European Cells and Materials Vol.55 2026 (pages 47–62) DOI: 10.22203/eCM.v055a04 ISSN 1473-2262

Review

OPTIMIZATION OF LABORATORY INFRASTRUCTURE AND SURGICAL WORKFLOW IN PRECLINICAL RODENT BONE RESEARCH: AN UNDERREPORTED BARRIER TO REPRODUCIBILITY

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Abstract

Preclinical surgical studies in rodent models play a pivotal role in elucidating the mechanisms of bone repair and regeneration. Similar to clinical practice, intraoperative and postoperative complications in preclinical studies significantly affect treatment outcomes and may even alter the overall experimental results. Well-established laboratory infrastructure and experimental procedures can reduce the risk of adverse events, such as intraoperative technical errors and postoperative infection. However, these aspects are often underreported or overlooked in the literature. In this study, fracture fixation in rats is presented as an example to highlight the essential but often unreported details, such as virtual surgical planning, preoperative rehearsal, disinfection protocols, intraoperative management, postoperative support, multidisciplinary collaboration, and research documentation. Supplementary and alternative solutions are also proposed for laboratories with limited resources. By applying virtual planning and rehearsal with three-dimensional (3D)-printed samples, the authors have performed 108 consecutive external fixation procedures for rat femoral fractures since 2020, with no intraoperative dropouts attributable to surgical technique errors. The operative time stabilized at 45.6 ± 3.8 minutes (mean $\pm$ standard deviation), reflecting low variability and a reproducible workflow. Systematic implementation of these strategies helps prevent postoperative complications, enhances animal welfare, and improves the reproducibility and translational potential of preclinical research.

Keywords: Orthopaedic procedures, animal model, three-dimensional printing, infection control, electronic lab notebook, environmental enrichment.

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Introduction

Rodents, particularly rats and mice, share numerous physiological and pathological features of the human musculoskeletal system, making them ideal models for investigating pathological processes and novel therapeutic interventions [1–5]. In the clinical setting, rigorous quality control is routinely implemented, and patient-reported outcomes can be assessed in real time. However, such quality control and evaluations are difficult to achieve or even unfeasible in animal studies. Consequently, minor oversights regarding risk factors residing in laboratory infrastructure, surgical procedures, and postoperative management may profoundly influence treatment outcomes, compromising the validity and translational potential. Therefore, meticu-

lous attention to detail throughout the entire study is essential to enhance the clinical relevance of preclinical surgical research.

Historically, the realistic simulation of clinical orthopaedic treatment in small animals was difficult, as only a few specialized surgical instruments were available. Over the past decade, the introduction of standardized fracture fixation devices for rodents has enabled the operative techniques to closely replicate the treatment scenarios in humans [6–9]. Moreover, the application of multimodal *in vivo* dynamic imaging has further facilitated the simulation of postoperative clinical follow-up [10].

Beyond standardized surgical implants, research workflows also have a significant impact on the reproducibility and translational value of preclinical studies. For example, the Planning Research and Experimental Procedures on Animals: Recommendations for Excellence (PREPARE) and Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) Guidelines for reporting animal studies emphasize that transparency in laboratory methodologies and perioperative procedures is pivotal for ensuring reproducibility [11–13]. These guidelines provide a critical framework for *in vivo* research, encompassing rigorous study design, detailed animal-related information, and comprehensive procedural reporting (such as anesthesia, analgesia, and humane endpoints).

However, scientific publications seldom provide a comprehensive account of complex experimental workflows. Certain micro-environmental and operational details can directly affect surgical success and complication rates, but are not fully addressed by the existing guidelines. In this study, the authors use the term microenvironment to denote underreported operating room factors that influence sterile-field integrity and the precision of surgical procedures, such as air quality, personnel traffic, attire, lighting, and temperature control. Other operational details include surgical planning, disinfection protocols, and postoperative management strategies [14].

Researchers and peer reviewers often prioritize the novelty of interventions and the significance of primary outcomes. Due to space limitations (e.g., word count) in many journals, preclinical studies involving complex surgical models tend to underreport procedural details. Moreover, under the intense pressure of publishing, researchers without prior clinical surgical experience may lack sufficient time or motivation to address overlooked aspects. For example, operative field management and sterile techniques are essential components of good surgical practice, but are nonetheless commonly underreported [15,16].

Furthermore, many standardized practices routinely implemented in clinical surgeries are difficult to replicate with high fidelity in preclinical settings [15,17]. These include specialized operating rooms, instrument sterilization between surgeries, personnel control in the surgical area, and intraoperative lighting (Table 1). Even in high-standard clinical institutions, preventing serious complications such as prosthetic infections remains a daunting challenge to date [18,19]. Consequently, deficiencies in preclinical laboratory infrastructure and workflow can substantially increase the risk of surgical failure and other adverse events, particularly in resource-limited research environments.

This manuscript examines critical but often underreported aspects of laboratory infrastructure and procedural workflow, using the rat femoral fracture model as a representative example. In addition, the authors provide recommendations and alternative solutions, particularly for laboratories operating with limited resources. By highlighting critical yet often-overlooked details, the article aims to reduce complications, enhance animal welfare, improve reproducibility, and strengthen the translational potential of preclinical orthopaedic research.

Optimization of Surgical Design through Digital Simulation and Three-Dimensional (3D) Printing

Virtual Planning Based on 3D Reconstruction and Simulation

Fracture reduction, precise osteotomy, and implant placement in small animals may present considerable technical challenges because of the limited operative field [14]. Minor intraoperative errors or unforeseen technical difficulties may prolong surgical time, increase tissue trauma, and lead to unnecessary animal suffering or death, thereby compromising the reliability of experimental outcomes. Therefore, moving beyond experience-based approaches to adopt preoperative surgical planning is essential to improving success rates, reducing animal use, and ensuring welfare.

In recent years, the adoption of preclinical imaging, computer-aided design (CAD), and 3D printing technologies has enabled researchers to design, simulate, and optimize surgical procedures prior to animal surgeries [20–22]. For example, the use of virtual design and 3D-printed reduction devices has been shown to reduce the operative time by up to 34 minutes in the osteosynthesis of canine tibial fractures [23].

The first step in refining surgical design involves acquiring and reconstructing 3D digital models of the target anatomical sites. In the author's laboratory, micro-computed tomography (micro-CT) is routinely employed to scan target bones (e.g., rat femur) at micrometer resolution (voxel size: 20–40 μm). The acquired data, typically in Digital Imaging and Communications in Medicine (DICOM) format, are imported into image-processing and CAD software such as 3D Slicer (version 4.10.2, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; www.slicer.org) and Meshmixer (version 3.5.474, Autodesk Inc, San Rafael, CA, USA; www.meshmixer.com) for skeletal reconstruction [24].

Within the virtual environment, anatomical parameters can be precisely measured to guide implant selection

Table 1. Comparison between clinical and preclinical orthopaedic surgeries, and potential impact of preclinical settings on the treatment outcomes.

