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Effect of Different Irrigation Solutions on Wound and Fracture Healing in a Rabbit Open Fracture Model—A Pilot and Feasibility Study

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ABSTRACT

This study compares the microbiological, radiological and histological effects of irrigation with povidone-iodine, hydrogen peroxide or saline on wound and fracture healing in an animal open fracture model. This study used an open fracture tibia model in a New Zealand White rabbit treated with debridement and irrigation 24 hours after the initial fracture. Irrigation was performed via gravity flow with either 20 mL of 0.9% saline, povidone-iodine 10% or hydrogen peroxide 3%, followed by rinsing with 100 mL saline. Tissue samples were taken before and after debridement for microbiological assessment of bacterial clearance and histological evaluation of wound inflammation. Radiographs were performed at intervals to assess the progress of fracture union. Eight weeks later, the tibia and surrounding tissues were extracted to histologically evaluate fracture and wound healing. All wounds healed well with no clinical evidence of infection. Reduction of the bacterial load was seen with irrigation by povidone-iodine. Fractures irrigated with povidone-iodine had a relatively faster radiological progression of fracture union than saline. There was no histological difference in wound and fracture healing among the tested solutions. This study provides evidence that povidone-iodine does not impair fracture healing. However, as this was a pilot study with a small sample size, a larger study is required to confirm statistical significance and clinical relevance.

Keywords: Antiseptic, fracture healing, irrigation, open fracture, wound healing

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INTRODUCTION

The incidence of open fracture is estimated at 30.7 per 10,000 persons per year and is bound to increase (Court-Brown et al., 2012). It is often associated with poor outcomes, with reported rates of 24% infection, 15% amputation and 11% non-union (Schade et al., 2021). These have a profound financial impact, costing up to £40,000 for treating infection and reconstructive procedures (Schade et al., 2021). Complications such as infection led to a six-fold increase in hospitalisation duration, decreased productivity and loss of financial earnings, where less than 50% of patients returned to work after one year (Flores et al., 2024; Hoekstra et al., 2017). These understandably cause substantial physical and psychosocial impacts on patients and significantly strain medical personnel and healthcare centres.

Hence, open fracture management focuses on preventing such complications from the outset. Studies and guidelines have been developed over the years to improve the outcome (Eccles et al., 2020). They advocate early antibiotic administration, adequate debridement, wound irrigation and fracture stabilisation. While irrigation mechanically removes foreign bodies and reduces the amount of potential pathological microorganisms, the choice of irrigation solutions is still subject to debate. A good irrigation solution is one that not only removes foreign materials but also kills harmful microorganisms without causing damage to host tissues. Antiseptic solutions such as povidone-iodine and hydrogen peroxide are widely used, as they are bactericidal and less selective, rendering them less susceptible to resistance. There have been concerns about their cytotoxicity, as reported by several in vitro studies (Kaysinger et al., 1995; Lineaweaver et al., 1985; Nicholson et al., 1998; Rueda-Fernández et al., 2022; Thomas et al., 2009). Nevertheless, irrigation of open fractures with antiseptics is a common clinical practice. In international surveys, up to a third of surgeons used antiseptics to irrigate severe open fractures, and almost half believed they were superior to saline (Petrisor et al., 2008; Puetzler et al., 2019).

Thus, our study investigates the effects of these antiseptics on wound and fracture healing *in vivo* in an experimental animal model. We designed a pilot study to assess the feasibility of this protocol. We hypothesised that povidone-iodine and hydrogen peroxide do not impair wound and fracture healing when used as irrigation solutions in a rabbit open fracture model.

MATERIALS AND METHODS

This pilot experimental animal study was conducted at the University Veterinary Hospital, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM). The study design was approved by the Institutional Animal Care and Use Committee UPM (approval number: UPM/IACUC/AUP-R056/2023) and conducted in accordance with the Animal Welfare Act 2015.

Animals

Four healthy male New Zealand White rabbits of the species *Oryctolagus cuniculus* (A Sapphire Enterprise, Selangor, Malaysia), aged eight to ten weeks old and weighing between 2.0 to 2.35 kg, were used in this study. Exclusion criteria were rabbits with ongoing infection and a previous history of injury or fracture. The rabbits were allowed to acclimatise for one week before surgery in the exotic animal ward of the University Veterinary Hospital UPM. They were housed in individual cages in a 12:12 light-dark cycle. They were given an antibiotic-free diet, Timothy hay enrichments and tap water ad libitum. The rabbits were allocated into three groups: Group 1 (control group)—normal saline (NS); Group 2—hydrogen peroxide (HPO); and Group 3— povidone-iodine (PI). At the start of the acclimatisation period, the rabbits' right hindleg fur was trimmed and cast with fibreglass (ALTOCAST™) (Figure 1). Venous samples were sent for a complete blood count and renal profile. Their body weights were measured and recorded.

