We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com

14

Histocompatibility of Acellular Matrix Bone with Osteoblast and Vascular Endothelial Cells

Gang Rui, Xuhong Jin and Shan Lin
The First Affiliated Hospital of Xiamen University
China

1. Introduction

The culture of stem cells is an essential element in tissue engineering.The base for the repairing of bone defect in tissue engineering is still its vascularization. Co-culture of the osteoblast and the vascular endothelial cell (VEC) can not only promote the proliferative activity of the osteoblast but can also accelerate the revascularization of the regenerating bone. Thus it can help to increase the ability of regenerating bone in the repairing of bone defect. The bone source and the site for supplying bone were limited in autografting, meanwhile it is difficult in the modeling of self-tissue, therefore its clinical application is limited. There is certain immunologic rejection in the isotype heterogenous bone grafting and the tissue engineering bone provides a new way for solving this problem. In this experiment, the osteoblast and the vascular endothelial cell were compounded by using the acellular rib of pig. Then the tissue engineering bone was constructed and implanted into the animal. Observe and study the biological compatibility, try to find out the pathological process of in vivo ossification, bone defect repairing and revascularizarion for the heterogenous bone grafting. Thus it will provide the theological base for the clinical application of tissue engineering bone. This chapter is divided into 3 parts: Part I: The culture of the seed cell in tissue engineering and the study of cellular compatibility, Part II: Study the effect of heterogenous bone acellular matrix (HBACM) on the biological compatibility between the osteoblasts and the vascular endothelial cells, Part III The experimental research of implanting the compound cell of heterogenous bone acellular matrix into the animal body.

2. General methods (Parts I - III)

2.1 Part I: The culture of the stem cells in tissue engineering and the study of cellular compatibility

The osteoblast (A group) and vascular endothelial cell (B group) of the rabbit was cultured, and then both of the cells were co-cultured (C group). The morphology and the growth of the cells in the 3 groups were observed under the inverted phase contrast microscope. Immunocytochemical stain of type I collagen was used to identify the osteoblast. Immunocytochemical stain of the vascular factor VIII was used to identify the VEC. The sections were made by the co-cultured cells. Three days later, observe the mixed growth of

the two kinds of cells after HE stain and Masson trichrome stain. Test the activity of ALP by using the method of paranitrobenzene phosphate. Observe whether the vascular endothelial would affect the ALP activity produced by the osteoblast. Analyse the growth and proliferation of the cells in the 3 groups.

2.2 Part II: Study the effect of heterogenous bone acellular matrix (HBACM) on the biological compatibility between the osteoblasts and the vascular endothelial cells

The fresh rib of the pig was defatted, deantigened and decellular by hydrogen peroxide solution, chloroform/methanol and Triton X-100, then it was made into the framework material of the acellular matrix and was observed under the scanning electron microscope. And then, the three groups of rabbit cells were compounded with the acellular rib of the pig individually. The adhesion of the cells with the material, the distribution of the cells in the pores of the material, and the growth, differentiation and proliferation of the cell were observed under the inverted phase contrast microscope and the scanning electron microscope. The biological compatibility was observed after HE stain, toluidine blue and Masson trichrome stain, after 5 days co- cultured. The cell cycle and the ploidy of the co-cultured three groups were tested by flow cytometer to understand the toxicity of the material to the cell on the 1d, 3d, 5d and 7d.

2.3 Part III: The experimental research of implanting the compound cell of the heterogenous acellular matrix into the animal body

The three groups of rabbit cells were marked with BrdU, and then they were compound with the decellular rib of the pig to repair the bone defect in the body. 27 New Zealand big-ear-rabbit weighted 2.5kilogram were selected and they were grouped into 3 groups with 9 in each group. Resect the 1.5cm long radius of the double forelimbs of the rabbit and prepare animal model of bone defect. The tissue engineering bone which had been compounded and cultured for 1 week by the heterogenous bone acellular matrix and the cells of each group was implanted to the left side. The heterogenous bone acellular matrix was implanted to the right side as a control. Select the material at 3 weeks, 6 weeks and 12 weeks to observe bone fracture. The development and the prognosis of the tissue engineering bone were observed by naked eye. X-ray examination was performed to understand the formation,change and moulding of the callus at the site with bone defect. The Brdu marked cells were traced and tested to understand the survival of seed cell in the experimental animal. The pathological process of in vivo ossification, bone defect repairing and revascularization were observed after HE, toluidine blue and Masson stain. And the immunohistochemistry of type I collagen was used to understand the secretion of type I collagen by each group of cells at various phase.The border between the tissue engineering bone and the normal bone was selected and the area of the vessel in each unit was measured by image analyzer.

3. Conclusion

1. The ossification activity of the osteoblasts was significantly increased ($P< 0.01$) by the vascular endothelial cells. The co-cultured cells had strong potency of proliferation.

2. The heterogenous bone acellular matrix had good biological compatibility, with low antigenicity, no cytotoxity or neoplastia ; The co-cultured cells had strong potency of proliferation in the extracellular matrix of the heterogenous bone acellular matrix.
3. The major ossification of the tissue engineering bone in the body was the cells cultured in vitro. There was synergistic action between the osteoblast and the vascular endothelial cell whether in vivo or in vitro. Fracture healing could be accelerated and the repairing of bone defect could be promoted by using the co-cultured cell as seed cell.

4. Part I: The culture of the seed cell in tissue engineering and the study of cellular compatibility

4.1 Introduction

The culture of seed cell is an essential element in tissue engineering. Osteoblast is the seed cell in tissue engineering, but the base for the repairing of bone defect in tissue engineering is still its vascularization. Co-culture of the osteoblast and VEC can not only promote the proliferative activity of the osteoblast but it they can also accelerate the revascularization of the regenerating bone. Thus, it can help to increase the ability of regenerating bone in the repairing of bone defect. To this end, we conducted related studies and reported as follows.

4.2 Materials and methods

4.2.1 Cell culture

4.2.1.1 The culture of osteoblast (group A) The calvaria cultures were taken from two 2-week-old newborn rabbits and the soft tissue was removed. After several times of rinsing in PBS, the soft tissue was cut into blocks of one mm^3 in size, which were then digested in 1:1 of 0.25% trypsin and 0.1% collagenase at 37 ℃ for 10 min. After the digestion terminated, the solution was centrifuged at 1200 r/min for 5 min and the supernatant was discarded. Then, the cells was suspended in 20% of fetal calf serum DMEM and inoculated in culture flasks, which were kept in the cell incubator at 37 ℃ with 5% of CO_2 and saturated humidity. The tissue blocks were digested using 1:1 of 0.25% trypsin and 0.1% collagenase at 37 ℃ for 20 min. The cells were collected via centrifugation and were inoculated in culture flasks. The above steps were repeated twice. Then, the culture medium was replaced every two to three days. The cells that became monolayer were used for subculture.

4.2.1.2 The culture of VEC (group B) The bilateral kidney cortex of the above two 2-week-old newborn rabbits were cut into fragments, which were then digested with 0.25% of trypsin at 37 ℃ for 20 min. After the digestion terminated, the solution was centrifugal at 1200 r/min for 5 min and the supernatant was discard. Then, the cells was suspended in 20% of fetal calf serum DMEM and inoculated in culture flasks, which were kept in the cell incubator at 37 ℃ with 5% of CO_2 and saturated humidity. Then, the culture medium was replaced every two to three days. The cells that became monolayer were used for subculture.

4.2.1.3 Co-culture of osteoblast and VEC (group C) Primary cultured osteoblast, which was in logarithmic growth phase, was inoculated by the ratio of 1:1 in the culture flasks. 20% of fetal calf serum DME were added in the flasks and kept in cell incubator at 37 ℃ with 5% of

CO_2 and saturated humidity. Then, the culture medium was replaced every two to three days. The cells that became monolayer were used for subculture.

4.2.2 The index of observation

4.2.2.1 Observation under the inverted phase contrast microscope Observe morphology and the growth of the cells in the three groups under the inverted phase contrast microscope.

4.2.2.2 Immunocytochemical stain The cells of group A and group B were inoculated with the density of 2×10^5 cells per well into the six-pore board with a built-in glass slide for making cell climbing slices. Three days later, immunocytochemical stain of type I collagen to identify the osteoblast for group A. Immunocytochemical stain of the vascular factor Ⅷ to identify the VEC for group B.

4.2.2.3 Histological observation The cells of group C were inoculated with the density of 2×10^5 cells per well into the six-pore board with a built-in glass slide for making cell climbing slices. Three days later, observe the mixed growth of the two kinds of cells after HE stain and Masson trichrome stain.

4.2.2.4 Test of the activity of alkaline phosphatase (ALP) The cells of groups A, B and C were respectively inoculated with the density of 3×10^3 cells per well into 96-pore board (12 pores each group). Test the activity of ALP by using the method of paranitrobenzene phosphate via a machine of UV / fluorescence / visible high efficiency analyzer (Perkin Elmer, USA). Observe whether the VEC would affect the ALP activity produced by the osteoblast.

4.2.2.5 Test of the cellular activity by the method of methythiazolyl tetrazolium bromide (MTT) The cells of groups A, B and C were digested and inoculated with the density of 4×10^3 cells per well into 96-pore board. 12 pores were taken on the 1st, 3rd, 5th and the 7th day, respectively. Added 5 mg ml^{-1} of MTT and reacted for four hours. After the liquid was discarded, dimethyl sulfoxide was added and oscillated for 10 min. An enzyme mark instrument (Perkin Elmer, USA) was used to obtain the adsorption at the wavelength of 570 nm and to analyze the growth and proliferation of the cells in the three groups.

4.2.3 Statistical methods

The result of the experiment was expressed by mean±standard deviation. The analysis of variance and q test of the measurement data among groups were done by using SPSS 10.0 software packet. This difference had statistic meanings ($P<0.01$).

4.3 Result

4.3.1 Observation under the inverted phase contrast microscope

Primary cultured osteoblast and VEC began to adhere to the wall four hours later and was adherent completely in 48 hours. Osteoblast displayed various shapes, some were spindle, and some were long triangles, with massive sharp horn like (Fig. 1a). VEC showed ovoidal and changed to typical 'flagstone' appearance when it fussed to monolayer (Fig. 1b). When VEC and osteoblast were co-cultured, they grew closely. There was no contact inhibition,

rejection and phagocytosis. The volume of osteoblast was large and the volume of VEC was relatively small (Fig. 1c).

