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Review Article

Experimental Trauma Models: An Update

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Treatment of polytrauma patients remains a medical as well as socioeconomic challenge. Although diagnostics and therapy improved during the last decades, multiple injuries are still the major cause of fatalities in patients below 45 years of age. Organ dysfunction and organ failure are major complications in patients with major injuries and contribute to mortality during the clinical course. Profound understanding of the systemic pathophysiological response is crucial for innovative therapeutic approaches. Therefore, experimental studies in various animal models are necessary. This review is aimed at providing detailed information of common trauma models in small as well as in large animals.

1. Introduction

Despite therapeutic advances in prehospital and intensive care, multiple trauma still remains the major cause of death in patients below 45 years of age [1]. Explanations may be found in the increasing mobility resulting in increased incidence of high-energy trauma during the last decades [2]. Immediate or early death following trauma is mainly caused by massive blood loss or severe head injuries [3]. During clinical course, multiple organ dysfunction remains a major problem [4].

Following severe injuries, a systemic immune response is induced [5, 6] trying to preserve the immune integrity [7]. An imbalance of the posttraumatic immune response determines an increased susceptibility to infection and sepsis, consequently leading to organ failure [8, 9]. Approximately, 27.5% of all trauma patients and 50% of patients developing a MODS decease during their treatment in the intensive care unit [10, 11]. Since suffered injuries representing the first hit cannot be influenced, treatment of patients with major injuries should minimize additional harm [7, 12]. Therefore, a profound knowledge of pathophysiological processes following major trauma is necessary. Since clinical studies are complicated by the heterogeneity of trauma populations [13], experimental studies using various animal trauma models are necessary. However, one has to be aware that transfer of results from experimental models to the clinical situation is limited [14].

This review provides an overview of commonly used trauma models in small as well as large animals. It should serve as an introduction and technical guidance of current experimental trauma models.

Although the mouse genome only matches approximately 80% of the human genome, specific advantages lead to common use of diverse mouse strains in experimental trauma studies [15]. Various knock-out mice are available allowing precise studies on certain mediators and receptors. Additional advantages in using mice are small size and low costs in acquisition and keeping, ease of handling, and ethical acceptance. Conversely, the small size leads to difficulties in operative techniques and limitations of perianaesthetic management. Due to the genomic distance to humans, further investigations in large animals are required before results from experimental studies can be transferred in clinical trials [4, 16].

Beside mice, studies using rat models have comparable advantages, including small size, cost, ease of handling, ethical acceptance, and availability. As compared to mice, shock models in rats are technically easier to perform [6]. Similar to mice, rats and humans share approximately 90% of the genome [16]. Despite this resemblance, genetic variations like knock-out models and transgenic rats as well as fewer

immunological tests are less available [4, 6]. The latter aspect may change in the future as more and more companies are starting to develop further rat genetic models.

Because porcine hemodynamic responses are similar to humans, numerous trauma models are established [4, 6]. Although pigs are easier to operate, more technical equipment is required [4]. Beside an equal metabolism response to drugs in pigs and humans, wound healing is described similar in pig and human skin [6]. On the other side, one should be aware of the increased costs based on the complexity needed.

Large animal studies are mostly limited to physiological and mechanistic investigations since cell- and mediator-specific molecular probes and reagents are rarely available.

Table 1 presents currently available animal models simulating relevant traumatic injuries.

2. Animal Models Simulating Hemorrhagic Shock

2.1. Murine Models. Trauma-hemorrhage in mice can either be performed pressure controlled or volume controlled. The established method of pressure-controlled hemorrhage usually requires a soft tissue trauma to implant arterial catheters. An adequate anesthesia during the surgical procedure is required. Currently, inhalation anesthesia using Isoflurane has become an established method [17–20]. After restraining the mice in a supine position, vessels are aseptically catheterized with a common size polyethylene tubes no. 10 (outer diameter 0.61 mm, inner 0.28 diameter mm) [17–22]. In the literature, different techniques are described using an isolated catheter in one femoral artery [21, 22] or two catheters in both femoral arteries [19, 20]. For venous fluid resuscitation, an additional venous catheter can be implanted [17, 18]. Less frequently, cannulation of the carotid artery is described [23, 24]. It has to be considered that the unavoidable soft tissue trauma already induces a systemic immune response [5]. Using an Isoflurane narcosis, animals can be allowed to be awaken after placement of catheters focusing on a simulation of the clinical reality [29]. However, guidelines of the respective country are to be recognized. In general, blood is withdrawn until a mean blood pressure of 35 ± 5 mmHg is reached [20, 21], measured via an arterial catheter using a blood pressure analyzer [17, 19]. The described pressure value should be reached within the first 5 to 10 minutes [17, 21, 22] and then kept between 30 to 90 minutes [20, 21, 23]. An anticoagulant, heparin, is often used to promote blood flow through the implanted catheters [21–23] but is described to affect the immune response [117]. At the end of this interval, animals are resuscitated. Some authors prefer to resuscitate the shed blood volume until the blood pressure has been corrected [23, 28]. More common, fluid resuscitation with Ringer's Lactate is used. The extent of this transfusion ranges from two to four times the exsanguinated blood volume within 30 minutes after completing trauma hemorrhage [17–22]. Catheters are then removed, the femoral vessels have to be ligated, and the incisions are closed. To provide adequate analgesia, Lidocaine should be applied to the incision sites [19].

