Experimental Study on the Treatment of Open Knee Fractures Combined with Seawater Immersion

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Abstract. *Objective:* To investigate the effects of different treatment methods on the therapeutic outcomes of open knee fractures combined with seawater immersion, providing references for the selection of appropriate treatment methods. *Methods:* Forty adult New Zealand white rabbits, weighing 2.20±0.25kg, were divided into four groups (10 per group) using a random number table: control group (Group A), primary internal fixation group (Group B), secondary internal fixation group (Group C), and external fixation group (Group D). After inducing open distal femur fractures, Group A's wound was left open for 2 hours, while Groups B, C, and D were immersed in seawater for 2 hours. Subsequently, Groups A and B underwent debridement followed by plate and screw internal fixation. Group C underwent debridement and external fixation, followed by plate and screw internal fixation after wound healing. Group D underwent debridement followed by trans-articular external fixation. Observations were made on wound healing, joint function recovery, and radiological and histological changes at the fracture end. *Results:* The wound healing time for groups with seawater immersion was significantly longer than Group A ($P<0.01$). Infection rates were 10% in Group A, 60% in Group B, and 20% in both Groups C and D. Group B had a significantly higher infection rate than the other groups ($P<0.05$). The affected knee joint width in the seawater-immersed groups was significantly greater than the healthy side and Group A ($P<0.01$), and the maximum extension angle of the knee joint was also smaller ($P<0.01$ and $P<0.05$, respectively). Among them, Group C had a better maximum extension angle than Group D ($P<0.05$), but no significant difference from Group B; furthermore, the minimum flexion angle of Group C was not significantly different from Group A but was significantly smaller than Groups B and D ($P<0.01$). Pathological healing grade analysis showed that Group B had a significantly lower fracture healing grade than Group A ($P<0.05$), with no significant differences among the other groups. *Conclusion:* Knee fractures combined with seawater immersion prolong wound healing time. Delayed internal fixation not only effectively reduces the infection rate of open knee fractures combined with seawater immersion but also promotes joint function recovery.

Keywords: Seawater immersion injury; Knee joint; Open fracture; Fracture fixation; Rabbit

1. Introduction

In modern naval warfare, amphibious operations, and various marine economic activities, fractures combined with seawater immersion are relatively common [1-3]. Preliminary research findings in China have addressed the characteristics and early treatment methods of open fractures accompanied by seawater immersion [4-5]. Some studies suggest that the infection rate of seawater-immersed open fractures and the poor formation rate of fracture-end callus are elevated. Using external fixation to stabilize fractures in long bones can effectively reduce the infection rate and enhance the level of medical treatment [6]. However, many fracture cases are often accompanied by articular fractures or the fracture ends are near the joints. In such cases, external fixation devices must span the joint to effectively stabilize the fracture. Due to the use of transarticular external fixators, the joint may remain immobile for extended periods, preventing early functional exercise, which could further impede joint function recovery. What is the impact of external fixators on joint function during their use? Can delayed internal fixation alleviate the adverse effects on joint function recovery brought about by transarticular fixation, while also reducing the infection rate of articular fractures combined with seawater immersion? To this end, we prepared an open knee joint fracture animal model in rabbits and observed the effects of different fixation methods on wound infection, joint function recovery, and fracture healing after an open knee joint fracture combined with seawater immersion. Our aim is to provide a scientific basis for selecting appropriate treatment methods for open articular fractures combined with seawater immersion, and to offer references for the treatment of casualties in naval warfare and maritime disasters.

2. Materials and methods

2.1. Animals and grouping

Forty adult rabbits, both male and female, weighing 2.20±0.25kg, were provided by the Animal Center of Daping Hospital, Army Medical University. Using a random number table method, animals were divided into four groups, with ten in each group. All were prepared as models of open knee fractures and treated differently according to their grouping. Group A served as the control group, Group B as the fracture with seawater immersion primary internal fixation group, Group C as the fracture with seawater immersion secondary internal fixation group, and Group D as the fracture with seawater immersion external fixation group.

2.2. Animal model preparation

After fur removal, animals were anesthetized with 1.5% pentobarbital sodium injection at a dose of 1ml-2ml/kg via the ear vein. A 2.5 cm longitudinal incision was made on the inner side of the rabbit's right knee. Muscles and ligaments were bluntly dissected to expose the knee joint and the distal end of the femur. The rabbit was then positioned in supine and fixed on an injury frame. Using a custom-designed vertical impactor, a fracture was inflicted using a 1.5×1.2cm inverted "L" shaped impact blade. The weight of the impactor was raised to a height of 50cm and then dropped freely, striking the handle of the blade, creating a model of open knee fracture with a femoral condylar split.