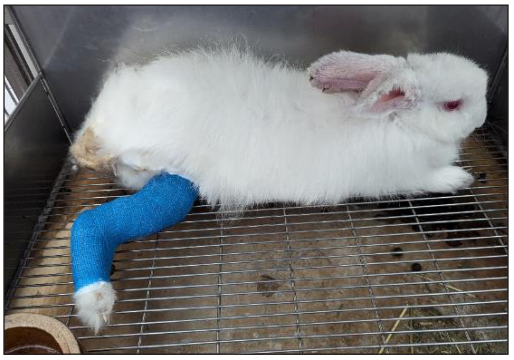


Figure 1. Rabbits allowed acclimatisation with the right hind leg cast one week prior to surgery

Irrigation Solutions

The irrigation solutions used in this study were sterile normal saline 0.9% (RinsCap®, Ain Medicare Sdn. Bhd.), povidone-iodine 10% w/v, containing 1% available iodine (Septidin 10%, AVANTE' Group Inc, USA), and hydrogen peroxide 3%.

Preparation of Open Fracture Model

The rabbits were anaesthetised with intramuscular injection (IM) of ketamine 20 mg/kg and midazolam 1 mg/kg. Anaesthesia was maintained with top-up doses of intravenous (IV) propofol 1–2 mg/kg, ketamine 1–2 mg/kg, and fentanyl 5–8 mcg/kg. Oxygenation was provided via facemask. The rabbits were placed in recumbency (Figure 2).

Using a cast cutter, a window measuring 5×2 cm was created over the anterior aspect of the rabbit's right hind leg cast. Through

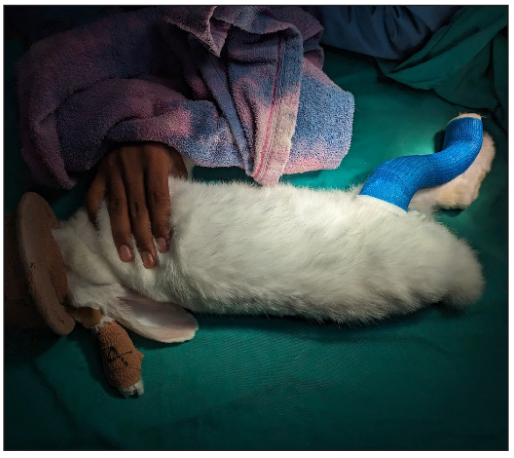


Figure 2. Rabbit positioned recumbent after general anaesthesia with mask oxygenation

this window, a longitudinal skin incision approximately 2 cm in length was made over the anterior surface of the right hind leg (Figure 3). The tibial diaphysis was exposed via the anterolateral approach, and an oblique osteotomy of the mid-tibia was done using an oscillating saw cooled with saline irrigation. No skin preparation was done, and non-sterile equipment was used to simulate an open fracture environmental contamination. The wound was then loosely packed with gauze, and bandaging was applied. This phase marked the surgical creation of the open fracture model. Analgesia was given post-operatively with subcutaneous (SC) meloxicam 0.5–1.0 mg/kg SID and tramadol 5 mg/kg TID.

(a) (b)

Figure 3. Surgical creation of tibia open fracture model: (a) Window marked and created over right hind leg cast for surgical access; (b) Incision and fracture creation done through the window

Surgical Debridement and Irrigation

Twenty-four hours after open fracture creation, definitive surgery was performed. General anaesthesia was administered as described above. Prior to debridement, tissue samples (fascia and muscle layer) of approximately 0.5 g were taken from the wound for microbiological and histological analysis. The cast over the right hind leg was removed with a cast cutter. The right hind leg was shaved, and surgical skin preparation was performed with povidone-iodine 10%. A sterile surgical drape was prepared over the right hind leg. Subsequent procedures were done using an aseptic technique with sterile instruments. The wound over the right hind leg was thoroughly debrided, removing unhealthy tissues. The wound and fracture ends were then irrigated with the respective solutions according to their groups. The control group received 20 mL of normal saline 0.9% (n = 1); the HPO group received 20 mL of hydrogen peroxide 3% (n = 1); and the PI group received 20 mL of povidone 10% (n = 2). Then, another 100 mL of 0.9% saline irrigation followed to rinse off the residual solution. All irrigations were performed at low pressure via gravity flow, approximately 5 cm above the wound. Post debridement, tissue samples 0.5 g were taken for microbiological and histological comparison. The tibia fracture was stabilised using a single intramedullary Kirschner wire inserted retrograde. The wound was then closed with