Category	Clinical orthopaedic surgery (human)	Preclinical surgical research (small animals)	Potential impact on surgical outcomes
Surgical planning	Supported by expert teams, device manufacturers, professional software, and 3D-printed models	Relies heavily on prior experience or information from published studies	Greater variability and higher failure risk
Operational complexity	Surgeries performed under direct vision, or with endoscopic magnification	Limited visibility and operating field, requiring fine operative techniques	Increased risk of technical errors
Surgical infrastructure	Fully equipped, dedicated operating rooms	May lack dedicated surgical facilities	Higher risk of infection
Environment control	Strict zoning and traffic control	Protocols are often inconsistent across laboratories	Poor sterility control and increased risk of infection
Instrument sterilization	Dedicated, sterilized instrument sets	Repeated use of instruments and limited sterilization capacity	Higher risk of cross-contamination
Consumables and draping	Standard use of sterile (disposable) materials	Materials and techniques vary across studies	Barrier integrity compromised
Intraoperative support	Advanced systems for temperature control and lighting	Advanced systems are often unavailable	Increased surgery- and anesthesia-related risks
Team composition	Trained surgeons and nurses	Greater involvement of researchers, students, and technicians	Variable skill levels and increased complication risk
Postoperative management	Professional care and supervised rehabilitation	Managed by research staff, it may lack adequate clinical or veterinary oversight.	Delayed detection and response to complications

and design [22]. The feasibility of the operative approach is also assessed through virtual simulations, enabling iterative optimization in a noninvasive and cost-effective manner, thus reducing the risk of intraoperative trial and error (Fig. 1**A**).

Preoperative Rehearsal Using 3D-Printed Models

Using digital models derived from imaging data, the authors employed 3D printing methods such as fused deposition modeling and selective laser sintering to prepare 1:1 scale bone models. These tangible and accurate models serve as intuitive training tools for rehearsal, allowing researchers to use surgical instruments to practice the procedure [25,26]. Hands-on practice often reveals technical limitations not apparent during virtual planning, such as operability of standardized bone defects and osteotomy, as well as the feasibility of fixation devices. Based on these insights, final adjustments to the surgical plan, implant, and instrument selection can be made prior to the actual *in vivo* experiment (Fig. 1**B**). Each operator can also fine-tune the process during rehearsal according to their own operating preferences.

Although the above process inevitably increases the workload for the research team during the planning phase, the upfront investment in design and validation helps mitigate the risk of later-stage failure due to procedural flaws, thereby laying a more robust foundation for future clinical translation. The authors have implemented virtual surgical planning and rehearsal using 3D models since 2020. In the subsequent 108 external fixation procedures for rat femoral fractures, no intraoperative dropouts attributable to surgical technique errors were recorded. Dropouts were defined as termination of the procedure and euthanasia due to failure in fracture induction, reduction, or stable fixation, which are essential to ensure postoperative weight-bearing with the operated limb. The mean operative time averaged at 45.6 minutes (45.6 ± 3.8, mean $\pm$ standard deviation), reflecting low variability and a reproducible workflow, thus supporting further postoperative multimodal imaging within a 90-minute general anesthesia window [10].

Table 2. Safety guidelines for environmental enrichment (EE) after rodent fracture fixation.

Category	Key items/actions	Conditions & monitoring
1. Baseline safety	• Tear-resistant, low-dust nesting • Semi-enclosed shelters • Social housing	Always allowed to provide baseline warmth, comfort, and natural behavior without device risk.
2. Application with caution (for general fracture surgeries)	• Climbing structures • Narrow tunnels • Wheels or elevated platforms	Introduce only with stable weight-bearing and wound status, and keep monitoring for 24–48 h after introduction.
3. Application with caution (for external fixation)	• Hanging toys or high platforms • Wire-mesh tubes • Hard-edged items or items causing collision/entrapment	Contraindicated during fixator presence to prevent injury or instability.
4. Dynamic staging	• POD 0–3: baseline warming & protection only • POD 3–14: gradual addition of low-risk items • ≥POD 14: escalation per imaging, gait, and veterinary approval	Stage EE introduction according to healing status and postoperative monitoring.
5. Monitoring	• Weight-bearing activity; Gait analysis • Inflammation/stress parameters: IL-6, TNF-, CRP, corticosterone • Wound and pin-tracts: redness, exudate • Imaging: X-ray or micro-CT if planned	Use quantitative criteria to assess EE safety and impact on recovery.
6. Withdrawal	• Remove EE items if gait, activity, wound, or fixation stability deteriorates • Document all changes	Log adjustments, metrics, and outcomes; Consult with a veterinarian doctor if necessary.

POD, postoperative day; IL, interleukin; TNF, tumor necrosis factor; CRP, C-reactive protein.

Table 3. Information categories recommended for electronic lab notebooks (ELNs) and the value for research.

Information category	Recommended ELN content	Value for surgical/digital research
Preoperative planning	Surgical design/procedure; 3D (CAD) model data.	Standardization and reproducibility.
Surgical environment	Working zones layout; Personnel traffic regulations; Surgical attire requirements.	Environmental sterility control.
Surgical instrumentation/consumables	Type of instrument/consumables; Cleaning and sterilization protocols.	Surgical quality assurance.
Intraoperative support	Core electric equipment; Disinfectants; Disinfection/sterilization protocols.	Reducing complications for data reliability.
Postoperative monitoring	Environmental enrichment and monitoring protocols.	Animal welfare improvement and injury prevention.
Protocol deviations/incidents	Description of unexpected events/corrective measures.	Transparency and enhanced data interpretation.

A more detailed template is provided in the **Supplementary Material**.

Novel Techniques for Optimization in Rodent Bone Research

Beyond preoperative design and rehearsal, there are novel techniques that can extend the workflow optimization to intraoperative and postoperative phases. For example, fixation devices can be coupled with implantable strain sensors, providing real-time biomechanical data at the fracture site [27,28]. Such a sensor-based solution could enable intraoperative evaluation of reduction quality, as well as early detection of implant construct failure after surgery.

