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Advances in experimental models of osteonecrosis of the femoral head

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ABSTRACT

Background: Osteonecrosis of the femoral head (ONFH) is a devastating disease affecting young adults, resulting in significant pain, articular surface collapse, and disabling dysfunction. ONFH can be divided into two broad categories: traumatic and non-traumatic. It has been established that ONFH results from an inadequate blood supply that causes the death of osteocytes and bone marrow cells. Nonetheless, the precise mechanism of ONFH remains to be elucidated. In this regard, preclinical animal and cell models to study ONFH have been established to assess the efficacy of various modalities for preventing and treating ONFH. Nevertheless, it should be borne in mind that many models do not share the same physiologic and metabolic characteristics as humans. Therefore, it is necessary to establish a reproducible model that better mimics human disease.

Methods: We systematically reviewed the literatures in regard to ONFH experimental models over the past 30 years. The search was performed in PubMed and Web of Science. Original animal, cell studies with available full-text were included. This review summarizes different methods for developing animal and cell experimental models of ONFH. The advantages, disadvantages and success rates of ONFH models are also discussed. Finally, we provide experimental ONFH model schemes as a reference.

Results: According to the recent literatures, animal models of ONFH include traumatic, non-traumatic and traumatic combined with non-traumatic models. Most researchers prefer to use small animals to establish non-traumatic ONFH models. Indeed, small animal-based non-traumatic ONFH modeling can more easily meet ethical requirements with large samples. Otherwise, gradient concentration or a particular concentration of steroids to induce MSCs or EPCs, through which researchers can develop cell models to study ONFH.

Conclusions: Glucocorticoids in combination with LPS to induce ONFH animal models, which can guarantee a success rate of more than 60% in large samples. Traumatic vascular deprivation combines with non-traumatic steroids to induce ONFH, obtaining success rates ranging from 80% to 100%. However, animals that undergo vascular deprivation surgery may not survive the glucocorticoid induction process. As for cell models, 10-6mol/L Dexamethasone (Dex) to treat bone marrow stem cells, which is optimal for establishing cell models to study ONFH.

The translational potential of this article: This review aims to summarize recent development in experimental models of ONFH and recommended the modeling schemes to verify new models, mechanisms, drugs, surgeries, and biomaterials of ONFH to contribute to the prevention and treatment of ONFH.

1. Introduction

Osteonecrosis of the femoral head (ONFH) is a refractory disease that can lead to debilitating hip pain and restrictions on physical activity in young adults. Common reasons for ONFH are long-term or excessive glucocorticoid use and proximal femoral neck fracture. ONFH has also been reported in people with alcoholism, sickle cell disease, AIDS and lupus [1,2]. These factors lead to ischemic changes in the femoral head, leading to osteocyte necrosis and damage to the trabecular bone [3]. In rare cases, ONFH resolves spontaneously, with femoral head collapse occurring in approximately 67% of asymptomatic patients and 85% of symptomatic patients [4]. Femoral head collapse, the final stage of

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ONFH, requires total hip arthroplasty, which brings a heavy economic burden to the patient and the health care system [2,4–6]. At the same time, hip arthroplasty is more suitable for middle-aged or older people, and postoperative complications are not uncommon. Hence, there is an urgent need to develop more effective treatments for ONFH.

Animal models exhibiting human ONFH play a pivotal role in preclinical experiments in our quest for more effective treatments. Meanwhile, establishing cell models to study ONFH helps explain this disease's complex etiology and pathological mechanism. So far, few preclinical animal and cell models have been established to assess the efficacy of various modalities developed for preventing and treating ONFH. Over the years, ONFH animal models have been established mostly on quadrupeds, including mice, rats, rabbits, dogs, pigs, sheep, goats, and horses. Investigation of bipeds has been limited to geese, chicken, ostriches and emus. However, many models do not share the same physiological and metabolic characteristics as humans [7,8]. Animal or cell models developed by different methods also exhibited different characteristics of human ONFH, while the success rates of these modeling methods vary widely.