Monosyn® 5/0 suture (B. Braun Surgical, Rubi, Spain). Gauze dressing and crepe bandage were applied over the right hind leg. Fibreglass cast was reapplied for further rotational stability, followed by window creation to facilitate wound inspection and dressing. A venous blood sample was taken from the contralateral hind leg for a post-operative haemogram.

Post-operative Treatment

Rabbits were isolated in their respective cages (Figure 4). Analgesia was given with SC meloxicam 0.5–1 mg/kg SID for up to five days and SC tramadol 5 mg/kg TID for up to two weeks, with further doses as clinically appropriate. IV cefuroxime 20 mg/kg 8-hourly was given as a post-operative antibiotic for three days. Oral metoclopramide 0.5 mg/kg BID, oral simethicone 20 mg/kg BID and oral multivitamin 0.2 mL SID were given for five days post-operatively to enhance gut motility and feeding. The rabbits were monitored clinically for signs of local or systemic infection. Weight measurements were performed weekly. Wounds were dressed daily with normal saline and non-adherent dressing until healing, and signs of erythema, swelling, exudate or dehiscence were observed. Casts were removed on day 42. At day 56 post-operatively, the rabbits were euthanised with IV propofol 10mg/kg and pentobarbital sodium 135–140 mg/kg. Tissue samples from the wound site were taken, and the right tibiae were extracted for histological analysis.

(a) (b)

Figure 4. Rabbits are placed in individual cages and allowed feeding and water ad libitum post-operatively

Radiological Analysis

Serial radiographs of the right tibia were taken immediately post-operatively and at 21, 42 and 56 days post-operatively. Anteroposterior and lateral views of each tibia were taken at each interval. Both the radiographic views provided a combined Radiographic Union Scale in Tibial fracture (RUST) score with a minimum score of four and a maximum of twelve (Table 1) (Whelan et al., 2010). Interpretation of the scores provided an estimate of the healing of the fracture (Table 2) (Leow et al., 2020).

Table 1
Radiographic union score for tibial fractures (RUST)

Score per Cortex	Radiographic Criteria	
	Callus	**Fracture line**
1	Absent	Visible
2	Present	Visible
3	Present	Invisible

Note. Two criteria are evaluated for each cortex (anterior, posterior, medial and lateral) from two radiographic views. The sum of all four cortical scores gives the total RUST score (Whelan et al., 2010)

Table 2
Interpretation of RUST score

Score	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Interpretation	Not healed		Possible non-union			Not non-union, not healed	Possibly healed	Healed	

Note. Union is defined as three cortices with a RUST score of 3, with a value higher than 10 indicating definite fracture healing (Leow et al., 2020)

Microbiological Analysis

Tissue samples of approximately 0.05 g collected pre-debridement and post-debridement were placed in 0.1 mL of saline in Eppendorf Tubes® with a dilution ratio of 1:10. Fluid taken from this tube was serially diluted with saline, subsequently inoculated onto agar plates and then incubated at 37°C for 24 hours. Colony-forming units (CFU) were counted, representing the bacterial count per gram of tissue (CFU/g). Colonies were also microscopically identified and further sub-cultured on agar plates to identify the type of microorganisms present.

Histological Analysis

Tissue samples collected pre-debridement and post-debridement were fixed in 10% neutral-buffered formalin and embedded in paraffin. 4µm thick tissue sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope for heterophils count to evaluate wound inflammation.

Tissue samples were collected after euthanasia for histological assessment of wound healing. These tissue samples were taken perpendicular to the initial surgical incision, incorporating the skin, fascia and muscle. They were subjected to similar processing as above, with additional Masson's trichrome stain for evaluation of wound healing (Table 3) (Sultana et al., 1970). The parameters assessed included the amount of granulation tissue, inflammatory infiltrate, collagen fibre orientation, pattern of collagen, and amount of early and mature collagen. The summation of all six parameters gave a total wound healing score. Good wound healing is denoted by scores of 16–19, fair 12–15 and poor 8–11.