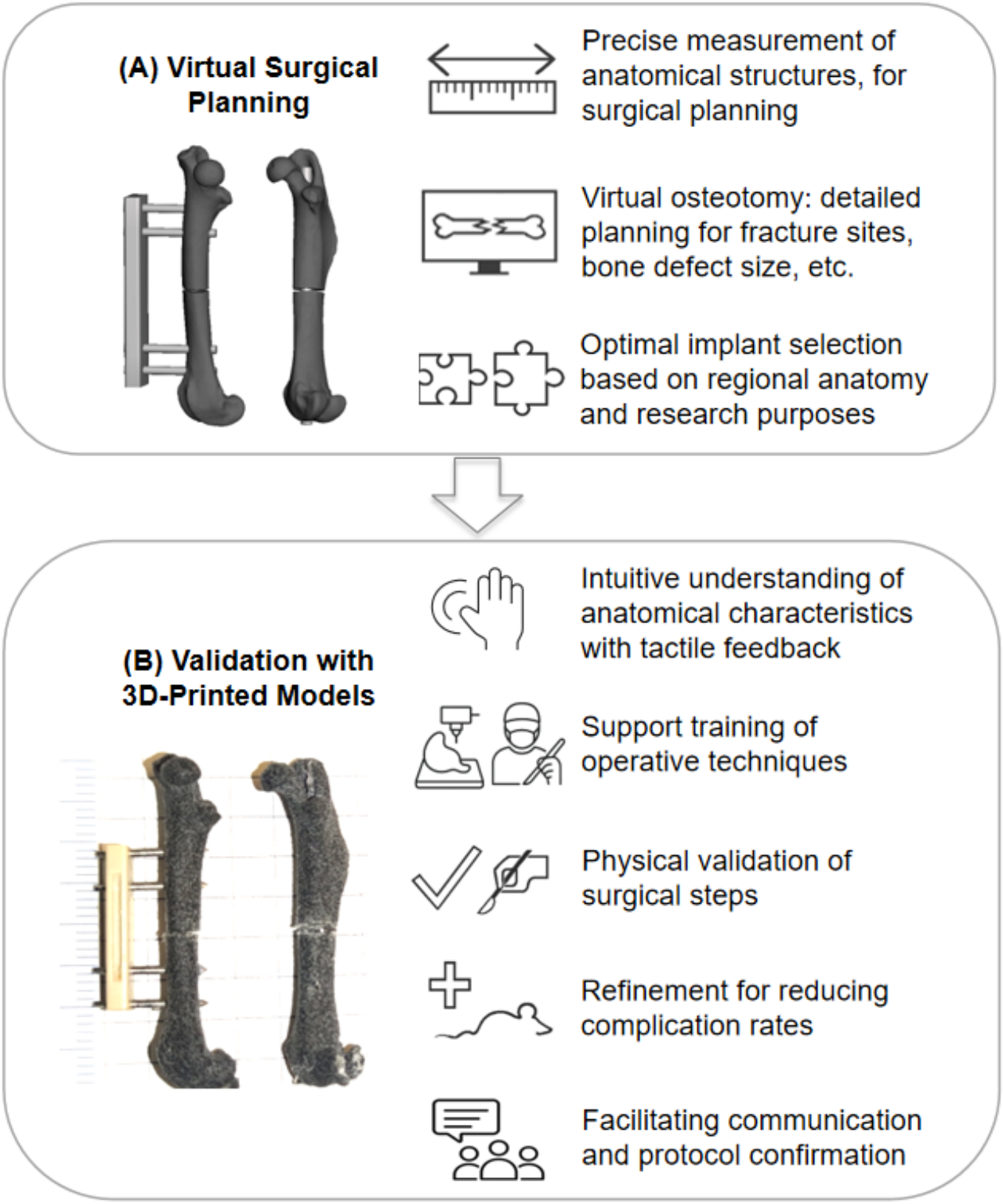

Fig. 1. Digital planning and 3D-printed rehearsal optimize surgical design and reduce technical variability. (**A**) Virtual surgical planning based on micro-CT reconstruction allows pre-measurement of anatomical parameters and pre-specification of acceptable alignment before live surgery. This reduces trial-and-error, shortens operative time, and improves first-pass success. (**B**) Preoperative rehearsal on 3D-printed models (1:1 scale) with actual instruments exposes constraints not apparent *in silico*, enabling final adjustment of approach and implant design prior to animal use. (**A**, left) 3D models of external fixation and intramedullary fixation for rat femur, segmented with 3D Slicer (version 4.10.2) and rendered in Meshmixer (version 3.5.474); (**B**, left) External fixation with standardized surgical device (RatExFix, RISystem AG, Switzerland) and intramedullary fixation with partially threaded Kirschner wire; Figure designed by authors (Y.S. and K.W.).

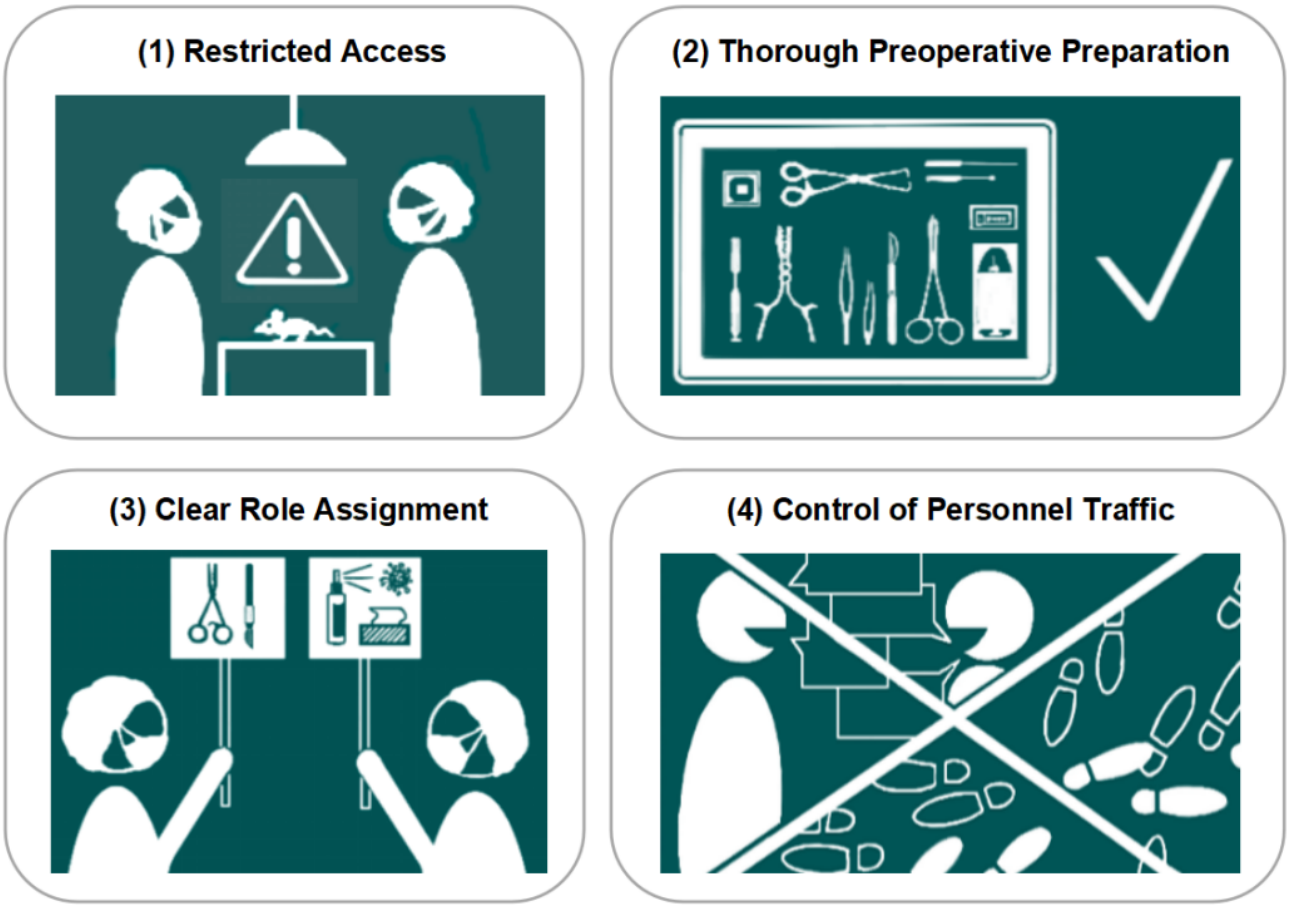

Fig. 2. Operating room discipline and traffic control mitigate airborne contamination and procedural error. Key operational rules for small-animal surgery rooms: (1) Restricted access for trained and role-assigned personnel only; (2) Thorough preoperative preparation, to verify sterile sets before incision; (3) Clear definition of intraoperative roles, including instrument handling, disinfection and imaging; (4) Controlling personnel movement and limiting door openings reduce turbulent airflow and airborne bioburden, protect sterile barriers, and decrease distractions that can trigger technical mistakes. Figure designed by authors (Y.S. and K.W.) and processed with Microsoft PowerPoint.

In parallel, microsensors embedded in implants may provide chemical monitoring, such as the application of pH sensors to detect acidic tissue environments during early postoperative infection [29]. Although the introduction of novel approaches poses substantial technical challenges in rodent surgeries, virtual design combined with 3D printing facilitates their application, thus supporting the optimization of the entire research flow.