Beside the pressure-controlled model, volume-controlled hemorrhage has also been used in a number of recent studies [23–25]. Volume-controlled bleeding is commonly performed with catheterization under anesthesia as described before. A weight-adapted blood volume is withdrawn through the implanted catheter. Less frequently used techniques induce volume-controlled hemorrhage by retro-orbital [26, 27] or cardiac puncture [118]. The latter procedure is performed using a 29-gauge needle causing additional trauma to the diaphragm [118]. In most studies, the shed blood volume ranges from 0.025 to 0.05 mL/g body weight representing 35–45% [36] of the estimated blood volume [23–25] while few studies withdraw up to 60% of the estimated blood volume [26, 27, 119, 120]. During the hemorrhage procedure, blood pressure and heart rate should be monitored [23, 24].

Trauma-hemorrhage models in mice are basically performed to analyze immunological questions. Especially pro- and anti-inflammatory cascades regarding possible medical interventions are currently focused [19, 20, 28, 121–125]. Referring to this issue, Kupffer-Cells, alveolar macrophages, and splenocytes are isolated or modulated to investigate the determining inflammatory influence measured by cytokines and other mediators [17, 18, 20, 28, 121–123].

2.2. Rat Models. Similar to murine models, trauma hemorrhage can be achieved by the two described procedures. Anesthesia is commonly performed by an inhalation narcosis with Isoflurane (5% induction, 2% maintenance) or Halothane (1.5–2% halothane in 100% oxygen) [29–33]. Following another technique, a single dosage of sodium pentobarbital (50–60 mg/kg body weight intraperitoneally [i.p.]) can be used [30, 34, 35]. The temperature during anesthesia and hemorrhage shock can be measured by a feedback heat pad or via rectal probe and is usually kept between 37.0°C and 37.5°C [29–32, 34]. In both, the pressure- as well as the volume-controlled method, arterial catheters should be implanted for blood removal and monitoring. Besides the described techniques in femoral vessels, the jugular vein or carotid artery can be used to withdraw blood or monitor blood pressure and heart rate [30, 34, 35]. Usually, polyethylene (PE-50) tubing (outer diameter 0.965 mm, inner diameter 0.58 mm) and a 50-gauge silicon catheter is used and should be placed within 30 minutes [29, 34, 35]. Following the pressure-controlled technique, a mean arterial pressure of 35–40 mmHg should be reached within the first 10 to 15 minutes and then kept for 90 to 120 minutes [29, 31, 33–35].

Following the volume-controlled model, 20 mL/kg body weight or a fixed volume (45% of the body weight) is withdrawn within the first 10 to 15 minutes [30, 37]. A total blood volume of 64 mL/kg body weight can be expected [36].

Similar to murine models, catheters remain in place for the duration of hemorrhage and resuscitation procedures. Animals can be allowed to awaken before starting the controlled bleeding. To prevent clot formation, the artery

Table 1: Available animal models simulating relevant traumatic injuries.

(a)

	Trauma hemorrhage	
	Pressure controlled	Volume controlled
Mouse	(i) Inhalation anesthesia [17–20] (ii) Catheterization with PE-10 tubing [17–22] (iii) BP 35 ± 5 mmHg for 30–90 min [20, 21, 23] (iv) Volume resuscitation [17–22, 28]	(i) Inhalation anesthesia [17–20] (ii) Catheterization with PE-10 tubing [17–22] (iii) 0.025–0.05 mL/g body weight (35%–60%) [23–27] (iv) Volume resuscitation [17–22, 28]
Rat	(i) Inhalation anesthesia [29–33] (ii) Catheterization with PE-50 tubing [29, 34, 35] (iii) BP 35–40 mmHg for 90–120 min [29, 31, 33–35] (iv) Volume resuscitation [33–35]	(i) Inhalation anesthesia [29–33] (ii) Catheterization with PE-50 tubing [29, 34, 35] (iii) 20 mL/kg body weight (45%) [30, 36, 37] (iv) Volume resuscitation [33–35]
Pig	(i) Complex anesthesia [4, 38–45] (ii) Orotracheal intubation and mechanical ventilation [41, 42, 44, 46] (iii) Complex catheterization [38–41, 43–45] (iv) BP 30–40 mmHg for 45–60 min [39, 40, 42, 47] (v) Volume resuscitation to BP 60–65 mmHg [49–51]	(i) Complex anesthesia [4, 38–45] (ii) Orotracheal intubation and mechanical ventilation [41, 42, 44, 46] (iii) Complex catheterization [38–41, 43–45] (iv) 25–35 mL/kg body weight (40%) [43, 45, 48] (v) Volume resuscitation to BP 60–65 mmHg [49–51]

(b)

