Locking plates in treating fractures:

Analysis of complications in 25 cases

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Abstract

BACKGROUND: The unique design of nail hole and screw of locking plate makes it possible to provide different fixation mechanisms in fracture fixation, which can provide diverse biological environments for bone healing, thus, lead to various healing patterns.

OBJECTIVE: To retrospectively analyze complications of locking plate treatment for fractures in 25 cases.

METHODS: The physical examination results, including injury mechanisms, whether multiple injuries, selection of internal fixation, operation principles, postoperative loading time, and X-ray films recheck, were analyzed subsequently. The numbers and reasons for internal fixation failure, bone nonunion or delayed union were investigated.

RESULTS AND CONCLUSION: Among the 8 cases of internal fixation collapse, 4 were caused by operation lapses, 1 was due to wrong selection of the internal fixation tool, 1 case took place by weight bearing too early, 2 cases were caused by postoperative infections; Among 11 cases of nonunion or delayed union, 6 were caused by severe primary injuries, and 5 by unsuccessful reduction. 2 cases of pain stemmed from hypodermal projection of proximal tibia LISS plate, 1 from postoperative infection. 1 case of long term to be exposed of plate end due to improper location of the plate during the operation was also involved. Essentially, locking plate just is a kind of internal fixation plate. Either the compression plate or the bridging plate, or the combination of the two could be applied in locking plate fixation. Through our study, we found that the pathway leading to the success was built upon an accurate cognition and skillful mastery of AO internal fixation technology, as well as the correct choice of the tool combined with the fine reduction and micro-damage operation.

INTRODUCTION

As a new tool with specialty for internal fixation, locking plate has shown its extensive clinical successes in China[1-5]. However, any efficient equipment of internal fixation would be bound to have various complications if improperly implemented. During the periods of June 2005 to June 2007, 25 cases with complications after locking plate fixation were analyzed and summarized in order to develop previous knowledge of actual effects regarding locking plate.

SUBJECTS AND METHODS

Design

A retrospective analysis.

Time and setting

The experiment was performed at the Department of Orthopedics and Traumatology, Jishuitan Hospital the Fourth Medical College of Peking University, from June 2005 to June 2007.

Subjects

A total of 25 patients were selected, including 19 males and 6 females, aged 18−79 years, mean aged 42 years. 16 cases suffered left side affections, and 9 at the right side. In the group, 7 cases had femur fractures (Positions: 3 cases of middle segment, 4 cases of distal segment; Types: 3 cases of type B fracture, 4 cases of type C fracture), 15 cases with tibia fractures (Positions: 7 cases of tibia plateau, 1 case of proximal segment, 3 cases of middle segment, 4 cases of distal segment; Types: 2 cases of type A fracture, 6 cases of type B fracture, 7 cases of type C fracture), and 1 case with humerus fracture (type B fracture); When classified from fractural features, 7 cases were simple fracture, 18 cases were comminuted fracture. When categorized by the trauma types, 21 cases were close injury, 4 cases of open injury (Among which, there was 1 case of Gustilo type Ⅱ, 2 cases of type Ⅲ B, and 1 case of type Ⅲ C); 4 cases of the group were reported with complicating fracture of other areas, and 1 case with vascular injury; Concerning the reasons: 14 cases are due to traffic accidents, 6 cases by high-level falls, 3 cases with striking injuries by the clubs, and 2 cases caused by twisted injuries. All 25 patients were dealt respectively with open or close reduction with different time periods of 6 hours to 7 days after the injuries, among which, 2 cases (femoral shaft fracture and humeral shaft fracture) were dealt with locking compression plate (LCP) fixation and autogenous bone graft due to the failures of previously internal fixations. Complications were observed in the follow-up of 6 to 24 months; among which, 7 cases had collapse of internal fixater, 1 with the plate broken, 8 cases were reported as nonunions, 6 cases were stated delayed unions, 2 cases with pain induced by hypodermal evection of tibia LISS plate, and 3 cases with infection of the operative site, among the three, 1 case developed to be exposure at the proximal end of the tibia LISS plate and 3 cases of valgus knee.

