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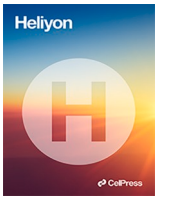

Review article

Significance and considerations of establishing standardized critical values for critical size defects in animal models of bone tissue regeneration

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ABSTRACT

Establishing animal models with critical size defects (CSDs) is critical for conducting experimental investigations engineering of bone tissue regeneration. Currently, a standardised protocol for establishing an animal CSDs model has not been developed. Furthermore, a consensus has not been reached regarding the critical values of CSDs. Successful establishment of animal models for CSDs is a complex process that requires researchers to meticulously consider a variety of factors such as age, species, bone defect size and anatomic location. The specific numerical values for CSDs in small animal models vary, and a clear definition of the critical value for large animal CSDs models in the literature is still lacking. This review consolidates the advancements in critical bone defects animal models by outlining the research landscape across variables, including animal species, age groups, bone defect sites, and sizes, to offer valuable guidance and a theoretical framework for the establishment of pertinent experimental animal models.

1. Introduction

Bone defects resulting from the disruption of bone integrity can be categorised into congenital malformations and acquired defects caused by inflammation, trauma, or tumours. Bone tissue possesses a certain level of intrinsic regenerative capacity. A defect is classified as a critical size defect (CSD) when its size exceeds the self-repair threshold, or if it fails to heal beyond 10 %. Nevertheless, some researchers define CSDs as minimal bone defects that are incapable of spontaneous healing and require secondary intervention [1]. Schmitz and Hollinger first coined the term “critical size defects (CSDs)” to refer to the smallest bone defects that cannot naturally heal within the animal’s lifecycle; they pioneered the development of an animal model for jawbone CSDs in 1986 [2]. However, Gosain indicated that CSDs in animal experimental studies was defined as the minimum size of bone defects that cannot heal naturally during

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experiments, since the research period was limited and did not cover the entire lifespan of the animals [3]. The values of CSDs vary with factors such as species, age, and anatomic location of defects [4], and these values are also influenced by environmental and nutritional factors. Despite a comprehensive understanding of CSDs, establishing standardised criteria for constructing animal models to study CSDs remains a challenge.

Bone defects significantly impact the aesthetics, function, and mental well-being of the patient, necessitating human intervention for repair. Autologous bone transplantation remains the gold standard of CSDs repair. However, this involves sacrificing bone tissue from a donor site that results in secondary injury. Researchers have been searching for alternative treatments for autologous bone transplantation for many years, yet these methods remain controversial [5].

Bone tissue engineering (BTE) is a promising research avenue aimed at addressing critical-sized bone defects by employing biological scaffold materials as substitutes for autologous bone transplantation [6]. Preclinical animal models are commonly used to study the processes of bone formation and repair, as well as to test novel interventions aimed at improving bone healing and regeneration [7]. The recapitulation of human disease is a core requisite of preclinical animal models, but this aspect is often neglected [8]. The vast majority of bone tissue engineering studies remain in the preclinical stage. This stage requires the successful construction of CSDs animal models to simulate the changes of the material under the complex microenvironment in vivo and to replicate the characteristics of corresponding human clinical diseases [9]. Consequently, the successful construction of CSDs animal models serves as a pivotal cornerstone for preclinical experiments and plays an indispensable role in advancing the field of bone tissue engineering [10]. In the realm of bone tissue engineering, Animal models of CSDs are commonly constructed using rabbits, mice, pigs, canines, and sheep (Fig. 1).

The successful replication of clinical conditions associated with CSDs in animal models relies on a multitude of factors, including species variability, age disparities, and the precise localisation and magnitude of the defects. Unfortunately, the absence of standardized guidelines [11] pertaining to these influencing factors has resulted in investigators making subjective judgments when constructing animal models of CSDs, thereby potentially compromising the accuracy and reproducibility of experiments and raising concerns regarding their ability to accurately replicate clinical scenarios [12].

Therefore, this article provides a comprehensive review of the current research on animal models of CSDs. Specifically, it examines various factors such as species, age, bone location, and size of bone defects, providing valuable assistance and theoretical support for experiments involving animal models of CSDs in the future.

2. Data and methods

2.1. Data sources

Data were gathered from PubMed searches and references from relevant studies using the keywords ‘critical size defects’, ‘bone regeneration’, ‘animal model’, ‘tissue engineering’.