	Traumatic brain injury		
	LFP	CCI	Weight drop
Mouse	(i) Anesthesia (ii) 2.0 diameter craniotomy [52] (iii) Installation of a fluid percussion device [52, 58] (iv) Impact on intact dura by a brief fluid bolus [60, 62, 63] (v) Injury magnitude: 3.6 atm [52]	(i) Anesthesia (ii) 3–5 mm craniotomy, dura intact [53–56] (iii) Installation of a pneumatically driven impactor (3 mm impounder) (iv) Velocity 5-6 m/sec; depth of 0.5–1 mm [53–56]	(i) Anesthesia (ii) Impact on exposed skull or intact dura [57] (iii) 250 g metal rod dropped from 2-3 cm [59–61]: (a) risk of skull fractures >3 cm [60] (b) inadequate trauma <2 cm [60]
Rat	(i) Anesthesia (ii) Craniotomy (4 × 2 mm) [30, 64, 65] (iii) Installation of a luer-lock connector to intact dura [30, 64, 65] (iv) Trauma induction with 2.4 bars [30, 64, 65]	(i) Anesthesia (ii) Craniotomy (diameter 6–10 mm) [66–68] (iii) Installation of a pneumatic cylinder [66–68] (iv) Impact velocity 4–8 m/sec [66–68]	(i) Anesthesia (ii) Fixation of a steel disc [69–71] (iii) 300–450 g weight drop from 1–1.8 m height (exposed skull) [69–71] (iv) 21 g from 35 cm height (exposed dura) [72]
Pig	(i) Complex anesthesia (ii) Complex craniotomy [73–75] (iii) Additional ICP monitoring [73–75] (iv) Trauma induction with 3–8 bars [73, 75]	(i) Complex anesthesia (ii) Complex craniotomy [73–75] (iii) Additional ICP monitoring [73–75] (iv) Injury induction on intact dura: (a) velocity of 3.5 m/sec [76, 77] (b) dwell time 400 ms [76, 77]	Commonly not performed

(c)

	Long bone fracture models		
	Open femoral fracture	Closed femoral fracture	Tibial fracture
Mouse	(i) Anesthesia (ii) Lateral exposure of femoral bone [78, 79] (iii) Fracture performance by osteotomy [78, 81]	(i) Anesthesia (ii) Initial stabilization: (a) intramedullary pin [80, 82]	(i) Anesthesia (ii) Less frequently performed [80] (iii) Fracture placement distal to keep fibula intact [80, 83, 84]

(c) Continued.

	Long bone fracture models		
	Open femoral fracture	Closed femoral fracture	Tibial fracture
	(iv) Stabilization by extramedullary fixation: (a) locking plate [80, 85] (b) pin clip device [80, 81] (c) external fixator [80, 89, 90]	(b) intramedullary screw [80, 82] (c) locking nail [86] (iii) Closed fracture by 3-point bending system [26, 80, 82, 87, 88]	
Rat	(i) Anesthesia (ii) Lateral exposure of femoral bone [91, 92] (iii) Medial exposure of femoral bone [91, 92] (iv) Fracture performance by osteotomy [98, 99] (v) Fracture stabilization: (a) intramedullary pin or K-wire [95, 96, 102–104] (b) combination of K-wire and 2 screws [99] (c) plate osteosynthesis [106, 107] (d) external fixator [91, 108, 109]	(i) Anesthesia (ii) Initial stabilization: (a) intramedullary steel pin [95–97] (b) intramedullary K-wire [95–97] (iii) Closed fracture by blunt guillotine: (a) 500–650 g steel weight [87, 97, 102, 105] (b) 14 cm drop height [87, 97, 102, 105]	(i) Anesthesia (ii) Closed fracture model [93, 94]: (a) 3-point bending system (b) 300 g weight from 20 cm height (iii) Open fracture model [100, 101]: (a) lateral exposure of tibia (b) fracture performance by osteotomy (iv) Fracture stabilization: (a) K-wire or modified needle [93, 101] (b) plate fixation [100, 110] (c) external fixator [111]
Pig	(i) Complex anesthesia (ii) Fracture is performed by captive bolt gun or osteotomy [112–115] (iii) Fracture remains commonly unstabilized [112–115] (iv) Intramedullary nailing is possible [116]	Not performed in trauma models	Not performed in trauma models

catheter is to be filled with normal saline containing 10–30 U/ml of heparin [34, 35]. As in mice, an immune response caused by heparin has been detected [126, 127]. To reduce postoperative pain, wounds should be flushed with 1% lidocaine throughout the surgical procedure [33]. At the end of the shock period, animals can be resuscitated by re-infusing of the shed blood or with two to four times the volume of the shed blood with Ringer's Lactate solution over 60 minutes [33–35].

Regarding the current literature, rat trauma-hemorrhage models are rather used for measurement of physiological parameters than analyzing the immune response [29–31, 128–130].

2.3. Porcine Models. Pigs undergoing surgery are usually fasted for at least 12 to 18 hours before starting anesthesia [4, 38–41]. Afterwards, a premedication 1 hour before surgery should be performed using, for example, Midazolam (0.1 mg/kg) with azaperone (4 mg/kg) and atropine (0.1 mg/kg) or a single dosage of Buprenorphine (0.03 mg/kg) [39, 41]. Anesthesia can be induced with a bolus dose of ketamine (10–20 mg/kg intramuscular), propofol (1–6 mg/kg intravenous), and piritramide (30 mg intravenous) administered via an ear vein [39, 41–43]. Other studies prefer the anesthesia induction with Isoflurane (5%) in a mixture of oxygen and nitric oxide (1 : 1) or halothane to facilitate oral intubation [38, 40, 43–45]. Beside an oral intubation, a tracheotomy is described [44]. Surgical anesthesia should subsequently be maintained with Isoflurane (1%–2% Isoflurane end tidal concentration) in a mixture of oxygen and nitrous oxide (1 : 2) or via total intravenous anesthesia applying propofol (6–8 mg/kg/h) and piritramide (30 mg bolus as needed) [40, 41, 44]. If necessary, neuromuscular blockade can be achieved with 0.1–0.3 mg/kg/h pancuronium after tracheal intubation to prevent spontaneous breathing or gasping [41, 42]. Intubation has to be performed with a 7.0 mm or 7.5 mm internal diameter cuffed endotracheal tube [46]. Following intubation, pigs are usually ventilated in a volume-controlled ventilation modus with 21%–50% oxygen, a respiratory rate of 12–14 breaths/min, an inspiratory to expiratory ratio of 1 : 2, and a tidal volume of 12 mL/kg body weight to

