Int J Clin Exp Med 2015;8(7):10619-10625
www.ijcem.com /ISSN:1940-5901/IJCEM0004751

Original Article

Experimental study of high-energy fractures delayed operation in promote bone healing

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Received December 12, 2014; Accepted June 22, 2015; Epub July 15, 2015; Published July 30, 2015

Abstract: To investigate role of delayed operation to stimulate growth of strong external callus in high-energy fractures, and explore a new way for bone healing. Twenty adult dogs were employed, and randomly divided into four groups, including group A-D. The dogs underwent osteotomy by wire saw in middle of femur, electric coagulation damaged surrounding periosteum, forming a 1 cm defect. Group A were internal fixed 14 days after osteotomy (higher-energy fractures delayed operation), Group B and C were internal fixed immediately (no delayed operation), Group D were internal fixed 14 days after osteotomy (delayed operation, but resected granulations around extremities). The results showed that groups of early fixed have no external callus growth and almost no growth in internal callus, these conditions leads to atrophy nonunion. On contrary, the porosis was strong and callus union was steady in group A and D, which have a delayed operation. In conclusion, early surgical fixation of high-energy fracture restrains external callus growth, easily lead to poor callus healing phenomenon of low-quality. Delayed surgical fixation can begin to repair soft tissues injury, stimulate external callus growth and improve fracture healing, so a small incision open reduction produce more robust growth effect than closed reduction.

Keywords: High-energy fracture, delayed operation, porosis, callus union, experimental study

Introduction

In clinical, the low quality healing as the main performance in poor callus growth often happens. Usually, it causing delays fracture healing, nonunion, non-violent plate fracture, and then fracture after take the plate. The reasons for the phenomenon of no callus are also controversial. Some scholars believe that instability is fixed, others believe that the fixed is too strong to the effect of stress shielding. Frost [1] believes that it caused by biological factors that are barriers to the formation of callus or callus calcification disorders. More accepted reason is damage to blood supply which caused by high-energy fractures. Smith [2] has found delayed surgery can stimulate a wealth of callus growth, significantly reduced the incidence of nonunion. In order to re-use of this phenomenon, we use experiments revealed the internal relations between delayed operation and growth of callus inherent ability, in order to explore a new way of enhance bone healing.

Materials and methods

Animals

Twenty normal adult dogs, 10-12 year old, no limit of male or female, 8-12 kg weight, 100-130 mm femur length, 10-12 mm diameter. All of the animals which provided by the People's Liberation Army Xijing Hospital, Fourth Military Medical University Experimental Animal Center. The dogs have no surgery area trauma, infection and deformity, and no other systemic disease, and unity for the same breeder rearing. All experimental procedures complied with the Fourth Military Medical University animal experiment guidelines. All the disposals were in accordance with the guideline of animal ethics.

Steel plate

Straight stainless steel plate are (Tianjin Ren Li Orthopedics equipment Co., LTD production) 6 holes ×80 mm, wide 12 mm, thickness 3 mm, changed for 8 holes ×65 mm, 8 holes ×75 mm as request.

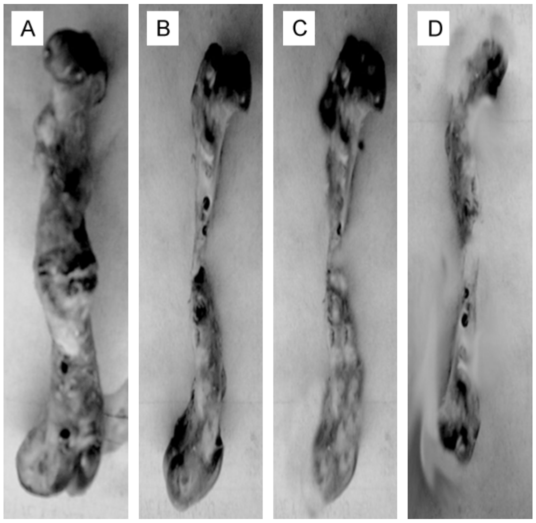

Figure 1. Gross observation of the callus formation. A. Group A; B. Group B; C. Group C; D. Group D.