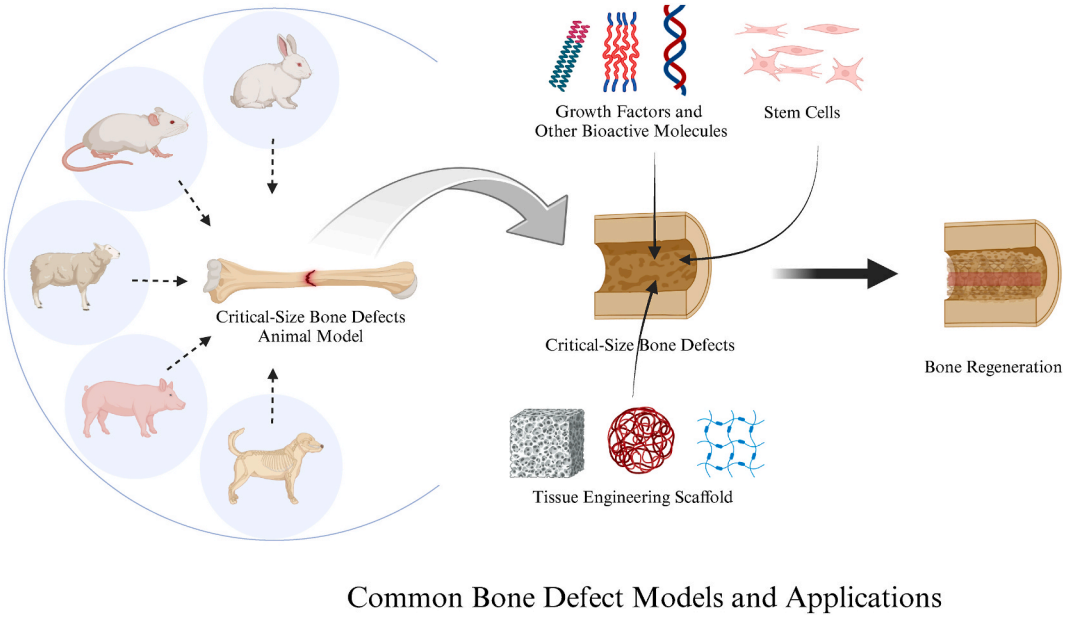

Fig. 1. Common bone defect models and applications.

2.2. Inclusion and exclusion criteria

The inclusion criteria included literature that presented reliable experimental content and well-substantiated arguments, literature that primarily elucidated the advantages and disadvantages of CSDs animal models, and literature that focused on the development, application, and progress in research on CSDs animal models.

The exclusion criteria included literature that was irrelevant to the content of this article and publications with repetitive conclusions.

2.3. Literature quality assessment and data extraction

The initial search yielded 1544 articles from the database. Titles and abstracts were screened, and full texts were reviewed when necessary to exclude low-quality, irrelevant, and duplicate studies. Ultimately, 85 articles highly relevant to the research on CSDs animal models and bone tissue engineering were selected for thorough review and inclusion in this study.

3. The CSDs of small animal model

Small animals, characterised by their low cost-effectiveness, high feasibility, and elevated ethical acceptance, are frequently employed as research subjects in CSDs models. Small animal bone defect models serve as the initial step in vivo evaluation of fundamental research or screening tests to determine the necessity of further preclinical animal testing. The successful establishment of small animal CSDs models plays a pivotal role in advancing basic research on the clinical applications of bone tissue engineering. Small animal CSDs models include rabbits, rats, and mice. The advantages and disadvantages of these models are presented in Table 1.

3.1. The CSDs of rabbit model

Rabbits, particularly New Zealand white rabbits, have been extensively used as research models for CSDs in several domestic and international studies. These studies focused on investigating different aspects such as bone replacement materials, osteogenic materials, and osteogenic mechanisms. Adult rabbits are commonly chosen as experimental subjects for the development of bone-defect models to ensure adequate bone maturation. Kaweblum demonstrated that New Zealand white rabbits reach skeletal maturity between the ages of 19 and 24 weeks based on examination of the tissue structure and imaging of the lower end of their thigh bones, upper end of their shin bones, and upper end of their calf bones [14]. Certain researchers exclusively select rabbits that are six months or older to guarantee bone maturity and employ X-ray imaging to track the progress of joint growth. Scholars commonly choose anatomic locations, including the ulna, radius, femur, tibia, parietal, and mandible, according to unique experimental criteria. Furthermore, the critical value and construction method of CSDs in animal models vary depending on the location of the bone defects.

3.1.1. Construction and application of rabbit ulna CSDs models

The adjacent bones support the rabbit ulna, eliminating the need for extra fixation and enabling bilateral comparison. A large number of studies have utilised rabbit ulnas to construct defects with diameter of 10–20 mm [15,16]. Based on this experience, some scholars have resected a 15 mm full-thickness bone in the middle of the ulna to form a segmental bone defect as the CSDs, which can be used to objectively evaluate the performance of bone tissue engineering alloy scaffolds and has been verified experimentally [17,18]. A 10 mm segmental CSDs at the ulnar midshaft was utilised to investigate the potential of honeycomb scaffolds to guide bone reconstruction [19]. A comprehensive assessment, including visual observation, imaging tools, and histological investigation, revealed complete healing of the bone defects in the groups with 14 and 15 mm defects 12 weeks after surgery. However, the bone defects in the groups with defects of 16 mm and larger did not exhibit any signs of healing. This study indicated that segmental bone defects in the midshaft of the rabbit ulna reached a critical threshold at a length of 16 mm. Nevertheless, Micic's research revealed that the diameter of ulnar CSDs is approximately 7 mm, with a length of 22 mm, which constitutes one-fourth of the total length of the rabbit ulna [20]. Micic argued that bone defects reaching or exceeding one-fourth of the total length of the ulna lack the potential for self-healing, and

Table 1
Advantages and disadvantages of small animal CSDs models.