Methods

Researching faculty consisted of formally trained surgeons who had mastered skills with clinical internal fixation, and participated in studying and discussing the case histories, physical examinations, analysis of injury mechanisms, detections concerning the existence of multiple injuries, selections of

internal fixation, operation principles, postoperative weight bearing times and X-ray rechecks of these cases.
With the results being recorded, these cases were classified as normal defined standards: ①Failure of internal fixation: Loosened, dissected or pulled out screws, plate rupture, and abnormal movement of fracture side or angulated deformity. ②Nonunion of fracture: No evidence was shown by X-ray or CT of osteotylus formation at 9 months after operation, which must be proved by nonexistence of radiographic findings with the incessant period of 2 to 3 months. ③Delayed union: No osteotylus were found at ends of the fracture 6 months postoperatively through X-ray recheck, yet with the gradual healing found in the following recheck[6-7]. ④Infections of operative sites: infection of the deep soft tissue was associated with operative incision within 1 year after the planting operation. Incisions broke spontaneously or by the surgeons, with purulent secretion or fever exceeded or equaled 38 Celsius degree, with local pain or palpation; deep incision infections were diagnosed by surgeons[8].

Main outcome measures

Numbers and reasons for internal fixation failure, bone nonunion and delayed union. Pre- and post-operative X-ray film of typical cases.

RESULTS

Numbers and reasons for fixation failure, bone nonunion and delayed union

Of 8 failures of internal fixations included in these 25 cases: 2 cases were femur shaft fractures, 2 cases were femural intercondyles fractures, 2 cases were tibia fractures, 1 case was humeral shaft fracture, and 1 case was humeral surgical neck fracture. Related reasons: 3 cases of femur fractures were due to operational lapses, which were caused by shortened length of screw tangential to the edge of the shaft; 1 case with fracture of the proximal tibia and 1 case of humeral shaft fracture were caused by insufficient length of the plate; 1 case with femoral shaft fracture and 1 case with humeral shaft fracture were due to weight bearing early postoperativly; and 1 case of humeral surgical neck fracture was due to infection that induced failure of the internal fixation.
The nonunion of 8 cases were all caused by dystrophic reasons, including 1 case with femoral shaft fracture, 6 cases with tibia fractures, and 1 case of radial and ulnar fracture. Among these 8 cases, 6 cases were severe ones with high energy trauma caused by car accident or machinery twisting; 2 cases of tibia fracture nonunion were caused by unsuccessful reduction during the operation. In 4 cases with delayed union, 1 case was femur fracture, 3 cases were tibia fracture. Among these 4 cases, two were caused by insufficient blood supply at the site of the fracture, and another 2 cases were caused by unsuccessful reduction during the operation. The pain in 2 cases with hypodermal evection of proximal piece of tibial LISS plate and 1 case with plate exposed were caused by excessive anterior position of tibial LISS plate during the operation.

Pre- and postoperative X-ray film of typical cases

Case 1, a female, aged 21 years, had intercondyle femur fracture caused by burst tire. Open reduction and internal fixation with LISS plate and lag screw were performed in the emergency room. Blood circulation was severely damaged with operation and the accident, which led to nonunion 12 months later (Figure 1).

a, b: Preoperative X-ray check

c, d: Nonunion at 12 mon after operation

Figure 1 Pre- and postoperative X-ray film of a 21-year- old female patient

Case 2, a female, aged 26 years, suffered from tibial and fibula fracture caused by high-level falls. Close reduction and internal fixation with LCP were operated. Nonunion was observed 15 months later due to unsuccessful reduction during the operation (Figure 2).

a, b: Preoperative X-ray check

c: Incision of the fixation with the MIPPO technique

d, e: Nonunion of the fracture due to unsuccessful reduction at 10 mon after operation

Figure 2 Pre- and postoperative X-ray film of a 26-year-old female patient

Case 3, a male, aged 59 years, had tibial and fibula fracture caused by batting. Close reduction and internal fixation was performed with LISS plate. Nonunion was observed 12 months later due to unsuccessful reduction of the fracture in operation (Figure 3).

a, b: Preoperative X-ray check

c: Large gap lying on the anterior side of the fractural ends due to unsuccessful reduction of the operation

d, e: The second operation with bone grafting. Insertion of soft tissue into the ends of the fractural site was confirmed during the operation. Bone grafting was performed after the clearance of the imbedded soft tissue

f, g: Union of the fracture 4 mon later

Figure 3 Pre- and postoperative X-ray film of a 59-year-old male patient

Case 4, a female, aged 69 years, fell from the bicycle and suffered from intercondylar fracture of femur. Open reduction was performed with LISS plate internal fixation. Due to unparallel relation of distal screw and articular facet, ecstrophy of knee joint is observed with walking difficulty. Proximal the second and forth screw have a little bit longer, resisting the opposite internal wall of cortex, do not locking into the plate. Since severe damage has been done to the fracture ends, healing is not seen 10 months postoperatively. Distal screwes ruptured, led to failure of internal fixation. The case expresses the fact that bone grafting in time was needed whenever delayed union was occurring in the reduction using locking plate (Figure 4).