4.3.2 Immunocytochemical stain

The immunocytochemical stain of osteoblast type I collagen and VEC factor VIII were both positive (Figs. 2 and 3). The cytoplasm revealed yellow staining and the cell nuclear blue staining. Results show that the primary cultured cells were osteoblast and VEC.

4.3.3 Histochemistry stain

The HE staining and Masson staining of the cells of group C showed the mixed growth of two types of cells with good cellular compatibility. The volume of osteoblast was large and the volume of VEC was relatively small (Fig. 4). VEC appeared red by Masson trichrome stain (Fig. 5).

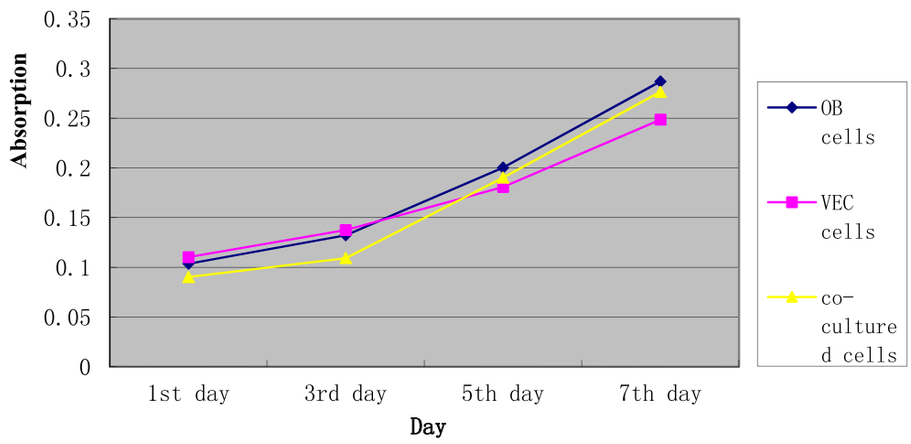


Fig. 1. Curves of cells viability in different groups

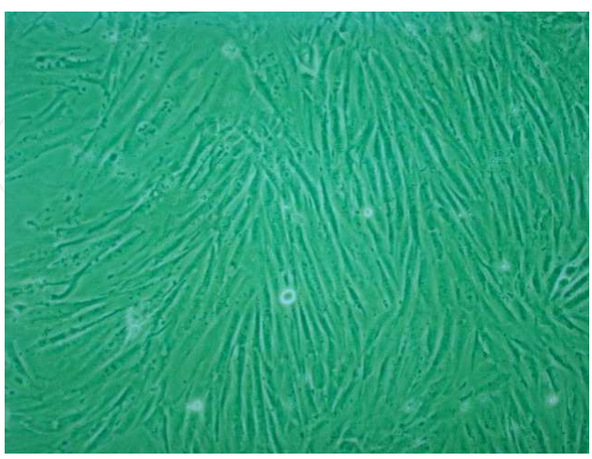


Fig. 1a. Observation under the inverted phase contrast microscope (100x). OB displayed various shapes, some were spindle, typical and some were long triangle, with massive sharp to monolayer.

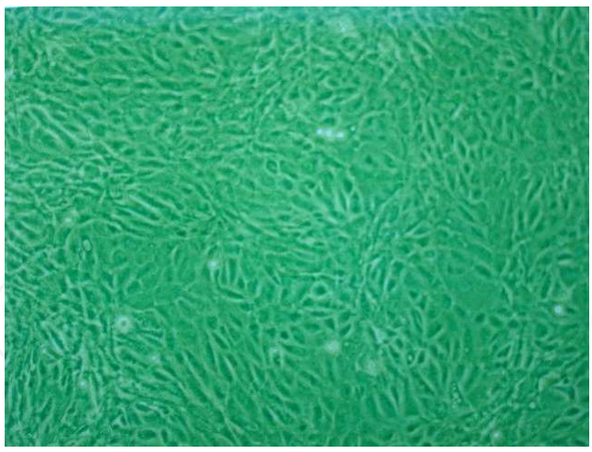


Fig. 1b. Observation under the inverted phase contrast microscope (100x). VEC showed ovoidal and 'flagstone' appearance horn like.

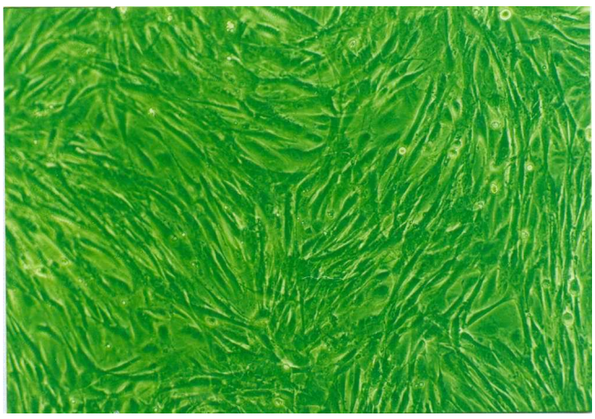


Fig. 1c. Observation under the inverted phase contrast microscope (100x). When VEC and osteoblast were co-cultured, cytoplasm revealed they grew closely. Nuclear blue.

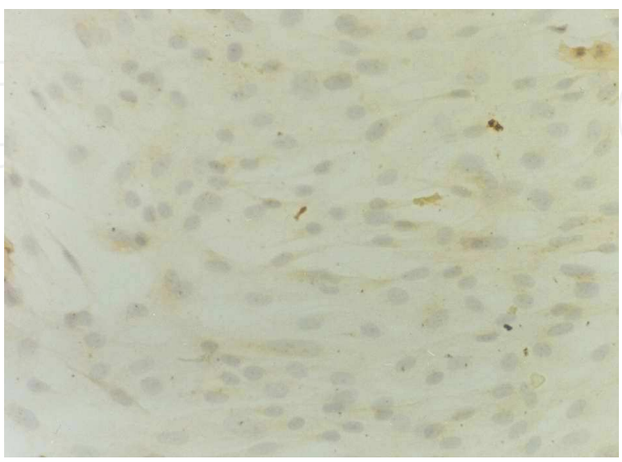


Fig. 2. The immunocytochemical stain of osteoblast type I collagen (100×). The result was positive. The cytoplasm yellow staining and the cell nuclear blue staining.

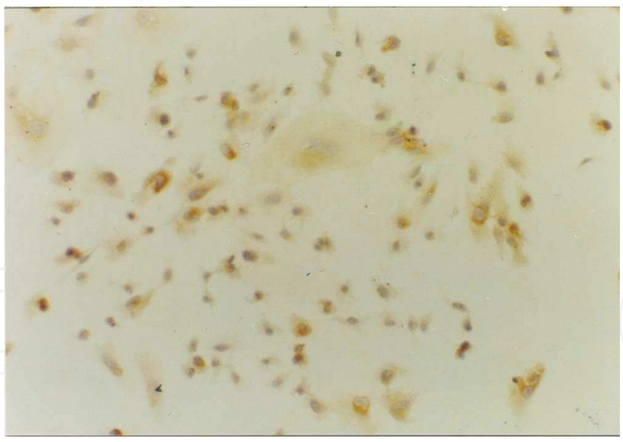


Fig. 3. The immunocytochemical stain of VEC factor VIII(100×). The result was positive. The cytoplasm revealed yellow staining and the cell nuclear blue staining.

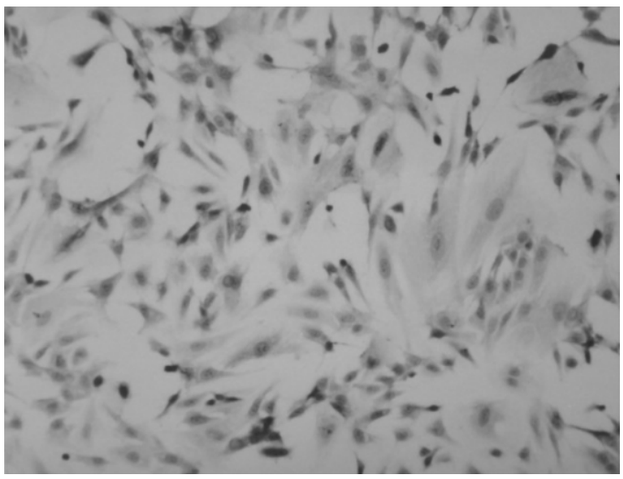


Fig. 4. HE staining of the cells of group C (100×). The HE staining showed the mixed growth of two types of cells with good cellular compatibility.

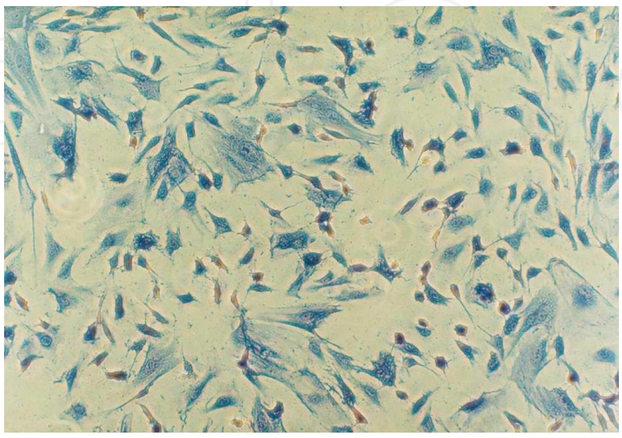


Fig. 5. Masson staining of the cells of group C (100×). The Masson staining showed the mixed growth of two types of cells with good cellular compatibility.

4.3.4 Test of the activity of ALP

After three days of growth, the activity of ALP in each group was as follows. Group A (0.1568 ± 0.0132) U/L, group B (0.1148 ± 0.0145) U/L, group C (0.5018 ± 0.0873) U/L and the F value was 202.811 (P <0.01). The ALP activities from high to low in order were group C, group A and group B. The ALP activity of group A was higher than that of group B (P<0.05). The ALP activity of group C was obviously higher than that of group A and group B (P<0.01).

4.3.5 Test of the cellular proliferation activity by MTT method

The activity of the cells is listed below (Table 1, Fig. 1). On the first day, group B> group A > group C, the difference was statistically significant (P <0.01, P <0.05). On the third day, the activity of group A and B was higher than that of group C, the difference was statistically significant (P <0.01) and there was no significant difference between group A and group B (P> 0.05). On the fifth day, results show that there was no significant difference among group A, B and C. However, on the seventh day, the activity of group A was higher than that of group B (P <0.01), and the activity of group C was higher than that of group B (P <0.01), the difference was statistically significant and there was no significant difference between group A and group C (P> 0.05).