Management of the Surgical Environment

Laboratory Management and Control of Personnel Traffic

Proper management of the operating environment is a critical factor in ensuring the reliability of preclinical experiments. Laboratory spaces should be clearly divided into distinct areas, including a preparation area, a surgical area, and a recovery area [14]. The surgical area must be maintained under strict hygienic conditions to minimize the risk of infection, and all personnel involved must adhere to stringent dress codes, such as wearing caps, masks, sterile gloves, and surgical gowns. Before surgery, it is critical to ensure that all necessary instruments, equipment, and other supplies are fully prepared and positioned in the surgical area. Only essential personnel with clearly defined responsibilities should be permitted to enter during the procedure (Fig. 2).

The importance of air quality in the surgical environment may have been underestimated in preclinical research. Frequent door openings and unnecessary movement within the room create turbulent airflow, which resuspends settled particles from floors, equipment, or clothing back into the air [30–32]. These factors are associated with increased airborne bacteria and particulate matter [32,33]. Personnel may also act as vectors of microbial contamination, and studies have shown a direct correlation between the level of physical activity and increased airborne microbial load [33–35].

Airborne particles can settle in surgical wounds, on instruments, or within the surgical field, becoming potential

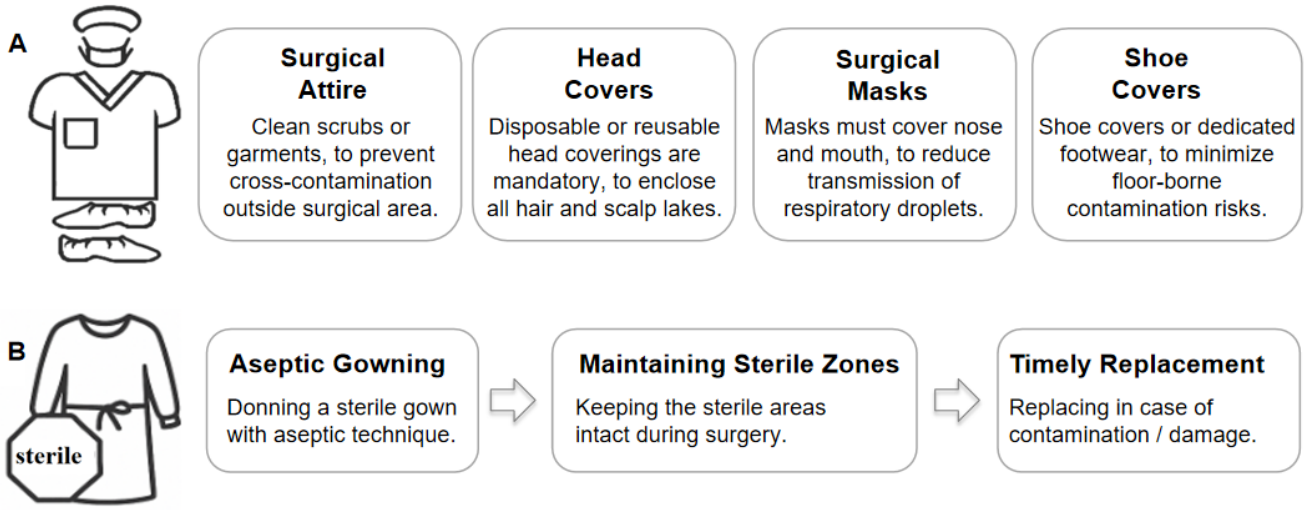

Fig. 3. Requirements for personnel attire in the surgical area, and proper use of sterile surgical gowns. (**A**) Protective attire on entry to the surgical area: clean scrubs, a cap fully enclosing hair, mask covering nose and mouth, and dedicated clean footwear/shoe covers, which are measures that lower baseline particulate and microbial shedding. (**B**) Sterile practice essentials: don a fluid-resistant, low-lint sterile gown after hand preparation; maintain sterile zones; replace attire immediately if contaminated or soaked, or after contact with non-sterile surfaces. These barrier integrity checkpoints directly impact surgical field sterility and downstream infection risk in orthopaedic procedures. Figure designed by authors (Y.S. and K.W.) and processed with Microsoft PowerPoint.

sources of infection. Although direct data on laboratory surgical environments are limited, research performed in clinical operating rooms has clearly demonstrated the importance of limiting personnel movement [31,32,36]. Excessive personnel traffic should therefore be recognized as an independent risk factor for surgical site infection (SSI), and the findings from clinical settings should be equally valuable in regulating preclinical surgeries.

Furthermore, excessive personnel and activity introduce additional risks beyond air quality issues, including (1) the risk of accidental contact, as higher numbers of people and uncoordinated movements raise the likelihood of contaminating sterile drapes, instruments, or the operative field; and (2) distraction of the surgical team, as movement and conversation can disrupt concentration, increasing the risk of procedural errors. Therefore, it is essential to limit the number of personnel and level of activity in the surgical room to the minimum necessary level (Fig. 2). In resource-constrained laboratory settings, mobile air purification devices may be used during surgical preparation as supplementary measures to improve air quality.

Appropriate Attire in the Surgical Area

Surgical attire comprises two key components: (1) dedicated surgical scrubs that should be worn upon entering operating areas; and (2) sterile gowns that must be donned after hand disinfection when performing sterile procedures (Fig. 3). These requirements are designed to create microbial barriers and define a relatively clean working environment, reducing the introduction of contaminants and minimizing pathogen transmission. Moreover, since exposure to blood or body fluids can compromise the barrier function and facilitate bacterial migration, the materials used for surgical gowns must possess adequate fluid resistance [36–38]. Once fluid penetrates the inner layers, the area is considered contaminated, and the gown should be replaced. Additionally, gown materials should be low-linting to minimize the generation and dispersion of airborne particles.

Sterile surgical gowns for animal surgeries may be either disposable or reusable. Laboratories can choose between the two, based on factors such as the anticipated fluid exposure, cost-effectiveness, and in-house sterilization capabilities. Polypropylene-based disposable gowns, which provide reliable protection, have been used in the author's laboratory. Regardless of the type used, strict sterility must be maintained during storage, transport, and use. Moreover, laboratories should implement clear dress code protocols and ensure that all staff are adequately trained in the proper use of surgical attire and adherence to protocols.

Disinfection of Surgical and Peripheral Areas

While skin disinfection at the surgical site is critical for preventing postoperative infection, it is equally important to manage adjacent skin and hair-bearing areas. These regions harbor microorganisms and debris that can be introduced into the surgical field via direct contact, instrument transfer, or air currents. Cleaning of peripheral areas, combined with proper draping to establish complete sterile barriers, is essential for reinforcing the aseptic field [39].