This review aims to provide cues for animal and cell experimental designs for ONFH preclinical research. We revealed animals that can be used for establishing ONFH models and compared their advantages and disadvantages. The methods for establishing animal models of ONFH are summarized with their corresponding success rates. In addition, we provided an overview of frequently used methods for establishing ONFH animal models, including rabbits, rats, and mice, mainly for non-traumatic models. Moreover, cell models to study ONFH are further discussed to illustrate the underlying mechanisms. Finally, we recommend well-established methods for ONFH modeling according to the needs of researchers.

2. Animal models of osteonecrosis of the femoral head

2.1. Evaluation of ONFH in animal models

Histopathologically, ONFH is defined as the presence of diffuse empty lacunae or pyknotic osteolytic nuclei in the bone trabeculae accompanied by and surrounding bone marrow cell necrosis. Animals with at least one osteonecrosis lesion in a stifle joint are considered positive for ONFH [9–12]. Moreover, the percentage of the necrotic area can be used to measure the extent of ONFH [4,13–15].

Up to now, many types of animals have been used to establish ONFH models. Most efforts in developing ONFH animal models have focused on quadrupeds, including mice, rats, rabbits, dogs, pigs, sheep, goats, and horses [7,16,17]. However, the bearing capacity of quadruped animals is not comparable to humans. Investigation of bipeds has been limited to geese, chicken, ostrich and emu [18,19]. Although the weight bearing of these animal models is close to human beings, their anatomical structure and physiological characteristics are not. Here, we list animals that can be used to establish ONFH models and their advantages and disadvantages (Fig. 1).

When developing ONFH animal models, it is essential to choose animals with similar physical and chemical properties to humans, such as pigs, dogs, sheep, etc. [7]. These animals are large, with femoral heads similar in size to humans, making it easier to observe necrotic areas. Furthermore, their suitability for traumatic surgery models has resulted in higher success rates. Nonetheless, large mammals have many shortcomings, including high costs, high requirements for feeding conditions and a relatively small number of specimens in a cohort, which make them unsuitable for establishing ONFH models.

In contrast, small animals exhibit different characteristics. The advantages of small animal models include low cost, convenient feeding

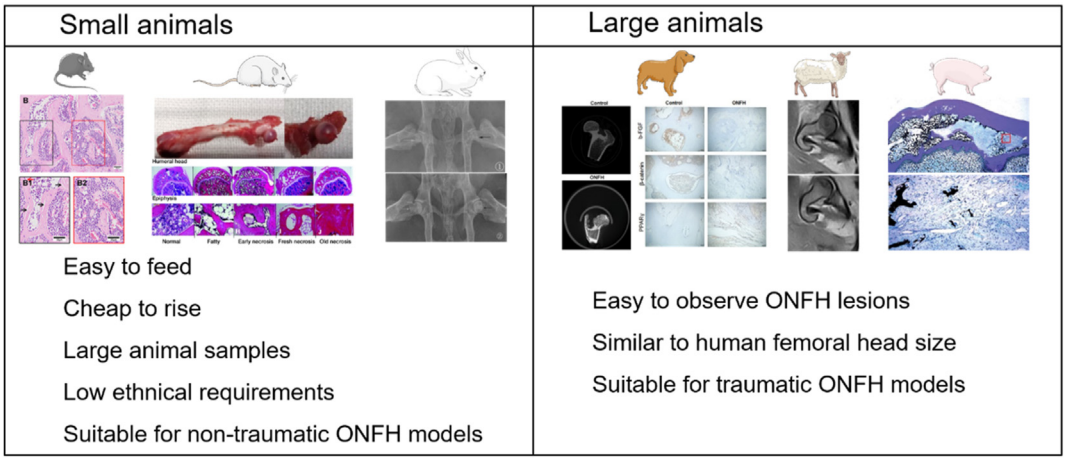

Fig. 1. The advantages of different animal models. Small animals are easy to feed, cheap to rear, suitable for non-traumatic ONFH models, and large samples can be tested with relatively low ethical requirements. ONFH lesions in large animals are similar to human femoral head size and are easy to observe, thus suitable for traumatic ONFH models.

The figures for ONFH models involved are quoted from:

Kamiya, Nobuhiro et al. "Development of a mouse model of ischemic osteonecrosis." Clinical orthopaedics and related research vol. 473.4 (2015): 1486–98. https://doi.org/10.1007/s11999-015-4172-6.