Groups	Culture time			
	1st day	3rd day	5th day	7th day
A	0.1037±0.0085	0.1323±0.0093	0.2003±0.0191	0.2869±0.0132
B	0.1203±0.0194**	0.1375±0.0055	0.1808±0.0099	0.2485±0.0116**
C	0.0803±0.0047*ΔΔ	0.1093±0.0129**ΔΔ	0.1902±0.0203	0.2765±0.0178ΔΔ

Table 1. Comparison of different cells viability (Compared with group A: * P<0.05, **P<0.01; Compared with group C: ΔP <0.05, ΔΔ P<0.01.)
Results show that the cells of group C proliferated slowly in the early stage and faster in the later stage.

Table 1. Comparison of different cells viability

4.4 Discussion

Osteoblast is the progenitor cell in tissue engineering; while VEC is rarely used as seed cell in tissue engineering. The three basic processes of bone graft are the vascularization of the graft, bone regeneration and bone-terminal fusion. The ultimate goal is to repair bone defects. The vascularization of the graft is the key step, which affects the total process of transplantation and repair, and it play a decisive role on the mode and effect of bone regeneration and fusion. Zhu, et al indirectly cultured osteoblast and VEC using nest dish culture method, in which the mediums of the two kinds of cells contact with each other, while the two kinds of cells do not contact. Results show that the medium of VEC can enhance the proliferation of osteoblast. However, in this paper, VEC was directly co-cultured with osteoblast. We observed whether the cellular compatibility of the two kinds of cells was good or not; what's the impact on the osteogenic activity of the osteoblast.

From the experimental results, immunocytochemical stain of type I collagen and the vascular factor VIII identified the osteoblast for group and the VEC for group B. Observation under the inverted phase contrast microscope, HE staining and Masson trichrome staining showed that the two kinds of cells mixed grew well, and the cells were well distributed. There was no contact inhibition, rejection and phagocytosis, explaining that osteoblast and VEC are with good cellular compatibility.

Brighton et al and Katagiri et al find in the experiments that the epidermal cells, endothelial cells and osteoblast may be the bone progenitor cells, which can behavior the characteristics of osteoblast after culture. For example, they can synthesize ALP, osteocalcin and type I collagen. The internal environment of body is maintained by the synergy of a variety of cells. And in vitro environment, osteoblast and VEC might have effects of mutual synergies. Lu et al find that the TGF-β release system can significantly promote bone marrow stromal cell proliferation and differentiation to osteoblast. Moreover, the number of surface cells, the activity of ALP and the osteocalcin production was significantly higher than that of the control group that is without the system. The bone morphogenetic protein, vascular endothelial growth factor (VEGF) and tetracycline have similar regulation with TGF-β to osteoblast. VEC can synthesize and secrete VEGF, thus significantly promote the activity of ALP in osteoblast.

Seen from the test of ALP activity by paranitrobenzene phosphate method, the ALP activity of group A was obviously higher than that of group B ($P < 0.05$); while the ALP activity of group C was remarkable higher than that of groups A and B ($P < 0.01$). Results show that VEC can significantly enhance the ALP activity in osteoblast, it further explained that VEC could increase the function of osteoblast, including the proliferation of osteoblast, and could promote osteoblast to transform to bone cell. The results were consistent with that of Ref.. On the other hand, osteoblast can secrete VEGF, FGF and other pro-angiogenic factors, which affect VEC to promote angiogenesis. In the bone marrow hemopoietic microenvironment, osteoblast plays a central role by producing colony-stimulating factor to promote the regeneration of hematopoietic stem cells and VEC.

Results of the MTT test show that activity of VEC on the 1st day and the 3rd day was strong. It began to decline on the 5th day. However, osteoblast began to proliferate fast on the 5th days. The proliferation activity of co-culture cells was lower than osteoblast and VEC on the 1st and the 3rd day. From the 3rd day, the two types of cells have good cellular compatibility and started into the logarithmic growth phase, thus demonstrating strong proliferation activity. On the 5th days, the cell proliferation activity obviously accelerated, and has no significant difference with osteoblast and VEC. On the 7th day, the proliferation activity of osteoblast and co-culture cell was remarkably higher than that of VEC. Whether these were due to the reason that the two kinds of cell competed for living sites on the board from the 1st to the 3rd day after co-culture, which affected the cell proliferation activity. It needs to further study. The experiment in this paper showed that the co-culture cells proliferated slowly in the early stage and faster in the later stage. Osteoblast and the co-culture cells are with strong proliferation, the proliferation of VEC is comparatively weak, indicating strong potency of proliferation of co-culture cells.

The experimental results show that, there was good cellular compatibility between the osteoblast and VEC. VEC could increase the function of osteoblast, and promote the proliferation of osteoblast. The VEC cultured in vitro may help the revascularization of the tissue engineering bone. Thus, it can provide normal nutritional, and the regulation of nerves and body fluid. Therefore, the co-cultured cells between osteoblast and VEC are expected to become the seed cells of the tissue engineering bone.

4.5 Conclusion

The conclusion section should be an independent paragraph. A reader who choose to read onlt the conclusion must be able to reasonably understand the topic. The conclusion should start with a sentence or two that describe the experiment before concluding results.

1. There was good cellular compatibility between the osteoblast and the VEC.
2. The ossification activity of the osteoblast was significantly increased by the VEC.
3. The co-cultured cells had strong potency of proliferation.

5. Part II: Study the effect of heterogenous bone acellular matrix (HBACM) on the biological compatibility between the osteoblasts and the vascular endothelial cells

5.1 Introduction

Tissue engineering is a science that uses cell biology and engineering principles to research and develop biological alternatives for the repair and improvement of wounded tissue and function. The histocyte that is cultured in vitro and with high concentration is amplified and is absorbed in a biocompatible extracellular matrix (ECM) that is degradable and can be absorbed by human body. The seeded cells continue to grow and reproduce during the degradation and the absorption process in the scaffold, and develop to corresponding new tissue and organ with original special function and morphology, then the expected purpose of wounds reparation and reconstruction function was attained. In this experiment, the osteoblast and the vascular endothelial cell (VEC) were compounded by using the acellular rib of pig. Observe and study the biological compatibility. Thus it will provide the theological base for the clinical application of tissue engineering bone.

5.2 Materials and methods

5.2.1 Preparation for the framework material for HBACM

Fresh pork ribs were selected and the soft tissue around was removed. Then the soft tissue was made into skeletons in size of 2.0cm × 0.5cm × 0.5cm. They were treated as the following steps: (1) the skeletons were immersed in 15% of H_2O_2 in at 37 ℃ for four hours and were washed repeatedly Double-distilled water; (2) the skeletons in the upper step were immersed in 1% of Triton X-100-Tris-HCl solution (V/V) with protease inhibitors added in it, and was oscillated at 4 ℃ for 48 hours; (3) circulating flushed for 12 hours in PBS; (4) the skeletons in the upper step were immersed into chloroform/methanol solution (3:1) at 4 ℃ overnight; (5) immersed in 0.02% Tris / EDTA (pH 8.0) solution, and was oscillated at 4 ℃ for 48 hours; (6) washed with distilled water continuously for 48 hours, then the acellular

disposal completed (7) the skeletons were freeze-dried and sterilized with ethylene oxide, for use, and then observe the degree of acellular under scanning electron microscope.

5.2.2 Cell culture

The co-culture osteoblast and VEC, the identification of osteoblast, VEC and the co-cultured cells were the same as the former.

5.2.3 Compound of the cell with HBACM

Experimental groups were as follows:

Group A: osteoblast compound with HBACM;
Group B: VEC compound with HBACM;
Group C: osteoblast + VEC compound with HBACM.

5.2.4 The indexes of observation

5.2.4.1 Observation under the inverted phase contrast microscope Observe the adhesion of the cells with the material, the distribution of the cells in the pores of the material, and the growth, differentiation and proliferation of the cell.

5.2.4.2 Histological observation The three groups of cells were compounded and cultured with the materials for 5 days. After being fixed with alcohol and stained directly by Masson trichrome, they were observed under optical microscope. Three of the materials in each group were taken and embedded in paraffin after decalcification to determine on histological sections. Then observe the biological compatibility after HE stain, toluidine blue and Masson trichrome stain.

5.2.4.3 Observation under the scanning electron microscope The three groups of cells were compounded and cultured with the materials for 5 days. One of the compound materials in each group were taken and rinsed in PBS for thrice, fixed with 2.5% glutaraldehyde, dehydrated with alcohol gradiently, and then fixed in iso-amyl acetate. After dried and coated with gold, the samples were observed by using a scanning electron microscope (JSM-5900) to test the adhesion, growth, proliferation and the matrix secretion of the cells on the HBACM.

5.2.4.4 Test by flow cytometer The three groups of cells were compounded and cultured on the culture plate with HBACM. Three compounds of cellular material were selected individually from each group on the 1d,3d,5d and 7d. Then they were digested. Collect and count the cells. The cells were staining in PI dye and were detected using a flow cytometer (Coulter Corporation, U.S.) to analyze the effect of HBACM on on cell cycle and DNA content. Then the DNA index (DI values), DI = 1.0 ± 0.15 was calculated as normal diploid cells. Analyze the cell cycle and the ploidy by flow cytometer to test the toxicity of the material to the cell.

5.2.5 Statistical methods

The result of the experiment was expressed by mean±standard deviation. The analysis of variance and q test of the measurement data among groups were done by using SPSS 10.0 software packet.

5.3 Result

5.3.1 Observation of HBACM under scanning electron microscope

The structure of the bone trabecular of HBACM was integrated, the bone lacunas were empty, and no residual cellular components were found (Fig. 1).

5.3.2 Observation under the inverted phase contrast microscope

When the three groups of cells were co-cultured with HBACM for 12 hours, the cells could adhere and grow in the pores and on the surface of HBACM. With the culture time gone, grew, differentiate and proliferate along the edge of HBACM pore gradually. The cells gradually extended, stretched across the pores, and the pseudopodium tended to contact with each other from the 5th day co-cultured with HBACM (Fig. 2).

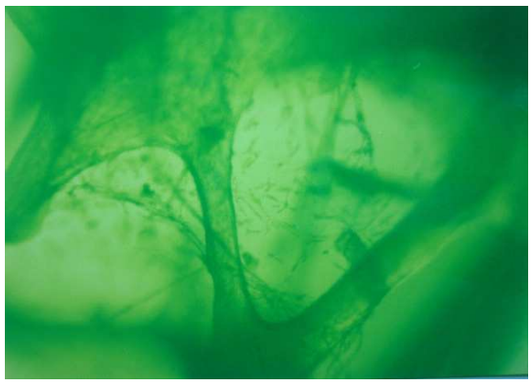


Fig. 1. Observation under the inverted phase contrast microscope (100×) when the osteoblast was co-cultured with materials for 5 days. The cells gradually extended, stretched across the pores.