Small animal species	Advantages	Disadvantages
Rabbit	The bone density closely resembles that of human bone. The molding process is facile. The procedure is well endured and showed resistance to infection.	The healing time for bones varies from that of human bones. Bone metabolism is more robust than human bone.
Rat	Good disease simulation capability. The genome closely resembles that of humans.	The small body size cannot fully replicate the healing process of human bones. The biomechanical loading environment is significantly different from that of humans.
Mouse	Low cost, brief growth period, high level of genetic similarity.	Molding operation presents challenges. No Haversian system—structural and functional units of cortical bone [13].

utilised the ulnar CSDs model to investigate the potential of a new hydroxyapatite bioceramic bone substitute material in managing segmental bone defects. Similarly, Galanis suggested that a defect in the ulna was deemed critical for non-spontaneous healing when it reaches approximately 2–2.5 times the ulnar diameter and explored the impact of combining autologous platelet-rich plasma with xenogeneic demineralised bone matrix on the healing process of ulnar CSDs [21].

3.1.2. Construction and application of rabbit radius CSDs models

The radius of the rabbit is supported by the ulna when constructing the bone defects. Maiti selected mature rabbits that were at least four months old and weighed approximately 2.5 kg for the experiment [22]. To construct an ideal rabbit radius CSDs model, a 15 mm bone defect was created in the middle of the radius, with the periosteum removed near the defect ends to prevent periosteal ossification. This study indicated that the healing process in rabbits with CSDs was accelerated by utilising autologous, allogeneic, and xenogeneic bone marrow mesenchymal stem cells on biological scaffolds [23]. However, some reports have indicated rabbit radial bone defects of different lengths, including 14, 18, 25, and 30 mm.

3.1.3. Construction and application of rabbit tibia and femur CSDs models

Many scholars have created circular bone defects with a diameter of 8 mm in the upper tibia of rabbits 5–6 months of age. In these studies, the cortical bone and periosteum on both sides of the tibia were removed to develop the CSDs model [24,25], which was utilised to investigate the impact of a polyolein ester preparation-guided bone regeneration membrane on bone formation. In a related study, as an ideal rabbit tibia CSDs model, a single cortical bone defect, with a diameter of 8 mm and depth of 6 mm, was introduced in the medial proximal surface of the tibia in rabbits to validate the efficacy of an injectable DNA-loaded nano-calcium phosphate paste as a bioactive bone substitute material in expediting the osseous healing process [26]. Recently, experiments in bone tissue engineering demonstrated that the use of porous scaffold materials in the construction of rabbit femoral CSDs promotes bone regeneration [27–29]. Hayashi et al. created a cylindrical bone defect with a diameter of 6 mm and depth of 3 mm in the rabbit femoral condyle and reported that defects of this size did not heal spontaneously and were filled with adipose tissue, which met the necessary requirements for the CSDs [30,31]. The researchers chose experimental rabbits weighing approximately 2.5 kg and created a cylindrical bone defect with a diameter of 6 mm and depth of 5 mm at the distal end of the femur, which met the necessary requirements for a CSDs. Some researchers manufactured a cylindrical bone defect in the femoral condyle measuring 6 mm in diameter and 10 mm in depth [32], which met the requirements of a CSDs. Cylindrical bone defects with diameters exceeding 6 mm are among the most prevalent CSDs in rabbit femurs.

3.1.4. Construction and application of rabbit parietal CSDs models

The parietal bone of the rabbit, characterised by its simplicity in operation and structure, as well as its extensive surface area, is widely applied in the construction of CSDs models. Delgado-Ruiz et al. concluded that for a round, full-thickness parietal bone defect in rabbits the CSDs was 15 mm, accounting for 71.42 %, followed by 8 mm (9.52 %) and 10 mm (9.52 %) [33]. Recently, researchers attempted to generate circular complete-layer bone defects on both sides of the midline of the parietal bone. For example, a CSDs model with a diameter of 8 mm was constructed to compare the bone regeneration and soft tissue formation abilities of different bone graft materials in a rabbit parietal defect model [34]. Additionally, as an ideal rabbit parietal CSDs model, a 10 mm diameter bone defect was established to assess the effect of incorporating collagen into granular bionic biphasic calcium phosphate on bone formation [35]. A 12 mm diameter CSDs model was constructed to evaluate the potential of porous composite scaffolds as bone graft substitutes for rabbit parietal defects [36]. Two parietal CSDs models, which were simultaneously constructed in the same animal, facilitated the comparison and examination of experimental findings. Nevertheless, numerous researchers maintained that the model could only be successfully constructed when the full-layer bone defect diameter of the rabbit parietal bone reaches 15 mm [37,38]. A singular defect model exclusively located at the central position of the cranial vault bone was utilised to explore the impact of platelet-rich fibrin (PRF) combined with bone marrow mesenchymal stem cells (MSC) on bone repair in rabbit cranial bone defects with critical size.