Nozaki, Yoshihiro et al. "Pravastatin reduces steroid-induced osteonecrosis of the femoral head in SHRSP rats." Acta orthopaedica vol. 83.1 (2012): 87–92. https://doi.org/10.3109/17453674.2011.641103.

Kang, Pengde et al. "Repairing defect and preventing collapse of femoral head in a steroid-induced osteonecrotic of femoral head animal model using strontium-doped calcium polyphosphate combined BM-MNCs." Journal of materials science. Materials in medicine vol. 26.2 (2015): 80. https://doi.org/10.1007/s10856-015-5402-x.

Gao, Runzi et al. "Three-dimensional-printed titanium alloy porous scaffold combined with trans-cinnamaldehyde for repairing osteonecrosis of the femoral head in a dog model." American journal of translational research vol. 12.3 1070–1079. 15 Mar. 2020.

Vélez, Roberto et al. "A new preclinical femoral head osteonecrosis model in sheep." Archives of orthopaedic and trauma surgery vol. 131.1 (2011): 5–9. https://doi.org/10.1007/s00402-010-1084-5.

Kim, Harry K W et al. "RANKL inhibition: a novel strategy to decrease femoral head deformity after ischemic osteonecrosis." Journal of bone and mineral research: the official journal of the American Society for Bone and Mineral Research vol. 21.12 (2006): 1946–54. https://doi.org/10.1359/jbmr.060905.

conditions, and a large number of specimens. In addition, studies based on small animals can more easily meet ethical requirements. Accordingly, small animals are more suitable for establishing non-traumatic models. Among various small animals, mice are the smallest, convenient for establishing conditional knockout mouse models. Unlike mice, rat and rabbit femoral heads are moderately sized for imaging analysis. These characteristics account for the widespread use of small animals to establish ONFH models.

Regarding bipedal ONFH animal models, emus and ostriches have been used to develop traumatic models and evaluate certain surgical modalities. Interestingly, the emu was found to hold attraction for systematic organ-level study of collapse mechanopathology. In an emu ONFH model, the parameters of liquid nitrogen cryosurgery were evaluated and optimized, including hold temperature, freeze duration, freeze/thaw repetition, and thaw duration. These parameters were investigated to determine their individual and combined effects on necrotic lesion morphology [20]. In another study, eighteen African ostriches were subjected to liquid nitrogen cryo-insult in the unilateral femoral head. The postoperative femoral head specimens exhibited changes in contour and articular cartilage degeneration [18]. Thus, bipedal ONFH animal models can also be used to simulate human ONFH.

Ethically, animal strains, gender, age, temperature and humidity of the breeding environment must be recorded in detail when establishing animal models to comply with FDA standards or other ethical criteria [21].

3. Methods for establishing animal models of osteonecrosis of the femoral head

Animal models of ONFH can be divided into three types: traumatic, non-traumatic and traumatic combined with non-traumatic models. The advantages, disadvantages (Fig. 2) and modeling methods (Table 1) are discussed below.

Moreover, trauma-induced ONFH animal models can be subcategorized into the following three major forms, i.e., surgical vascular deprivation, physical insult and chemical insult–induced traumatic ONFH models.

3.1. Surgical vascular deprivation-induced ONFH animal model

The surgical vascular deprivation-induced ONFH animal model is induced by damage to the vasculature around the femoral head [45]. Nishino et al. [46] established an adult dog model of ONFH by dislocating the hip joint and ligating the medial and lateral circumflex femoral arteries and veins. At 2 and 4 weeks, 80% of the animals showed widespread ONFH detected by MRI. Overall, the success rates of surgical vascular deprivation-induced ONFH models ranged from 60% to 100% [21,22–25,46]. The advantages of this method include a high success rate, easy-to-observe results, the concentration of the necrotic lesions in femoral head and the large range of ONFH stages that could be achieved. However, the difficulty of the procedure, the severity of trauma, the