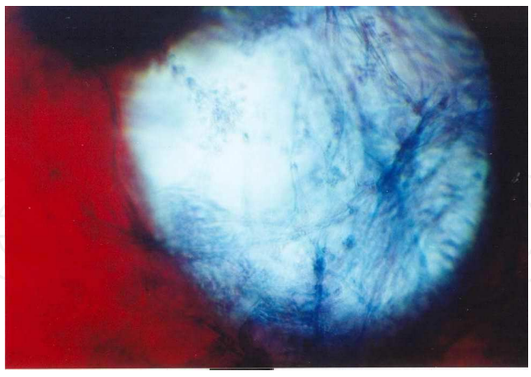


Fig. 2. Observation under the inverted phase contrastmicroscope (100×) after Masson staining for compound of the co-cultured cells and the material. The cells and the materials attached closely and connected into a network.

5.3.3 Histological observation

The HBACM of compound cell was directly Masson stained. Results show that the cells in each group tightly adhered with materials. They gathered into a group, connected into a

network, and there were a large number of proliferations of the cell on the surface and in the pores of HBACM. The number of cells adhesion on HBACM in osteoblast + VEC group was the maximum (Fig. 3). Stained histological sections showed that the cells were closely attached along the edge of materials, and the histocompatibility of cells and HBACM was good.

5.3.4 Scanning electron microscope results

After cultured with HBACM for five days, the cells were tightly adhered on the surface of HBACM. Osteoblast appeared spindle-shaped or polygonal, the adjacent cells connected to each other with processes (Fig. 4). VEC was oval-shaped, with angular protrusions, and was closely attached to the materials (Fig. 5). The co-cultured cells were showing their own form, and the two kinds of cells tightly combined with each other. The cell compatibility and compatibility between the cells and HBACM were good, with network collagen attached around (Fig. 6). A plenty of cells adhered on the surface of the materials of osteoblast + VEC group, and much collagen formed. However, less collagen formed on the surface of VEC group.

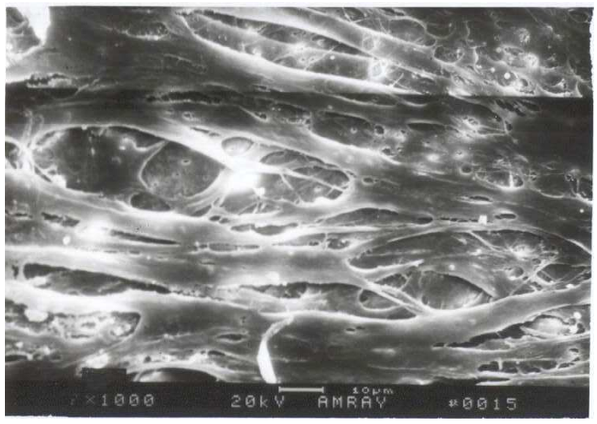


Fig. 3. The compound of osteoblast cells and the material (scanning electron microscope, 1000×)

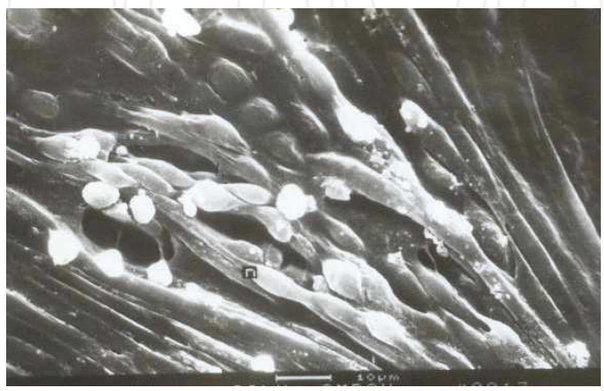


Fig. 4. VEC compound with the material (scanning electron microscope, 1200×)

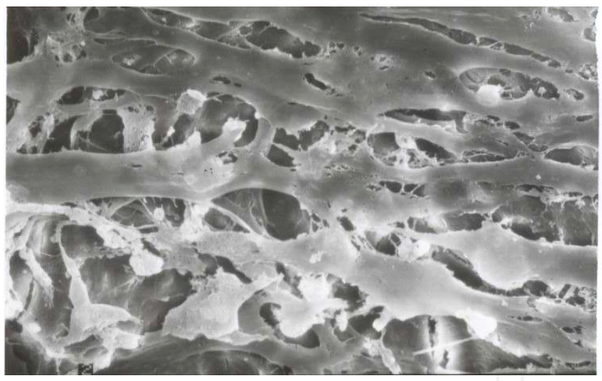


Fig. 5. The co-cultured cell compound with the material (scanning electron microscope, 1000×)

5.3.5 Test by flow cytometer

The three groups of cells were compounded and cultured with the material for one, three, five, seven days, respectively. The testing results of cell cycle and ploidy level are listed Table 1. The S phase cells of the co-cultured group on the 1st and the 3rd day was higher than that of the single cell group, no heteroploid cells were found. The effect of HBACM on the cell cycle and the ploidy of the three groups of cells are listed on Table 1.

Group / Cell cycle		Group A	Group B	Group C	Control group
Pre-synthesis stage of DNA (G_1, %)	1 day	67.4	71.5	57.9	68.8
	3 day	74.5	80.5	70.2	73.9
	5 day	67.1	66.3	68.2	66.7
	7 day	53.3	69.9	67.8	63.6
Synthesis stage of DNA (S, %)	1 day	24.6	18.7	29.5	20.6
	3 day	12.3	12.1	20.6	13.5
	5 day	19.1	15.7	19.7	17.8
	7 day	23.2	16.8	21.0	19.9
Pre-mitosis and mitosis (G_2/M, %)	1 day	8.0	9.8	12.7	10.6
	3 day	13.2	7.4	9.2	12.6
	5 day	13.8	18.0	12.1	15.5
	7 day	23.5	13.3	11.3	16.5
DNA content of G_1 stage	1 day	93.0	100.4	101.9	98.2
	3 day	97.5	91.5	105.6	96.8
	5 day	119.7	108.8	112.3	113.7
	7 day	118.2	117.6	122.4	119.8
DNA index	1 day	0.95	1.02	1.04	
	3 day	1.01	0.95	1.09	
	5 day	1.05	0.96	0.99	
	7 day	0.99	0.98	1.02	

Table 1. The influence of the HBACM to the cell and DNA index of three group cells

5.4 Discussion

As a kind of artificial extracellular matrix (ECM), scaffolds provide three-dimensional space for the growth, reproduction and metabolism of the cells. The biocompatible of scaffold directly affects the adhesion, growth and proliferation of the cell. Different kinds of ECM are widely used nowadays, they are: biodegradable polymer; ceramic material; composite materials and natural extracellular matrix material (NECM).

Heterogeneous bones, which are obtained from animals such as pigs and cattle, have a rich resource and lower cost, and are easy to access and process. Moreover, the bone tissue retains the dense pore structure naturally and is with good effect of scaffold, which provides three-dimensional space for the proliferation, differentiation and the osteogenesis of the cells. At the same time, the bone tissue is with high bone induction activity and degradation rate matched well with the growth rate of new bone. Thus, it has great potential for developing as bone substitute material. However, if the untreated heterogeneous bones were implanted into human body, there will be a strong immune rejection. Hence, a variety of physical, chemical treatments are used to eliminate the antigen as far as possible, and simultaneously retain the useful biologically active substances and its bone induction activity, as well as its mechanical strength. Those are also the research focus of the heterogeneous bones tissue engineering transplantation. This study focused on the transplantation of the heterogeneous bones and the elimination of the cells antigen, and then used the heterogeneous bones as a superior scaffold for bone tissue engineering.

5.4.1 Biological compatibility of HBACM

Bone extracellular matrix is the intercellular substance of calcified bone tissue, and is consist by organic and inorganic ingredients. The organic ingredients are mainly: collagen fibers and some amorphous material, which are both the secretion of bone cells. Inorganic components are mainly calcium salt, which exist mostly as hydroxyapatite crystals. Low-permeability method was selected to produce HBACM in this experiment. The cells were burst and the membrane structure was damaged. Other methods were also used to extract the cell membrane, cytoplasm, organelles and other ingredients. Only the cell was extracted, and the inorganic and organic ingredients were retained. Results show that the cells of the three groups could be attached to the HBACM and grew. The reasons maybe as follows: the natural pore system was not significantly damaged; the original trabecular bone, the trabecular bone gap and the lumen system was retained; the three-dimensional structure of the bone salt still existed. This natural structure was conducive to cell adhesion, growth and provide generous interior space and surface for the secretion of ECM. Therefore, the acellular bone matrix showed excellent biocompatibility.

5.4.2 Antigenicity of HBACM

It is generally believed that transplantation antigen of heterogeneous bones present in bone cells, hematopoietic cells, white blood cells, red blood cells, plasma, blood vessels and bone matrix. To transplant heterogeneous bones, we should reduce or eliminate the antigenicity of transplanted bones. After a series of physical and chemical treatment, the rib of pig was made as HBACM, which did not contain cells under the scanning electronic

microscopy. Thus, it was of low antigenicity. Observation from the inverted phase contrast microscope, the three groups of cells grew along the edge of the pores of the material. The cells gradually extended, stretched across the pores, and the pseudopodium tended to contact with each other, they gradually grew, differentiated and proliferated. Stained histological sections and the result of scanning electron microscopy showed that the cells were closely attached, gathered into a group, and connected into a network. These indicated that the compatibility between cells and the compatibility between cells and HBACM were good, without any mutual exclusiveness. These further explained that there were none or extremely low antigenicity when using this method to prepare acellular bone matrix.

5.4.3 Cytotoxicity of HBACM

Flow cytometry could be used to determine DNA content and proliferation activity of the cells, and could analyse the cell cycle and the ploidy level. When the body suffers from cancer or precancerous lesions with malignant tendency, the DNA content of the cells changes abnormally, leading DNA aneuploid. Flow cytometry can detect aneuploid cells by measuring DNA content, which has been an effective tool making cancer for the diagnosis of tumor cytology. In this study, flow cytometry was used to determine the ploidy level and the DNA content of the three groups of cells that co-cultrued with acellular matrix scaffold in vitro. No aneuploid cells were found, indicating the scaffolds were without cytotoxicity and tumorigenesis. We could see from the result of cell cycle analysis that the cells of each group had normal growth cycle in the acellular matrix scaffolds. The S phase of the co-cultured group on the 1st and the 3rd day was higher than that of the single cell group, indicating high proliferation of the cells on scaffold materials when osteoblast was co-cultured with VEC.