3.1.5. Construction and application of rabbit mandibular CSDs models

Studies indicated that CSDs had been used more frequently in the mandible than the maxilla [39]. An animal model with a mandibular defect is more effective in replicating the specific chewing pressure and cellular environment observed in the human maxillofacial region [40]. However, the irregular structure of the mandible leads to variations in the location and shape of bone defects. Lopez investigated the bone regeneration capacity of 3D printed bioactive ceramic scaffolds in a narrow bone defect setting [41], whereas Soares examined the histomorphology and density changes of several bone graft materials following bone defect surgery that created a square CSDs model of 10 × 10 mm at the mandibular branch and in front of the front angular notch of the mandible [42]. Some researchers created a cylindrical defect with a diameter of 10 mm in the mandibles of rabbits to investigate radiation injury to the maxillofacial bone [43]. A cylindrical defect with a diameter of 8 mm was created in the mandible of rabbits to investigate the potential of nanocrystalline porous biphasic calcium phosphate ceramic balls [44]. Researchers created a box-shaped bone defect measuring 10 mm × 5 mm × 3 mm in the mandible of rabbits to confirm that the process of bone repair was accelerated by utilising a combination of insulin-loaded microspheres, nano-hydroxyapatite/collagen and a poly lactic-*co*-glycolic-acid (nHAC/PLGA) scaffold [45]. Wang et al. formed box-shaped bone defects of different sizes in the mandible of a rabbit and concluded that the dimensions of box-shaped CSDs created in the rabbit mandible through an intraoral approach were 8 mm × 3 mm × 2 mm [46]. Additionally, certain researchers created a 13 mm × 9 mm rectangular CSDs model [47], which extended through the entire thickness of the bone, to investigate whether large mandibular bone defects can be cured by employing an alternative material to autografts, such as porous biphasic calcium phosphate. The rectangular CSDs model was also used to examine the biological response of porous biphasic calcium phosphate in different oral environments, including dental, periodontal, and bone environments. The current research results of rabbit

CSDs models are listed in Table 2.

3.2. The CSDs of rat model

Rats, as predominant small rodents, which are widely used to construct CSDs model, possess numerous advantages, including a higher cost-benefit ratio, ease of control, and simpler feeding protocols. The parietal, mandibular, and femoral bones are commonly used as construction sites for CSDs models in rats (Fig. 2).

3.2.1. Construction and application of rat parietal CSDs models

Researchers frequently utilised eight-week-old rats to induce bone defects in the parietal region. A successful model of a full-thickness bone defect with a 5 mm diameter was established by utilising a surgical drill to create a hole in the rat parietal bone, exposing the dura mater [48]. Wahnert and Kiyoch established a CSDs model, a circular full-layer bone defect with a diameter of 5 mm in the parietal bone of rats, to assess the effects and biological characteristics of biphasic phospho-niobium pentoxide nanocomposites and spongy™ materials for bone defect repair [49,50]. Cao established a CSDs model by creating two 5 mm diameter and 1 mm thick defects on both sides of the sagittal line of the cranioorbital bone in rats to investigate the potential use of a puerarin-based drug delivery system in combination with scaffold materials for repairing bone defects [51]. A circular bone defect, with a 10 mm diameter in the parietal bone, was established to demonstrate that composite scaffold materials can provide a suitable osteogenic microenvironment by releasing oxygen to enhance bone regeneration [52]. Some researchers have claimed that the behaviours and characteristics of adult rats, specifically those aged three months, may not accurately represent the circumstances and attributes of older rats. Therefore, young rats aged from 11 to 12 weeks and elderly rats aged from 22 to 24 months were divided into groups based on the size of their bone defects which were 3 mm, 5 mm, or 7 mm [53]. Finally, this study revealed that the critical value of parietal defects in adult rats was 5 mm, whereas the value decreased to 4 mm in elderly rats. Based on the above information, the critical value of CSDs was 5 mm in the parietal bones of adult rats aged over 8 weeks.

3.2.2. Construction and application of rat mandibular CSDs models

A successful model of bilateral non-segmental bone defects, each with a diameter of 4 mm, was created in the mandible of rats using a surgical drill [54]. The process of bone regeneration was assessed by utilising micro-computed tomography and mechanical testing,

Table 2
Summary of rabbit CSDs models.

Model construction site	Critical value size	Research Application and significance
ulna	10 mm bone defect	Honeycomb scaffolds can guide bone reconstruction.
	15 mm bone defect	Alloy scaffolds exhibite the corrosion resistance and osteogenesis properties.
	The length of the bone defect reaches or exceeds 1/4 of the total length of the ulna	The novel hydroxyapatite bioactive bone replacement material has the function of regulating bone remodeling.
	The length of the ulna defect is about 2–2.5 times the diameter of the ulna	Autologous platelet-rich plasma combined with xenogeneic demineralized bone matrix can promote bone healing.
radius	Most of them were not uniform, but they were all larger than the bone defects of 14 mm in length	Autologous, allogeneic and xenogeneic bone marrow mesenchymal stem cells on biological scaffolds can promote faster healing of bone defects.
tibia	Round bone defect 8 mm in diameter	Guided bone regeneration membrane prepared by polyglycolate can promote bone formation.
	Unilateral cortical bone defect with diameter of 8 mm and depth of 6 mm	The DNA-loaded nano-calcium phosphate paste can significantly accelerate the healing of bone defects.
femur	Cylindrical bone defect of 6 mm × 3 mm	3D printing calcium phosphates overcome the limitations of conventional materials.
	Cylindrical bone defects larger than 6 mm in diameter	To explore the potential of porous scaffolds in mechanical, angiogenesis and bone regeneration.
parietal	Single CSDs model: The diameter of the circular defect was 15 mm	To explore the effect of PRF combined with MSC on bone repair of skull defect.
	Two CSDs models were constructed at the same time: diameter of 8 mm, 10 mm and 12 mm	To study the bone regeneration and soft tissue formation ability of different bone graft materials in rabbit skull defect.
mandible	10 mm × 10 mm full thickness square bone defect	To investigate the osteogenic ability of different bone graft materials in different bone defect environments.
	Full thickness cylindrical defect with diameter of 8 mm and 10 mm	Nanocrystalline porous biphase calcium phosphate ceramic balls have the potential to promote bone regeneration. The full thickness cylindrical defect of 10 mm in diameter can be used as a radiation-related bone defect model.
	Segmental bone defect of 10 mm × 5 mm × 3 mm	The insulin-loaded microsphere-loaded nHAC/PLGA scaffold significantly accelerated bone healing.
	Box-shaped bone defect of 8 mm × 3 mm × 2 mm	This model may provide a clinically relevant base for future tissue engineering efforts in the mandible.
	Full thickness bone defect of 13 mm × 9 mm	To explore the effect of porous biphasic calcium phosphate on bone repair in different oral environments.