For rat femoral surgeries, in which the surgical site is close to paws and tail, the authors perform: (1) preoperative washing of adjacent non-surgical regions with warm

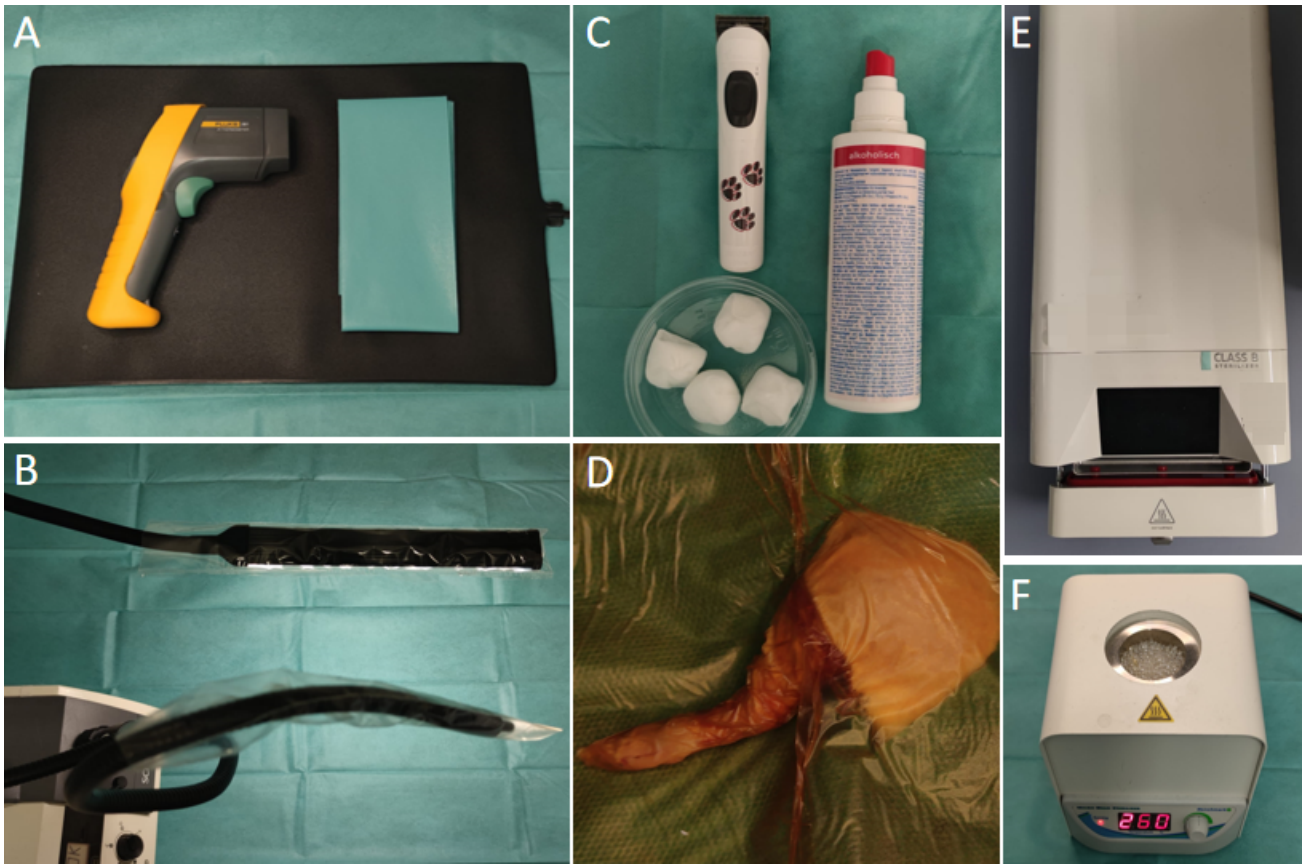

Fig. 4. Thermoregulation, lighting, hair removal, draping, disinfection, and sterilization: practical levers for reducing anesthesia time, improving precision, and lowering infection risk. (**A**) Heating pad and thermometer for maintaining normothermia (36–38 °C), with a protective drape layer to prevent thermal injury, to reduce anesthetic complications and speed recovery. (**B**) Shadow-free surgical lighting (gooseneck and supplementary LED lamp) with sterile covers to provide tissue contrast and shadow reduction, thereby lowering intraoperative risk. (**C**) Electric clippers and disinfectant, to minimize microabrasions during hair removal, and to reduce SSI risk during skin preparation. (**D**) Sterile drapes and adhesive film to isolate high-bioburden areas (paw, tail, perineum) from the field; once placed, drapes are not moved from contaminated to clean zones. (**E**) Autoclave sterilization for instruments between animals, also allowing high-temperature cycles (134 °C with 7–15 minutes exposure) to limit intraoperative prolongation of anesthesia. (**F**) Glass-bead sterilizer for cleaning of small instruments (260 °C, 30–60 seconds), which is not a substitute for full sterilization (for contingency-only).

water (36–38 °C) after hair removal at surgical sites; and (2) drying with sterile gauze and application of skin disinfectants (e.g., 10 % povidone-iodine, 2 % chlorhexidine gluconate, 70 % ethanol or isopropanol), followed by covering with sterile materials. Both adhesive films and sterile drapes can be used to prevent further contact with the surgical field or instruments [40,41]. In cases where equipment must be used near the surgical area, or adjusted intraoperatively but cannot be disinfected (e.g., electric drills and surgical lighting), sterile transparent covers can serve as protective barriers.

To further reinforce aseptic conditions of surgical sites before incision, loose hair or skin debris should be checked again and removed after the drying of the disinfectant. This can be done by gently wiping with sterile gauze from the center of the surgical site outward. For areas adjacent to the surgical field, such as the margins of the hair-removal zone, swabbing with sterile gauze soaked with the aforementioned disinfectants can be performed, taking care not to carry contaminants back into the disinfected zone [14,39].

When external fixation devices are applied for femoral fractures, the entire connecting bar and portions of the screws remain outside the skin after surgery, making the postoperative cleaning and disinfection essential. On the day of surgery and postoperative day 1, dried blood exudate on the implant surface can be cleaned with skin disinfectants, followed by secondary cleansing and drying with sterile saline and gauze.

Preoperative Preparation Involving Electric Equipment

Thermoregulation: Preventing Perioperative Hypothermia

Maintaining normothermia is critical for rodent surgeries because small animals are susceptible to rapid heat loss due to their high surface area-to-volume ratio [14]. General anesthesia further exacerbates heat loss by sup-