With the research and development of biodegradable scaffolds with three-dimensional and multi-space structure, the bioactive bone transplantation materials formed by the three kinds of cells and acellular matrix bone shows a broad application prospects.

5.5 Conclusion

It is preferred not to use abreviations in the conclusionsor at least use the fuul description once, at the first conclusion.

1. Heterogenous bone acellular matrix has good biological compatibility.
2. Heterogenous bone acellular matrix was with low antigenicity.
3. Heterogenous bone acellular matrix had no cytotoxity or neoplastia.
4. The co-cutured cells had strong potency of proliferation. heterogenous bone acellular matrix

6. Part III: Implanting the compound cell of heterogenous bone acellular matrix in vivo: An animal study

6.1 Introduction

Autografting and isotype heterogenous bone grafting have been widely used in clinic for many years. Autografting is the best method of bone graft because of the bioactive

molecules, living cells, and blood supply in the grafted tissue. However, the bone source and the site for supplying bone were limited in autografting, meanwhile it is difficult in the modeling of self-tissue, and therefore its clinical application is limited. There is certain immunologic rejection in the isotype heterogenous bone grafting, which worried many people. At the same time, the bone source is limited. Since the 1980s, with the rapid development of cell culture technology, tissue engineering bone provides a new way for solving this problem. In this study, heterogenic acellular extracellular matrix was compound with cell to construct tissue engineering bone, which was implanted into the animal. Then the bone defect repairing was observed.

6.2 Materials and methods

6.2.1 Mark the cell with BrdU (5 - bromodeoxyuridine)-labeled cells

400 μL of BrdU was added into 200 mL of serum-free F_{12} culture medium. 35 bottles of 3 generations and 5 generations of rabbit osteoblast, VEC and the co-cultured cells were taken with the culture medium being discarded, and then, the serum-free F_{12} culture medium marked by BrdU were added (6mL per bottle). The bottles were kept in the cell incubator at 37 ℃ with 5% of CO_2 and saturated humidity for one hour. After that, the supernatant was discarded and the cells were digested and collected, which was washed for thrice with culture medium to remove BrdU that had not incorporated cells.

6.2.2 The compound cell of heterogenous bone acellular matrix

30 blocks compound cell of heterogenous bone acellular matrix (pork ribs) with the size of 2.0cm × 0.5cm × 0.5cm were divided into three groups (10 blocks each group). Group A was compound with osteoblast, Group B was compound with VEC, and the Group C was compound with co-cultured cells. The three groups of cells marked with BrdU were inoculated with the density of 6×10^5 cells per block into HBACM, respectively. One block of each group was taken for in vitro testing, and the remaining 27 blocks were used for implanting into the animal body.

6.2.3 Animal experiments

6.2.3.1 Animal grouping 27 New Zealand big-ear rabbits weighted 2.5 kilogram were selected and they were grouped into three groups with nine in each group. Groups of A, B, C were HBACM compound with osteoblast, VEC and co-cultured cells marked by BrdU. Animal grouping is listed as follows.

Time Group	3rd week	6th week	12th week	Total
osteoblast group	3	3	3	9
VEC group	3	3	3	9
Co-cultured cells	3	3	3	9
Total	9	9	9	27

Table 1. Animal grouping of the New Zealand rabbits

6.2.3.2 Preparation for animal model of bone defect Resect the 1.5cm long radius of the double forelimbs of the rabbit and prepare animal model of bone defect. The tissue engineering bone which had been compounded and cultured for one week by the heterogenous bone acellular matrix and the cells of each group was implanted to the left side. The heterogenous bone acellular matrix was implanted to the right side as a control. Select the material at 3 weeks, 6 weeks and 12 weeks to observe bone fracture.

6.2.4 The index of observation

6.2.4.1 Observation of gross morphology Three, six and 12 weeks after the operation, three animals in each group were sacrificed and the radius were dissected. Observe the development and the prognosis of the tissue engineering bone at various phases by naked eye.

6.2.4.2 X-ray examination of bone defect After three, six, and 12 weeks of the operation, samples were taken, respectively. The X-ray (CR) radiography was used to observe the healing of bone defects, and in order to understand the conjugation of the implanted tissue engineering bone and the peripheral normal radius, also to understand the formation, change and moulding of the callus at the site with bone defect.

6.2.4.3 Trace and test the BrdU marked cells The paraffin sections was prepared in three and six weeks, respectively, for BrdU immunohistochemistry stain, to detect BrdU marked cells, then to understand the survival of seed cell in the experimental animal. After HBACM and BrdU marked cells was co-cultured in vitro for one week, one tissue engineering bone in each group was taken for the production of paraffin section, in order to detect the compound cell of heterogenous bone acellular matrix in vitro.

6.2.4.4 Routine histological examination About 1.5 cm of the samples was selected from three experimental animals at various phases. The samples were taken with the implanted bone tissue engineering as the center and some normal bone on both ends. After fixed, decalcified, dehydrated and embedded, the samples were made into paraffin sections for HE, toluidine blue, Masson staining.

Pathological changes (such as growth and differentiation of the cells in vivo, inflammatory cell infiltration, degradation of the scaffold, rebuilding and remodeling of callus, recanalization of canal, and the revascularization) of HBACM compound with the cells in each group were observed under optical microscope.

6.2.4.5 Immunohistochemistry of type I collagen The paraffin sections of the sixth weeks was taken for type I collagen immunohistochemistry stain, to understand the secretion of type I collagen by each group of cells at various phase.

6.2.4.6 Image analysis Masson stains at the 3rd and the 6th week were selected and the border between the tissue engineering bone and the normal bone was detected by an image analyzer to measure the area of the vessel in each unit. The revascularization of tissue engineering bone in each group was observed.

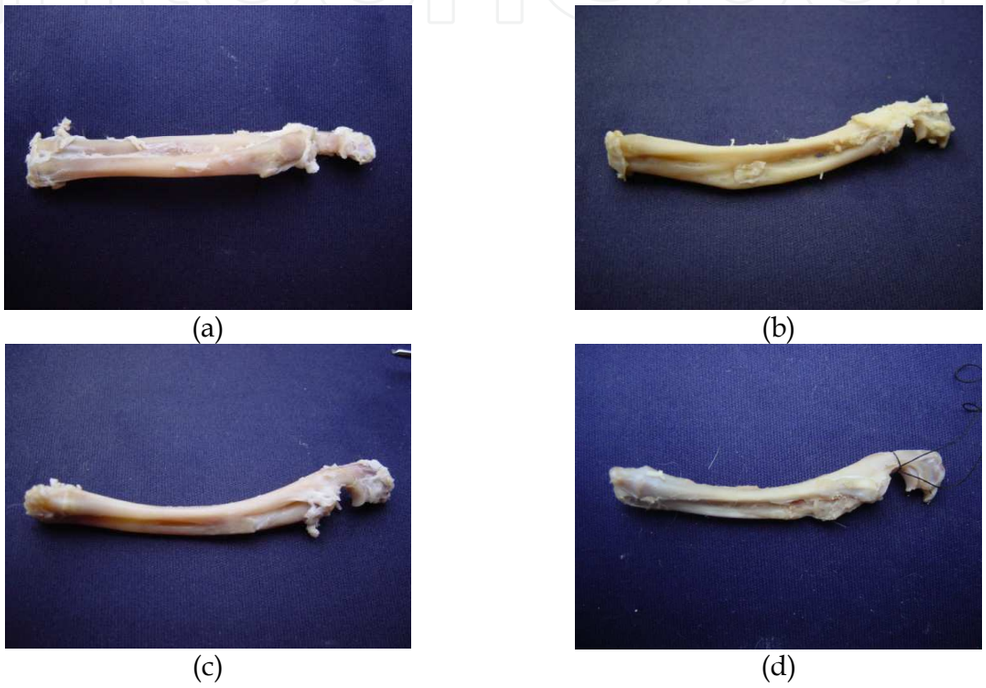
6.2.5 Statistics method

The result of the experiment was expressed by mean±standard deviation. The analysis of variance and q test of the measurement data among groups were done by using SPSS 10.0 software packet.

6.3 Result

6.3.1 Observation of gross morphology

The samples were taken and observed at the 3rd, 6th, and 12th week, respectively. Results show that the speed and degree of repairing the bone defect by tissue engineering bone from high to low in order were: that of the co-cultured cell group, that of the osteoblast group, that of the VEC group and that of the simple framework material group (Figs. 1a, 1b, 1c, and 1d).



(a) (b)

(c) (d)

Fig. 1. Observation of gross morphology in the 6th week: (a) osteoblast group, (b) VEC group, (c) co-cultured cell group, (d) simple framework material group

6.3.2 X-ray examination of bone defects

We could see from the X-ray examination that the speed and degree of repairing the bone defect by tissue engineering bone from high to low in order were: that of the co-cultured cell group, that of the osteoblast group, that of the VEC group and that of the simple framework material group (Figs. 2a, 2b, 2c, and 2d).

6.3.3 Trace and test the BrdU marked cells

One week after compound in vitro, we could see from the immunohistochemical staining result that cells compound and grew well with HBACM, the cells attached to the pores of HBACM, closely adhere to the edge of HBACM and grew (Fig. 3). When the bone was implanted for three weeks and six weeks, the marked cells could still be detected in HBACM in vitro, and the new cartilage could also be observed (Figure 4).

6.3.4 Routine histological examination

In the 3rd week, a large number of cartilages formed in tissue engineering bone. On the juncture with the normal bone, the formation of cartilage zone could be seen. osteoblast and osteoclasts coexisted in the pores of HBACM, in which the capillary grew (Fig. 5). In the 6th weeks, the pores in HBACM gradually grew confluent, the blood vessel increased in the pores, and the cartilage cells gradually transformed to bone cells (Fig. 6). In the 12th weeks, the marrow between tissue engineering bone and normal bone recanalized, and the fracture healed (Fig. 7).