Extracted tibiae were stored in 10% neutral-buffered formalin for three days and decalcified in 10% formic acid solution for 8–14 days before further processing. The samples were embedded in paraffin, sliced longitudinally, perpendicular to the diaphyseal axis, into 4 μm thick sections, and stained with H&E and Masson's trichrome. These bone sections were scored for fracture healing based on the quantity and type of predominant tissue, i.e., fibrous, cartilaginous, immature or mature bone, in ascending order of bone healing (Table 4) (Huo et al., 1991).

Table 3
Histological scoring system for wound healing

Number	Histological Parameter
1	Amount of granulation tissue (profound-1, moderate-2, scanty-3, absent-4)
2	Inflammatory infiltrate (plenty-1, moderate-2, a few-3)
3	Collagen fibre orientation (vertical-1, mixed-2, horizontal-3)
4	Pattern of collagen (reticular-1, mixed-2, fascicle-3)
5	Amount of early collagen (profound-1, moderate-2, minimal-3, absent-4)
6	Amount of mature collagen (minimal-1, moderate-2, profound-3)

Note. Interpretation: Good (16–19); Fair (12–15); Poor (8–11) (Sultana et al., 1970)

Table 4
Histological scoring system for fracture healing

Score	Findings at the fracture site
1	Fibrous tissue
2	Predominant fibrous tissue with minimal cartilage tissue
3	Cartilage tissue and fibrous tissue in a uniform manner
4	Predominant cartilage tissue with minimal fibrous tissue
5	Cartilage tissue
6	Predominant cartilage tissue with minimal immature bone
7	Immature bone and cartilage tissue in a uniform manner
8	Predominant immature bone with minimal cartilage tissue
9	Bone healing with immature bone
10	Bone healing with matured bone

Note. Cartilaginous and bony tissue types are associated with progressive stages of fracture healing (Huo et al., 1991)

Data Measurement

Radiological scoring was recorded by an orthopaedic surgeon who was blinded to the treatment groups. Microbiological and histological analyses were performed by a veterinary pathologist who was blinded to the treatment groups.

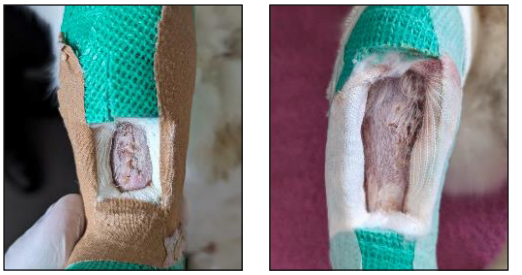
RESULTS

General Observations

All wounds healed well by 7 to 10 days after surgery (Figure 5). There were no signs of infection, such as erythema, exudate, swelling or wound dehiscence. There were two mortalities: (1) one death (HPO group) on day 10 due to gut stasis and (2) another (PI group) on day 39 due to bronchopneumonia, likely infection from an environmental source. Increasing analgesic frequency and duration up to a minimum of two weeks resulted in reduced stress and improved gut motility in subsequent rabbits. All the rabbits in the PI and control group showed normal appetite, bowel and urinary functions, and locomotion up until their death or euthanasia.

Microbiological Evaluation

Only one wound tissue sample in the PI group demonstrated a bacterial colony pre-debridement, which was reduced by 2.8×10^3 CFU/g following irrigation with povidone-iodine (Table 5). The colony culture grew S*taphylococcus aureus*. The other wound tissue samples did not detect any CFU.



(a) (b)

Figure 5. Clinically healed wound of two different rabbits at (a) two weeks and (b) eight weeks

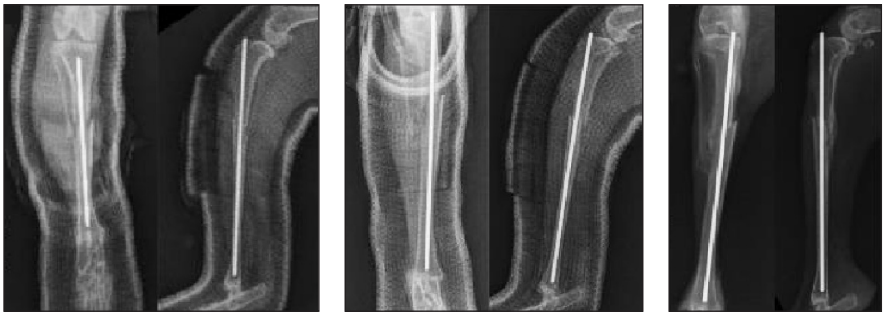
Table 5
Microbiological evaluation of tissue sample before and after debridement