Upon successful preparation of the model, animals in Group A were left untreated for 2 hours, while animals in Groups B, C, and D were fixed on a specially designed rabbit frame and placed over a plastic bucket (diameter 20 cm, height 25 cm) filled with seawater, immersing both lower limbs in the seawater for 2 hours. The seawater temperature was maintained at 20-22°C using a water bath. The seawater was sourced from the Quanzhou sea area of the Taiwan Strait, with primary parameters being NaCl 26.518g/L, MgCl2 22.447g/L, MgSO4 3.305 g/L, CaCl2 1.141 g/L, KCl 0.725g/L, and an osmotic pressure of 1250-1350 mOsm/L. After 2 hours, wounds were immediately debrided, fractures were reduced, and fracture fixation was performed accordingly.

2.3. Fracture fixation and post-operative care

All animals underwent debridement prior to fracture fixation, which involved removal of necrotic skin, muscle, joint capsule, fat pad, and periosteum until tissue showed active bleeding. Wounds were irrigated with saline (0.45% hypotonic saline) and immersed in iodine for 5 minutes. For Groups A and B, internal fixation was carried out using plate and screws. The wound was primarily closed, sterile dressings applied, and dressings were changed regularly. Group C underwent external fixation using a tubular plaster cast, leaving the wound open with sterile dressings applied. A window was made in the local area of the wound for periodic dressing changes. Once the wound healed, a secondary open reduction and internal fixation using plate and screws were performed. Group D received transarticular external fixation. The wound was left open with sterile dressings applied and a window made in the wound for periodic dressing changes, followed by secondary suture.

Postoperatively, animals were housed in separate cages. They received a combination of antibiotics through the ear vein: 800,000 units of sodium penicillin, 5ml of 0.5% metronidazole injection, and 5ml of 0.2% levofloxacin lactate sodium chloride injection, twice daily for 5 consecutive days.

Any animals that died within 7 days post-surgery were replaced. If wound infection persisted 5 days after the anti-infective treatment, subsequent interventions included wound debridement, keeping the wound open with regular dressing changes, secondary suturing, and continued use of sensitive antibiotics. For Groups A, B, and C, where the animals had joint mobility, passive joint exercises were conducted for half an hour daily once the wounds healed. After 4 weeks of observation, animals were euthanized through venous air injection.

2.4. Outcome measures

2.4.1 Observation of wound healing duration across all groups. Healing criteria: Well-apposed skin wound, dry scab formation, no redness, swelling, or exudation, and no signs of ischemic necrosis.

2.4.2 Calculate the infection rates (number of infections in animals postoperatively housed for $\geqslant$7 days) and degree of infection. Assessment criteria: Mild is characterized by skin redness and edema at the wound site with tissue fluid exudation; Moderate presents as skin ischemic necrosis with fissures, purulent secretion, with a normal blood supply in the wound's deep tissues; Severe entails extensive skin necrosis, copious purulent discharge, ischemic necrosis of deep soft tissues, potentially extending to the bone tissue of the knee joint.

2.4.3 Joint functional recovery: Comparison of appearance, knee joint width, limb length, and joint mobility between the affected and healthy limbs at 4 weeks post-operation.

2.4.4 Observation of fracture healing in each group: X-rays of the affected tibia in anteroposterior and lateral views were taken routinely at 0, 2, and 4 weeks post-operation, focusing on the fracture fixation and healing at the fracture ends. After completing the 4-week observation, animals were euthanized by venous air injection. Knee joint and distal femur specimens were obtained, decalcified post-fixation, and stained with hematoxylin and eosin (HE). Under microscopy, granulation tissue, fibrous tissue, cartilaginous callus, and new bone formation at the fracture ends were examined, followed by pathological grading:

Grade 0: Only granulation or fibrous tissue present between the fracture ends.

Grade I: Initial healing dominated by cartilage with a minimal bony callus.

Grade II: Adequate healing with equal proportions of cartilaginous and bony callus.

Grade III: Predominantly bony healing with a large bony callus and minimal cartilage.

2.5 Statistical Methods

The quantitative data results are presented as mean ± standard deviation (□ s). Statistical analyses were performed using SPSS 13.0 software. Limb measurement data were analyzed using paired T-tests and one-way analysis of variance (ANOVA). Infection rates were compared using the chi-squared test, and tissue healing pathological grades of the callus were compared using non-parametric tests. A P-value of <0.05 was considered statistically significant, while a P-value of <0.01 was considered highly statistically significant.