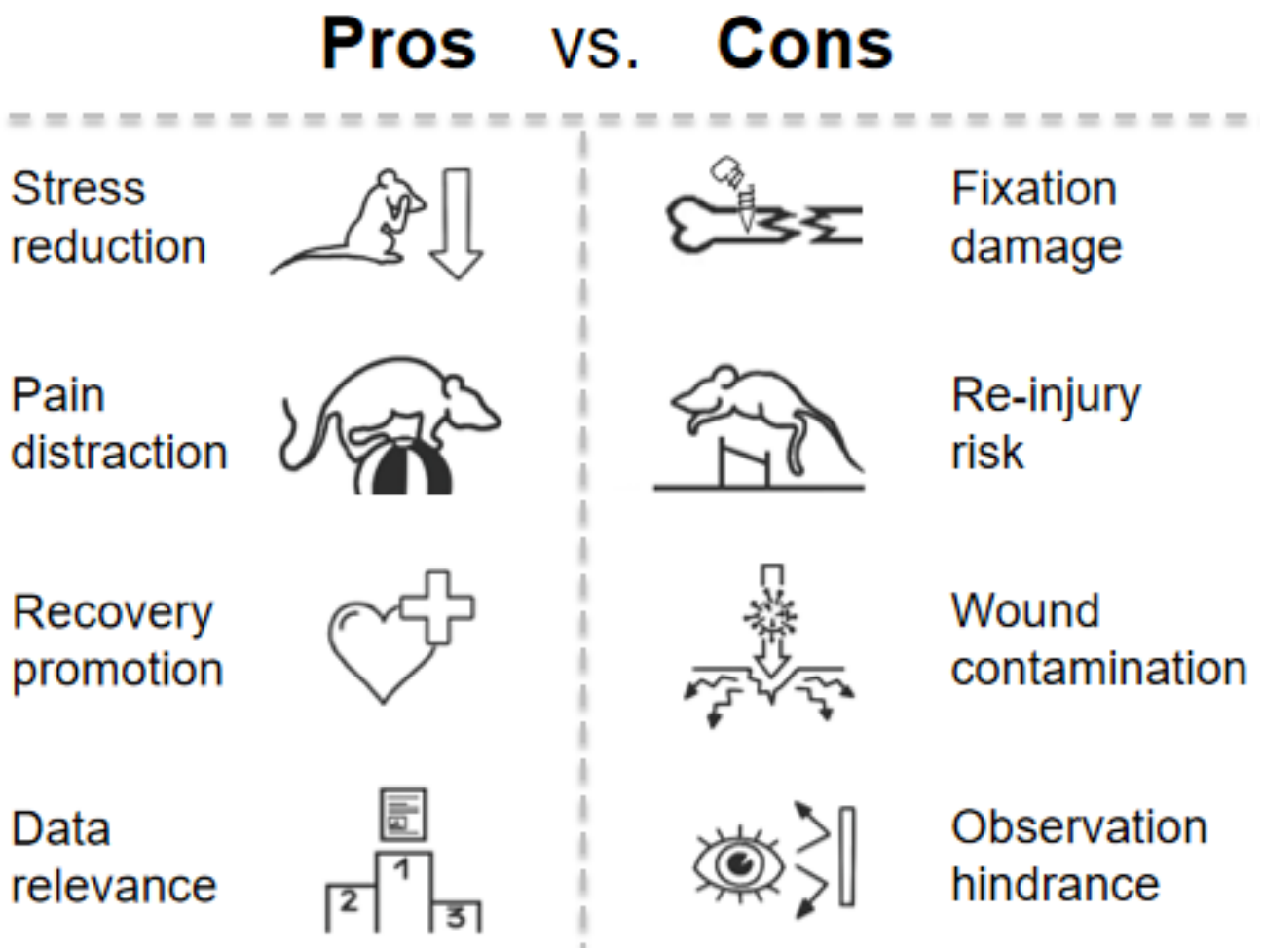

Fig. 5. Environmental enrichment (EE) after orthopaedic surgery: stage-wise implementation balancing welfare gains with device safety. Concept and caution: EE (e.g., nesting, shelters, social interaction when appropriate) improves welfare (Pros), but must be individually tailored and staged, especially for fracture fixation models to avoid adverse impacts on hardware (collision, entrapment) or wound irritation (Cons). Operational guidance: (1) start with low-risk items (warm nesting, low-profile shelters) in the immediate postoperative period; (2) avoid climbing structures or narrow passageways until radiological confirmation of cortical bridging; (3) monitor for weight-bearing activity, wound condition, and fixator stability; and (4) remove or modify any item causing adverse signs or hindering observation.

pressing thermoregulatory mechanisms and inducing peripheral vasodilation [42–45]. In addition, surgical exposure and the evaporation of irrigation fluids contribute to hypothermia (core body temperature <36 °C).

Intraoperative hypothermia can result in a wide range of adverse effects, including delayed drug metabolism (leading to prolonged anesthesia recovery), cardiovascular disturbances (e.g., arrhythmias), coagulopathy (increasing bleeding risk), immunosuppression (raising infection risk), delayed wound healing, and overall prolonged recovery time [42,44–47]. Therefore, preventing hypothermia is a critical component in perioperative management.

Many research facilities may lack specialized devices for monitoring core body temperature (intra-abdominal or rectal) during surgery. The authors recommend the following supplementary and alternative approaches to mitigate the risk of hypothermia: (1) minimizing heat loss by shortening anesthesia and surgical durations to reduce incision exposure, and by using pre-warmed fluids for irrigation; (2) protecting animals during transport by wrapping them in aluminum foil or warming blankets to conserve heat; and (3) applying external heat sources, such as thermostatically controlled heating pads or circulating-water blankets [14,45,48].

It is important to maintain heating devices within a safe temperature range (e.g., 36–38 °C), and to place a protective layer between the animal and the device to prevent thermal injury (Fig. 4**A**). When using heating lamps, a safe distance must be maintained to avoid burns or excessive drying [43]. Temperature-monitoring devices should be implemented to detect potential overheating or equipment failure.

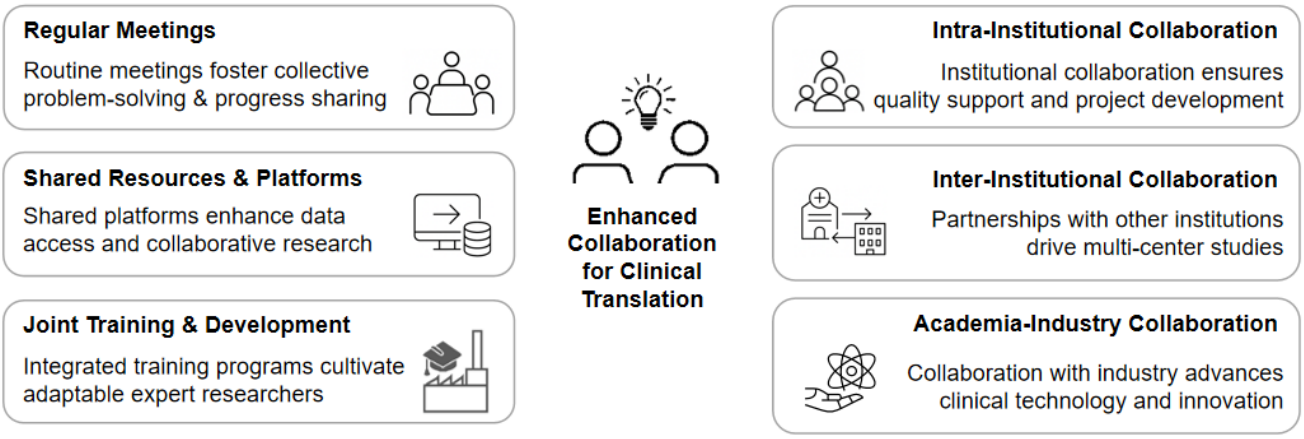

Fig. 6. Multidisciplinary collaboration links planning, execution, and evaluation to raise reproducibility and translational value. The diagram illustrates practical strategies that strengthen collaboration throughout the research lifecycle, from project design to execution and translational impact. Practical pathways to team-science in preclinical orthopaedics involve integrating bone surgery, veterinary care, imaging, biomechanics, biomaterials, and data documentation support. Examples include joint protocol design, shared access to imaging core facilities, and scheduled cross-disciplinary reviews of implant selection and welfare metrics.

Surgical Lighting: Ensuring Adequate Visualization

High-quality surgical lighting is essential for clear visualization of anatomical details during surgery, such as blood vessels or peripheral nerves, thereby facilitating accurate dissection, hemostasis, and suturing [49–51]. This is especially important when operating on delicate and small-scale rodent anatomy. Furthermore, reducing shadows within the surgical field enhances visual comfort, decreases fatigue for surgeons, and may shorten surgical time, thereby reducing anesthetic risks [52].

Mobile surgical lamps or adjustable desktop gooseneck lamps can serve as the primary light source (Fig. 4**B**). Surgical headlamps are also recommended to offer coaxial illumination and remain effective during head movement. In laboratories with limited resources, alternative lighting, such as non-surgical headlamps, can also improve surgical field visibility [53]. However, these devices must provide sufficient and adjustable brightness, a color temperature close to natural daylight (to aid tissue color differentiation), and minimal shadow interference.