maintain normocapnia defined as an end-tidal pCO_2 of 35–45 mmHg measured by continuous capnography [41, 42, 44]. Similar to the small animal studies described above, femoral arteries and veins are catheterized through groin incisions (catheter size: 7 French) under aseptic conditions [41, 44, 48]. Through an additional surgical incision, external jugular veins and carotid arteries can be identified and cannulated (catheter size: 8 charrière/FG, length 400 mm or 9 French) [38–41, 43–45]. A 5 to 7.5 French catheter is commonly positioned in the pulmonary artery for continuous monitoring of pulmonary and carotid artery pressures [41–43]. To guarantee stabilization of cardiopulmonary physiological parameters following the surgical procedure, a recovery period of 15–90 min for temperature control or volume application is described before initiating hemorrhage [39–41, 48]. Body temperature can be measured with a nasopharyngeal or an endotracheal temperature probe and should be maintained between 38.0 and 39.5°C using an external heating device, for example, a Bair-Hugger blanket [38, 41, 42, 46]. Intravenous fluids used during the surgical procedure should be warmed to 37°C reducing the risk of intraoperative hypothermia [41]. At the end of the recovery period, hemorrhagic shock is induced following one of the described techniques. Based on the pressure-controlled model, blood should be withdrawn until a mean blood pressure of 30–40 mmHg is reached (max. 55 mmHg) and then held for at least 45 to 60 minutes, depending upon the depth of shock and lethality desired [39, 40, 42, 47]. Following the volume-controlled model, 25–35 mL/kg (approximately 40% of the blood volume) are withdrawn with a constant rate over a 15 minute period assuring an adequate shock state [43, 45, 48]. During hemorrhage procedure and following resuscitation, animals should be kept under anesthesia as described above. Standard volume resuscitation using Lactated Ringers solution and additional colloid fluids aim at a mean arterial pressure to 60–65 mmHg, the average baseline blood pressure of anesthetized pigs [49–51].

In summary, porcine models are commonly used to analyze different volume resuscitation forms [42, 43, 47]. Moreover, this model is used to assess the effects of interventions including hypothermia and innovative haemostatic products [39, 131].

3. Animal Models Simulating Traumatic Brain Injury

Severe head injury is known as one of the most frequent major injuries in polytrauma [132]. Moreover, if accompanied with hypotension and ischemia resulting in secondary brain injury, patients' outcome is described worse [4]. Consequently, animal models are designed to simulate this additional impact analyzing pathophysiology and potential interventions.

3.1. Murine Models. Traumatic head injury in mice can be performed as an isolated injury or as an additional trauma beside trauma-hemorrhage [53–56, 59, 60]. While isolated brain injury models are widely used, a combined trauma is technically challenging and therefore rarely represented in the current literature [53, 54]. In case of trauma-hemorrhage, catheters are placed in the described techniques before initiating the head injury model [53, 54].

Three experimental head injury models are performed in mice. The lateral fluid percussion (LFP) has been established as a standard experimental model of traumatic head injury with high reproducibility and accurate association between injury severity and outcome [60, 62]. The injury consists of changes in blood flow and metabolism, an altered ionic homeostasis, breakdown of the blood-brain barrier, reactive astrocytosis, and ongoing necrotic and apoptotic cell death at the injury site [63]. Moreover, traumatic axonal injury in the subcortical white matter is achieved using this model [63]. Originally described, LFP involves a 4.8 mm craniotomy centered over the left parietal bone, 4.0 mm lateral to the sagittal suture [62]. As expected, the initial model had to be adapted to accommodate smaller body weight, skull size, and skull thickness of a mouse [52]. Consequently, a 2.0 mm diameter craniotomy, 0.5 mm lateral to the sagittal suture and 0.5 mm caudal to bregma, has to be created carefully [52]. After trepanation of the skull, injury is performed by a fluid percussion device: this device is comprised of a plexiglas cylinder (32 cm long and 2 mm in diameter) filled with distilled water [52, 58]. A pendulum from a known height impacts the piston of a saline-filled reservoir, forcing a brief fluid bolus on the intact dural surface [60, 62, 63]. An injury magnitude of 3.6 ± 0.1 atm has to be created [52]. Compared to the following models, the lateral fluid percussion is rarely performed in murine models.

The "controlled cortical impact" (CCI) model uses a pneumatically driven impactor to produce traumatic brain injury. It is mimicking acute subdural hematoma, axonal injury, concussion, blood-brain barrier dysfunction, and coma [56, 60]. After placing the anesthetized mouse in a stereotaxic head-holder frame, a 3–5 mm craniotomy is performed over the left parietotemporal cortex (between midline, bregma, and lambda) using a dental drill [53–56]. The bone flap is removed [53, 54] without damage to the dura mater [56]. CCI injury is conducted by a 3 mm flat-tip impounder deployed at a velocity of 5-6 m/sec and a depth of 0.5–1 mm [53–56]. A brain temperature microprobe can be inserted through a burr hole [53, 54]. Brain temperature should be maintained between 36.5 and 37.5°C during the experiment [53, 54].