Trial grouping

Twenty adult dogs randomly divided into four groups, including group A-D. Each group underwent 0.15 ml/kg dose intramuscular injection of anesthesia in the line item department with Sumianxinzhusheye II. After routine sterilization drape, the dogs were taken unilateral thigh straight incision along the femur, about 7 cm, subcutaneous skin incision under sterile conditions and deep fascia, separate the septum intermuscular laterale, revealing the femur. Then we underwent in the middle line of the femoral transverse osteotomy with a wire saw and electric coagulation full damage to the surrounding periosteum, the extent of damage 2.5 cm long. The standard model of transverse fracture of the femur has been obtained, and each group has the same amount of soft tissue injury.

Group A with a fine Kirschner wire after osteotomy through the bone marrow in order to maintain stable contact with clients, and then sutured incision, body in a natural state. Fourteen days later, we exposed the broken ends of fractured bone again, amputated 10 mm of it, fixed with 8-hole plate and kept 10 mm gap between the bone ends. The operation is close to the cortical strip, keeping the continuity with granulation and the surrounding tissue. Group D is the same operating procedures as group A, except after the fixation resect granulation around the broken ends of fractured bone completely. Group B, after the destruction of the periosteum, was amputated stump 10 mm, fixed with 8-hole plate and kept 10 mm gap between the bone ends. Group C is the same procedures as group B, but no electric condensate damage the periosteum which limited to stripping damage.

Postoperative managements

The dogs were intravenous dripped celmetin (2.0 g) once before awake, dogs were freely moving and the limbs were mostly suspended in the early stages. One dog were sacrifice in each group after 4 weeks and two were sacrifice after 8 weeks (A, B group were tissue labeling in oral tetracycline 1 week before). Then two were sacrifice after 12 weeks.

Outcome measures and assessments

Gross observation: On the animal after the operation, observe general conditions such as food and activity, local wound healing, limb swelling, and the callus formation of the gross specimen.

X-Ray examination: Take the CR-X-ray examination separately in 3 weeks, 6 weeks, 9 weeks and 12 weeks after operation, Observe quantity of the callus proliferation, put a transparent grating fitted to the X-rays (Surveyed grids of the grating were squares with all sides a millimeter long), count the number of callus and with grids' number, and compare the changes of callus among groups.

Histomorphological specimen fabrication and microscope observation: The animals were sacrificed in 3 weeks, 6 weeks, 9 weeks and 12 weeks after operation. Take the femur specimens for paraffin analysis, observe and analysis tetracycline fluorescent mark

Statistical method

The statistical data was analyzed with SPSS 13.0 statistical software, the comparison among groups used t test. The results showed with x±s. The difference was statistically significant ($P<0.05$).

Results

Postoperation observation

Gross observation of the callus formation indicated that the defects of the A group have been