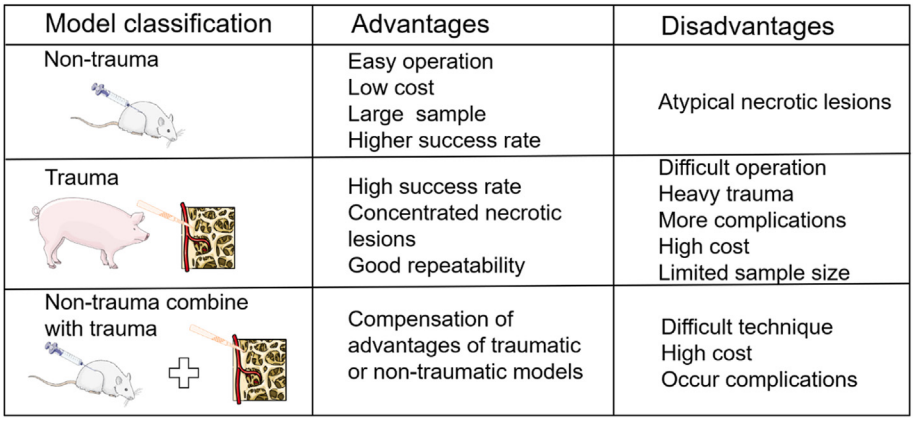

Fig. 2. Methods for Establishing Animal Models of ONFH. These methods include non-traumatic, traumatic, and non-traumatic combined with traumatic approaches. Easy operation, low cost, large sample and higher success rate represent the main advantages of non-traumatic animal models. The main disadvantage is the formation of atypical necrotic lesions. On the other hand, traumatic ONFH animal models have a high success rate, good reproducibility, and concentrated necrotic lesions. Disadvantages of traumatic models include difficult operation, heavy trauma, more complications, high cost, and limited sample size. Traumatic combines with non-traumatic methods include benefits of both approaches. However, this type of animal models is difficult to establish and associated with high costs and risks of complications.

Table 1
Methods for establishing animal models of ONFH.

Model classification	Method	Characteristics	Success rate	Ref.
Trauma	SVD	Damage of vascularity and blood circulation around the FH.	80 ± 20%	[7,21,22–25]
	PI	Repeated cryo-insult-rewarming procedure under radiographic guidance.	60 ± 20%	[18,26–28]
	CI	Direct intraosseous injection of pure ethanol.	100%	[19]
	SVD + PI	Damage of vascularity and blood circulation around the FH, combined with repeated freeze–thaw cycles.	95%	[16]
	SPO	Hyperbaric/Oxygenation/Spontaneously/Hypertensive/Standing		
	GC	Reflect characteristics of clinical GC-induced ONFH.	15–90%	[7,29]
	LPS	Induce immune stimulatory factors.	9%	[7,30–32]
	HS	Induce serum sickness ONFH by injection of HS.	60–90%	[8,17,32,33]
	GC + LPS	Simulate the inflammatory host state, initiate intravascular coagulation and eventual ONFH.	50–90%	[7,30,31,29, 34–37]
	GC + HS	Induce vasculitis, causing breakdown and obstruction of small arteries.	90%	[38,39]
	ALC	Simulate alcohol-related ONFH in humans.		[40,41]
	DYS	Simulate human caisson disease, most lesions were diaphyseal.		[42,43]
	CHE	Simulate human hemolytic disorders associated with thrombosis.	10–20%	[17,44]
Trauma combines with non-trauma	GC + SVD	Achieve higher ONFH incidence than either method alone.	nearly 100%	[24]

Abbreviation: ONFH: osteonecrosis of the femoral head; FH: femoral head; SVD: surgical vascular deprivation; PI: physical insult; CI: chemical insult; SVD + PI: surgical vascular deprivation combined with physical insult; SPO: spontaneous; GC: glucocorticoid; LPS: lipopolysaccharide; HS: horse serum; GC + LPS: glucocorticoid combine with lipopolysaccharide; GC + LPS: glucocorticoid combine with horse serum; ALC: alcohol; DYS: Dysbarism; CHE: chemically induced; GC + SVD: glucocorticoid combine with surgical vascular deprivation.

increased incidence of surgical complications, high costs and limited sample size represent significant limitations. Frederic Shapiro et al. induced ischemic ONFH in ten three-week-old piglets by tying a silk ligature around the base of the femoral neck (intracapsular) and cutting the ligamentum teres. Magnetic resonance imaging was to observe the hips, which made it easier and clearer to observe the incidence of ONFH, which reached 100% [22]. Nevertheless, the whole process of this modeling approach is too expensive, and the experimental animals experience heavy trauma and surgical complications, which are against ethical principles for animal experimentation.