Rabbit	Group	Bacterial count (CFU/g)		Culture	
		Pre-debridement	Post-debridement	Pre-debridement	Post-debridement
1	HPO	0	NA	0	NA
2	PI	3.2×10^3	4.0×10^2	*Staphylococcus aureus*	*Staphylococcus aureus*
3	NS	0	0	0	0
4	PI	0	0	0	0

Note. The bacterial count and the microorganism culture before and after debridement
HPO = hydrogen peroxide; PI = povidone-iodine; NS = normal saline; NA = not available

Radiological Evaluation

RUST scores to evaluate fracture healing were determined from four cortices seen on anteroposterior and lateral radiographic views of the tibia (Figure 6) (Whelan et al., 2010). Criteria for scoring included the presence of a callus and visibility of the fracture line. A score of eight or less implied possible non-union; nine was indeterminate, while ten and above signified a healed fracture.



(a) (b) (c)

Figure 6. Anteroposterior and lateral radiographic views of the right tibia were taken: (a) post-operative; (b) at three weeks; and (c) at eight weeks. Radiographic healing was assessed using the RUST method, giving a score of 4, 6 and 8, respectively

On days 21, 42, and 56, both rabbits in the PI group noted comparable or slightly higher scores than the control (Table 6). No score was recorded for the HPO group due to the rabbit's premature death.

Table 6
Radiological evaluation of fracture healing at post-operative day 1, week 3, 6 and 8

Rabbit	Group	RUST score at a time interval			
		D1	Week 3	Week 6	Week 8
1	HPO	4	NA	NA	NA
2	PI	4	5	8	NA
3	NS	4	5	6	6
4	PI	4	6	7	8

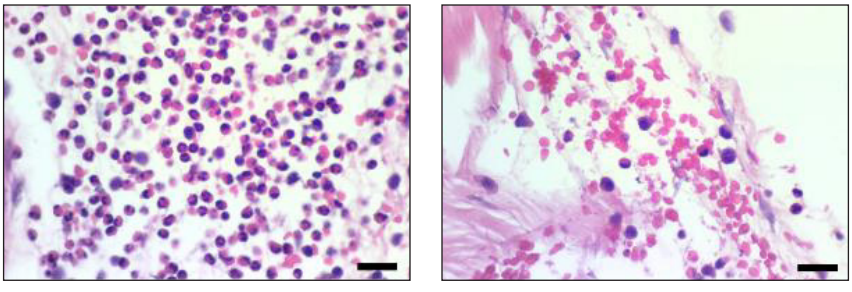
Note. HPO = hydrogen peroxide; PI = povidone-iodine; NS = normal saline; RUST score = Radiographic union scale in tibial fracture; NA = not available

Histological Evaluation

Histological examination of tissue samples under light microscopy found a reduction of heterophils count in both the PI and control groups post-debridement (Figure 7). However, one of the samples in the PI group noted a moderate amount of heterophils post-irrigation despite recording a nil heterophil count pre-debridement (Table 7).

Both the PI and control groups demonstrated good wound healing, although one tissue sample in the PI group had a fair score due to the lack of cutaneous tissue for a complete assessment (Figure 8, Table 7).

Macroscopically, all the extracted tibiae demonstrated features of bone union (Figure 9). Histologically, all rabbits in the PI and control groups showed evidence of bone healing by the sixth and eighth weeks, with predominant immature bone noted (Figure 10, Table 7). There was no evidence of bone infection. No tissue sample was available for histological evaluation from the HPO group due to the rabbit's premature death.



(a) (b)

Figure 7. Wound tissue histology showing heterophils count (a) pre-irrigation and (b) post-irrigation. H&E stain, magnification ×800, bar: 20 µm

Table 7
Histological evaluation of wound inflammation, wound healing and fracture healing

Rabbit	Group	Death/ Euthanasia	Heterophils count		Wound healing score	Fracture healing score
			Pre-irrigation	Post-irrigation		
1	HPO	D10	NA	NA	NA	NA
2	PI	D39	2+	1+	18/20	7/10
3	NS	D62	3+	2+	17/20	8/10
4	PI	D58	NA	2+	15/20	8/10

Note. HPO = hydrogen peroxide; PI = povidone-iodine; NS = normal saline; NA = not available; 1+ = low, < 10/HPF; 2+ = moderate, 10-100/HPF; 3+ = high, > 100/HPF; HPF = high power field