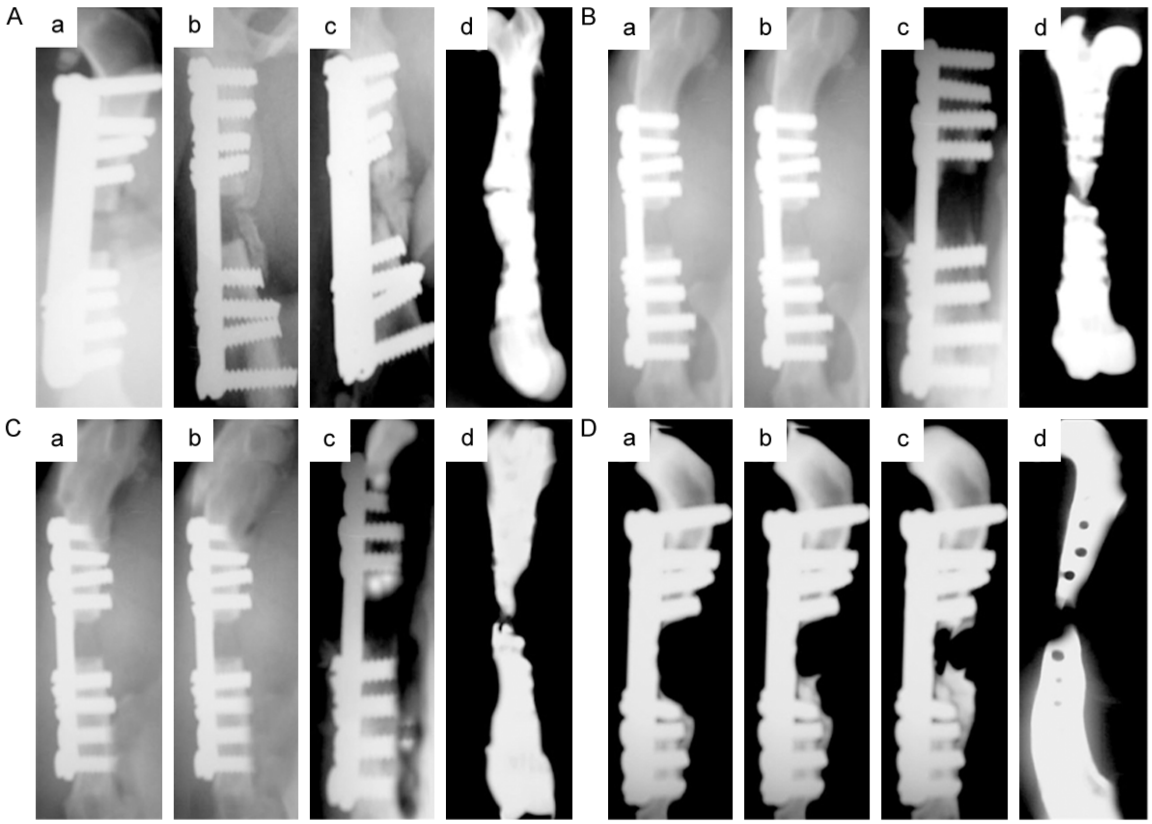

Figure 2. Postoperative X-ray analysis of the callus formation in each group. A. Group A; B. Group B; C. Group C; D. Group D. The 'a', 'b', 'c' and 'd' represents 3 weeks, 6 weeks, 9 weeks and 12 weeks postoperation, respectively.

repaired totally (**Figure 1A**). While the callus formation gross observation of the group B-D showed a very poor repair (**Figure 1B-D**).

Postoperative X-ray analysis shows that group A presented weak callus shadow after 3 weeks, thickness of callus increased greatly after 9 weeks ($P<0.05$), and has been completely filling fracture gaps (**Figures 2A**, **3A**). Group B-D hadn't presented callus until 12 weeks ($P>0.05$; **Figures 2B-D**, **3A**). The growing number of callus for group A presented two rise periods and two platform periods. The first rise period appeared in the initial period to 3 weeks, and the platform period was between 3-6 weeks. The second rise period was from 6 to 9 weeks, and the second platform period began after 9 weeks (**Figure 3A**).

Analysis of samples after sacrifice

The samples of 12 weeks sacrifice dogs shown that in group A the extremities did not shrink, callus was a large spindle-shaped, close to the rendezvous, compared with group A-C ($P<0.01$, **Figure 3B**, **3C**). For group A, only left with a thin layer of zona cartilaginea, extremities was stable, callus number was 3532 mm^3, computed tomography (CT) value is greater than 1300 Hu (**Figure 3B**, **3C**). In group B the extremities was atrophied, a small conical callus extended to the fracture gap at the cacumen , there were cicatricial connections between the two cacumen and the Steel plate was loose, the number of new callus is 270 mm^3, CT is less than 500 Hu (**Figure 3B**, **3C**). Group C without external callus, but no significant bone atrophy of the extremities, the plates had no obvious loosening, callus of fracture gap was a semicircular accretion into the centre, formed a Join callus situation, new bone callus number of 261 mm^3, CT is less than 500 Hu (**Figure 3B**, **3C**). In group D the extremities was mild atrophy, with virtually no external callus growth, plate has been loosened, the number of new callus is 50 mm^3, and CT is less than 500 Hu.