PRF, platelet-rich fibrin; MSC, mesenchymal stem cells; nHAC/PLGA, nano-hydroxyapatite/collagen and poly lactic-*co*-glycolic acid.

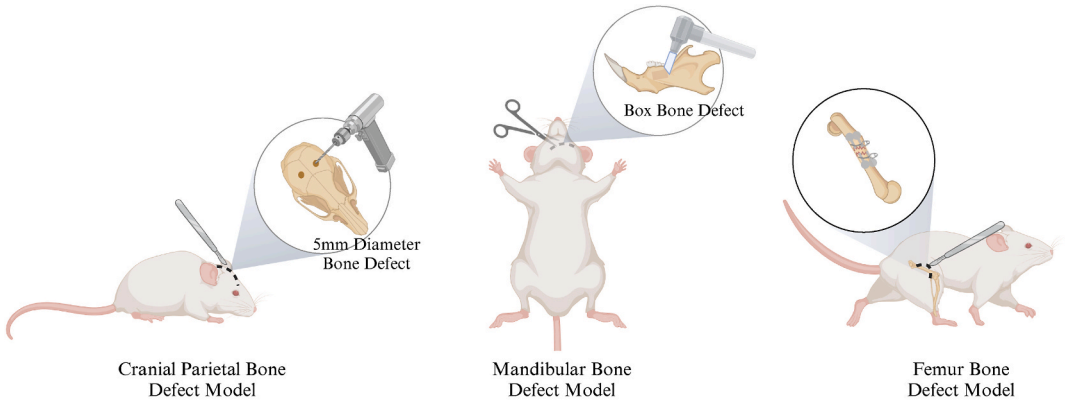

Fig. 2. CSDs model in rats.

following a duration of eight weeks. This study demonstrated that the process of bone regeneration could be improved by employing a scaffold made of polysaccharides crosslinked with smooth agonist (SAG), vascular endothelial growth factor (VEGF), and bone morphogenetic protein 6 (BMP-6). A bone defect measuring 6 × 2 mm, which was limited to a single surface and had a rectangular form, met the criteria for bone tissue research. Micro-computed tomography did not reveal any indication of spontaneous bone regeneration [55]. Furthermore, the authors asserted that this particular box bone defect is highly adaptable to a variety of materials, which could enhance the assessment system for bone regeneration techniques in maxillofacial drilling defects. This transition facilitated the shift from animal experiments to clinical trials and decreased costs related to experimentation. In an experiment conducted by Miguez, mandibular defects with a diameter of 5 mm were created as CSDs to investigate the effect of hesperidin on the development and regrowth of mineralised tissue [56]. A circular mandibular defect measuring 4 mm in diameter was utilised to investigate how regeneration of the craniomaxillofacial region could be enhanced by employing 3D-printed tissue engineering bone structures [57].

3.2.3. Construction and application of rat femur CSDs models

A femur CSDs model with a segmental bone defect length of 10 mm was used to investigate the effect of the particle size of Herafill, which is a bioabsorbable bone void-filling material primarily composed of calcium sulphate, calcium carbonate, triglycerides, and gentamicin [58], on bone healing as a bone graft substitute. Radiographic analysis of the femur confirmed the absence of apparent bone healing in the control group after eight weeks. A 5 mm segmental CSDs at the midshaft of the femur was utilised to investigate the

Table 3
Summary of rat CSDs models.

Model construction site	Critical value size	Research Application and significance
parietal	A bone defect of 5 mm	Study on the mechanism of umbilical cord mesenchymal stem cells overexpressing wnt10b to promote the healing of skull defect. To explore the effects and biological characteristics of nano-composite materials and spongy materials in repairing bone defects. Investigating the combined impact of angiogenesis and osteogenesis of the puerarin porous composite scaffold to enhance the repair of bone defects. Oxygen generating scaffolds regenerate critical size bone defects.
mandible	A bone defect of 4 mm in diameter	SAG/VEGF/BMP-6 polysaccharide scaffold can promote bone regeneration and is a suitable substitute for bone transplantation.
	Box bone defect 6 mm × 2 mm in size	To explore the feasibility of 3D printing technology to prepare tissue engineering bone structure to promote craniomaxillofacial regeneration. Box bone defects were highly compatible with a variety of materials, which can optimize the bone regeneration evaluation system and accelerate the transformation of clinical trials.
femur	A bone defect of 10 mm in length	Radiology and other methods proved that there was no obvious bone repair of 10 mm bone defect.
	A bone defect of 5 mm in length	Calcium carbonate apatite honeycomb porous scaffold can promote bone regeneration.
	A bone defect of 4 mm in length	The experimental model of rat femur induced by Masquelet-induced membrane technique was successfully constructed.