3.2. Physical insult–induced ONFH animal models

Overall, physical insult–induced ONFH animal models have been associated with a success rate ranging from 40% to 80% [18,26–28]. In this respect, an established ONFH rabbit animal model induced by microwave heating (heating the femoral heads at 55 °C for 10 min) yielded a good collapse rate in the short term. Results indicated that the treated region showed low density and cystic changes in X-ray photographs, while ONFH and repair occurred simultaneously at 4 and 8 weeks, as confirmed histologically, and the collapse of femoral heads was observed in 69% of cases at 12 weeks [26]. Cryogenic and thermal insults have been increasingly adopted as laboratory vehicles for inducing ONFH, and they are the main aspects of the resulting repair response that reasonably replicate those of naturally occurring ONFH lesions [47]. In cryosurgery, the freezing process causes direct cellular and vascular injury. With hyperthermia treatment between 43 and 45 °C, the tissue temperature mildly raised for a certain period is expected to induce cell death by affecting membrane fluidity, cytoskeleton, protein and nuclear structure, and disruption of DNA replication [48]. Importantly, it is easily confined in the local tissue resulting in the devitalization of uniform tissue within the microwave radiation field. Besides, it is easy to control the heating temperature and time with a low animal death rate, high success rate, and good repeatability [17].

3.3. Chemically-insult induced ONFH animal models

Direct intraosseous injection of pure ethanol remains the mainstay in developing chemically-insult induced ONFH animal models. This approach has been used to establish a sheep ONFH model [19]. Partial necrosis was documented over 12 weeks in all animals with macro-texture and microcirculation changes. This model yielded a high ONFH incidence, with a modeling success rate up to 100%, but only early-stage ONFH with a medium complication incidence, with 2 out of 10 sheep.

3.4. Surgical vascular deprivation in combination with physical insult induced ONFH animal models

Surgical vascular deprivation in combination with physical insult-induced ONFH animal models has been associated with a high success rate, reaching 95% [16]. A new emu ONFH model was developed surgically, using a combination of ischemic (vessel ligation) and cryogenic (liquid nitrogen) insults. Of nineteen emus allowed free-roaming pen activity to study the natural history of such lesions, eighteen developed an osseous structural failure, and sixteen exhibited incapacitating lameness at an average duration of 11.75 weeks after the surgical insult. In this model, bone freezing has been shown to induce osteoblast/osteocyte death, but repeated freeze–thaw cycles were necessary to achieve vascular destruction. The highly invasive nature leads to massive osteonecrosis associated with femoral head collapse and sub-capital fractures. Moreover, other disadvantages should be considered, including the difficulty of the modeling procedure, high costs, and serious complications.

Non-traumatic ONFH animal models can be categorized into spontaneous, steroid-induced, or steroid-associated, lipopolysaccharide-induced or combination-induced, horse serum-induced, alcohol-induced, and dysbarism ONFH models.

3.5. Spontaneous ONFH animal models

Factors contributing to spontaneous ONFH models include oxygenation, hypertension and upright standing [49]. Interestingly, Mihara et al. fed rats severally in high and low cages 5–15 weeks after birth. High rat cages were designed with the feeding apparatus placed up high to ensure that the rats had to stand on their hindlimbs to feed. In contrast, rats in the low cages could not stand up. Results indicated a significantly higher ONFH incidence of 40% when the rats were forced to stand [50].

3.6. Steroid-induced ONFH animal models

Corticosteroids are pivotal risk factors in ONFH development, providing a theoretical basis for exploring the molecular mechanisms of ONFH and related treatments. Many researchers have described steroid-induced ONFH model in rabbits by administering methylprednisolone (MPS) intramuscularly. A rabbit model was induced by 20 mg/kg MPS administered once intramuscularly, with 83% incidence of ONFH in the proximal metaphysis histologically 3 weeks after induction [51]. Otherwise, the same model yielded 70% ONFH incidence at the distal one-third and proximal one-third femur histologically at 4 weeks, yet 20% of rabbits died after the MPS induction [52]. The advantages of the steroid-induced approach include easy operation, low cost, and a large number of samples. Thus, steroids are widely used for developing ONFH animal models. Limitations include the poor and inconsistent success rates of these models and the dispersed formation of necrotic lesions in the femoral head. Due to heterogeneity in the dosage of steroids and the method of administration, success rates of steroids-induced ONFH models range from 15% to 90%.