Paraffin slices analysis

Paraffin slices showed there were a large number of active fibroblasts within the granulation hyperplasia (**Figure 4A**) for group A. When fixed

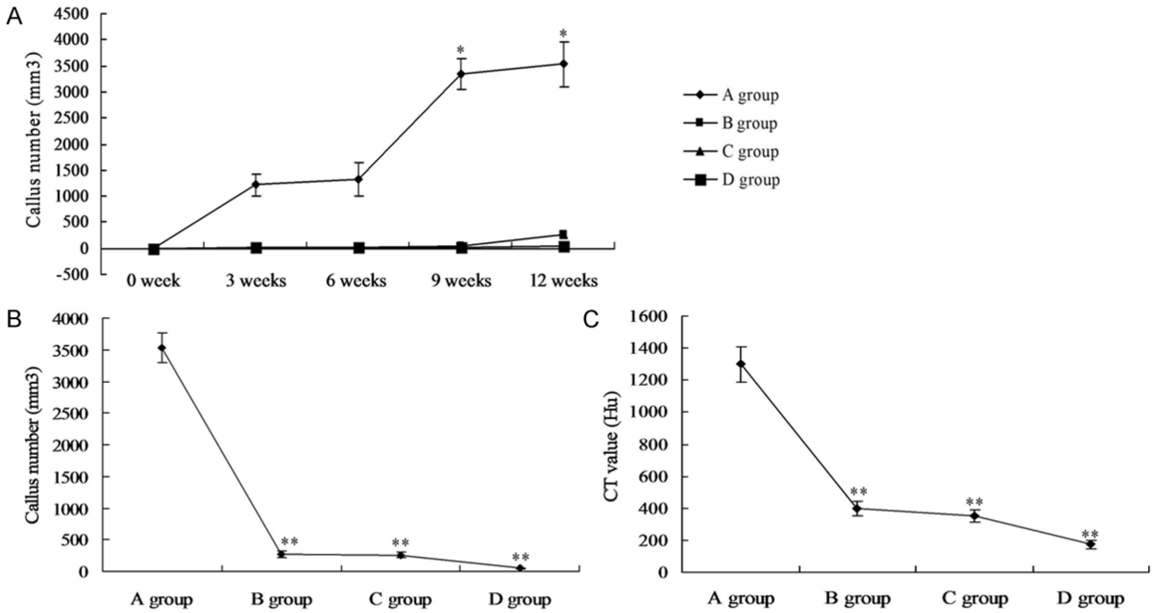

Figure 3. Callus quantity and number after 12 sacrifice in 12 weeks post-operation in every groups. A. Callus quantity changes in every period for group A-D. B. Callus number analysis; C. CT value analysis.

with steel plate, the active fibroblasts formed bone callus tissue samples after 4 weeks (**Figure 4B**), mature callus tissue after 9 weeks, mature bone tissue after 12 weeks. Group B showed mature fiber cells after 4 weeks, has begun to scar (**Figure 4C**, **4D**), and scar tissue between 9 and 12 weeks.

Plastic slices analysis

Take 9-week postoperation specimens to make callus tissue plastic slice and display by tetracycline fluorescent marker. Trabecular of Group A was mature and bulky (**Figure 5A**), while trabecular of Group B-D were sparse small and not mature (**Figure 5B-D**).

Discussion

The essence of high-energy fractures is excessive soft tissue injuries cause no callus growth, no callus growth causing failure of the extremities of bridge grafting, then these lead to nonunion [3, 4]. According to above reasons, we designed the same amount of man-made damage of soft tissue in order to simulate high-energy fractures, showed the source and the area of callus growth with bone defects. The separated extremities was clearly shown that when the fracture was healing the extremities occurs two sets of fracture callus moves forward to the center. One was the internal callus in the fracture cross-section, showing direct growth of the extremities. The other was the external callus outside of in the fracture cross-section which derived from granulation tissue of os endochondrale.

The internal callus moved from the fracture cross-section to the center semicircularly. Group C did not damage the periosteum shows that callus, the callus was small scope, low density, appear late, slow growth, CT was less than 500 Hu. If the fracture gap is small, stable fixed, it could produce small callus healing which just filled the fracture gap. Group B had no internal callus growth according to the damage to the periosteal, which showed that the growth of internal callus also depends on the healthy surrounding tissues.