Hair Removal

Once a surgical incision is made, animal hair can easily become a source of SSI. Therefore, preoperative hair removal is a critical step in infection prevention during orthopaedic procedures [54]. The removal area must exceed the anticipated incision length to establish a broad and hair-free zone (e.g., 2.5 cm beyond the incision margin in rats). This precaution prevents adjacent hair from entering or contaminating the sterile field during intraoperative skin traction or movement. For limb surgeries (e.g., femoral, knee, or tibial procedures), circumferential removal may be required.

The authors routinely use electric clippers with fine-toothed blades (Fig. 4**C**). Compared to razor blades, electric clippers effectively remove hair while minimizing microabrasions to the skin, which can serve as foci for bacterial colonization [54,55]. If chemical removal is needed, it can be applied after initial clipping, followed by gentle scraping with a smooth and blunt metallic instrument.

To minimize the risk of bacterial recolonization on exposed skin, hair removal should be performed as close to the time of surgery as possible [55]. Hair removal should be performed in the preparation area, rather than the operating area, to limit contamination from airborne hair particles. After clipping, all loose hair must be thoroughly removed using a vacuum, lint roller, or damp sterile gauze. The skin can then be cleaned using the aforementioned disinfectants, followed by drying for a minimum of 2 minutes. Non-operative regions can be covered with sterile drapes or materials to establish a sterile surgical field (Fig. 4**D**).

The goal of draping is to physically isolate potential contamination sources from the disinfected surgical field through the use of sterile towels or wraps [39]. Body areas with high microbial burdens (e.g., mouth, nose, perineal region, paws, and tail) should be effectively excluded from the sterile field. Laboratories may select the type of drapes based on their specific needs and capabilities. Regardless of material, draping must be performed using strict aseptic techniques. Once placed, sterile drapes must never be moved from contaminated areas toward the center of the sterile field. All surgical team members should receive training in draping techniques to ensure effective coverage and isolation.

In femoral fracture surgeries, the fixation device may inadvertently trap residual hair or debris, which can become sources of postoperative infection. Therefore, a meticulous

inspection of the entire operative field and all anatomical layers should be performed before wound closure. The authors routinely employ a minimum of 40 mL of sterile saline for thorough irrigation during inspection to reduce the infection risk.

Instrument Sterilization and Glove Management

Sterilization of Surgical Instruments

Routine orthopaedic procedures in small animals often involve surgical exposure of bone and implantation of hardware or foreign materials (e.g., screws and bone grafts), which substantially increase the risk of SSI. Therefore, all instruments and materials in contact with the surgical field or introduced into body cavities must meet stringent sterilization standards before use.

When performing surgeries on multiple animals in a single day, the optimal approach is to prepare a separate and fully sterilized instrument pack for each animal to minimize the risk of cross-contamination [39]. If resource limitation necessitates the reuse of certain instruments, thorough decontamination should be performed, followed by autoclave sterilization between surgeries. It is critical to recognize that any instrument reused without completing a full sterilization cycle presents a risk of infection for subsequent animals.

In rodent fracture surgery, fixation devices (e.g., titanium screws) are often supplied in single sterile packages containing quantities sufficient for multiple procedures [10]. When several operations are scheduled on the same day, opening such a package may result in unused implants being unnecessarily exposed to the operating room environment, thereby increasing the risk of contamination. To minimize this risk, the authors routinely reallocate the number of implant components in each sterilized set, according to the specific requirements of the planned procedure. For instance, when stabilizing femoral fractures with external fixation, only one connecting bar and four screws are prepared per surgery [10].

In cases of intraoperative contamination, contaminated instruments must be replaced with sterile backups or undergo rapid sterilization to mitigate the risk of cross-infection. Before sterilization, all visible organic material must be removed, followed by rinsing with sterile fluids. Traditional autoclaving at 121 °C may require 30 minutes for steam exposure, which can significantly extend the surgical and anesthesia duration if performed intraoperatively. When equipment permits, the authors recommend using high-temperature cycles (132–135 °C) to reduce sterilization time to 7–15 minutes (Fig. 4**E**) [56,57].

In resource-constrained preclinical environments, glass bead sterilizers may serve as a temporary alternative for small surgical instruments, provided that instruments are thoroughly cleaned and disinfected beforehand (Fig. 4**F**) [39,58]. The authors operate such devices at 260 °C for approximately 30–60 seconds. However, the method is not a replacement for proper sterilization and should only be used for intraoperative contingencies.

Intraoperative Glove Management

Surgical gloves serve as the primary barrier between the operator's hands and the sterile operative field. However, they are not impermeable. Minor perforations may occur during bone reduction, implant handling, or wound closure, often going unnoticed. The risk of perforation increases with longer surgical durations. Laine and Aarnio [59] demonstrated that single gloving results in a 13-fold higher risk of contamination compared with double gloving. To minimize contamination, double gloving should be a standard practice for all orthopaedic procedures.

Even in the absence of obvious perforation, gloves should be intermittently disinfected with appropriate disinfectants during surgery and replaced at strategic intervals, based on procedural duration and contamination risk [60,61]. For early detection of perforations, Meakin *et al*. [62] reported a significantly higher sensitivity with colored indicator gloves compared to standard gloves (83 % vs. 34 %, $p < 0.001$), indicating the benefits of using the novel gloves during surgery.

Gloves must be changed immediately under the following circumstances: (1) after contact with non-sterile surfaces (e.g., adjusting surgical lamp without sterile cover, touching non-sterile equipment, or contacting areas beyond the sterile field); (2) when perforation or puncture is detected; (3) before critical steps, such as implant placement; and (4) routinely, for example, when the procedure exceeds 90 minutes [59–61,63–65]. Although disinfectants may temporarily reduce surface contamination, they should be regarded as interim measures only. Changing gloves remains the most reliable and appropriate practice for maintaining sterility.

Postoperative Environmental Enrichment: Balancing Welfare Needs and Safety Risks

Environmental enrichment (EE) refers to providing conditions that promote the expression of natural behaviors and enhance physical activity in laboratory animals [66–69]. EE plays a vital role in mitigating abnormal behaviors and stress, and is a key component of the "Refinement" principle within the 3Rs (Replacement, Reduction, and Refinement) framework [70,71]. In the context of orthopaedic recovery, animals may experience pain, discomfort, restricted mobility, and, in some cases, temporary individual housing. Under these circumstances, appropriate and safe enrichment strategies, such as the provision of nesting materials, shelters, and social interaction, may offer multiple benefits (Fig. 5).

Importantly, EE protocols should be tailored to the surgical model and dynamically adjusted according to the stage of recovery [72,73]. Certain enrichment interventions

may increase the risk of postoperative complications, especially with fracture fixation devices [6,10,74,75]. For example, narrow passageways or climbing structures may result in impact on fixation devices during movement, leading to hardware loosening or displacement. Therefore, although EE offers clear rehabilitative advantages, its implementation should follow a cautious and stepwise approach with safety as the highest priority (Fig. 5). More detailed safety guidelines for EE after rodent fracture fixation are provided in Table 2.

Following the introduction of any EE intervention, animals must be monitored with increased frequency and attention. In case of adverse signs attributable to EE (e.g., loosening of fixators, entrapment, or wound irritation), the respective enrichment item should be removed or modified, and veterinary consultation should be initiated if necessary [76–78]. Key monitoring points for the application of EE include: (1) safe interactions between laboratory animals and enrichment objects; (2) stability of internal or external fixation devices; (3) abnormal signs during weight-bearing activities, such as worsening lameness and progressive swelling of the surgical limb; (4) wound complications including redness, discharge, or dehiscence; and (5) general behavior, activity level, food intake, and body weight changes. Similar to preoperative planning, the optimization of EE involves expertise beyond routine bone research and can benefit from a comprehensive team with multidisciplinary expertise.