3. Results

3.1. General observations

After surgery, the animals consumed food and water normally but exhibited limited activity due to knee joint fixation. All animals in Group A survived post-operatively, while 4 in Group B, 1 in Group C, and 1 in Group D died. Any animal that died within 7 days post-surgery was replaced.

3.2. Wound Healing and Infection Status

Animals that displayed signs of infection post-surgery were immediately cleaned, treated regularly with dressings, and given antibiotic treatments. By the end of the observation period, all animals in Group A had healed, while 3 in Group B, and 1 each in Groups C and D, had not. Among the healed animals, the average healing time for Group A was 4.90±1.79 days, compared to 8.43±2.44 days for Group B, 7.50±1.69 days for Group C, and 7.33±1.00 days for Group D. A one-way ANOVA indicated $F=6.577$, $P=0.002$, showing that the healing times in the saline-soaked injury groups were significantly longer than in Group A.

Group A had an infection rate of 10%, Group B 60%, and both Groups C and D 20%. Using a 2xK table split and pairwise comparison, Groups A, C, and D could be combined, resulting in a chi-squared value of 7.064 with a P-value of 0.014 ($P<0.05$). This suggests that the infection rate in Group B (primary internal fixation group) was significantly higher than in the other groups. The infection levels for each group are presented in Table 1.

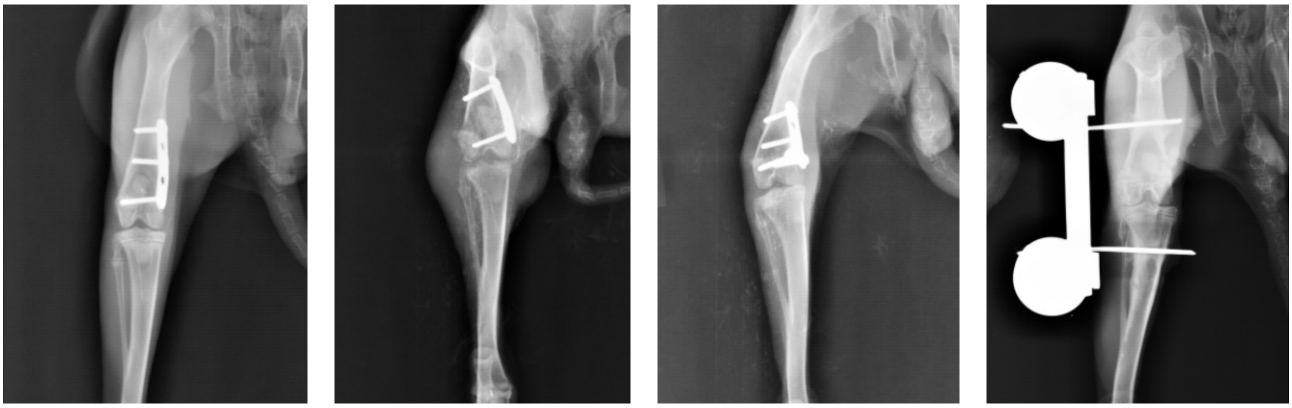
Table 1: Infection and Healing Status of Each Group

Group	Mild Infection (cases)	Moderate Infection (cases)	Severe Infection (cases)
A	1	0	0
B	1	3	3
C	0	0	0
D	1	5	0

Table 2: Limb and Knee Joint Conditions of Each Group 4 weeks Post-injury ($\overline{X} \pm s$)

Group	Knee Joint Width (cm)		Limb Length (cm)		Maximum Extension Angle of Knee Joint (°)		Minimum Flexion Angle of Knee Joint (°)	
	Healthy Side	Affected Side	Healthy Side	Affected Side	Healthy Side	Affected Side	Healthy Side	Affected Side
A	1.80□0.14	1.92□0.20▲	27.47□1.55	26.60□1.60▲	169.80□4.13	162.8±9.40▲▲	21.2±2.10	23.00±2.45
B	1.86±0.46	2.62□0.58▲▲**	27.95±1.57	25.65±2.85▲▲	169.40±1.35	147.10±21.10▲▲*	21.20±3.766	30.10±7.38▲**
C	1.82±0.41	2.56□0.49▲▲**	28.25±1.31	26.40±2.05▲▲	170.4±2.50	146.50±18.15▲▲*	20.20±1.93	23.7±3.23▲△△
D	2.12±0.33	2.54□0.39▲▲**	27.9±1.17	25.63±1.94▲▲	172.00±2.58	129.30±15.81▲▲**△☆	21.40±2.27	31.30±4.60▲▲**☆☆

Compared with the healthy side: ▲ P<0.05, ▲▲ P<0.01; compared with group A: * P<0.05, ** P<0.01; compared with group B: △ P<0.05, △△ P<0.01; compared with group C: ☆ P<0.05, ☆☆ P<0.01.