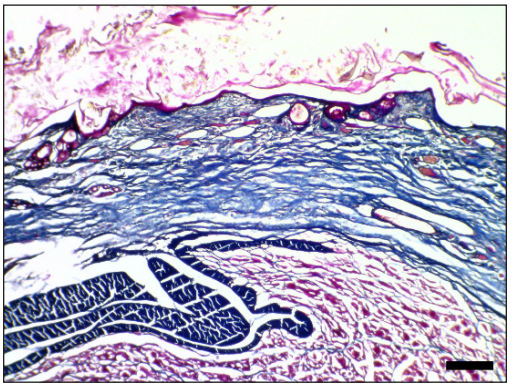


Figure 8. Histology of tissue from the previous wound site after eight weeks shows moderate mature collagen with horizontal orientation. Masson's trichrome stain, magnification ×800, bar: 20 µm

(a) (b)

Figure 9. The extracted tibia was processed for histological evaluation: (a) macroscopic features of fracture union indicated by callus formation and immobile fracture site; (b) longitudinal cut section at fracture site showing callus formation

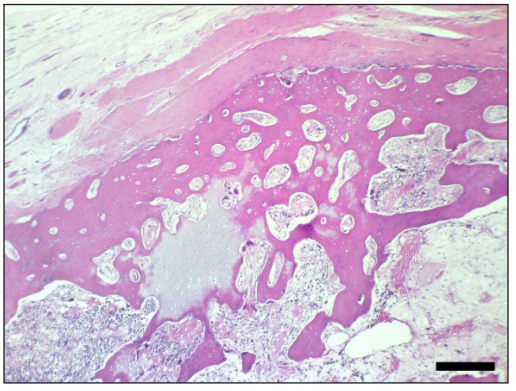


Figure 10. After eight weeks, bone histology at the fracture site shows a predominant amount of mature bone interspersed with cartilage tissue. H&E stain, magnification ×200, bar: 100 µm

DISCUSSION

There have been a substantial number of studies on the effects of antiseptics on bacterial count and wound healing. Despite their ability to reduce the number of bacteria on wound surfaces, they are also known to be detrimental to the viability and functions of cellular units essential to wound and fracture healing, such as fibroblasts, osteoblasts, and osteoclasts (Bhandari et al., 2001; Kaysinger et al., 1995; Lineaweaver et al., 1985; Thomas et al., 2009). Even *in vivo* studies showed a rebound in bacterial count

48 hours and 14 days after the initial irrigation (Owens et al., 2009; Penn-Barwell et al., 2012). This rebound could be theorised to result from the toxic effects of these solutions, thus allowing the proliferation of bacteria in damaged host tissues. However, these studies cannot account for the complex interplay of host immune response and regenerative capabilities of cellular tissues *in vivo*. Earlier *in vivo* studies on fracture healing described the effect of povidone-iodine, hydrogen peroxide and chlorhexidine in a rat fracture model, but these were closed fractures with no bacterial contamination (Husodo et al., 2016; Özbay, 2021). Therefore, we designed our study protocol to replicate an actual open fracture scenario, where the effectiveness of antiseptics in promoting wound and fracture healing depends on its ability to clear harmful bacteria while avoiding cytotoxicity to host tissues.

We chose rabbits, considered closest to human phylogeny after primates, for the study due to their similarity to humans in terms of bone attributes, such as spontaneous Haversian remodelling, bone metabolism, bone mineral density and resistance to fracture (Sengupta & Dutta, 2020; X. Wang et al., 1998). Axial forces and bending moments of rabbit tibia also approach that of humans (Reifenrath et al., 2012). They have a systemic and local inflammatory response comparable to that of humans, and antibiotics demonstrate similar pharmacokinetics. These characteristics make them popular candidates for bone infection and pharmacology studies (An et al., 2006; Bottagisio et al., 2019). The size of the rabbit tibia and the limited soft tissues surrounding the bone allow a more accessible surgical approach and implant insertion, which resembles that of the human orthopaedic scenario (Pearce et al., 2007). In addition, rabbits are easier to handle due to their docile nature and size. Their size allows for the standardisation of fracture creation and repeatability of protocol at a lower cost.