SAG, smoothened agonist; VEGF, vascular endothelial growth factor; BMP-6, bone morphogenetic protein 6.

potential of calcium carbonate apatite honeycomb porous scaffolds to promote bone regeneration [59]. Zeng et al. established an experimental model of a rat femur by constructing a 4 mm bone defect in the middle part of the rat femur to study the potential benefits of the masquelet-induced membrane technique [60], a two-stage bone defect treatment method, which is also considered one of the preferred approaches for repairing severe limb bone defects [61,62]. The current research results of rat CSDs models are listed in Table 3.

3.3. Construction and application of mouse CSDs models

Sanchez-Casanova created a 4 mm-diameter hole in the parietal bone of a 5-week-old mouse and studied the initiation of osteogenesis in bone defects [63]. Nevertheless, a parietal CSDs model with a 5 mm diameter was used to investigate the osteogenic impact of bacterial cellulose-modified polyhydroxyalkylate (PHB/BC) scaffolds [64]. A 3.5 mm segmental femur defect, which accounted for approximately 25 % of the length of the femur, was considered the CSDs for mouse femur [65]. The current research results of mouse CSDs models are listed in Table 4.

4. The CSDs of large animal model

Small animals are cost-effective, feasible, and have a strong ethical acceptance, making them frequently used as research models for CSDs. However, large animals exhibit physiological similarities to humans in terms of anatomical structure, vital signs, and healing processes, leading to their frequent selection for preclinical research [66]. Large animals pose several challenges to experimentation, such as the need to create appropriate environments, significant financial investments, and restricted ethical and moral approval. Typical examples of large animals use for this research include sheep, canines, and pigs. The current research results of large animal CSDs models are listed in Table 5.

4.1. The CSDs of sheep model

Sheep exhibit docility [67], which is a practical advantage often lacking in preclinical experimental animals. They also possess an easily manageable feeding routine, demonstrate a bone macrostructure and weight similar to those of adult humans, and offer the benefits of convenient surgical treatment at low cost [66]. Sheep aged 6–7 years or older are very similar in terms of bone mineral content, macrostructure, microstructure, remodeling capacity, and biomechanical properties, and thus can serve as a reliable substitute for investigating human clinical problems [67]. The sheep CSDs model has proven to be invaluable for evaluating both conventional and innovative approaches in bone tissue engineering [68]. Hence, the sheep CSDs model exhibits significant research value.

4.1.1. Construction and application of sheep tibia CSDs models

The femoral cortex is comparatively narrower than the tibia, which may lead to failure to construct the CSDs model [69]. Studies have shown that the soft tissue coverage of the tibia is significantly lower than that of the femur. To prevent spontaneous healing of a 30 mm segmental defect, researchers have proposed a method that involves excising the periosteum in the muscle septum and the tibial periosteum within 10 mm of the defect on both sides. The control group did not undergo any interventions, except for the use of a bridge plate to connect the fractured ends. Ultimately, the observations revealed that the bone defect did not show signs of healing, thus meeting the criteria for a CSDs model [70]. Marcondes created a 30 mm segmental bone defect on the right tibia of 18 sheep to establish an animal model for studying segmental bone defects in bone tissue engineering [71].

4.1.2. Construction and application of sheep scapula CSDs models

The main body of the sheep's scapula is flat and thin, but the caudal margin exhibits a bulge or thickening on the medial side. The caudal margin is a thick bony structure that offers ample space for experiments. The central portion of the sheep scapula is characterised by a flat thin structure, whereas the lower edge exhibits pronounced protrusion or thickening on the inner side. The caudal edge is a robust bony structure that provides abundant bone resources for conducting tests. Five bone defects, each with a diameter and depth of 8 mm, known as CSDs, were created in the scapula of sheep by employing trephine drilling. This approach creates five bone defects, which allows for comparison within the same animal and minimises the need for a larger number of animals [72].

4.2. The CSDs of canine model

Canines exhibit robust adaptability and excellent resilience, rendering them highly suitable for utilisation in animal

Table 4
Summary of mouse CSDs models.

Model construction site	Critical value size	Research Application and significance
parietal	Bone defect of 4 mm and 5 mm in diameter	To study the mechanism of inducing osteogenesis in bone defect. Bacterial cellulose modified PHB/BC scaffold has osteogenic effect.
femur	About 25 % of the length of the femur	The critical bone defect of the femur was greater than 25 % of the length of the femur.

PHB/BC, polyhydroxyalkanoate.

Table 5
Summary of large animal CSDs models.