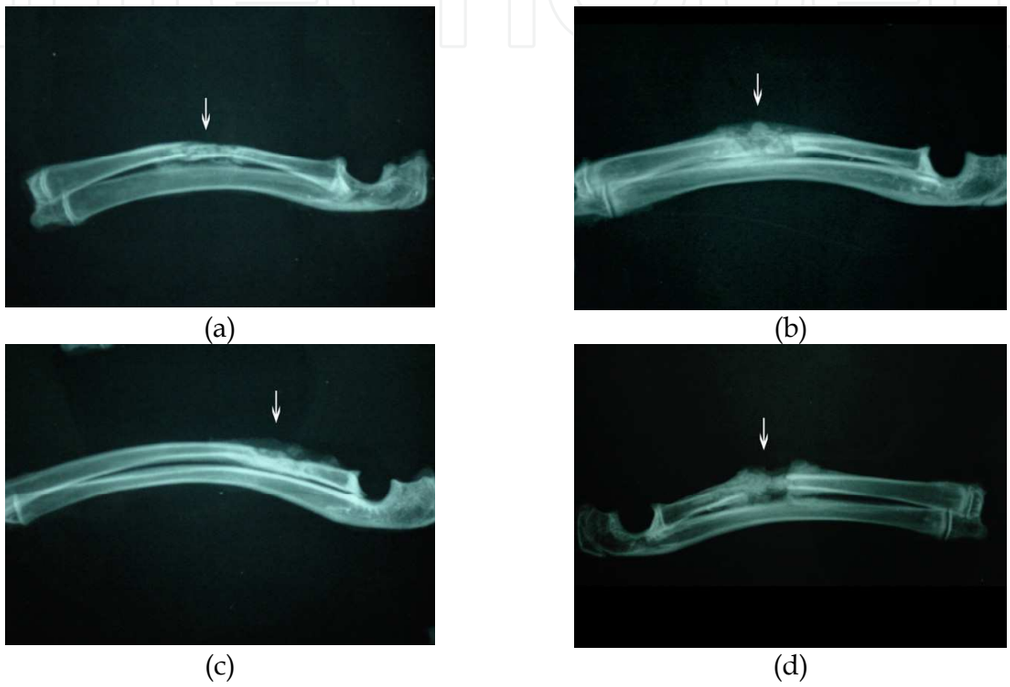


Fig. 2. X-ray examination in the 3rd week: (a) osteoblast group, (b) VEC group, (c) co-cultured cell group, (d) simple framework material group

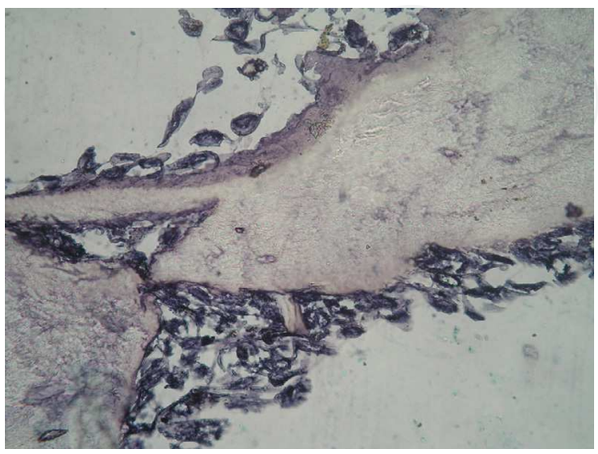


Fig. 3. BrdU immunohistochemical staining (400×) osteoblast closely adhere to the edge of HBACM and grew.

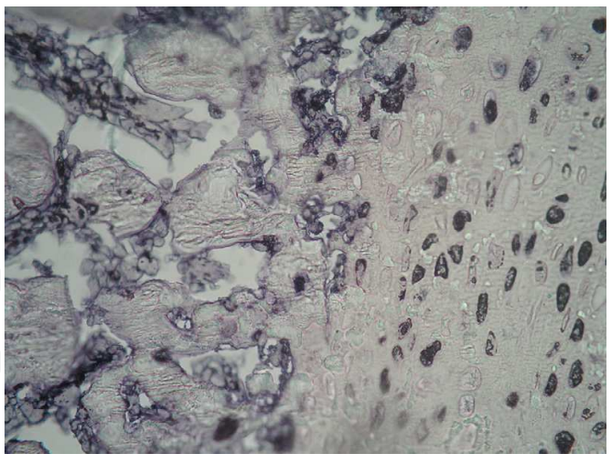


Fig. 4. BrdU immunohistochemical staining (400×). When the bone was implanted for three weeks, marked cells could still be detected in HBACM, and the new cartilage could also be observed.

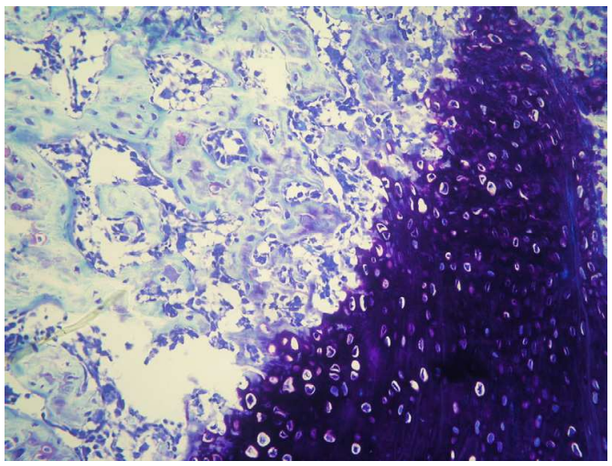


Fig. 5. Toluidine blue in the 3rd week (200×) On the juncture with the normal bone, the grewformation of cartilage zone could be seen in the pores. osteoblast and osteoclasts coexisted in the pores.

6.3.5 Immunohistochemistry of type I collagen

After the three groups of cells compound with HBACM were implanted in vivo, type I collagen was expressed (Fig. 8), while VEC group was lower expressed.

6.3.6 Image analysis

The results of image analysis in the 3rd week were as follows. osteoblast group (41.20 ± 7.37), VEC group (47.54 ± 5.71), co-cultured cell group (50.54 ± 3.86), co-cultured cells group> osteoblast group, the difference was statistically significant ($P < 0.05$). In the 6th weeks, osteoblast group (28.52 ± 6.52), VEC group (50.24 ± 10.11), co-cultured cell group (66.98 ± 7.72), co-cultured cell group> VEC group> osteoblast group, the difference was statistically significant ($P < 0.01$).

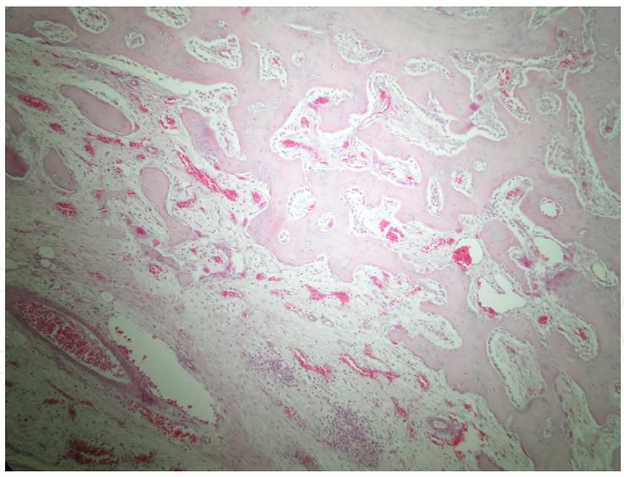


Fig. 6. HE stain in the 6th week (100×). In the 6th weeks, the pores in HBACM gradually confluent, the blood vessel increased of HBACM.

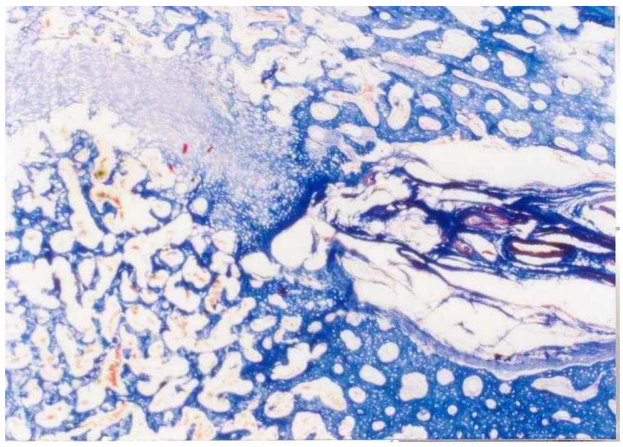


Fig. 7. In the 12th weeks, medullary cavity formed in the tissue engineering bone.

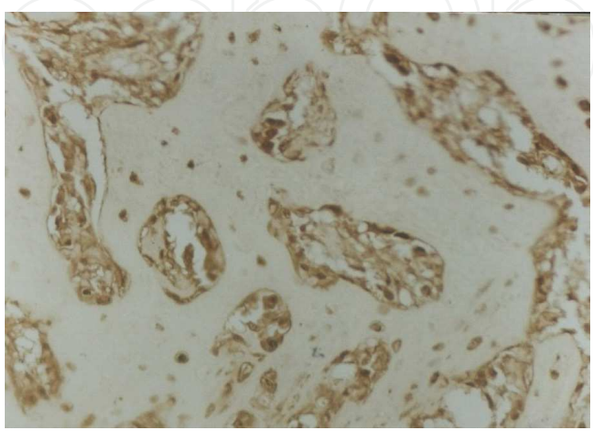


Fig. 8. Immunohistochemical of type I collagen in the 6th week

6.4 Discussion

As the research development of cell biology, molecular biology, and biological engineering and materials science, tissue engineering was born as a new discipline. "Tissue engineering" was put forward in 1987 by the National Science Foundation (USA), which refers to the science that uses cell biology and engineering principles to research and develop biological alternatives for the repair and improvement of wounded tissue and function. The basic premise of modern tissue engineering is to guide the tissue repair and regeneration by the application and the control of cell tissue and the micro-environment. It is a thoroughly new therapeutic mode to repair bone defects by using the methods and tools of tissue engineering, which has broad application prospects. With the rapid development of the current research of bone tissue engineering, tissue engineering bone has become one of the most promising tissue engineering achievements that will be come into use in clinical applications, which will lead a revolutionary change in the treatment of the fracture healing and the bone defects caused by trauma, tumor resection and malformation correction.