3.7. LPS-induced ONFH animal models

As an inducer of ONFH, lipopolysaccharide (LPS) has been shown to induce immune stimulatory factors. LPS activates vascular endothelial cells, platelets, monocytes/macrophages, and components to lead to their hypercoagulable and/or hypofibrinolytic states partly through the reduction of endothelial expression of thrombomodulin. Overexpression of tissue factor in the LPS model suggests the existence of causal material for intravascular coagulation, whereas that in the steroid-induced ON model was not apparent. These findings could indicate that LPS induces immune stimulatory factors would be useful for elucidating the pathogenesis. Irisa et al. developed an ONFH rabbit model induced by a single low-dose (10ug/kg) LPS intravenous injection that showed a multifocal ONFH in the treated rabbits after 4 weeks [53]. Organized thrombi in the intraosseous small-sized arteries and arterioles were frequently seen in and around the necrotic tissues. However, the reported success rate of a single administration of the LPS-induced ONFH animal model is less than satisfactory (9%) [7,30–32].

3.8. Horse serum-induced ONFH animal models

Current evidence suggests that the success rates of horse serum-induced ONFH animal models range from 60% to 90% [17]. A rabbit serum sickness ONFH model was induced by an intravenous injection of 10 ml/kg of sterile heat-inactivated horse serum at room temperature, administered twice with a 3-week interval, yielding findings similar to the immune complex deposition seen in patients with systemic lupus erythematosus (SLE). At 72 h, no ONFH was found. The incidence escalated to 86% after 1 week and dropped to 64% after 3 weeks [32,33].

3.9. Steroids in combination with LPS-induced ONFH animal models

ONFH models induced by steroids in combination with LPS are associated with a relatively higher success rate, ranging from 50% to 90% [7,30,31,29,34–37]. Interestingly, this method can simulate an inflammatory state in the host. Nowadays, many researchers use Qin et al.'s protocol to establish a steroid-associated ONFH model in rabbits. The protocol involves a single low-dose LPS (10ug/kg) intravenous injection followed by three intramuscular injections of high-dose MPS (20 mg/kg) at intervals of 24 h to assess preventive strategies against ONFH [10]. In Qin et al.'s study, 6 weeks after induction, 93% of the rabbits developed ONFH, and no rabbits died throughout the experimental period. The high ONFH incidence and absence of mortality in rabbits treated with this inductive protocol suggested its effectiveness for evaluating the therapeutic efficacy of interventions developed to prevent steroid-associated ONFH.

3.10. Steroids in combination with horse serum-induced ONFH animal models

Steroids combined with horse serum-induced ONFH animal models have achieved high success rates (up to 90%) [7]. For example, Matsui et al. [38] described a rabbit ONFH model induced by employing a combined protocol of hypersensitivity vasculitis caused by horse serum and administration of high-dose corticosteroids. Fourteen of twenty specimens (70%) exhibited histological evidence of ONFH in the femoral metaphysis, including marrow necrosis (n = 7) and marrow and trabecular necrosis (n = 7), while no ONFH was observed in groups treated with horse serum or steroid alone. Vasculitis induced by this process was complicated by the introduction of steroids, inhibiting the synthesis of collagen and elastic fibers.