a, b: Preoperative X-ray check

c, d: Unparallel relation between distal screw and the articular facet, which leads to postoperative knee ecstrophy. The second and forth screw is improperly used

e, f: Failure of internal fixation at 13 mon after operation, the patient is unable to walk

g: CT scan of the failure

Figure 4 Pre- and postoperative X-ray film of a 69-year old female patient

Case 5, a male, aged 38 years, had left tibial and fibular fracture caused by car accident. Close reduction was performed with LISS fixation. Due to forward locating, the plate got evection hypodermally, and caused pain. Ulceration was seen 2 months after the operation, which led to revelation of the plate (Figure 5).

a, b: Excessively anterior locating the plate causes pain and local swelling, 2 cm of plate can be seen

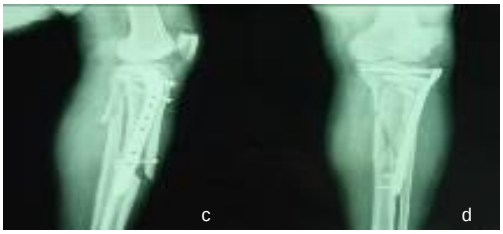
c: Revelation of LISS plate of 8 cm in a long term report

Figure 5 Pre- and postoperative X-ray film of a 38-year-old male patient

Case 6, a male, aged 68 years, had fracture of tibia and fibula caused by fallen from the bicycle. Open reduction with LISS internal fixation was performed at the local hospital. The micro-movement of fractural site and pain were reported, with

inability to walk postoperatively. 5 months later, abnormal activity of the fractural ends in the operation could be found. This case well illustrate that failure of internal fixation can be caused by problem of plate length and interval distance between the screws (Figure 6).

a, b: Preoperative X-ray check



c, d: Due to shortened length of the plate and wrong interval distance, internal fixation failure occurred

Figure 6 Pre- and postoperative X-ray film of a 68-year-old male patient

Case 7, a male, aged 36 years, had open intercondylar fracture of femur caused by car accident. Close reduction is performed after the wound got healed, internal fixation with LISS. However, due to operative lapse, proximal screw got tangent relation with the lateral cortex, making the screws infirm to fix. Failure was discovered 1 week after operation (Figure 7).

a, b: Preoperative X-ray check

c: Wrong locking of proximal screw lead to internal fixation failure

Figure 7 Pre- and postoperative X-ray film of a 36-year-old male patient

DISCUSSION

With various biological modes engendered by the different fixation mechanisms through the unique design special holes and screws of locking plate, different patterns of fractural healing can be induced[9]. Specifically, with the cone-shape locking mechanism of plate and screw, LCP can be used as not only the compression plate, but also the bridging plate, or even the combination of the two. LCP could be fixed outside the periosteum without direct contact of the bone itself, which mimics those internally installed external fixater, and with advantages of shortened distance from crank arm to the bone, it greatly decreases unsatisfying impairment to the blood circulation of the fracture side, and avoids the loss of the fracture reduction without perfect premoulding of the plate. When it comes to bridging the severe fragment, LCP can reduce the influence to the peripheral soft tissues, diminishing the infection probability, and is conducive to the secondary healing of the fracture with the decreased refracture rate. By the aforementioned points, we can conclude that locking plate had widespread indication.

However, as a novel technique and internal fixation tool, it brings surgeons unexpected problems simultaneously. One apparent problem is that insufficient cognition and inadequate learning and training would nonetheless produce improper operation and consequent unsatisfying effects. Through the analysis, we considered that the results of treatment using locking plate can be affected by the following conditions, and all combined could have integrative negative effects.

