted surgery is future tendency. Therefore, an injectable scaffold is suitable to fulfill such requirement. A liquid support matrix that polymerized to a gel would be shaped easily and molded for custom reconstruction or augmentation, and much less invasive without an open surgical procedure. Besides, a liquid material may incorporate various therapeutic agents such as bone morphogenetic proteins to enhance bone regeneration. In this study, we will analyze different injectable scaffolds and discover if these injectable scaffolds mixed with adipose-derived stem cells (ASCs) or bone marrow stem cells (BMSCs) can repair the craniofacial defect effectively.

Materials and Methods: In this research, at the begining we should be familiar with the harvest isolate and purify the ASCs and BMSCs from different species such as dog, porcine. Osteogenic differentiation of ASCs and BMSCs were evaluated by alkaline phosphatase activity (ALP), Alizarin red stain, ALP stain and RTPCR of bone specific mRNA. In first experiment, we develop a new thermogelling, injectable scaffold – hyaluronic-acid-g-chitosan-g-NIPPAm hydrogel (HACPN). In-vitro osteogenesis, ectopic bone formation and orthotic bone regeneration are evaluated by combination of HACPN and bone marrow stem cells. In second experiment, nature hydorgel- fibrin glue made by fibrinogen and thrombin was used as an injectable hydrogel to improve the disadvantages of non-absorbable properties and possible toxic metabolites created by synthetic scaffolds. The ability of in-vitro osteogenesis and cranial bone defect regeneration were compared by mixing the ASCs or BMSCs with fibrin glue +/- Mastergraft. In third experiment, due to the weak strength and less growth factor of fibrin glue, three-dimensional PCL scaffold made by selective laser sintering (SLS) added with ASCs and injectable platelet rich plasma(PRP) which contained multiple growth factors was used to reconstruct the load-bearing mandibular defect.

Results: The in-vitro study showed the ASCs and BMSCs both had the potential of osteogenic differential by stain, ALP activity and RT-PCR of specific osteogenic protein. The first experiment demonstrated that HACPN hydrogel was a good injectable biomaterial for bone marrow stem cells for in-vitro study. Besides, the nude mice study proved the ectopic bone formation by the combination of HACPN hydrogels and BMSCs. The pig skull defect reconstruction model showed that the HACPN/BMSCs also have the potential of orthotic bone regeneration. The second experiment showed the fibrin glue+/-Mastergraft can be as a good scaffold for ASCs and BMSCs for in-vitro study and in-vivo pig skull bone regeneration demonstrated that the fibrin glue/Mastergraft/BMSC or ASCs have the best outcome, followed by fibrin glue/BMSCs and fibrin glue/ASCs has the least result. The third experiment showed modification of the laser-sintered PCL scaffold by PRP indeed enhances the affinity and osteogenic potential of pASCs. The pASCs spread well in the PRP/lsPCL scaffold. The ALP activity, mineralization, real-time PCR of Cbfa1, ALP, and OCN were also performed well in this group. The animal study also proved the dense bone formation with stiffness close to normal bone.

Conclusion: The injectable scaffolds can be as effective cell carriers for bone defect reconstruction. They also can be combined with Mastergraft or laser-sintered PCL scaffolds for enhancing the quality and strength of bone regeneration.