3.11. Alcohol-induced ONFH animal models

Alcohol-induced ONFH animal models are useful for elucidating the pathogenesis of alcohol-related ONFH in humans and evaluating different therapeutic protocols [40,54–56]. A rat model of alcohol-induced ONFH was used to assess the relationship between the pro-inflammatory response via Toll-like receptor (TLR) and the development process of ONFH in rats [9]. The researchers fed male Wistar rats with a Lieber–DeCarli liquid diet containing 5% ethanol (experimental group) or dextran (control group) for 1–24 weeks. No ONFH was observed in rats fed with the control diet, while ONFH was observed in three, four and six rats at 1, 2 and 4 weeks after alcohol feeding, respectively. The incidence of ONFH at 4 weeks in alcohol-treated rats was significantly higher than in the control rats. However, no relationship was found between the pro-inflammatory response induced via TLR4 and the development of alcohol-induced ONFH. Furthermore, Ikemura et al. evaluated the morphological changes in bone marrow fat cells and the changes in the serum lipid levels in alcohol-treated rabbits [57]. They found morphological and hematological abnormalities in lipid metabolism in the experimental rabbits after alcohol administration. Moreover, these findings were more apparent in rabbits treated with high-dose alcohol (30 ml/kg per day) than those treated with low-dose alcohol (15 ml/kg per day).

3.12. Dysbarism ONFH animal models

Over the years, dysbarism animal models have been established to investigate the pathogenesis, diagnosis, and treatment of dysbarism ONFH [17]. Lehner et al. established a dysbarism ONFH in adult sheep after 12- and 24 h of exposure to compressed air (2.6–2.9 atm absolute) for 2 months. At 7 months, juxta-articular regions of some long bones showed histological evidence of apoptosis of osteocytes and fatty marrow fibrosis, although, as in human caisson disease, most lesions were diaphyseal.

3.13. Chemically-induced ONFH animal models

Chemically induced animal models are useful for studying ONFH induced by sickle-cell disease and other hemoglobinopathies, which have success rates of 10%–20% [9]. Exposure of rats to 2-butoxyethanol (BE) has been associated with hemolytic anemia, disseminated thrombosis, and infarction in multiple organs, including bone [58–60]. A rat model of ONFH was induced by daily doses of 250 mg BE/5 ml water/kg of body weight for four consecutive days [44]. At 24 days after induction, osteonecrosis in the femur was observed histologically in 1 of 8 animals, while changes in the femurs were confined to the diaphysis. This model is therefore useful for studying ON induced by sickle-cell disease and other hemoglobinopathies.

3.14. Traumatic factors combine with non-traumatic factors induced ONFH animal models

Interestingly, using a combination of traumatic and non-traumatic factors to establish ONFH animal models can reportedly yield higher ONFH incidence than either method alone, reaching nearly 100% [24]. Recently, Kuroda et al. described a new ONFH rabbit model induced by intramuscular injection with 40 mg/kg methylprednisolone and vascular occlusion of the capital femoral epiphysis by electrocoagulation [61]. The rabbits started to develop ONFH around 4 weeks, and ONFH was confirmed within 8 weeks. At 12 weeks, femoral head collapse was observed in 2 out of 5 animals. At 24 weeks, all specimens exhibited ONFH changes. The advantages of this method are the possible compensation of other advantages of traumatic or non-traumatic models, as the etiology did not parallel to humans and necrotic lesions might not confine to the femoral head. However, disadvantages include technical difficulty, high cost, and increased risk of complications.

Taken together, we conclude that the success rates of non-traumatic ONFH models are generally significantly lower than traumatic ONFH modeling. Unlike the success rates of non-traumatic models, which vary from 9% to 90%, the success rate of the traumatic model can reach 100% [7,21,22–25]. Modeling by a drug combined with operation may yield better performance, and the femoral head necrosis lesions formed by the model are concentrated, typical and easy to observe. However, surgical models have not been widely used due to high technical requirements, trauma severity, the high number of surgical complications, high costs, and limited samples. Non-traumatic ONFH models by glucocorticoid induction are easy to operate due to the low cost and large sample numbers, and the experimental data is more representative and statistically significant, which is much more suitable for laboratory application. Although the success rates of drug models are not high and the foci of femoral head necrosis formed are scattered, animal models induced by drug agents can be used to explore the molecular mechanism and treatment efficacy of femoral necrosis.