Degree of impairment on blood circulation at the fracture end

Blood circulation is the criteria to judge whether an internal fixation method is applicable or not, since it can indicate the activity of the fractural piece, and thus remains as a crucial step in the healing progress. The difficulty of determining the condition of blood circulation at the fracture end makes the technique of micro-damage momentous in the clinical practice. Influence is huge when osteonecrosis is triggered as either traumatic or iatrogenic one. The severe fracture introduced by the high-energy injury, repetitive close reduction or the rudely open reduction could not only damage the blood circulation at the fractural site, but also promotes the formation of sequestration. When the piece of the fracture needs absolute stability internal fixation for a long time, traditional compress plate mode could be selected with bone grafting operation in order to healing well[9]. But the only way without the grafting would be using locking plate to process bridging fixation under the condition that blood circulation or the local hematoma has not been destroyed, otherwise, disunion would occurred to the fracture. Therefore, some scholars thought that this system can only be effective when certain active piece of fracture exists[10]. Thus, it is important to have preoperative plan with full recognition of the mechanism, and many choices depend on the specific circumstances before the operation. In the group, there were 7 cases of nonunion, 6 of which were caused by severe injuries that lead to comminuted fractures, within which, 4 were open fracture, and 1 case was combined with vascular injury. 5 of the 7 cases had been processed with close reduction, 2 cases with open reduction. Due to serious vascular injury at the site, not only vitality of local bone decreased, but also disunions were accompanying. Thereby we agreed that when choosing indications, AO principle of internal fixation was the tenet to follow, which can be illustrated as accurately selection of compression plate or bridging plate method after the reduction of intraarticular fracture or metaphyseal fracture. However, locked intramedulllary nail was preferred for shaft fracture fixation. The principle of combination fixation was

applied when multiple fractures take place within one piece of bone, and to fix it with compression plate method for intra-articular fracture after open reduction, and using bridging plate for severe metaphyseal and shaft area fracture after close reduction. The principle should not to do comprehend the coincidental using two kinds of screws to perform the fixation. However, the above procedures must be rechecked to have bone grafting at anytime in case disunion or delayed union predisposition takes place (Case 1).

Status of fracture reduction

Locking plate makes it possible for the application of MIPPO technique, which demands that anatomical reduction in intra-articular fracture, alignment reduction in the fracture of metaphyseal and shaft area. Since thrypsis fragment can assume bigger strain than the simple one, the latter one requires more precise reduction. In order to have ideal amount of strain, decreasing the gap between the pieces of fracture less than 0.5 to 1 mm was suggested. Rigid internal fixation, if performed in the situation with gap between the fracture sides, would stop the formation of osteotylus with increasing probability of nonunion. Neither anatomic reduction nor stable fixation can be applied in the severe thrypsis, both of which, if done albeitly, would do further damage to the blood circulation, ultimately leading to nonunion. However, to process without anatomic reduction does not equals to lower the standards of reduction, satisfying contraposition and alignment between the two main pieces of the fracture was necessary. When repeated reduction was unsatisfactory, what is possible is that soft tissue has been intercalation between the fragments. Severe fracture, inadequate reduction, absorption of the fracture sides would increase the gap, blocks the bridging of osteotylus and causes delayed union or nonunion. If the defect of cortical occurred, delayed healing or infirm healing would be seen, and would result in the rupture of the plate; since to which the asymmetrical stresses was posted. Thus, we suggest that if judgment of stuck soft tissue is unclear or unsatisfying reduction occurs, percutaneous reduction by leverage or incision reduction under direct vision should be performed first. 5 cases with tibial nonunion (3 simple types, 2 comminuted types) and 1 case nonunion with segment fracture of femur in the group were due to excessive huge gap, which were confirmed by the detection of setting in muscular tissue of case 2, and bone defect in case 3. If bone defect has taken place of the fracture site, healing process would slow down, and long term stress would make plate rupture or internal fixation fail. The instance of this kind in the group was the intercondyles fracture that led to rupture of the distal screws of LISS plate (Case 4). Since the shape of LISS was designed accordingly to the anatomic structure after satisfying reduction, if it is reduced inadequately or with unmatching alignment, the plate would easily go dispositioned, that leads to unsatisfying screw location and intensity of fixation, which were often seen as complications of screw off-position or projection of the plate. 3 cases in the group have obvious hypodermal projections of proximal end of LISS plate, which led to the postoperative pain. In case 5 we discovered long term revelation of the plate to the outside. Although in 4 cases of the group, the internal fixation remained stable, the unsatisfying reduction of the tibia fracture triggered delayed unions for all.