Multidisciplinary Collaboration

The preceding sections have outlined the critical roles of infrastructure and experimental workflow in ensuring research quality, animal welfare, and reproducibility in orthopaedic surgical studies. These aspects encompass digital planning, intraoperative asepsis, and postoperative management. While their implementation facilitates the simulation of clinical scenarios and enhances translational potential, it often exceeds traditional disciplinary boundaries and the technical capabilities of a single laboratory team.

Preclinical orthopaedic studies frequently require the integration of expertise from bone and joint surgery, veterinary medicine, imaging, biomechanics, biomaterials, and more related fields. Establishing multidisciplinary collaboration is therefore essential for overcoming the limitations of individual teams by enabling experts from diverse fields to contribute their unique perspectives [79]. This ensures that each phase of the research, from planning to execution and evaluation, is carried out with specialized expertise. However, successful collaboration does not occur spontaneously and instead requires cultivation and active management.

In preclinical studies, certain advanced laboratory resources and infrastructure may demand significant financial investment and specialized maintenance, such as dedicated operating rooms, *in vivo* imaging platforms, biomechanical testing systems, and simulation-based computational tools. Multidisciplinary collaboration facilitates shared access to these core facilities and equipment, enhancing research efficiency while avoiding redundant expenditures (Fig. 6).

Enhancing Translation with Electronic Lab Notebooks

Traditional paper notebooks have limitations in capturing the complexity of methodological information, particularly often-overlooked laboratory and experimental details. Furthermore, paper records hinder efficient data management, searching, sharing, and long-term preservation. For surgical studies involving numerous parameters and multimedia evidence, these shortcomings can be addressed using electronic lab notebooks (ELNs) to ensure research quality and reproducibility [80,81].

ELNs provide a structured environment for documenting the multifaceted details discussed throughout this paper, thereby improving research transparency and facilitating clinical translation. For example, ELNs enable researchers to embed diverse data types within a single record, including 3D model data, tables, and checklists for surgical preparation, as well as a textual description of postoperative observations. Although implementation requires careful platform selection, template design, and personnel training, it enables consistent recording of critical parameters to minimize omission.

The integration of ELNs supports broader digital research initiatives, as structured records streamline the extraction of information according to guidelines such as PREPARE and ARRIVE, and hold potential for future computational analyses and data mining efforts. Importantly, standardized digital documentation also enables transparent reporting, aligning with FAIR (Findable, Accessible, Interoperable, Reusable) data principles [82]. Moreover, digital documentation can enhance every phase of the preclinical workflow, and ELNs have been explicitly recommended as a cornerstone of data management in preclinical animal research [83].

Various ELNs are available, each with specific features, such as openBIS ELN LIMS (ETH Zurich, Zurich, Switzerland) and eLabFTW (Deltablot, Villejuif, France) [84,85]. The authors have developed and applied the open-source ELN Herbie, which can be tailored to any scientific discipline and process by formal semantic descriptions in ontologies [86]. This enables consistent annotation, improved data interoperability, and enhanced reproducibility across experiments. The ontology underlying our ELN implementation is currently under development and is intended for public release in the near future to support transparency and reuse. Table 3 summarizes the categories of information mentioned in previous sections for ELN documentation. A more detailed template is provided in the **Supplementary Material**, covering applications across preoperative, intraoperative, and postoperative phases.

In animal studies involving musculoskeletal surgeries, details of laboratory infrastructure and experimental workflow are frequently underreported or overlooked in publications. However, these confounding factors may critically influence physiological responses and treatment outcomes. Failure to report or control these variables can hinder reproducibility, exacerbating the "reproducibility crisis" in preclinical research [87–91]. Freedman *et al*. [92] estimated an annual cost of irreproducibility in USA to be approximately 28 billion USD.

Such oversights in laboratory and experimental details may partly explain why many promising therapies in animal models ultimately fail in clinical trials. For example, tumor-growth patterns in mice can be significantly influenced by housing temperature, which undermines interlaboratory reproducibility if unreported [93]. In preclinical bone studies, design flaws, substandard surgical environments, inadequate aseptic techniques, and insufficient postoperative management may significantly increase the risk of complications. These issues also contradict the "Refinement" of the 3Rs.

Conclusions

This study focuses on frequently underreported yet critical technical elements that impact research quality, reproducibility, animal welfare, and translational potential in orthopaedic research. Recognizing that not all laboratories have access to ideal and clinically benchmarked facilities, the authors recommend supplementary and alternative approaches based on surgical experience and relevant clinical literature.

This work may serve as a practical reference for researchers engaged in preclinical orthopaedic studies, encouraging greater attention to essential components during experimental planning and more transparent reporting in scientific publications. Through the utilization of complementary and alternative approaches, combined with a multidisciplinary workflow, the authors have effectively prevented intraoperative technical failure and postoperative infection in rat fracture fixation surgeries.

The authors' recommendations are primarily derived from surgical workflows for rat femoral fracture fixation, which may not fully generalize to other species, skeletal sites, or soft tissue procedures. Digital planning and ELN adoption may face barriers (software access, availability of input devices, training, and data governance constraints) in resource-limited laboratories. Moreover, the reported metrics (e.g., intraoperative dropouts) reflect our team's specific experience and infrastructure, and should be validated across independent settings. Nevertheless, the underlying principles, such as preoperative rehearsal, rigorous asepsis, quantified monitoring, and digital documentation, are broadly applicable and amenable to context-specific adaptation.

Availability of Data and Materials

Not applicable.

Author Contributions

YS wrote the manuscript. OW and HH supported laboratory and experimental management. CCL, HYQ, and SG provided support for 3D design and printing. KW provided support for figure design. SA and RWL provided support for writing the manuscript. CE provided help and advice for ELN. BZP, JBH and RWR supported and coordinated the project management. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

Ethics Approval and Consent to Participate

Not applicable.

Acknowledgments

The authors express sincere thanks to colleagues for their kind support: Prof. Gerhard Schultheiß, Sarah Vieten, Andrea Matzen, Dr. Michelle Rothaug, ZTH and VHH team (Central Animal Facility, Kiel University); Dr. Björn Wiese, Dr. Thomas Ebel, Monika Luczak, Dr. Katharina Philipp, Annette Havelberg, and Anke Borkam-Schuster (Institute of Metallic Biomaterials, Helmholtz-Zentrum Hereon); Dr. Andrey Pravdivtsev, Eva Peschke, Farhad Haj Mohamad, Charbel Assaf, Yenal Gökpek, Jaime Andres Pena, Timo Damm, Dr. Sanjay Tiwari and Prof. Claus-C. Glüer (Section Biomedical Imaging, Department of Radiology and Neuroradiology, University Medical Center Kiel, Kiel University); Yannik Michalsky and Prof. Andreas Seekamp (Department of Orthopedics and Trauma Surgery, University Medical Center Kiel, Kiel University); Fabian Bäumler (Department of Functional Morphology and Biomechanics, Zoological Institute, Kiel University).

Funding

The research is funded by the Helmholtz Association and the IDIR-Project (Digital Implant Research; a cooperation financed by Kiel University, University Hospital Schleswig-Holstein, and Helmholtz-Zentrum Hereon).

Conflict of Interest

The author(s) declare no conflict of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.22203/eCM.v055a04.

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Editor's note: The Scientific Editor responsible for this paper was Martin Stoddart.

Received: 22nd May 2025; **Accepted**: 29th October 2025; **Published**: 30th January 2026