日本財団 助成事業
The Nippon Foundation

2001年度日中医学協会共同研究等助成事業報告書

—中国人研究者・医療技術者招聘助成—

平成14年3月15日

財団法人　日中医学協会

理　事　長　殿

招聘責任者氏名　諏訪　文彦　㊞

所属機関名　大阪歯科大学

職　　名　教　授

所　在　地〒573-1121　枚方市楠葉花園町8-1

電話　072-864-3012　内線

1．招聘研究者氏名　金　岩

所属機関　中国西安第四軍医大学・口腔医学院組織病理教研室　職名　教　授

2．研究テーマ　組織工学的骨移植の血管再生についての研究

3．日本滞在日程

① 自平成13年6月24日
至平成13年7月　1日

② 自平成13年11月12日
至平成13年11月19日

4．研究報告書

別紙報告書作成要領により、添付の用紙で研究報告書を作成して下さい。

研究発表中または研究中の本人のスナップ写真を添付して下さい。

＊研究成果を発表する場合は、発表原稿・抄録集等も添付して下さい。

＊発表に当っては、*日中医学協会助成金*による旨を明記して下さい。

Revascularization of Transplanted Bone Constructed through Tissue Engineering

Researcher: Yan Jin(金岩),
Cooperation Institute(Chine): Department of Oral Histology & Pathology, Stomatological College, the Fourth Military Medical University, Xi'an, PR Chine
Cooperation Institute(Japan): Department of Anatomy, Osaka Dental University
Supervisor: Prof. Fumihiko Suwa
Collaborator: Yuan Liu(刘源)

Abstract:

The aim of this study was to investigate bone formation, by employing a tissue engineering method, and the effects of a combined pattern of biomaterials and bone marrow stem cells on osteogenetic ability. The project consisted of four sections: Bone formation potentiality of bone marrow stem cells (MSCs) in vitro; biological behavior of MSCs in three different complexes combined with biomaterials; in vivo bone formation induced by rhMBP-2 and TGF-β combined with CBB and PEO-PPO-PEO complexes; observations of the bone healing and revascularization, which was enhanced by tissue engineered bone in vivo. Our results showed that: SD rat MSCs have great bone formation potentiality; seeding MSCs on the mixed CBB and PEO-PPO-PEO various complexes were the best for the construction of tissue engineered bone; observations of the bone healing and revisualization enhanced by tissue engineered bone in vivo. Our results showed that the CBB and PEO-PPO-PEO complexes are best for in vitro bone construction and rhBMP-2, TGF-β can greatly enhance the speed and quantity of bone formation.

Keywords: Revascularization, Tissue Engineering, Bone, rhBMP-2, TGF-β

Introduction:

The aim of tissue engineering is to investigate and restore tissue and organ

substitutes. In this study, bone formation was investigated by employing a tissue engineering method. The effects of a combined pattern of biomaterials and bone marrow stem cells on osteogenetic ability were also studied.

Materials and method:

1. SD rat MSCs were cultured in vitro by an explant method, then were cultured in mineralization-conditioned medium for 5～10 days. Cell proliferation ability and alkaline phosphatase (ALP) activity were observed. The bone formation potentiality of MSCs in mineralization-conditioned medium was investigated by von-Kossa staining. The secretion of collagen type I was investigated by an immunocytochemistry method;
2. SD rat MSCs were subcultured to 3rd passage and cultured in mineralization-conditioned medium for 5 days. Three different kinds of complexes were constructed; ① ceramic bovine bone (CBB) and poly(ethylene oxide)-poly(propylene oxide)-poly(ethylene oxide) (PEO-PPO-PEO) were mixed together, and the MSCs were then seeded on the complex; ② MSCs were resuspended in liquid PEO-PPO-PEO and then mixed with CBB. ③ MSCs were combined with CBB and then mixed with PEO-PPO-PEO; ④ MSCs were mixed with CBB as a control. The biological behavior of the MSCs in the different groups were investigated at 5 and 10 days. The proliferation ability of the MSCs was investigated by cell counting. The attachment and growth of MSCs on the materials were studied by SEM. ALP activity and the expression of collagen type I were measured by an immunocytochemistry method;
3. MSCs were cultured, expanded and induced in vitro, as mentioned above. CBB was ground and sieved. Cells were resuspended with PEO-PPO-PEO and then mixed with CBB. There were two groups; ① combined with rhBMP-2 50μg/ml and TGF-β 50ng/ml, and ② combined with rhBMP-2 50μg/ml. Then the complexes were implanted subcutaneously in the backs of nude mice and investigated histologically at 2w, 4w and 6w intervals;
4. An SD rat model of critical size calvarial defects will be established and artificial bone made of MSCs-ceramic-PEO-PPO-PEO will be used to repair these defects (study still in progress).