Animal species	Model construction site	Critical value size	Research Application and significance	Advantages	Disadvantages
Sheep	tibia	30 mm segmental bone defect	Studies showed that the CSDs model can be formed by removing the periosteum within 10 mm on both sides of the defect, while the CSDs model cannot be successfully constructed otherwise.	Easy feeding and molding operation. The bone structure closely resembles that of human bone.	Easy to be influenced by the environment, exhibiting poor adaptability. Prone to illness and challenging to nourish.
	scapula	A defect of 8 mm in diameter and depth	The model facilitates intra-animal comparison and reduces the number of animals studied.		
Canine	mandible	not have a unified view	The data of mandibular CSDs lack homogeneity.	The organic and inorganic composition, as well as the biological characteristics, closely resemble those of human bone.	The challenges in social ethics are quite significant.
	radius	30 mm segmental bone defect	rhBMP-2/polylactic acid sustained-release microsphere composite scaffold can promote bone regeneration.		
	femur	A 40 mm segmental bone defect	Angle-stable interlocking nails can refine the critical-sized femoral defect model in canines and reduce the total number of animals.		
		A 15 mm × 10 mm saddle-typebone defect	Hydroxyapatite-processed biomaterials can guide bone regeneration.		
Pig	mandible	5 cm^3 volume bone defect	The results showed that the critical size of mandibular defect in adolescents was 5 cm^3	A lamellar bone resembling human bone. Bone remodeling capability and bone regeneration rate are essentially equivalent to that of human bone.	The demand for an optimal feeding environment is high, and the cost of pig feed is substantial. The biological resemblance to humans is lower compared to typical laboratory animals.
	parietal	A bone defect of 30 mm in length	Mesenchymal cells combined with 3D bone conduction scaffolds can promote skull defect repair.		
		Bone defect with external diameter of 12 mm, internal diameter of 10 mm and height of 10 mm	It is helpful to construct a standardised preclinical 3D bone defect model of pig skull.		

rhBMP-2, recombinant human bone morphogenetic protein-2.

experimentation. Canines possess a highly beneficial trait in that the biochemical composition of their bones closely resembles that of humans. The microstructure and remodeling of canine bone tissue differ from those of human bone tissue [73]. However, both canine and human bone tissues share similarities in terms of organic and inorganic material characteristics, weight, and density [74]. Recently, canines have been used as CSDs models for bone regeneration research.

4.2.1. Construction and application of canine mandibular CSDs models

Chao established a CSDs model by surgically removing the bilateral anterior premolars and first molars, creating a defect that was 10 mm wide and 4 mm high [75]. This study explored how bone defects surrounding implants could be accelerated in healing by utilising bone morphogenetic protein 2 peptide with a composite material consisting of hydroxyapatite (HAp), β-tricalcium phosphate (TCP), and collagen (Col) scaffold materials. Huh demonstrated that canine mandibular defects failed to repair when the periosteum was absent, and the length of the bone defect exceeded 15 mm [76]. However, if the periosteum is preserved, the canine mandibular defects must exceed 50 mm in length to ensure proper healing. A 30 mm segmental bone defect was created in a canine's mandible to serve as a CSDs model for evaluating the biomechanically outperform of 3D printed plates based on generative design [77].

Nevertheless, a review revealed that regardless of the geometric shape of the canine mandibular defect (cylindrical, rectangular, box-shaped, or s-shaped), the available data on canine mandibular CSDs lacked uniformity [78].

4.2.2. Construction and application of canine radius and femur CSDs models

Research on the canine long bone CSDs model is relatively limited. Zhou et al. constructed models of critical and super-CSDs for canine long bone defects based on the measured diameters of the middle radii of adult experimental canines [79]. The critical and supercritical defect sizes were 30 and 50 mm, respectively. This study indicated that the super-CSDs model could be repaired utilising recombinant human bone morphogenetic protein 2 (rhBMP-2)/polylactic acid sustained-release microsphere composite scaffold materials. A 40 mm segmental bone defect, which is a CSDs model in the mid-diaphyseal femur of canines, was established to investigate whether the use of angle-stable interlocking nails could refine the critical-sized femoral defect model in canines and reduce the total number of animals [80]. A 15 mm × 10 mm saddle-type CSDs was utilised to investigate the potential of bovine hydroxyapatite-processed biomaterials to guide bone regeneration [81].

4.3. Construction and application of pig CSDs models

Sun employed 4-month-old pigs [82], which possesses the advantages of a stratified bone structure and a bone regeneration rate comparable to that of humans, to construct a CSDs model. Defects with volumes of 3, 5, or 7 cm^3 were deliberately created in the molar root tip and mandibular angle areas of two pigs simultaneously, and the buccal periosteum was ensured to remain intact. In an additional four pigs, bone defects ranging from 3 to 5 cm^3 were randomly formed in the root tips and mandibular corners of both mandibles and the buccal periosteum was removed. Computed tomography and histological investigation revealed that regardless of the extent of the defect, bone deficiencies in the apical area of the molar with a maintained buccal periosteum successfully healed, but the bone defects in the mandibular angle area were not fully repaired. After the buccal periosteum was removed, the bone defect in the apical part of the molar did not fully heal, whereas the bone defect in the mandibular angle exhibited less recovery in comparison to the former. The study concluded that a 5 cm^3 bone defect in the mandibular angle region of pigs, with the removal of the buccal periosteum, was a suitable CSDs model for studying mandibular defects in teenagers. A bone defect 30 mm in diameter, which is a CSDs model in pig parietal bones, was established to investigate whether parietal bone defects could be repaired utilising mesenchymal cells and 3D bone conduction scaffolds [83]. In research on the graft repair of bone defects, Moest established a reliable preclinical model using pig parietal tissue by creating bone defects with specific dimensions [84]. These defects had an outer diameter of 12 mm, inner diameter of 10 mm, and height of 10 mm. The CSDs of pig craniofacial bones vary based on shape, size, breed, age, and location [85].