Problems regarding screws application

It remains controversial of the how many screws should be used on the two fracture ends to keep the stability of the injury. Most agree on the principle of fixing 2 to 3 screws bilaterally if the fracture occurs on the lower limbs. Concerning the dominant role of rotational stress in the fractures of humerus or the forearm, 3 to 4 screws was suggested to the each side[11-13]. Gautier claims that for simple fracture, fixation of 2 screws on every bone piece with penetration of 3 layers of cortical part was a must, and for the thrypsis, 2 screws with 4 layers was the premise to guarantee the stability. Types of the fracture and the principles of the internal fixation determine the length of the plate, the number and positions of the screws. Gautier also agrees that only when proper length ratio (plate length/fractural length) and density of screw distribution (number/holes for screws) are obtained, can proper healing of fracture be seen. He deems that the length ratio for the thrypsis should be 2 to 3, and 8 to 10 is the right value for the simple fracture; the density for the screw should be less than 0.5 to 0.4[9]. The aforementioned means that stable fixation of the fracture was established on the basis of correct plate length and screw intervals. The sequence of screwing would also affect the reduction effect. When jointly using internal fixation principle, lag screw is preferred to press the pieces of the fracture, and successively finish the fixation with locking screw. Some scholars hold the opinion that different internal fixation principles should not be applied to the same fracture, absolutely stable lag screw that was used in relatively stable locking plate system would interrupt the minimal-movement of the fracture side, increase the probability of nonunion or lead to delayed union[14]. Thus, in order to reduce the stress of the plate, 2 to 3 holes near the ends of the simple fracture should be left without screws, yet holes around the ends of the comminuted fracture should be accomplished with screws, and together with longer plate to increase the stability. The failures caused by screws pulled out in the group of 1 nonunion of femoral fracture and case 6 with tibial thrypsis were due to shorter plate length with inadequate number of fixed screws.

No osteoporosis was found in the group. Although, theoretically, the locking plate can be applied to these indications, but with decreasing intensity, most scholars suggest using longer plate with bi-cortical screw which has longer working length and more stability along the longitudinal and lateral side. Gautier suggests plate be molded to have the directions of the screws divergently or convergently arranged which can increase the intensity of attraction[15-17].

Handling internal fixation tool by the operator

One of the advantages regarding locking plate was the satisfying locking mechanism provided by the screw and plate, which forms the angular stability. However, the operator could not sense precisely the attraction power showed by the screw of this locking mechanism. The problem would be worse if powerdriver was used, even when sometimes the screws were fixed outside the plate. Such constant illusion would shorten the length of the plate used and decrease the number of the screw needed, which would have the patients begin weight bearing earlier than ideal, and the screws would be cut or pulled out. We discovered that the two methods that can solve the problem: the first was to lengthen the distal incision properly, with

confirmation by touching that the plate is right at the central of the bone shaft; the second was to overlap the bone shaft, plate and the guide bar under the X-ray, which would also guarantee the best location of the fixation. The 3 failures of internal fixation in the group were all due to tangent location of screw to the lateral cortex that made screw pullout (Case 7).

Lapses of the operation

The dexterity of the surgeons in the mastery of the internal fixation principle, operating rules, and advanced techniques would directly influence the healing procedure of the fracture. Lapses can be classified as follows: ①The surgeons is not equipped with the skillful mastery of the MIPPO, and rudely proceed the operate without anatomic reduction, damaging the regional blood circulation with bridging fixation. ②Special clipping torsion driver are not in use by some surgeons, thus, spiral ties would be easily impaired, and internal fixation would be loosen. ③When no cooling water exists, the thermal dynamics affects the peripheral bones which could decrease the fixation intensity. ④If length measurement was not precisely, repetitive replacement of the screws would enlarge the diameter of the hole, reducing the intensity. ⑤When performing MIPPO, directly placing the plate onto the bone without using the flat end screw that controls the distance between the two would certainly damage the circulation of blood and ultimately affects the healing. ⑥If the unicortical screw was excessively long, it would hold the screw to interior wall of the opposite side of cortex, which lead to inadequate locking. In vitro experiment shows that inadequate contact would provide no better stability than the complete one, and clipping torsion screwdriver does not guarantee the compact locking between the plate and screws. ⑦Spiral markings were destroyed when progress rate changes during the bit penetrates the opposite side of cortex using self-drill screw and pulling device, which could stimulates the soft tissue with disappointing intensity of fixation. ⑧Ka “a” b indicates that from the lab result, when using targeting unit for screwing, certain vertical plate was needed. If the deviation of the angle was larger than 5 degrees, then the screws could not be well locked into the plate, which leads to loosening and failure of the fixation[18]. Therefore, simplifying the procedure of the operation would only result in the increasing of failing probability. Strict conforming to the rules was the necessity of every surgeon. ⑨Even expected result of reduction is obtained, tibial LISS plate is inclined to be fixed unnecessarily forward, which would lead to hypodermal projection, and regional symptoms would occur. Undoubtedly, the fixation effect would be affected. ⑩Parallel relationship should be generated between the first screw used in LISS plate with the articular surface of distal femur or tibial plateau, otherwise varus or evert with the knee joint would be observed. One case of such with fracture of the distal femur was observed in the group, which should be emphasized to draw the attention of our surgeons (Case 4).