The "weight-drop model" uses the gravitational forces of a free falling weight to produce traumatic brain injury by contusion, cortical cell loss, cerebral edema formation, blood-barrier dysfunction, and apoptotic cell deaths [59–61]. The impact of the free falling weight is delivered to the exposed skull or the intact dura [57]. In brief, a surgical incision is initially made over the cranium. A burr hole (4 mm diameter, located 1 mm lateral and 1 mm posterior to the bregma) has to be performed using a surgical drill to expose the dura mater [59, 61]. This preparation step can be skipped, and the weight-drop injury is directly performed to the exposed skull [60]. However, a 250 g metal rod is dropped from a 2-3 cm height onto the exposed skull or dura mater [59–61]. Falling heights over 3 cm are likely to

result in unwarranted skull fractures or immediate lethal posttraumatic respiratory depression while falling heights less than 2 cm cannot guarantee an adequate traumatic brain injury [60].

3.2. Rat Models. Traumatic head injury in rats can be performed as an isolated injury impact or as an additional trauma beside trauma hemorrhage as well [30, 64–67, 69, 72]. As compared to mice, a combination of traumatic brain injury and trauma-hemorrhage is more frequently performed in rats since surgical procedures are technically less challenging [30, 64, 66, 69]. Due to the difference between the brain's diameter of rats compared as to mice (dorsoventral diameter: 10 mm in rats versus 5.5 mm in mice), the technique has to be adapted [56].

The LFP represents an established model in rats but requires considerable adaptations to guarantee an adequate brain trauma. Initially, anaesthetization has to be gained before placing the rats' head in a stereotactic frame. Afterwards a 4-5 mm craniotomy is trephined into the left-sided skull between the lambda, bregma, and 2–4 mm lateral to the sagittal suture [30, 64, 65]. With the intact dura, a luer-lock hub is placed over the exposed dura and bonded in place, that is, with cyan acrylic adhesive [30, 64, 65]. Subsequently, the luer-lock connector is cemented to the skull and the anchoring screw using dental cement [30, 64, 65]. During this procedure, animals should be placed on heating pads preventing hypothermia by maintaining the body temperature between 37.2°C and 37.5°C [30, 65]. After cannulation of the vessels for later trauma hemorrhage, the luer-lock is connected to a fluid percussion injury device. The brain trauma has to be induced with 2.4 bars (2.0–2.6 atm) [30, 64, 65].

The CCI model in rats produces the required brain injury comparable to mice. Initially, rats are positioned in a stereotactic frame under adequate anesthesia [66–68]. After a midline incision, the scalp and temporal muscle are reflected and a 6–10 mm diameter craniotomy has to be performed using a dental drill [66–68]. The dura mater is kept intact over the cortex [66, 67]. Injury is induced by a pneumatic cylinder with a 3–9.5 mm impactor-tip at a velocity of 4–8 m/sec [66–68]. Consequently, a 1–2.5 mm cortical compression is achieved [66–68]. During this procedure, rats are kept normothermic (37.0°C) using a feedback-regulated heating pad or lamp [66–68].

Using the "weight drop model", the skull has to be explored by a midline scalp incision after securing the anaesthetized rat in a stereotactic frame [69, 70, 72]. With the intact skull, a round steel disc (1 cm diameter, 3 mm thickness) is fixed by surgical cement or bone wax onto the central area of the skull [69–71]. Animals are placed and secured in prone position on a piece of foam (height 5 cm) under a Plexiglas tube [69, 71]. A 300–450 g weight is dropped freely from a height of 1–1.8 m onto the centre of the steel disc [69–71]. Rebound impacts have to be prevented by moving the animals immediately after the first impact or using an inflexible rope tied to the weight [69, 71]. Similar to the murine model, "weight drop" can be performed directly to the dura mater. In this model, a 21 g weight from 35 cm hits onto a piston (diameter 4.5 mm) resting on the dura [72]. If required, an ICP monitoring can be conducted by a previously inserted spinal needle (e.g., 24 gauges) within the lateral ventricle [72]. Trephined bone flaps should be replaced after trauma [72].

3.3. Porcine Models. Although rodents are mostly used in head injury models, several important structural and cellular differences between human and rodent brains exist. The lissencephalic rodents' brain differs from the gyrencephalic humans' brain resulting in varying biochemical characteristics [76, 133]. Conversely, the swine brain has more similarities to humans': its gyrencephalic structure, glial-to-neuronal structure, and extent of white matter tracts are similar to humans [76, 134] resulting in comparable biomechanical characteristics [133]. The large size of swine allows extended monitoring similar to treatment of humans during an intensive care stay.

The LFP in swine is initiated by placing the anaesthetized animal in a head stabilizer. The head is shaved and prepared with povidone iodine solution [73]. Afterwards, a 1 cm craniotomy is performed over the left front parietal area approximately 1 cm lateral to the midline and 1 cm rostral to the bregma [74, 75]. A hollow bolt is installed flush with the intact dura [74, 75]. Another craniotomy on the contralateral side allows introducing catheters for measurement of ICP and intracerebral temperature [73–75]. Therefore, the catheter is placed in the subdural space. Additionally, another 4 French catheter can be implanted in the superior sagittal sinus for serial sampling of cerebral venous blood gases and electrolytes [73, 75]. During the procedure, 10–15 mL/kg/hour of crystalloid fluid should be infused to stabilize hemodynamics [74, 75]. Finally, the fluid percussion device is connected to the hollow bolt mentioned before. Thereafter, a 30–60-minute stabilization period is started [73–75]. The traumatic brain injury is performed with 3–8 atm (3.04–8.11 bar) directly to the dura after removal of the implemented ICP probe [73, 75]. Subsequently, this probe is reinserted and recalibrated usually within 2 minutes after trauma impact [75]. In contrast to rodent models, additional trauma-hemorrhage is started simultaneously [73–75].

