Clinical Observations on Repair of Non-Infected Bone Nonunion by Using Mineralized Collagen Graft

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Recent advances in tissue engineering have expanded toward the new discipline of in vivo tissue engineering, which uses autologous cells immediately derived from the patient within the surgery. This report presented an evaluation on the clinical effect of mineralized collagen (MC) artificial bone graft incorporated with autologous bone marrow for the treatment of non-infected nonunion. In this clinical case report, 23 patients suffered from non-infected nonunion were treated with MC bone graft combined with autologous bone marrow and proper operational processes. The patients were followed-up and clinical and radiologic evaluations were performed to determine healing effects. At the last follow-up, every patient completed a medical outcome study (MOS) 36-item short form health survey (SF-36) questionnaire. The patients were followed-up for 12–26 months. All the bone nonunions reached clinical healing in 6.4±0.7 months. X-ray films showed fractures were healed without rotation, angular or shortening deformity. SF-36 scores were as follows: physiological function was 53.8±28.7, physical pain was 48.7±25.4 and general health was 62.3±20.2. As a result, MC bone graft combined with autologous bone marrow could achieve satisfactory results in the treatment of non-infected bone nonunion.

Keywords: Non-Infected Nonunion, In Vivo Tissue Engineering, Mineralized Collagen, Autologous Bone Marrow.

1. INTRODUCTION

Conventional tissue engineering utilizes living cells, biomimetic scaffold and suitable biochemical and physiochemical factors to build biological substitutes that restore, maintain, or improve tissue function.1–3 Cell, scaffold and growth factor were therefore considered as three essential elements for tissue engineering.4–6 Many biomimetic scaffolds has been developed by engineering and materials approaches and have been used in clinics.7,8 For example, mineralized collagen has been fabricated into scaffolds for bone defect repair.9–11 However, living cells limited clinical applications of tissue engineering. Autologous cells might not be available for those suffered from genetic disease or seriously illnesses, and elderly persons, since such patients do not have sufficient healthy cells to establish cell line.12 Moreover, culturing and proliferation of cells also take a lot of time.13 Allogeneic cells exist immunogenic problems and ethical issues.14

Recent advances in tissue engineering have expanded toward the new discipline of in vivo tissue engineering, which uses novel biomaterials that are able to recruit, program and disperse autologous cells in situ to target locations for tissue maintenance or regeneration.15 In vivo tissue engineering also uses autologous cells immediately derived from the patient within the surgical process, rather than culture and proliferate cells in vitro, or obtain seeding cells from commercially available cell-line or cell-bank. For in vivo tissue engineering, the fresh autologous cells are incorporated with the scaffold prior to the application and fate of cell would be controlled by the scaffold.15,16 Along with the cells, extracellular matrixes containing a lot of cytokines are also incorporated into the scaffold, thereby constitute bioactive repair material.17 In vivo tissue engineering is a novel practical clinical protocol in conformity with the essential principles of tissue engineering, and avoids above mentioned disadvantages.

Non-infected nonunion is always an important clinical problem that affect bone healing.18 Bone grafting has been...
used in the treatment of bone nonunion for a long time and achieved encouraging results.\textsuperscript{8–21} Therefore, autologous bone was identified as the “gold standard.” However, limited availability and donor site complications of the autologous bone grafting is obvious.\textsuperscript{22} Allogeneic bone grafting may cause immune rejection, and the source of allogeneic bone is limited.

According to the conception of in vivo tissue engineering, biomimetic artificial bone graft incorporated with fresh autologous bone marrow collected intraoperatively provides an ideal solution for the treatment of non-infected nonunion. In this clinical case report, artificial bone grafts made of biomimetic porous MC were employed as the scaffold, and the fresh autologous bone marrow extracted from the patients were incorporated with the MC scaffold, so as to build biological substitutes for the treatment of bone nonunion. From August 2008 to August 2012, 23 patients suffered from non-infected nonunion were treated by this therapeutic method. Patients were followed-up routinely in the outpatient clinic, where radiologic evaluations were performed to determine healing effects and whether MC could be used as an alternative for autologous iliac crest bone graft in the treatment of non-infected nonunion. The details of the observations are reported.

2. MATERIALS AND METHODS

2.1. Artificial Bone Graft

MC bone graft used in the present work was a commercially available artificial bone repair material “Bon-Gold” purchased from Beijing Allgens Medical Science and Technology Co., Ltd. Briefly, this MC bone graft was prepared by two main steps.\textsuperscript{23} Firstly, mineralized type I collagen fibrils were synthesized via a self-assembling process that hydroxyapatite (HA) crystals grew within collagen fibrils and collagen triple helices self-assembled. During such biomimeralization process, the nucleation and growth of HA were regulated by collagen fibrils, which was similar to the natural process of bone mineralization in animals. Then the mixed solution was freeze-dried thoroughly and produced porous MC. The product was cut into small granules and sterilized by irradiation of $^{60}$Co before use.