5. Limitations and perspectives

The successful construction of CSDs animal models requires taking multiple factors into consideration. Environmental and nutritional conditions influence the significance of CSDs, which vary across species, age groups, defect sizes, and bone locations. Despite the conceptual clarification of CSDs, standardised critical values specific to each species are still lacking. In some cases, researchers may lack a clear understanding of the concept of CSDs and the associated critical values, which has led to construction of non-standardised animal models, diminished reproducibility of experimental outcomes, and limited persuasiveness of the experimental data. The construction of animal models of CSDs serves as an experimental foundation for bone tissue engineering research, which is conducive to elucidating the repair mechanism of bone tissue defects to improve the difficulty of bone tissue defect repair in the clinic. This article provides a comprehensive summary of the recent domestic and international studies on animal models of CSDs. It specifically focuses on animal species, age groups, bone defect sites, and defect size. Researchers are supposed to prioritise the concept of CSDs, clarify the essential importance of appropriate animal models for CSDs, and improve the success rate of constructing CSDs animal models, thus advancing bone tissue engineering research. The construction of animal models for CSDs still presents some challenges. Some incomplete research on the development of relevant animal models for CSDs has hindered the identification of the critical values for some CSDs. Primates such as monkeys and apes exhibit some advantages, including physiological systems, vital signs, and healing cycles that closely mimic those of humans, which make them suitable for implementation in preclinical experimental models. However, specific animal models of primate CSDs has not been developed, necessitating further investigation. Existing studies have primarily focused on constructing models for long bone defects, whereas our understanding of constructing models for specific anatomic sites remains limited. For example, research on constructing CSDs models in the joint regions and the maxilla is limited.

Traditional techniques such as HE staining, immunohistochemistry staining, and Micro-CT have been widely used in the study of CSDs for quite some time. The traditional methods used in these studies can only observe a limited number of morphological and structural parameters. The novel techniques, such as imaging mass cytometry and high-throughput analysis techniques, can be used to conduct more detailed and comprehensive studies of CSDs at the cell and tissue, protein, and molecular levels.

Correctly addressing the ethical issues related to the construction of animal models of CSDs is paramount. Some researchers failed to fully recognise their responsibilities. First, some researchers do not fully adhere to the principles of replacement, reduction, and refinement (3R) because of the lack of standardised surgical protocols for constructing animal models of CSDs when establishing these models. For example, some defect sizes used by researchers have been tailored to their material functional research, without fully considering the 3R principles of animal experiments and the reproducibility of animal experiments in constructing rabbit ulnar and radial CSDs. Second, differences in shape, size, species, age, and anatomic location result in a lack of homogeneity in the data, which seriously affects the construction of animal models for CSDs, such as occurs with respect to canine mandibles, porcine craniofacial bones, and other CSDs animal models. Additionally, species with high genetic diversity and difficult-to-regulate genetic factors have been employed directly in animal experiments to construct CSDs models. However, this approach has led to animal abuse and poor reproducibility of research results. Thus, the methods for constructing CSDs models ought to be changed to include other methodologies such as in vitro experiments, computer simulations, and machine learning, to avoid unnecessary utilisation of animals.

Therefore, anyone who constructs bone tissue engineered CSDs animal models is supposed to adhere strictly to animal ethics guidelines and laws. The reliability and repeatability of CSDs in animal models, as well as the scientific utilisation and reasonable protection of animals, are prioritized without compromising experimental requirements.

In the future, efforts are focused on improving relevant studies, which will provide subsequent researchers with more comprehensive experimental foundation for constructing CSDs animal models.

6. Conclusions

Strict adherence to ethical rules and laws regarding animal experiments is essential when preclinical research on bone tissue

engineering is conducted. The concept of CSDs is clearly understood to ensure that the constructed animal models meet the requirements of CSDs.

Animal species are selected based on the size of the experimental sites, the environment, and the cost, as different species require variously in relation to these factors. Small animals are prioritized to efficiently achieve the main objectives of the experiment and optimize data collection when researchers are faced with limited resources. Subsequently, large animals may be selected for clinical experiments based on the analysis of experimental data. Animals, identified through X-ray imaging to be in a stable stage of growth and development, are the optimal choices for constructing CSDs models. Furthermore, the methodology for model construction is optimized by selecting the appropriate size and location of bone defects. For example, the bone defects model can remain stable, even in the construction of CSDs involving the calvarium, ulna, and radius where fixation devices are not used. Finally, the comprehensiveness and rigorousness of the experiment are enhanced by carefully selecting appropriate experimental animals and anatomical sites for modeling, as well as establishing a blank control group.

Data availability statement

No data was used for the research described in the article.

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It is a review paper.

CRediT authorship contribution statement

Jian Wei: draft manuscript preparation, data collection. **Xiao Chen:** manuscript proofreading. **Yingjiao Xu:** data collection. **Lijuan Shi:** data collection. **Menglian Zhang:** data collection. **Minhai Nie:** manuscript proofreading. **Xuqian Liu:** study conception and design, manuscript proofreading.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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