A B C D

Figure 1: Radiographic representation 4 weeks post knee joint fracture surgery.

A: Control group, fracture of the knee joint healed; B: Immediate internal fixation group, postoperative infection with non-union fracture, osteolytic resorption, and significant soft tissue swelling; C: Delayed internal fixation group, fracture end healed; D: External fixation group, good fracture healing observed with callus growing along the Kirschner wire.

3.3. Joint Function Recovery Status

By the end of the observation period, the knee joint widths on the affected side in Groups B, C, and D were significantly larger than those on the healthy side and the affected side of Group A (P<0.01). The length of the affected limbs was also notably shorter than the healthy ones (P<0.01). The maximum extension angle of the affected knee joints was less than that of the healthy side (P<0.01) and less than Group A (P<0.05). The minimum flexion angle of the knee joint was also inferior to the healthy side (P<0.05). Specifically, the maximum extension angle of the knee in Group C was greater than that in Group D (P<0.05) but showed no significant difference from Group B. Furthermore, the minimum flexion angle in Group C showed no significant difference from Group A but was much smaller than Groups B and D (P<0.01). The limb lengths and knee joint status across groups can be seen in Table 2.

3.4. Radiographic observations

Postoperative open knee joint fractures in animals were consistently examined using anteroposterior and lateral plain radiographs to monitor fracture healing and any signs of infection. At 4 weeks post-operation, Group A showed no evident fracture lines and no radiographic signs of bone infection. Group B had 3 cases displaying clear signs of osteomyelitis, characterized by non-union fracture ends, osteopenia, and blurry bone margins. There was evidence of bone resorption and necrosis at 4 weeks post-surgery. Additionally, one case in Group B showed a fracture line that had not yet healed. Both Groups C and D had one case each with a noticeable fracture line but no clear signs of bone infection. Radiographically, the rate of bone end union in Group A was 100%, Group B was 60%, and both Groups C and D were at 90%.

3.5. Histopathological observations

Macroscopic observations: Postoperative immersion in seawater resulted in more pronounced early-stage localized soft tissue edema and exudation in all groups compared to the control group. Infected animals showed localized erythema, raised nodules, and, in some instances, tofu-like purulent discharge. Exposed bone ends, osteolytic destruction, and in some cases localized skin and patellar ligament dry necrosis were observed. In contrast, uninfected cases had no significant macroscopic abnormalities.

Table 3: Bone Tissue Healing Grades for Each Group (count)