Performing the CCI model, anaesthetized animals have to be fixated in a stereotactic frame. Afterwards, a 15-mm burr hole is made centered anterior to the coronal suture and lateral to midline over the frontal lobe [76, 77]. The bone is carefully removed to expose the intact dura mater. Additionally, ICP monitor and brain temperature probes should be inserted through a 2 mm burr hole contralaterally [76, 77]. Furthermore, swines are observed for 1 hour after ICP insertion for hemodynamic stabilization [76, 77]. Following preparation and stabilization, the head in the stereotactic frame is placed in the CCI device. The head injury is created using a depth of depression between 9 and 12 mm due to the injury severity required [76, 77]. A velocity of 3.5 m/sec and a dwell time of 400 ms are commonly used [76, 77].

The previously described "weight drop" is not used in porcine trauma models.

4. Animal Models Simulating Additional Thoracic Trauma

Blunt thoracic trauma is common in polytrauma patients and associated with an increased risk of organ failure [135]. Therapy is mostly limited to supportive therapy. Moreover, there is only limited correlation between the volume of the affected lung and severity of pulmonary dysfunction [136]. Therefore, a profound understanding of the underlying mechanisms is required. To date, the mechanism by which acute lung failure is mediated is still not completely elucidated. Several models of blunt lung injury in rodents as well as in large animals are described. Basically, lung injury is induced either by mechanical stress, inhalation, or ventilation induced.

4.1. Rodent Models. In murine models of lung injury, trauma can be induced by a single blast wave centered on the thorax [137]. A newer study reports pulmonary blast injuries caused by laser-induced stress waves with high controllability and reproducibility [138]. Severity of chest trauma regarding physiological parameters depends on peak pressure applied to the thorax as well as on distance between blast wave generator and chest wall [137]. Commonly used is a peak pressure of ~0.75 bar with a distance of 2 cm for 3.4 ms [139]. Beside blast waves, lung contusion is also induced by weight drop with a defined weight from a defined height with a protective shield to prevent blunt cardiac injury [140, 141]. Energy applied to the chest ranges from 1.8–2.7 J in rats [141]. In further rat studies, this model was combined with instillation of acid and small gastric particles through an endotracheal tube to simulate lung contusion with gastric aspiration [142]. Another murine model uses a cortical contusion impactor strucking the chest from the lateral aspect with a defined velocity of 5.8 m/s, depth of penetration of 6.3 mm, and therefore an energy of 152 J/m^2 resulting in a reproducible pulmonary contusion [143]. Moreover, early studies to investigate lung injury used a captive bolt gun [144, 145].

In other rodent models lung injury is induced by ventilation with high tidal volumes (30 mL/kg body weight) or negative-pressure cycled ventilation ranging from $-25\,\text{cm}\,H_2O/0\,\text{cm}\,H_2O$ to $-10\,\text{cm}\,H_2O/-3\,\text{cm}\,H_2O$ [146]. For experimental acute lung injury, intravenous injection of oleic acid is used in rats as well as in mice [147, 148]. Lung fibrosis models use intravenous application of bleomycin [149].

4.2. Large Animal Models. Blunt lung injury can also be induced by a captive bolt gun in pigs [150]. In large animals, trauma induced by inhalation is more commonly used. Inhalation injury is described in sheep as a single injury [151] as well as combined with a burn injury [152]. Commonly used are anesthetized sheep subjected to a 20–40% total body surface area third-degree burn and 48 breaths of cotton smoke (<40°C) [151–153]. In sheep, ventilation-induced lung injury is described as well using increased tidal volumes (20 mL/kg body weight for 15 minutes in preterm lambs) as compared to rodents [154].

5. Animal Models Simulating Additional Long Bone Fracture

Beside head and thoracic injuries, long bone fractures are critical in polytrauma patients [155]. Additional to in vitro studies and clinical trials, animal models are used to extent knowledge of fracture repair and systemic immunological influences on multiple or single traumatized patients [80]. Differences in anatomy and metabolism of animals compared with humans must be considered in interpreting the results. Furthermore, the experimental design must be aware of the considerable influence of animals' age referring to fracture healing [156]. Therefore, only animals with completed bone growth should be used to mimic fracture healing in human adults [80].

5.1. Murine Models. Although mice and other small rodents possess only a primitive bone structure without a Haversian system, fracture healing processes similar to larger mammalians [157]. Therefore, murine models are used to investigate bone healing or regeneration [80]. Several murine models using long bone fractures are commonly described analyzing therapeutic options of different stabilization techniques or the systemic influence of an additional impact in trauma models. Due to the small size of mice, only large long bones, especially tibia and femur, have been studied [158].