Results:

1. After 5 days of culture in mineralization-conditioned medium, the cell proliferation ability was greatly decreased, the ALP activity was enhanced, and collagen type I staining was positive. When cultured up to 10 days, von-Kossa staining showed there were calcium depositions with a nodule shape;
2. These results clearly demonstrated that at the same time interval, the proliferation abilities of groups ① and ③ were better than groups ② and ④. The results of the

SEM study showed that the attachment, growth and secretion of collagen in group ① were better than it was in the other groups. The results of the immunocytochemistry showed that the expression of collagen type I in group ① was better than the other groups, and there were no differences within the other group. In regard to the ALP activity, there were no obvious changes between group ① and ③, and no differences between group ② and ④, but there was more positive staining demonstrated in group ① and ③ than in group ② and ④. The cell numbers, attachment and growth, the expression of collagen type I and the ALP activity at 10 days were all better than those at 5 days.

3. At 2w, the implantations were white, smooth and flexible, about 1.0-1.5 μm thick, surrounded by fibrous membrane and had irregular disc-like shapes. Histological results showed that there were bone matrix-like tissues formed inside the complexes, and blood capillaries were seen inside the tissue. The tissue formations in group ① were better than those in group ②. At 4w, the results were almost the same as those at 2w, except that there was more bone matrix-like tissue formation. The investigation of the 6w groups is still in progress. At each time point, all of the groups combined with rhBMP-2 50 μg/ml and TGF-β 50 ng/ml had more bone formation than those combined with rhBMP-2 50 μg/ml only.

Conclusions:

1. SD rat MSCs have great bone formation potentiality and can be used in the formation of bone constructed by tissue engineering;
2. Seeding MSCs on the mixed CBB and PEO-PPO-PEO complexes were the best for construction of tissue engineered bone;
3. Adding rhBMP-2 50 μg/ml and TGF-β 50 ng/ml can greatly enhance the bone formation ability of the mixed CBB and PEO-PPO-PEO complexes.

References:

1.Li Shi-Bao, Yang Wei-Dong, Tang Li-Hui, et al. Preparation and application of temperature dependent hydrogel. J Fourth Mil Med Univ 2001 ; 22(4) : 306-308.

2.Minamide A, Tamaki T, Kawakami M, et al. Experimental spinal fusion using sintered bovine bone coated with type I collagen and recombinant human bone morphogenetic protein-2. Spine 1999 ; 24(18) : 1863-70.

3.Lin FH, Liao CJ, Chen KS, Sun JS. Preparation of a biphasic porous bioceramic by heating bovine cancellous bone with Na4P207.10H20 addition. Biomaterials 1999 Mar ; 20(5) : 475-84.

4.Cao YL, Rodriguez A, Vacanti M et al. Comparative study of the poly(glycolic acid), calcium alginate and pluronic in engineering of autologous porcine cartilage [J]. J

Biomater Sci Polymer Edn. 1998 ; 9(5) : 475-487.
5.Yang Weidong, Chen Fulin, Mao Tianqiu, et al. Fabrication of cartilage with injectable synthetic hydrogel. J Pract Stomatol 1999(Sept) ; 15(5) : 378-380.
6.Lee YM, Park YJ, Lee SL, et al. Tissue engineering bone formation using chitosan/tricalcium phosphate sponges. J Periodontol 2000 ; 71(3) : 410-7.
7.Minamide A, Tamaki T, Kawakami M, et al. Experimental spinal fusion using sintered bovine bone coated with type I collagen and recombinant human bone morphogenetic protein-2. Spine 1999 ; 24(18) : 1863-70.
8.Despina D. Deligianni, Nikoleta D. et al. Effect of surface roughness of hydroxyapatite on human bone marrow cell adhesion, proliferation, differentiation and detachment strength. Biomaterials 2001 ; 22(1) : 87-96.

Anticipate completion date:March 15th, 2002.