Group	0	I	II	III
A	0	0	2	8
B	3	1	3	3
C	1	1	4	4
D	1	0	5	4

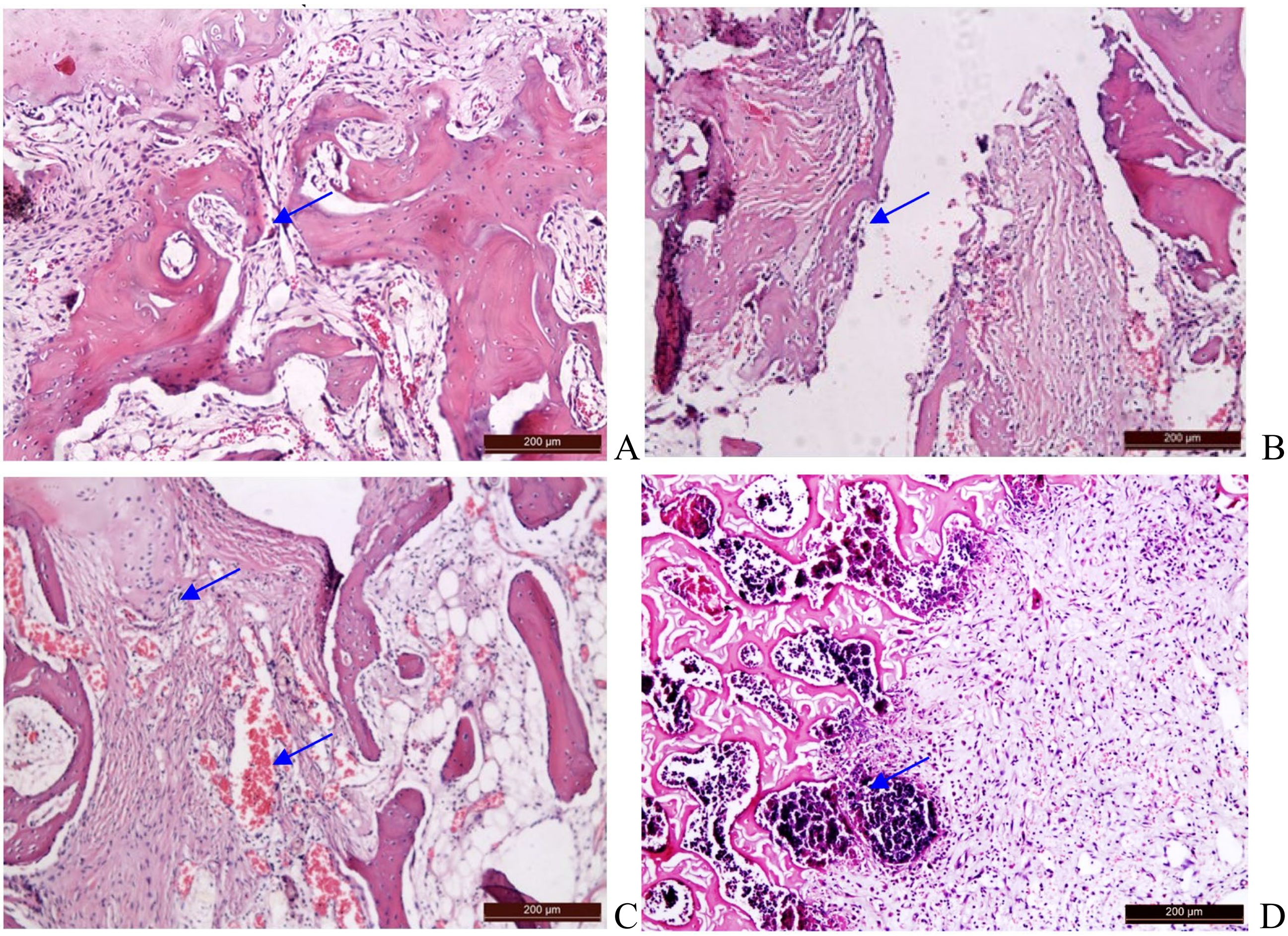


Figure 2: Histopathological representation 4 weeks post knee joint fracture surgery (100x).

A: Control group, the fracture site showed osseous union with primary bone callus (arrow); B: Immediate internal fixation group, non-union fracture ends with fibrous tissue growth on both ends (arrow); C: External fixation group, good fracture healing with bone callus, cartilage callus, fibrous tissue, and numerous blood vessels (arrow); D: Osteolytic destruction with extensive inflammatory cell infiltration (arrow).

Microscopic observations: Following the grading standards for fracture healing, Group A had 8 cases that achieved osseous union, with the remaining 2 also showing good healing. Groups B, C, and D all exhibited cases of non-union or poor healing. Specifically, Group B had 3 non-union cases. The tissue healing grades of surviving animals 4 weeks post-surgery are presented in Table 3. Using non-parametric statistical methods to assess differences in healing rates among the groups, Group B had a significantly lower union rate compared to Group A ($\chi 2=5.885$, $P=0.015$). No significant statistical differences in healing rates were observed between the other groups. Following fracture infection, a large infiltration of inflammatory cells within the bone marrow and osteolytic destruction was observed (Figure 2).

4. Discussion

With the continuous advancement of China's maritime capabilities and marine resource development, coupled with the strengthening of coastal defense, the incidence of maritime trauma and seawater immersion injuries is expected to increase. Specifically, in potential coastal military conflicts, the occurrence of explosive injuries in modern naval warfare will surge, resulting in a significant number of open fractures caused by related shrapnel. Consequently, the number of seawater-immersed fracture victims will also rise. Addressing open fractures combined with hypothermia and seawater immersion will be crucial in maritime logistical support.