As a tubular bone with consistent scales (length 15 mm, outer diameter 1.5 mm), the femur is commonly used in different fracture models [80]. The fracture required can be performed open or closed: basically, closed fracture models create minor soft tissue injury since splitting of the huge quadriceps muscle covering the femur can be avoided. Nevertheless, a contusion of the muscle while performing the fracture results in an simultaneous crush injury. After disinfection of the leg, an intramedullary pin, locking nail, or intramedullary compression screw is implanted to guarantee postoperative stabilization [80, 82, 87, 88]. Afterwards, the closed diaphyseal fracture is produced using a 3-point bending system including a blunt guillotine with a 500 g steal weight [26, 80, 82, 87, 88, 159]. Postoperative X-rays verify diaphyseal fracture and correct intramedullary stabilization [88]. To stabilize a closed femoral fracture, different intramedullary techniques can be used. Initially, an intramedullary pin is implemented: under sterile conditions, a 4 mm medial parapatellar incision is performed and the patella is dislocated laterally [80, 82]. After drilling a hole (0.5 mm) into the intracondylar notch, a stainless steel wire (diameter 0.25 mm) is applied for intramedullary fracture stabilization [80]. Migration of the pin can be prevented by wedging the distal end of the intramedullary canal [80]. Alternatively, a "locking nail" or compression screw can be implemented preventing lack of rotational stability of the pin. For nailing, a modified 24-gauge needle can be used. Initially, the distal needle ends have to be flattened. Rotation stability is now achieved by canting the distal ends intramedullary [80, 86]. Using the previously implemented pin as a guide wire, the modified needle is implemented after producing the femoral fracture [80, 86].

Finally, the guide wire is removed. Beside the rotationally stable nailing technique, a small compression screw (length 18 mm, diameter 0.5 mm) has been engineered [80, 82]. This osteosynthetic design guarantees additional axial stability [80, 82]. Similar to the techniques described above, a guide wire is used before performing the fracture and implantation of the screw. A possible disadvantage might be found in the manipulation of the endosteum and bone marrow caused by the intramedullary stabilization.

In open fracture models, the femur is fractured under visible control [80]. The skin is incised laterally along the femur [78, 79]. After incision and splitting of the fascia lata, muscle bellies of the overlying quadriceps and hamstring muscles are parted to gain access to the femoral diaphysis [79, 81]. The fracture is created in the middle of the femur by an osteotomy or manually after weakening the bone by several drill holes [78, 81]. Thus, the open fracture model produces a major soft tissue trauma, which may have influence on the vascularisation and, therefore, perfusion of the femur [80]. In contrast to closed fracture models using intramedullary stabilization systems, open fracture models stabilize the femoral fracture by extramedullary fixation techniques like locking plate, a pin-clip device, or even an external fixator [78, 79, 85]: to attach an external fixator, initially a pair of 0.55 mm diameter drill holes is angled 7° perpendicular on each of the proximal and distal fragments. After the fixator pin insertion, the fixator bar is only applied loosely to perform the mentioned osteotomy [85]. As the distance between the proximal and distal holes in the fixator bar differs from the corresponding distance in the drilling jig, fracture compression can be achieved by down sliding the fixator bar [85]. Beside the considerable soft tissue injury, performing an open fracture the fixators' design can restrict the physiological activity of injured mice [80, 85].

The pin-clip device presents another open fracture model combining an intramedullary pin with an extramedullary clip [80, 81]: after drilling a hole (0.5 mm) into the intracondylar notch, a distally flattened 24 gauge needle is implanted intramedullary. Afterwards, the femur is exposed through a lateral approach and an osteotomy is created in the middle of the femur. After reduction of the gap and reposition of the bone ends, a metallic clip, bridging the osteotomy, has to be implanted ventrodorsally [80, 81]. In comparison to the fixator method, the pin-clip technique causes less mobility restrictions [80].

Plate osteosynthesis represents the third extramedullary fixation technique in open femoral fractures in mice [80]. Through a lateral approach between the vastus lateralis and biceps femoris muscles, the plate is fixed to the anterior aspect of the midfemur [89, 90]. It is fixed to the bone with four screws using a compression guide to guarantee fracture compression [90]. Two different plate designs, a rigid plate and a flexible plate, allow either stable or standardized flexible stabilization of the femur [80, 89, 90]. Nevertheless, the periosteum and thus perfusion and nutrition may be damaged by the external plate fixation and affect thereby the callus formation at the implant site [80].

Compared to femoral fracture models, tibia fracture is less commonly performed [80, 83, 84]. This may refer to the triangular structure of the tibia in connection with the fibula and its curved major axis. Both anatomical characteristics demand enhanced requirements to implants used to guarantee a stable fracture fixation [80, 84]. Furthermore, fractures should only be placed distal to keep the fibula intact avoiding more than one callus formation [80]. Although access to the tibia requires less soft tissue injury, the described anatomic disadvantages prevent tibial fracture commonly used in experimental studies.

5.2. Rat Models. Similar to murine models rats are used investigating fracture healing. However, due to the missing Haversian system, intracortical remodeling cannot be detected in rats representing an essential difference to humans [160]. Closed femoral fracture in rats is performed after intramedullary stabilization with a steel pin or K-wire [95–97]. The diaphysis of the pinned femur is fractured with a three-point bending device [95–97]. Commonly, a blunt guillotine ramming system is used with a dropped steel weight of 500–650 g from a drop height of 14 cm [87, 97, 102, 105]. If greater soft tissue damage is required, a 0.94 kg steel weight is dropped from 15.3 cm delivering increased impact force [161]. The fracture as well as the healing process can be documented radiographically [105].