The current study performed definitive debridement 24 hours after the initial 'injury' in contrast to other *in vivo* studies where debridement was performed two to six hours later (Cheng et al., 2015; Owens et al., 2009; Penn-Barwell et al., 2012). Moreover, none of the studies evaluated fracture healing. Owens et al. (2009) performed irrigation with surfactants and antibiotics, such as bacitracin, castile soap and benzalkonium chloride, which have largely fallen out of favour in current practice. The 24-hour period in our study more accurately reflects real clinical scenarios, especially in developing countries or even developed countries with limited resources or in polytrauma patients whose resuscitation attempts precede surgical debridement, where often, the time to first debridement may extend more than 24 hours (Hadizie et al., 2022; Mener et al., 2020; Yusof et al., 2013).

The solutions used in the current study are the standard formulations found in clinical use. Iodine has long been known for its bactericidal properties. However, its use alone is limited by its low solubility in water and the caustic effect on skin and mucosal membranes. Complexing iodine with a polymer thus makes it stable and less irritating to tissues. We used povidone-iodine 10% solution with 1% available iodine for our study, the commonly

available commercial option. A full-strength povidone-iodine 1% destroyed all fibroblasts in an *in vitro* study (Lineaweaver et al., 1985). Another study demonstrated toxic effects on embryonic chick tibia and osteoblasts with povidone-iodine above one-tenth of the usual strength and with hydrogen peroxide from as low as one-thousandth of the usual strength. Removal of the offending solutions did not cause a return of the cellular functional activity (Kaysinger et al., 1995). However, the findings of *in vitro* studies do not necessarily correlate with those of *in vivo* studies. A clinical study of appendicectomy wounds in children noted a reduced wound infection rate using 1% povidone-iodine but a worse outcome with a 5% concentration (Viljanto, 1980). Even a 10% povidone-iodine solution applied for 15 minutes was safe for decontaminating bone grafts (Bauer et al., 2011; Yaman et al., 2007). These may be partly due to factors such as inactivation of the active solution by body fluids such as blood (Docherty et al., 2005).

Hydrogen peroxide is another commonly used antiseptic solution. Its bactericidal property is conferred by the generation of hydroxyl free radicals, which attack essential cell components. The usual concentration is 3%, as lower concentrations may be less effective, especially in the presence of organisms with catalase or peroxidase enzymes (McDonnell & Russell, 1999). However, 3% or even lower concentrations are cytotoxic *in vitro* (Kaysinger et al., 1995; Lineaweaver et al., 1985; Nicholson et al., 1998; Rueda-Fernández et al., 2022; Thomas et al., 2009). On the other hand, *in vivo* studies noted conflicting results. Gruber et al. (1975) reported accelerated healing of experimental animal wounds and human skin graft donor sites. Tur et al. (1995) described increased vascular perfusion in ischemic ulcers in guinea pigs after applying hydrogen peroxide cream. This contradictory effect may be partly explained by catalase enzymes in normal tissue, which partially degrades the available hydrogen peroxide (Brown & Zitelli, 1993). Data from the current study regarding the effect of hydrogen peroxide is unavailable due to the premature death of the rabbit in the HPO group. The cause of the premature mortality was gut stasis. Increasing the analgesic dose frequency and duration and providing oral prokinetic agents improved gut motility in subsequent rabbits.

Overall, *in vivo* studies comparing the efficacy of irrigation solutions on bacterial clearance and wound healing are limited (Cheng et al., 2015; Owens et al., 2009; Penn-Barwell et al., 2012). Cheng et al. (2015) induced a rat open femur fracture model by using blunt force and leaving the wound exposed for two hours before debridement and irrigation with saline, iodophor and hydrogen peroxide. They showed significant bacterial clearance with all tested solutions and the least wound inflammation by saline (Cheng et al., 2015). Interestingly, they achieved a high bacterial count ($> 10^5$ CFU/mL) from the environmental exposure before debridement. Our study could not obtain consistent contamination despite the environmental exposure and use of non-sterile equipment for fracture creation. This disparity may be due to the different methods of fracture creation; a blunt force may induce greater tissue damage, which renders it more susceptible to bacterial colonisation. An

improvement to our study will be the direct inoculation of microorganisms during fracture creation. In our PI group with recorded CFU pre-debridement, povidone-iodine reduced bacterial count, but S*taphylococcus aureus* was still cultured post-irrigation. It is doubtful if the remaining bacteria could cause a clinically significant infection, as the wound healed well. The histological evaluation noted similar wound healing scores between the PI and control group, although a full study will be required for a proper analysis.