Seawater is characterized by its high osmolarity, high sodium content, alkalinity, and low temperature. Its osmotic pressure is 43 times that of human plasma; sodium ion concentration is three times higher than that in plasma; its pH is alkaline. Except in tropical regions, the water temperature in various seas is generally below 20°C, significantly lower than the normal human body temperature. The high osmolarity and alkalinity of seawater can elevate the local osmotic pressure of open fracture wounds, leading to cell dehydration and interstitial edema. It can directly stimulate the release of inflammatory mediators such as IL-8, TNF, and NO, enhance lipid peroxidation reactions, inhibit the activity of cell membrane Na-K-ATPase, suppress coagulation mechanisms, exacerbate microvascular bleeding in injured tissues, and dilate blood vessels, aggravating tissue edema. This further reduces peripheral perfusion. After immersion, the improvement of peripheral perfusion can cause reperfusion injury, leading to cardiovascular system damage [7]. Heat loss in water is three times greater than in air of the same temperature, and local wounds immersed in seawater below body temperature further exacerbate tissue energy metabolism disorders [8]. All these local and systemic pathophysiological responses are detrimental to fracture healing, resulting in slower endochondral ossification, higher rates of poor callus formation, and reduced fracture healing strength [5]. In this study, the bone fracture ends in group A were mainly osteogenic calluses, while those in the seawater-immersed groups were predominantly granulation tissue, fibrous tissue, and cartilage tissue. The healing effect in group B was significantly lower than that in group A, with a statistically significant difference, while the healing grades in groups C and D were also lower than that in group A, but with no significant statistical difference. This indicates that seawater immersion results in reduced fracture healing quality, which is more evident when primary internal fixation is performed.

Seawater contains a plethora of bacteria, with 100,000 bacteria per 1 ml of seawater, especially Vibrio vulnificus, which can easily lead to wound infections. The complex physicochemical factors of seawater exacerbate the inflammatory response in open fractures, facilitating bacterial proliferation [9]. In this experiment, even with primary internal fixation and standardized postoperative care, the infection rate in group B was significantly higher than in the control group, confirming the increased infection rate of open knee joint fractures immersed in seawater, indicating the significant impact of seawater immersion on infection occurrence in open knee joint fractures. Moreover, for rabbit knee joints combined with distal femoral open fractures, the infection rate in group B was significantly higher than in groups C and D, while the infection rates in groups C and D were slightly higher than in the control group but without statistical significance. This suggests that it's inappropriate to choose primary internal fixation for seawater-immersed knee joints combined with distal femoral open fractures as it increases the risk of infection. Within 3 days after seawater-immersed open fractures, bacteria proliferate rapidly, and the body's immunity is at a low level, insufficient to prevent rapid bacterial growth. Closing the wound at this time impedes wound drainage and forfeits the opportunity for delayed debridement, hindering the body's natural defense against bacteria, which is detrimental to infection prevention and treatment. Moreover, internal fixation further damages tissue blood supply, reducing its resistance to bacterial invasion, while internal devices shelter bacteria, exacerbating infection onset and progression. Using external fixation or waiting for infection control before performing delayed internal fixation can reduce soft tissue damage in the initial post-injury period, minimize local disturbances, facilitate wound observation and drainage, and enable adjustments based on X-ray findings, effectively reducing the infection rate of seawater-immersed open knee fractures.

Ordinary long bone fractures usually do not exceed the joint in external fixation, allowing early joint function exercises with minimal impact on joint recovery. Some studies suggest that external fixation is the preferred treatment for open fractures with seawater immersion that doesn't require trans-joint fixation [6]. However, for open fractures of the knee joint and distal femur with seawater immersion, external fixation must be performed across the joint. This immobilizes the knee at a fixed angle, preventing functional exercise and potentially leading to joint functional decline, affecting joint recovery. Moreover, external fixation has drawbacks like pin tract infections. In our study, the maximum extension angle of group D (external fixation group) was lower than that of groups B (primary internal fixation group) and C (delayed internal fixation group) ($P<0.05$). The minimum flexion angle of group D was not as good as that of group A (control group) and group C, with no significant difference from group B. This indicates that long-term trans-knee joint external fixation affects joint function recovery more than internal fixation. Delayed internal fixation first involves thorough debridement and external fixation, followed by early internal fixation and joint function exercises once infection is controlled, benefiting both infection control and early joint function recovery.

5. Conclusion

For open knee joint fractures combined with seawater immersion, early external fixation should be employed to control infections and promote wound healing. Once infections are managed, delayed internal fixation should be conducted, combined with early joint function exercises, to reduce infection rates and improve knee joint recovery levels.

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