To generate an open femoral fracture in rats, medial and lateral approaches to the diaphysis are described [91, 92]: after anaesthetization medial right thighs are incised under aseptic conditions and the femoral biceps muscle overlying the semimembranous and adductor muscles are carefully retracted to expose the midshaft of the femur [92]. More commonly, the lateral approach is used: a skin incision across the lateral aspect of the thigh is performed. The fascia latae between the gluteus superficialis and biceps femoris muscles is split, exposing the femur [91, 98]. Similar to murine models, the femoral fracture is technically simulated by an osteotomy or fractured manually after weakening the bone [98, 99]. To stabilize the fracture, an intramedullary pin or K-wire is used. A medial parapatellar incision (1 cm) is performed under aseptic conditions in the right hind limb. The patella is dislocated laterally. The medullary canal is entered through the intercondylar notch and reamed with a 21-gauge needle. A stainless steel pin or K-wire (0.71–1.25 mm diameter) is inserted into the canal secured in the greater trochanter by tamping [95, 96, 102–104]. The distal portion of the pin is cut flush within the intercondylar notch. The patella is reduced, and soft tissue and skin are closed in two layers by resorbable sutures [95, 96]. Although rotational and axial stabilization cannot be guaranteed by this technique [99], experimental nailing systems or compression screws are not described in closed fracture models in rats. In the literature, two experimental nailing systems are described requiring an open fracture model [98, 99]. The system combines a retrogradely implemented K-wire with two centrally perforated screws [99]. These screws are implemented after predrilling (1.5 mm drill) on each side of the osteotomy before inserting the K-wire. Another experimental nailing technique uses a steel nail which is placed antegrade after reaming of the medullary cavity [98].

Reaming is performed from the osteotomy site in proximal direction through the top of the greater trochanter and in distal direction to the level of the condyles until a diameter of 1.6 mm is achieved [98].

Plate osteosynthesis represents another extramedullary fixation technique in open femoral fractures [106, 107]: after a lateral approach to the femur as described above, a 16-mm long, 4-5 hole, titanium plate (e.g., AO Synthes) is applied to the shaft. The bone has to be predrilled with no tap, and the plate is attached with 1.2-1.3 mm screws, which are not fully tightened yet. Afterwards, mid-diaphyseal femoral fracture is induced by osteotomy at the midpoint of the plate with a small saw. The 4 screws are then tightened to fixate the plate [106, 107]. Beside trauma models, plate fixation is frequently used in bone union/non-union studies [162, 163].

External fixators are frequently used in trauma as well as union/non-union studies [91, 108, 109]: using 0.9 mm drill bit, four holes are drilled into the rat's femur. Four pins (0.65 mm inner diameter, 1.2 mm outer diameter, 25 mm long) are manually inserted after predrilling [91, 108, 109]. An external fixator bar is attached to the implemented pins with an offset of 6 or 15 mm [91]. Using a saw, an osteotomy is created afterwards.

Beside femoral fractures, experiments using tibial fractures are performed in rats. Similar to the femur, open [100, 101] and closed [93, 94] models exist. The closed fracture model involves a three-point bending system, in which a 300-g weight from a height of 20 cm drives a blunt guillotine onto the tibial diaphysis [93, 94]. For open fracture or segmental bone defect, the tibia is exposed by a lateral longitudinal incision and the defect required is created in the midshaft using a saw [101]. Fractures can be stabilized intramedullarily with K-wire or modified needles [93, 101], with plate fixation [100, 110] or less frequently using an external fixator [111].

5.3. Porcine Models. Porcine models are frequently used in analyzing femoral fractures since morphological similarity to human [164]. Experimental femoral fracture is commonly used in polytrauma models simulating an additional trauma beside trauma-hemorrhage, head injury, or abdominal trauma [112–115]. In contrast to rodent models, femoral fractures are commonly performed by a captive bolt gun. Following anesthesia, the left femur is operatively exposed through a longitudinal muscle-splitting incision [114]. Afterwards, the captive bolt is fired against the femur producing an open femoral fracture [112–115]. Blood vessels should not be hit to avoid distinct bleeding [114]. Any bleeding should be measured if trauma-hemorrhage is added in a polytrauma model [112]. Computed tomography or X-ray can be helpful to analyze the fracture extent [113, 165]. Beside the captive bolt-induced fracture, osteotomy can be conducted to mimic a femoral fracture [116]. Yet, a three-point bending system with vertical load has only been described in vitro using fresh frozen porcine femora [165]. In polytrauma models, the induced femoral fracture remains unstabilized [112–115] although intramedullary nailing has been described [116]: this nail is designed with a diameter of 12 mm and a length of 10 cm. Initially, the femur is reamed with a flexible drill. After a midshaft osteotomy, the nail has to be placed and locked proximally and distally of the fracture. X-Rays should be provided verifying an adequate osteosynthesis.

6. Conclusion

Although advances in diagnostics and therapy improved outcome of trauma patients in the last decades, further innovative approaches are required. Actually, a wide variety of experimental trauma models can be found. Based on the problem addressed by a researcher, the optimal model should be carefully considered. The conflict between clinical comparability, standardization and therefore reproducibility remains a major problem for every investigator. However, complicating factors such as age, gender, medical histories, and so fourth essentially require clinical trials verifying experimental results. In general, rodent models and especially mice should be used to answer questions regarding molecular mechanisms while physiological processes are more commonly investigated in large animals due to the similarity to humans.

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