6.4.1 Trace and test the Brdu marked cells

The structure of BrdU is similar with thymidine, it is characteristic is that the methyl, which connects the fifth carbon atom and pyrimidine ring of phase Ⅲ in thymine, is substituted by bromide atom. During the synthesis process, BrdU can specifically incorporate into DNA at S phase, which is the same as thymine. Simultaneously, there is no cross-reaction between BrdU and thymine, and it is without radioactive material contamination. Therefore, BrdU has been used to mark cells in both in vivo and in vitro, and has been widely applied into the research of cell kinetics, as its high accuracy, rapidity and safety for marking cells. Generally speaking, it can be detected in vivo within eight weeks, and will not be longer than 12 weeks. In this study, BrdU was used to mark cells that cultured in vitro. After compound the marked cells with HBACM, in vitro test and in vivo test for implantation at the 3rd week and at the 6th week were done. Results show that cells compound and grew well with HBACM, the cells attached to the pores of HBACM, closely adhere to the edge of HBACM and grew. When the bone was implanted for three weeks and six weeks, the marked cells cultured in vitro could still be detected in HBACM, and the new cartilage could also be observed. These indicated that the histocompatibility between HBACM and cells was good; and it further indicated that the major ossification of the tissue engineering bone in the body was indeed due to the cells cultured in vitro.

6.4.2 Pathological process of bone tissue engineering repairing bone defects

Fracture healing is a complex process, which is continuous and is the result of the mutual synergistic effect between osteoblast and osteoclasts. The research results in this experiment showed the pathological process after the tissue engineering bone was implanted into animal body. The endochondral bone formation is the main effect during the fracture healing process. Under the effect of osteoblast, HBACM gradually osteoblasted outward; gradually transformed the fibrous tissue among the fracture ends and the pores of HBACM into cartilage. Then the pores grew confluent. At the same time, the surface of bone trabecula in HBACM began to change into small cavities, which gradually interconnected to form larger cavities. Vascular formed, the chondrocyte gradually proliferated, calcified and

ossified in the larger cavities. The fracture healing process completed when the larger cavities grew confluent and recanalized with autogenous marrow cavity.

6.4.3 Comparison of the osteogenic ability of bone tissue engineering in vivo

Type I collagen is the major extracellular matrix secreted by osteoblast. It is expressed from the beginning of cell proliferation, and achieved to the maximum at synthesis stage of matrix. The results showed that after the three groups of cells compound with HBACM were implanted in vivo, type I collagen was expressed (Fig. 8), while VEC group was lower expressed. From the gross specimen and X-ray results, we could see that the speed and degree of repairing the bone defect by tissue engineering bone from high to low in order were: that of the co-cultured cell group, that of the osteoblast group, that of the VEC group and that of the simple framework material group. These indicated that fracture healing could be accelerated and the repairing of bone defect could be promoted by using the co-cultured cell as seed cell. Simultaneously, the simple framework material group could also achieve fracture healing, which indicated that the HBACM might contain bone morphogenetic protein, with a strong cross-species induction of osteogenic activity; namely, induced undifferentiated mesenchymal cells differentiate into cartilage or bone, its effect was non-species-specific and could repair bone defects.

6.4.4 Comparison of revascularization ability of bone tissue engineering in vivo

There has been a significant progress in the research of bone tissue engineering. However, with the experiment object becoming large-scale, the research of bone tissue engineering bone is facing the key problem -- rapid vascularization. Like other types of transplanted bone, adequate blood supply is the decisive factors to ensure tissue engineering bone to survive in vivo. Current research on the reconstruction of the blood supply in the new tissue engineering bone is still at the initial stage. The main methods are as follow: (1) combined transplantation of VEC and osteoblast (2) the use of VEGF to promote the growth of blood vessel (3) the application of microsurgical techniques in the revascularization of tissue engineering bone, including ①osteoblast + biomaterials + tissue flap with vascular pedicle embedded; ②osteoblast + biomaterials + vascular bundle implantation. Zhengfu Fan, et al prepared a deep facial flap animal model with a nameless vascular pedicle in forelimb. When the facial flap was applied to cover the biomaterials of compound cells to repair the 1.5 cm of radial bone defect, the speed of revascularization was obviously higher than that of the control group without facial flap after the repairing of bone defect was finished. Casabona, et al further designed a bio-engineered prefabricated flap. Human bone marrow stromal cells which were cultured for two weeks were delivered into the latissimus dorsi of athymic mice by a porous hydroxyapatite ceramic model. Eight weeks after the implantation, histologic examination revealed the presence of spongious bone tissue with rich blood supply. A simple myocutaneous flap was thus transformed into a composite osteomyocutaneous flap. Although the flap can cover any area or any known blood vessels, and had the possibility of preshaping the graft to the exact characteristics of the defect. However, two operations should be taken in vivo. The image analysis showed that the number of revascularization of tissue engineering in co-cultured cells group in the body in the 3rd week was higher than that of the osteoblast group, the difference was statistically significant ($P < 0.05$). In the 6th week, the number of revascularization from high to low in order was: that of the co-cultured cells group, that of the

VEC group, that of the osteoblast group, and the difference was statistically significant ($P < 0.01$). The combined transplantation of VEC and osteoblast were used in this experiment and the same result was obtained, which made the new bone tissue engineered bone to entirely replace the autogenous bone graft entirely possible, and provide a excellent experience for tissue engineered bone to be implanted in vivo.

It can be seen, the activity of osteoblast could be enhanced, the fracture healing could be accelerated, the repairing of bone defect could be promoted and the revascularization of tissue engineering bone could be improved by using the co-cultured cell osteoblast and VEC as seed cell. There was mutual synergistic action in vitro and mutual promoted effects in vivo between the osteoblast and the VEC

6.5 Conclusion

This in vivo animal study suggests that:

1. The osteogenesis process of the regenerating bone was the formation of endochondral bone regeneration.
2. The major ossification of the regenerationg bone occurred to the cells cultured in vitro.
3. There was synergistic action between the osteoblast and the VEC whether in vivo or in vitro.
4. Fracture healing could be accelerated and the repairing of bone defect could be promoted by using the co-cultured cell as seed cell.

7. References

[1] Enncking WF, Campanacci DA. Retrieved human allografts: a clinical- pathological study [J]. J Bone Joint Surg (Am), 2001, 83: 971-986.

[2] Boone DW. Complications of iliac crest graft and bone grafting alternatives in foot and ankle surgery [J]. Foot Ankle Clin, 2003, 8: 1-14.

[3] Xiao Y, Mareddy S, Crawford R. Clonal characterization of bone marrow derived stem cells and their application for bone regeneration [J]. Int J Oral Sci, 2010, 2(3): 127-135.

[4] Prioleau MN,Gendron MC,Hyrien O.Replication of the chicken beta-globin locus:early-firing origins at the 5'HS4 insulator and the rho-and beta A-globin genes show opposite epigenetic modifications [J]. Mol Cell Biol, 2003, 23: 3536-3549.

[5] Hakeda Y.Action of glucocortieoid on bone-forming and bone-resorbing cells [J]. Clin Calcium, 2006, 16: 1817-1822.

[6] Grishchuk EL, Ataullakhanov FI. In vitro assays to study the tracking of shortening microtubule ends and to measure associated forces [J]. Methods Cell Biol, 2010, 95: 657-676.

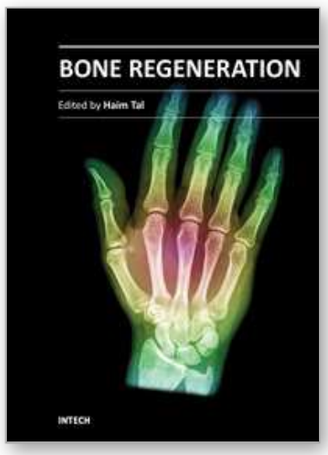
[7] Okada E, Yamanaka M, Ishikawa O. New insights into the mechanism of abnormal calcification in nephrogenic systemic fibrosis-gadolinium promotes calcium deposition of mesenchymal stem cells and dermal fibroblasts [J]. J Dermatol Sci, 2011, 62(1): 58-63.

[8] Isaac J, Nohra J, Lao J, Jallot E, et al. Effects of strontium-doped bioactive glass on the differentiation of cultured osteogenic cells [J]. Eur Cell Mater, 2011, 21: 130-143.

[9] Zhiming Yang, Zhengfu Fan, Huiqi Xie, et al. Vascularization and its relationship to osteogenesis in transplantation of tissue engineered bone to repair seg ment defect of long bone [J]. Chinese Journal of Microsurgery. 2002; 25 (2): 119
[10] Takahashi N.Bone and bone related bioehemieal examinations.Bone and collagen related metabolites.Regulatory mechanisms of osteoclast differentiation and function [J]. Clin Calcium, 2006, 16: 940-947.
[11] Tao Sheng-xiang, Chen Zhen-guang, Xie Yun, et al. An experimental study on revascularization after vascularized bone allograft [J]. Chinese Journal of Microsurgery, 2008, (2):119-121.
[12] Bishop AT, Pelzer M. Vascularized bone allotransplantation: current state and implications for future reconstructive surgery [J]. Orthop Clin North Am, 2007, 38:109 - 122.
[13] Weinan Zhu, Zhiming Yang, Xiuqun Li, et al. Experimental study on rabbit periosteal osteoblasts and renal vascular endothelial cells indirect co-culture in vitro [J]. Chinese Journal of Reparative and Reconstructive Surgery. 2002; 16(5): 307
[14] Rui gang, Wang Zhesheng, Lv Gang, et al. Study on biological behavior of osteoblast and vascular endothelial cell cultuer [J]. Chinese Journal of Reparative and Reconstructive Surgery. 2004, 18 (4): 327-330
[15] Brighton CT, Lorich DG, Kupcha R, et al. The pericyte as a possible osteoblast progenitor cell [J]. Clin Orthop, 1992, (275): 287
[16] Katagiri T, Yamaguchi A, Komaki M, et al. Bone morphogenetic progenetic protein-2 converts the differentiation pathway of C2C12 myoblasts into the osteoblast lineage [J]. J Cell Biol, 1994, 127(6 Pt 1): 1755-1766
[17] Lu L, yaszemski MJ, Mikos AG. TGF-betal release from biodegradable polymer microparticles: its effects on marrow stromal osteoblast function [J]. J Bone Joint Surg Am, 2001; 83-A Suppl 1(Pt 2): S82
[18] Oldham JB, Lu L, Zhu X, et al. Biological ctivity of rhBMP-2 released from PLGA microspheres [J]. J Biomech Eng, 2000; 122(3): 289
[19] Murphy WL, Peters MC, Khon DH, et al. Sustained release of vascular endothelial growth factor from mineralized poly(lactide-co-glycolide) scaffolds for tissue engineering. Biomaterials [J], 2000; 21(24): 2521
[20] Schwartz Z, Lohmann CH, Wieland M, et al. Osteoblast proliferation and differentiation on dentin slices are modulated by pretreatment of the surface with tetracycline or osteoblasts [J]. J Periodontol, 2000; 71(4): 586
[21] Zhiming Yang. Basis of tissue engineering and clinic [M]. Chengdu: Sichuan science and technology press, 2000: 134-134
[22] Winn SR, Hollinger JO. An osteogenic cell culture system to evaluate the cytocompatibility of Osteo-et, a calcium sulfatebone void filler [J]. Biomaterials. 2000; 21: 2413-2425.
[23] Burg KJL, Porter S, Kellam JF. Biomaterial developments for bone tissue engineering [J]. Biomaterials, 2000; 21: 2347-2359.
[24] Grane GM,Lshaug SL,Mikos AG,et al. Bone tissue engineering [J]. Nature Medicine. 1995, 1(12): 1322-1324
[25] Chen VJ, Ma PX. The effect of surface area on the degradationrate of nano-fibrous poly (L-lactic acid) foams [J]. Biomaterials, 2006, 27(20):3708-3715.
[26] Cheung AY, Lau KT, Lu TP, et al. A critical review on polymer-based bio-engineerd materials for scaffold development [J]. Composites: Part B 2007;38:291-300.