2.2. Patients

Between August 2008 and August 2012, a total of 23 patients suffered from non-infected bone nonunion were treated by using MC bone graft. There were 16 male and 7 female patients with the age from 16 to 64 years old (mean age: 32.4 years old). The nonunion sites were: 7 cases at middle-lower of tibia, 6 cases at middle-lower of femur, 1 case at proximal ulna, 1 case at radial shaft, 2 cases at humeral and 6 cases at clavicle. The time interval from injury to therapy was 9\textendash}38 months (average: 15.6 months). There were 0\textendash}2 times of surgeries for each patient before the operation and the average time was 1.1. Among those undergone surgeries: 16 cases were treated by internal fixation, wherein 4 cases were performed by intramedullary interlocking nail, 10 cases by limited contact dynamic compression plate, 2 cases by Kirschner wire fixation; as well as 4 cases were performed by external fixation. According to Weber-Cech classification,\textsuperscript{21} there were 8 cases of abundant blood supply—active nonunion wherein 2 cases of the elephant foot shaped bone nonunion, 3 cases of horseshoe nonunion and 3 cases of malnutrition; and 15 cases of poor blood supply—no activity of nonunion, wherein 3 cases of comminuted bone nonunion, 7 cases of bone defect nonunion and 5 cases of atrophic nonunion.

2.3. Surgical Techniques

Each patient was treated with a specific operation plan according to his/her bone nonunion condition.

For those reliably fixed by previous surgeries: minimal invasive incision was created to get to the nonunion site, scar connective tissue was thoroughly scraped, hardened necrosis bone was clearly removed and sclerotic bone was chiseled until reaching normal bone tissue; the medullary cavity was then dredged until the fresh blood exuded; the MC bone graft was implanted into the nonunion site and medullary cavity to ensure that the implant was infiltrated by fresh blood.

For those internally fixed by intramedullary nail: the distal or proximal locking screws were removed according to specific situations, the static fixation was therefore changed to dynamic fixation; and then the artificial bone graft was implanted as above mentioned.

For those without any fixation or unstably fixed: the fracture was fixed by a reliable method, such as intramedullary nail, meanwhile the artificial bone graft was implanted as above mentioned.

Postoperative treatment: rehabilitation therapy was carried out step by step. Muscle contraction and relaxation, flexion and extension training was carried out in early period of post-operation, non-weight-bearing exercises were done in mid-term, and weight-bearing exercises were gradually performed in later period. Until obvious continuous callus was showed on X-ray film, full weight-bearing walking exercises started.

2.4. Follow-Up Evaluations

All the patients were followed-up routinely in outpatient clinics for radiologic evaluation. At the last follow-up, every patient completed a medical outcome study (MOS) 36-item short form health survey (SF-36) questionnaire.

2.5. Radiologic Evaluation

At the 1st day and the 1st, 3rd, 6th, 9th and 12th month after the operation, X-ray examinations were taken to dynamically observe the growth of callus. The clinical
healing standard for bone fracture was cited to evaluate the treatment effect: local tenderness disappeared, no longitudinal percussion pain, the wounded limb is loadable, and X-ray film shows that the broken ends of the fractured bone was connected by callus and the gap disappeared.

3. RESULTS
Patients were followed-up for 12~26 months and an average of 17.5 months. All the patients recovered well post-operation without fever, and there were no inflammatory responses, such as obvious edema and exudation, at the wounds. Rejection reaction did not occurred, and the incisions were properly healed. All the patients achieved clinical healing in 6.4±0.7 months, and they were then weight bearable.

SF-36 scores were as follows: physiological function was 53.8±28.7, physical pain was 48.7±25.4 and general health was 62.3±20.2.

Typical case: male patient, 32 years old, left tibia and fibula fractured by a traffic accident. In previous surgery performed by other hospital, his tibia was treated with external fixation and fibula was treated with Kirschner wire intramedullary fixation (Fig. 1). 15 months post-operation, the fractures did not heal (Fig. 2). Then, in our hospital, MC bone graft was implanted into the nonunion site and medullary cavity of the tibia via a minimal invasion without replacement internal fixation, and the fibula was left without any treatment. The wound healed well post-operation. X-ray examinations were taken at the 1st day, 1st month, 6th month and 9th month post-operation (Fig. 3). The fracture lines blurred at the region where MC bone graft implanted, and osseous union gradually formed. Moreover, osseous union gradually appeared at the same horizontal section of the fibula. However, the fracture at the upper of the fibula without implantation of MC bone graft remained unhealed. The fixation was removed.