The external callus was opposite to the plate, around two peripheral fracture was two discrete half arc. In the beginning, a small gap between the cortex of bone and the external callus, the external callus gradually thickened and extend meet with the cortex of bone, and then filling the gap of bone fracture and integrate within the internal callus. Group A had a good external callus (some showed the early callus bridge which directly appeared in the bone defects), after 3 weeks in X-rays could have significant performance. The density of callus was significantly increased after 6 weeks, a

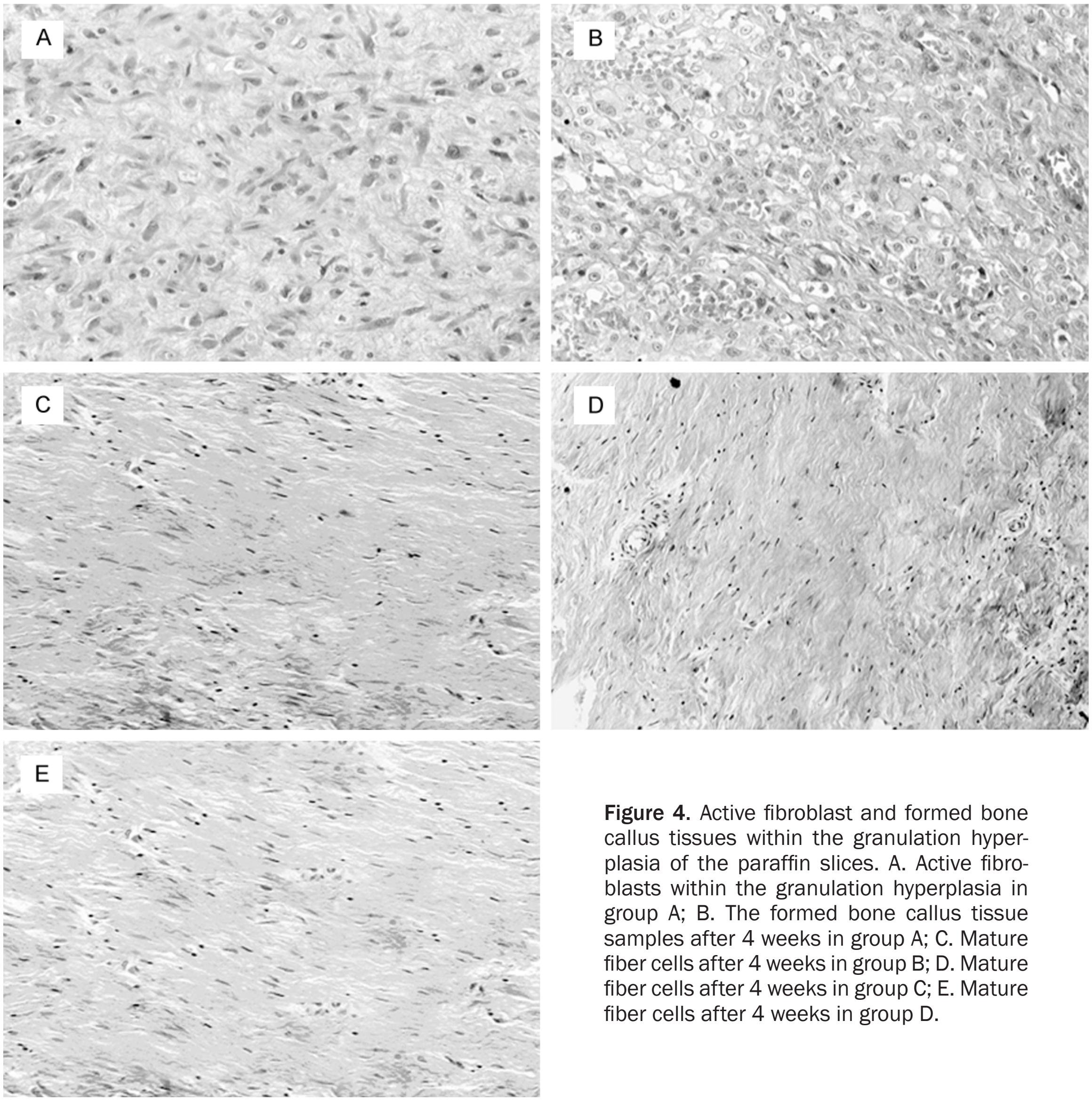

Figure 4. Active fibroblast and formed bone callus tissues within the granulation hyperplasia of the paraffin slices. A. Active fibroblasts within the granulation hyperplasia in group A; B. The formed bone callus tissue samples after 4 weeks in group A; C. Mature fiber cells after 4 weeks in group B; D. Mature fiber cells after 4 weeks in group C; E. Mature fiber cells after 4 weeks in group D.

slight increase in the thickness of the callus. The thickness of callus was greatly increased after 9 weeks, and the gap was completely filled fractures, CT was greater than 1300 Hu. The callus grown external along the fracture, and then inserted into the fracture gap, this is the basis of callus healing. Even if the extremities were separated, stable fixed does not affect their growth. And the extremities which were surrounded by external callus had no atrophy and resorption, because a large number of osteoblast external callus had active osteoblast activity, the ends of the bones could absorb and coupled new bone formation effectively at the same time. Group B of early stage surgery was no external callus, the extremities atrophied, scar connected, forming a nonunion. It had shown that early surgery of high-energy fractures can inhibit the formation of external callus.

Comparison of group A and B has shown delay surgery can start good outside callus growth in the exclusion of periosteum, which contained in the process of the formation of granulomatous inflammation stimulated by the preoperative bone movement. Derived from bone end and the granulomatous second injury inflammation, callus size depends on granulomatous number [5, 6]. Granulation is precursor of callus, for Group D, granuloma resection lead to no callus growth which further confirmed this point. If the concept for the treatment of high-energy fractures, the callus can quickly filling the postop-