[27] Wiria FE, Leong KF, Chua CK,et al. Poly-epsilon-caprolactone/hydroxyapatite for tissue engineering scaffold fabrication via selective laser sintering [J]. Acta Biomater. 2007; 3(1): 1-12.
[28] Zhiming Yang. Tissue engineering and Experiment Surgery [J]. Chinese Journal of Experiment Surgery, 2002, 19(2): 103-104
[29] Linbo Wu, Jiandong Ding. Advances in Fabrication Methodology and Technology of Three-Dimension Porous Scaffold for Tissue Engineering [J]. Journal of Functional Polymers, 2003, 16(1): 91-96.
[30] Hillsley MV, Frangos JA. Reviews:Bone tissue engineering: The role of interstitial fluid flow [J]. Biotechnology and Bioengineering, 1994, 43: 573-581.
[31] Suzuki M, Guimaraes MV, Marin C, et al. Histomorphometric evaluation of alumina-blasted/acid-etched and thin ion beam-deposited bioceramic surfaces: an experimental study in dogs [J]. J Oral Maxillofac Surg. 2009; 67(3): 602-607.
[32] Mikos AG, Sarakinos G, Leite SM, et al. Lamimated three-dimensional biodegradable foams for use in tissue engineering [J]. Biomaterials, 1993, 14: 323-330
[33] Cheung HY, Lau KT, Lu TP, et al. A critical review on polymer-based bio-engineerd materials for scaffold development [J]. Composites: Part B 2007; 38: 291-300.
[34] Daculsi G, Goyenvalle E, Cognet R, et al. Osteoconductive properties of poly (96L/4D-lactide)/beta-tricalcium phosphate in long term animal model [J]. Biomaterials. 2011, 32(12): 3166-77.
[35] Sirin Y, Olgac V, Dogru-Abbasoglu S, et al. The influence of hyperbaric oxygen treatment on the healing of experimental defects filled with different bone graft substitutes [J]. Int J Med Sci. 2011, 8(2):114-25.
[36] Ginebra MP, Espanol M, Montufar EB,et al. New processing approaches in calcium phosphate cements and their applications in regenerative medicine [J]. Acta Biomater. 2010, 6(8): 2863-2873.
[37] Di Sha, Yingling Jiang, Jingheng Gao, et al. Preparation of porcine long bone acellular bone extracellular matrix (ABECM) and preliminary measurement of its biomechanical and histological quality [J]. Chinese Journal of Practical Aesthetic and Plastic Surgery. 2000, 12, 11 (6): 331-334
[38] Brown KLB, Montreal M. Bone and cartilage transplantation in orthopedic surgery [J]. J Bone Joint Surg (Am). 1982; 64: 270-279
[39] Isaac J, Nohra J, Lao J, Jallot E, et al. Effects of strontium-doped bioactive glass on the differentiation of cultured osteogenic cells [J]. Eur Cell Mater, 2011, 21: 130-143.
[40] Merkel De, Mccuire WL, Ploidy, Proliferation activity and prognosis DNA flow cytometry of solid tumors [J]. J Cancer, 1990, 65(5); 1194-1205
[41] Shan Lin, Qingshui Yin, Yu Zhang, et al. Prepartion and cytotoxicity evaluation of the digital coral hydroxyapatite artifical bone in vitro [J]. Chinese Journal of Bone and Joint Injury, 2011, 26 (2): 23-26.
[42] Shan Lin, Qingshui Yin, Yu Zhang, et al. Prepartion and Characteristics of digital coral hydroxyapatite artifical bone [J]. The Orthopedic Journal of China, 2010, (24): 2039-2041.
[43] Ewers R, Goriwoda W, Schopper C, et al. Histologic findings at augmented bone areas supplied with two different bone substitutematerials combined with sinus floor lifting [J]. Report of one case: Clin Oral Implants Res, 2004, 15(1): 96-100.
[44] Khan SN, Cammisa FP Jr, Sandhu HS, et al. The biology of bone grafting [J]. J Am Acad Orthop Surg, 2005, 13: 77-86.

[45] Zijderveld SA, ten Bruggenkate CM, van Den Bergh JP, et al. Fractures of the iliac crest after split-thickness bone grafting for preprosthetic surgery: report of 3 cases and review of the literature [J]. J Oral Maxillofac Surg, 2004, 62: 781-786.
[46] Shan Lin, Qingshui Yin, Yu Zhang, et al. Prepartion and cytotoxicity evaluation of the digital coral hydroxyapatite artifical bone in vitro [J]. Chinese Journal of Bone and Joint Injury, 2011, 26 (2): 23-26.
[47] Shan Lin, Qingshui Yin, Yu Zhang, et al. Prepartion and Characteristics of digital coral hydroxyapatite artifical bone [J]. The Orthopedic Journal of China, 2010, (24): 2039-2041.
[48] Taboas J M, Maddox P H,et al. Indirect Solid Free Form Fabrication of Local and Global Porous, Biomimetic and Composite 3D Polymer-ceramic Scaffolds [J]. Biomaterials, 2003, 24: 181-194.
[49] Ginebra MP, Espanol M, Montufar EB,et al. New processing approaches in calcium phosphate cements and their applications in regenerative medicine [J]. Acta Biomater. 2010, 6(8): 2863-2873.
[50] Mistry S, Kundu D, Datta S, et al. Comparison of bioactive glass coated and hydroxyapatite coated titanium dental implants in the human jaw bone [J]. Aust Dent J. 2011; 56(1): 68-75.
[51] Yunyu Hu, Prospection of repair of osteoarticular injury [J]. J Trauma Surg. 2000, 3 (2): 129-130
[52] Kuo YC, Yeh CF. Effect of surface-modified collagen on the adhesion, biocompatibility and differentiation of bone marrow stromal cells in poly(lactide-co-glycolide)/chitosan scaffolds [J]. Colloids Surf B Biointerfaces, 2011, 82(2): 624-31.
[53] Meng B, Chen J, Guo D, et al. The effect of titanium particles on rat bone marrow stem cells in vitro [J]. Toxicol Mech Methods, 2009, 19 (9): 552-558.
[54] Grishchuk EL, Ataullakhanov FI. In vitro assays to study the tracking of shortening microtubule ends and to measure associated forces [J]. Methods Cell Biol, 2010, 95: 657-676.
[55] Okada E, Yamanaka M, Ishikawa O. New insights into the mechanism of abnormal calcification in nephrogenic systemic fibrosis-gadolinium promotes calcium deposition of mesenchymal stem cells and dermal fibroblasts [J]. J Dermatol Sci, 2011, 62(1): 58-63.
[56] Isaac J, Nohra J, Lao J, Jallot E, et al. Effects of strontium-doped bioactive glass on the differentiation of cultured osteogenic cells [J]. Eur Cell Mater, 2011, 21: 130-143.
[57] Zhiming Yang, Tissue Engineering. Chemical Industry Press: Beijing, 2002, 210
[58] Dan Jin, Guoxian Pei, Bin Chen, Yu Qin. Angiogenesis and neurotization study of bone tissue engineering [J]. Journal of Clinical Rehabilitative Tissue Engineering Research. 2001, 8 (5): 18-19
[59] Zhengfu Fan, Zhiming Yang. Study on the vascularization of the transplants and the relationship of vascularization and osteogenesis in transplantation of tissue engineered bone to repair segmental defect of long bone. Proceedings of the Second National Academic Conference of tissue engineering. 378-386.
[60] Casabona F, Martin I, Muraglia A, et al. Prefabricated engineered bone flaps: an experimental model of tissue reconstruction in plastic surgery [J]. J Plast Reconstr Surg. 1998, 101(3): 577



Bone Regeneration
Edited by Prof. Haim Tal

ISBN 978-953-51-0487-2
Hard cover, 340 pages
Publisher InTech
Published online 04, April, 2012
Published in print edition April, 2012

Bone is a specialized connective tissue, most prominently characterized by its mineralized organic matrix that imparts the physical properties that allow bone tissue to resist load, to support functional organs, and to protect highly sensitive body parts. Bone loss and bone damage may occur as a result of genetic conditions, infectious diseases, tumours, and trauma. Bone healing and repair, involves integrative activity of native tissues and living cells, and lends itself to the incorporation of naturally derived or biocompatible synthetic scaffolds, aimed at replacing missing or damaged osseous tissues. There are several modalities of bone regeneration including tissue engineering, guided bone regeneration, distraction ontogenesis, and bone grafting. This book concentrates on such procedures that may well be counted among the recent outstanding breakthroughs in bone regenerative therapy.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Gang Rui, Xuhong Jin and Shan Lin (2012). Histocompatibility of Acellular Matrix Bone with Osteoblast and Vascular Endothelial Cells, Bone Regeneration, Prof. Haim Tal (Ed.), ISBN: 978-953-51-0487-2, InTech, Available from: http://www.intechopen.com/books/bone-regeneration/histocompatibility-of-acellular-matrix-bone-with-osteoblast-and-vascular-endothelial-cells

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2012 The Author(s). Licensee IntechOpen. This is an open access article distributed under the terms of the Creative Commons Attribution 3.0 License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

IntechOpen

IntechOpen