Problems of postoperative recovery

Lawrence discovers that weight bearing only can be applicable when healing was expressed in X-ray check, no matter what kind of supracondylar fracture of femur is[19]. However, we do discover in our group that, 5 cases of tibia fracture in which the patients did not strictly perform the weight bearing for 6 month after the operation showed no signs of healing, and show it 2 months latter after yet performed weight bearing. Thus, with this novel internal fixation theory, the enforcement of postoperative recovery and weight bearing should be determined by the degree of operational stability and possible amount of the stress associated with the ends of the fracture site. Seide confirms that the stability of locking plate was 3.3 times larger than that of the traditional plate in resisting the rotational force, which illustrates that stress remains relatively small[20]. We suggest that longer plate should be used for the simple fracture, since shielding of stress may lead to delayed union. Since the application of locking plate is the recent progression, the healing mechanism is under further exploration.

In conclusion, although many advantages can be seen from the comparison between the locking plate and the traditional plate, such as low mobility of complications (4.5%)[20], By no means it can be guarantees the healing of the fracture treated with locking plate. AO internal fixation tool cannot replace the delicate AO internal fixation technology. However, the healings has lot to do with the types of the fracture, preference of the operators, and updates of cognitions and techniques besides the application of new internal fixation tools. Only by taking into the consideration of well-rounded comprehension of AO fixation principle, a mastery of AO technology, carefully planned preoperative scheme, meticulous selection of cases, and correct application of locking plate in operation, could we increase the healing rate, and avoid the occurrence of aforementioned complications as well.

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锁定钢板置入治疗骨折：25 例并发症分析

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摘要

背景：锁定钢板独特的钉孔和螺钉设计使其在骨折固定过程中可以利用完全不同的固定机制，给骨折的愈合提供不同的生物学环境，引起不同的骨折愈合模式。

目的：锁定钢板治疗骨折后出现并发症的 25 例回顾性分析。

方法：对患者体检结果，其受伤机制、是否多发损伤、内固定物选择、操作方法、术后开始负重时间及术后复查的 X 射线片逐一进行分析。主要观察内固定物失效、骨折不愈合及延迟愈合例数及原因。

结果与结论：8 例内固定物失效的病例中，4 例由于手术操作失误，1 例由于内固定物选择失误，1 例由于术后负重过早，2 例由于术后感染导致内固定物失效；11 例骨折不愈合或延迟愈合的患者中，6 例由于原始损伤严重，5 例由于术中复位不佳引起不愈合或延迟愈合。2 例 LISS 钢板近端突起于皮下引起疼痛，1 例术后伤口感染，钢板近端长期外露，是由于术中钢板的放置位置不良。应用锁定钢板固定系统治疗骨折成功的关键是操作者充分理解 AO 内固定原理，掌握 AO 内固定技术，强调术中对骨折的良好复位和微创操作。只有良好的术前计划、选择适当病例，正确使用锁定钢板，才能提高骨折愈合率，避免并发症的发生。

关键词：固定器；钢板；骨折；并发症；愈合
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As the Official Journal of the American Association of Hip and Knee Surgeons, The Journal of Arthroplasty brings together the clinical and scientific foundations for joint replacement. This peer-reviewed journal publishes original research and manuscripts of the highest quality from all areas relating to joint replacement or the treatment of its complications, including those dealing with clinical series and experience, prosthetic design, biomechanics, biomaterials, metallurgy, biologic response to arthroplasty materials *in vivo* and *in vitro*.
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