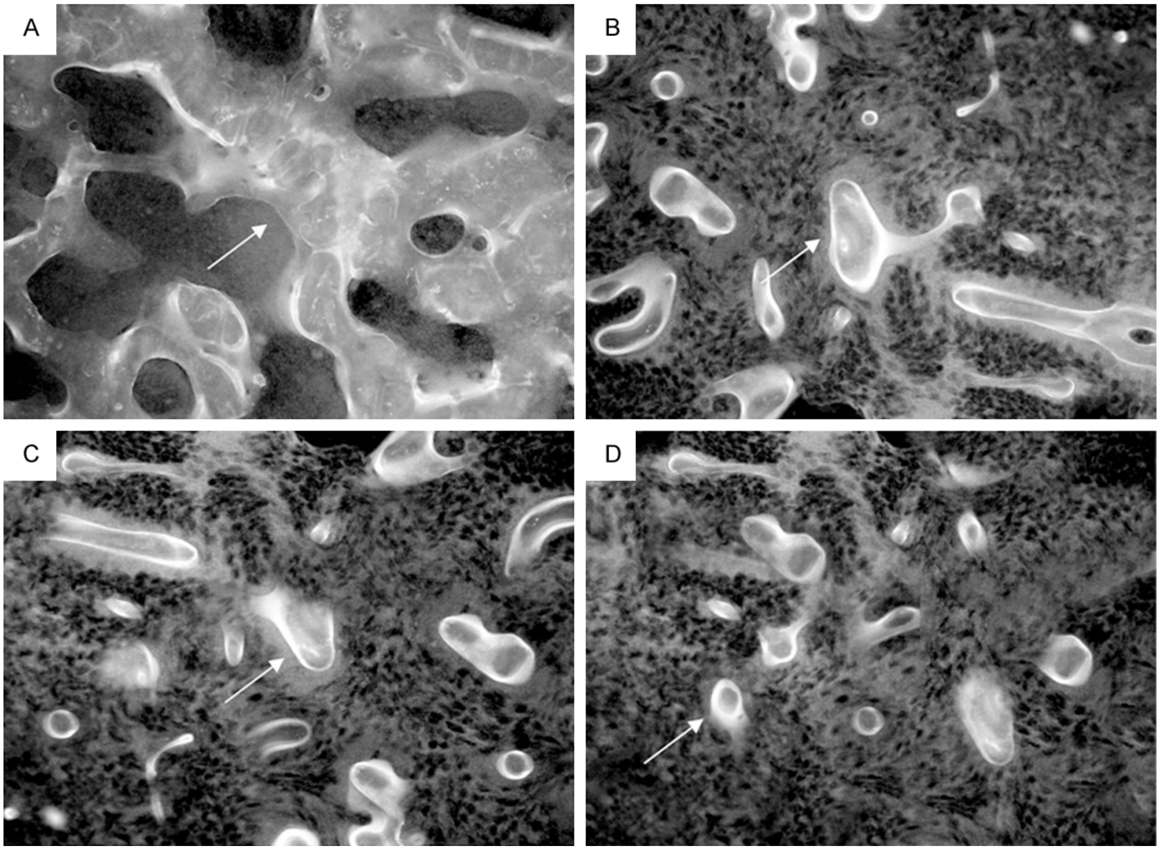

Figure 5. Plastic slices analysis of trabecular in every group. A. Group A; B. Group B; C. Group C; D. Group D.

erative legacy bone defect, fast bag around large dead bone bridge, done quickly in the opposite side of the steel plate mechanical support, stabilize the fracture end early and effectively, thus can prevent the non-union bone .

Although the group D was implemented delay surgery, but resected the granulation has formed around fracture end, which has no callus growth. It indicated that the delay surgery callus derived from the generated granulation tissue, the granulation tissue around fracture end includes various primitive cells on the healing, which are the callus seeds for future growth. Delay surgery cultivated and stored the callus precursor, and make callus formation. Early surgery did not store callus precursor, so have no outside callus growth. The protection of the hematoma, which we always advocated actually protects the original early fracture of granulomatous inflammation. Budge or strain stimulation also stimulates the more fracture healing inflammatory granulation, strong fixed to suppress the inflammatory granulation of further, thereby inhibiting the outside callus.

Zhang et al. [7] found that delayed surgery can stimulate the growth of callus, but must cooperate with low hardness steel plate, and outside callus will be reduced along with the degrees of plate steel increase. Another study [2] also indicates that delayed operation can stimulate a wealth of callus growth and significantly decrease the incidence of nonunion, which is consistent with our results. Furthermore, the delayed operation has also been utilized in acute cholecystitis, cardiac operation, appendicitis, and act. For our experiment, there is a huge gap between the two complete bone separations, there was no interaction between the bones, the bone growth behavior in the bone defect is totally generated with the growth of granulomatous behavior before the operation, the stable fixation and bone separation has no influence on this behaviour.