Our results showed a similar radiological score between the PI and control groups, with slightly faster healing in the PI group beginning week three and a higher final score at week eight. Macroscopically, all extracted tibiae appeared united. Histologically, callus at the fracture sites in both groups found a mixture of immature bone and small amounts of cartilaginous tissue. Conversion of cartilage to woven bone suggests good fracture healing, implying minimal or no toxicity to bone *in vivo*. However, a larger sample size will be needed for a definite analysis. Our study can also provide a correlation between the radiographic RUST scale and histological data of fracture union for further validation of the scale (Leow et al., 2020; Whelan et al., 2010).

The positive effect of povidone-iodine on fracture healing can be attributed to the direct antimicrobial activity of PI and other mechanisms that contribute to bone and wound healing. A bacterial load of more than 10^5 has a strong predisposition to clinical infection in an open fracture (Sen et al., 2000). Naturally, a reduced bacterial load will lead to a favourable environment for bone healing, with reduced inflammatory stress brought on by substantial infection. Even subclinical colonisation of bacteria in deeper tissues can elicit an inflammatory response such as vasculitis, which interferes with healing (Piérard-Franchimont et al., 1997). In addition, iodine was found to activate macrophages and modulate their cytokine and growth factor secretions, which then regulate mesenchymal stem cell (MSC) proliferation and differentiation. In a favourable environment, in the absence of infection, macrophage production of cytokines, including bone morphogenetic proteins (BMP) such as BMP-2 and BMP-6, is increased (Moore et al., 1997). These osteoinductive cytokines are critical to bone healing (Champagne et al., 2002). *In vitro*, BMP-2 increased osteoblastic activity and differentiation even after an initial retardation (Schmidlin et al., 2009). Of note, this effect was only observed if there were osteogenic cells in the adjacent area, the most important source of which is the periosteum (Knight & Hankenson, 2013). Furthermore, iodine stimulates the upregulation of transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF), which have roles in endothelial cell migration and angiogenesis, while downregulating the production of interleukin 6 (IL-6), which reduces congestion and oedema (D. Wang et al., 2022; L. Wang et al., 2017). This effectively converts the inflammatory phase of healing to the proliferative phase, which is beneficial for wound healing. However, the positive effect of PI in this study could also be explained by variability due to the small sample size. Therefore, a larger sample needs to be studied to confirm the significance of the findings.

The preliminary results suggest PI as a viable adjunct for irrigation in open fractures. A cost-effective approach is ideal, given the enormous economic burden of treating open fractures and their complications. Since irrigation is already standard practice in treating open fractures, optimising the irrigation solution is an obvious choice, considering it is one of the cheapest and easily modifiable variables, without relying on expensive methods to improve the outcome. The results of this study can also be applied to treating fracture-related infections.

The current study does have limitations. We did not investigate different fracture types, concentrations and contact times of the irrigation solutions. Testing these would present ethical concerns due to the huge sample involved and the difficulty in achieving standardisation. Our open fracture model does not encompass other factors, such as polytrauma injury, multiple surgical treatments, and soft tissue damage, which may be seen in clinical scenarios and affect the outcome. Further biomechanical wound tensile and bone strength testing may provide information regarding the functional outcome. There was inconsistent microbial contamination during the open fracture creation phase, which can be improved by directly inoculating a fixed quantity of bacteria. We performed tissue biopsies for organism culture and detection, which is considered the gold standard (Serena et al., 2021). However, these may be subject to sampling error, and fastidious organisms that are difficult to culture may be missed. Newer and more advanced techniques, such as bioluminescence or fluorescence imaging, can provide real-time quantification of bacteria and repeated measurement. The effects of the irrigation solutions were studied in a diaphyseal open fracture model. Peri-articular fractures, in the presence of articular cartilage, may behave differently. A critical-size bone defect or significant loss of periosteum, often seen in high-grade open fractures, is outside the scope of this study. This experimental model also exhibits a translational barrier. Although there is good representation similarity in terms of micro and macro anatomy and molecular and biochemical interactions, there are differences which may not translate directly (Marmor et al., 2020). Rabbits have different bone shapes and sizes, flexed limbs with different weight loading and faster bone turnover, which may not be fully representative of bone healing response in adult humans (Li et al., 2015). The parameters evaluated, such as *ex vivo* bone histology, provided valuable information to guide treatment strategy. However, there is no comparable analysis in humans, which is impossible in a clinical setting.

CONCLUSION

These findings suggest that povidone-iodine may be a safe irrigation solution for open fractures. However, given the small sample size, further research with larger cohorts must validate these results before clinical recommendations can be made.

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