4. DISCUSSION
The definition of bone nonunion is not yet unified, but the commonly used definition is proposed by FDA at 1986: at least 9 months after injury or fracture, and there were no further healing trend for 3 months.24 In those cases, all the patients were in line with the definition. Bone nonunion may be caused by many factors, including: systemic factors (smoking, drugs, nutrition, etc.), local factors (unstable fixation, impaired blood supply, bone defect, infection, etc.), human factors (improper treatment method, etc.). These factors often cause bone nonunion together, thus leading to the treatment more difficult. There are many treatment methods for bone nonunion, and bone grafting has 100 years of history and achieved encouraging results. Although some scholars pointed out that bone graft is not necessary in the treatment of bone nonunion,25,26 but most of scholars considered that the bone graft played a positive role in the treatment of bone nonunion.27,28

The commonly used bone graft in clinics includes autologous bone, allograft bone, and artificial bone. The autologous bone was considered to be “gold standard,” since it has fast osteogenesis rate, does not require special storage and does not cause any immune response. But the autologous bone still has many disadvantages: 1, obtaining autologous bone adds new operation site to the patient, which elongates operation time and increases bleeding amount, thereby increasing the operation risk and donor site morbidity; 2, the autologous bone does not possess specific shape and structure; 3, for pediatric patients, autologous bone quantity is limited, it is difficult to meet the complex
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Fig. 3. Osseous union gradually formed after been treated by implantation of MC bone graft combined with autologous bone marrow. (a) X-ray film 1 day post-operation, MC bone graft was implanted into fracture site of the tibia and the fibula was left without treatment, (b) X-ray film 1 month post-operation, the fracture lines blurred at the fracture line of the tibia and osseous union locally formed, while the nonunion at the fibula did not heal, (c) X-ray film 8 months post-operation, the fracture lines blurred at the fracture line of the tibia and osseous union formed, while the nonunion at the fibula still did not heal, (d) X-ray film 10 months post-operation, the nonunion of the tibia completely healed and the fracture line disappeared, while the nonunion at the fibula still did not heal.

clinical needs; 4, the cortical bone is very limited, too much extraction can lead to bone fracture. In view of the above disadvantages, people try to find new bone substitute materials. Along with the development of tissue engineering and biotechnology, artificial bone, as a kind of new material, has entered clinics. Artificial bone has many advantages: 1, it does not contain exogenous growth factors, so immune rejection would not be induced; 2, it reduces or avoid the damage to the donor site; 3, it has good biological compatibility. The ideal artificial bone should possesses: good bone conduction, bone induction, biological compatibility, degradability in vivo, similar structure to the bone tissue, operation and usage convenience.25 Peng et al. applied bio-derived bone transplantation in orthopedics with tissue engineering technique, after 7-year follow-up, all the ten cases were healed, and bone union was completed within 3.0 to 4.5 months in 9 cases.10

In this case report, the MC artificial bone graft is in conformity to these characteristics. MC made up of type I collagen and hydroxyapatite was prepared by in vitro biomimetic mineralization process, it therefore has the same chemical components and microstructure to the natural bone tissue, and such MC bone graft possesses good biocompatibility and is able to provide favorable microenvironment for attachment and proliferation of osteoblasts.31-33 Therefore, the biomimetic MC artificial bone graft is good for promoting bone healing,9,11 which was also demonstrated by the typical case in this report: the tibia and the area of fibula with bone grafting achieved good healing effect, and the area without bone grafting did not heal.

In the treatment of bone nonunion, preoperative diagnosis is very important. For the patient whose nonunion was unfixed or unstable fixed, firstly, reduction of fracture was performed and reliable fixation was chosen or the unstable fixation was replaced, and this step was tried to be performed by minimally invasive surgery; then the MC bone graft was implanted into the broken end of the fracture via the minimal invasion. For those whose fracture end was stable no matter fixed with external or internal fixture, they were only treated by implanting the MC bone graft into the bone defect area and intramedullary cavity. Two patients have been treated with intramedullary nail fixation in previous surgeries, in this operation, we removed distal or proximal locking screws, scraped fibrous callus at the bone fracture end, and implanted the MC bone graft. They were weight loaded and dynamically compressed at early stage post-operation, and finally achieved fracture healing.34 For the patients suffered from bone nonunion with internally fixed by intramedullary nail, the static fixation should be changed to dynamic fixation. Because stress stimulation is necessary for the formation of bone callus at the nonunion
region, while such stress would be produced by broken ends within a dynamic fixation.

In this case report, favorable treatment effects were achieved by simple implantation of the MC bone graft. In the implantation process, we emphasized the minimal invasion to protect the soft tissue and blood circulation. The operations were performed under direct vision to make sure the bone grafting sufficient and effective. In the surgeries, the treatments prior to bone grafting included scraping scar of connective tissue, cleaning necrosis bone clearly, chiseling sclerotic bone until reached normal bone tissue, and dredging medullary cavity until fresh blood exuded. After these treatments, the MC bone graft was implanted into the nonunion site and medullary cavity, and make sure the implant was infiltrated by the fresh blood. In this way, the MC bone graft, fresh blood and bone marrow were mixed together to form a mixture comprised of scaffold, cells and growth factors in vivo, thus meeting the requirement of three essential elements for bone fracture healing. This operation method according to in vivo tissue engineering can significantly reduce operation time, minimize operation wounds, lighten economic burden for the patients, and shorten hospitalization time. But it is required to judge the stability of existing fixation condition accurately and reliably prior to the operation to ensure fracture end was stable.

5. CONCLUSION

In summary, the treatment of bone nonunion by using MC bone graft combined with autologous bone marrow obtained satisfactory treatment effects. MC artificial bone graft could serve as an alternative of autologous iliac crest bone graft in the treatment of non-infected nonunion.

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References and Notes

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