According to the experimental results we can infer that the nutrition of osseous tissue comes from the around soft tissue, so soft tissue must provide fracture healing materials, health soft tissue promote the growth of callus by providing adequate fracture healing materials. Obviously damaged soft tissue with high energy has greatly lost transmission capacity of healing materials [8, 9]. It results in the bone tissue inflammation (and exudate bone induced factor), the around soft tissue did not provide adequate primitive cells. So it can't produce strong bone entrainment, granulation tissue generated from the overdue primitive cells without induction, and are prone to develop scar and stop healing. Delayed surgery repaired the soft injury, restored their due transmission capacity of healing materials, and prestored a number of primitive cells, all of which are consistent with the former studies [10-14]. The inflammation of the lining the induced factor for the second time just induced primitive cells which has been stored, and produced the high growth in this experiment. If the small incision was implemented for high-energy fracture, it will produce a better and more robust growth effect than closed reduction.

In conclusion, early surgical fixation of high-energy fracture restrains external callus growth, easily lead to poor callus healing phenomenon of low-quality. Delayed surgical fixation can begin to repair soft tissues injury, stimulate external callus growth and improve fracture healing, so a small incision open reduction produce more robust growth effect than closed reduction.

Acknowledgements

In this study, analysis of tissue sections from the Fourth Military Medical University Pathology Laboratory collaborative implementation.

Disclosure of conflict of interest

None.

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