4. Classification of commonly used models for osteonecrosis of the femoral head

Since traumatic approaches are deleterious to animals, engendering many serious side effects and being more costly than non-traumatic ONFH animal models, more and more researchers prefer to use small experimental animals to establish non-traumatic ONFH models. Indeed, small animal-based non-traumatic ONFH modeling can more easily meet ethical requirements. Over the years, rabbits, rats, and mice have been used to establish animal models of ONFH. Here, we summarize the commonly used small animal models and specific modeling methods (Table 2). Since the success rates of the non-traumatic ONFH models are related to the type of agent used in animal experiments, drug dosage and the frequency of administration, conclusions have been drawn based on the findings observed.

Table 2
Commonly used models for ONFH.

Animal type	Drug	Dosage	Frequency	Delivery cycle	Administration	Observation time	Success rate	Ref.
Japanese white rabbit	MPS	1 mg/kg	Single dose		IM.	4 W	0%	[7,62]
		5 mg/kg					42%	
		20 mg/kg					70%	
		40 mg/kg					96%	
	1.HS	10 ml/kg	Single dose	3 weeks	IV.	2/4/8 W	54%	[63]
		7.5 ml/kg	Single dose	2 weeks	IV.			
	2.MPS	45 mg/kg	Once a day	3 days	IM.			
	1.LPS	40ug/kg	Once a day	2 days	IV.	1/3/7/14/21D	36.2%	[29]
	2.PS ACE	20 mg/kg	Single dose		IV.			
	PS ACE	20 mg/kg	Single dose		IV.		30%	
	LPS	100ug/kg	Twice a day	1 day	IV.	4/6 W	60%	[64]
	MPS	20 mg/kg	Once a day	3 days	IM.			
	MPS	20 mg/kg	Single dose		IM.	2 W	75%	[65]
	MPS	20 mg/kg	Single dose		IM.	2/4/8/12 W	70%	[66]
New Zealand white rabbits	1.LPS	10ug/kg	Once a day	1 day	IV.	2/4/6 W		[35]
	2.PS	20 mg/kg	Once a day	3 days	IM.			
	1.HS	10 ml/kg	Single dose	2 weeks	IV.	2 W		[4,67]
		6 ml/kg	Once a day	2 days	IV.			
	2.MPS	20 mg/kg	Twice a week	2 weeks	IP.			
SD rats	1.LPS	4 mg/kg	Once a day	2 days	IV.		73.30%	
	2.MPS	60 mg/kg	3 times a day	1 day	IM.			
	1.LPS	20ug/kg	Twice a day	1 day	IV.		83.30%	
	2.MPS	40 mg/kg	Once a day	3 days	IM.			
	1.HS	10 ml/kg	biweekly	4 weeks	IP.		90%	[68]
	2.MPS	40 mg/kg	Once a day	3 days				
	MPS	40 mg/kg	Once a day	3 days			15%	
	PS ACE	12.5 mg/kg	twice a week	4 weeks	IM.	4 W	71.43%	[7,69]
	MPS	20 mg/kg	3 times a week	3 weeks	IM.	6 W		[70]
Wistar rats	1.LPS	2 mg/kg	Once a day	2 days	IV.	2/4 W	66.7%	[71]
	2.MPS	20 mg/kg	Once a day	3 days	IM.			
SHRSP/Izm rats	MPLS	4 mg	Single dose		SC.	2 W	100%	[72]
BALB/cJ mice	DEX				PO.	12 W	45%	[12]
							8%	
							50%	
C57/BL6 mice	LPS	20ug/kg	Once a day	2 days	IP.	4 W	80%	[37]
	MPS	40 mg/kg	Once a day	1 day	IM.			

Abbreviation: MPS: methylprednisolone; HS: horse serum; LPS: lipopolysaccharide; PS ACE: prednisolone acetate; PS: prednisolone; DEX: dexamethasone; IM.: intramuscular injection; IV.: intravenous injection; IP.: intraperitoneal injection; SC.: subcutaneous injection; PO.: oral administration; W: week; D: day

4.1. Rabbits

Rabbits are one of the most used experimental animal models, mainly established by glucocorticoid induction. The reported success rates of the rabbit ONFH model induced by 1, 5, 20, and 40 mg/kg MPS were 0%, 42%, 70%, and 96%, respectively [62]. Compared to MPS (20 mg/kg) alone, lipopolysaccharide(40 μg/kg) in combination with MPS (20 mg/kg) induced ONFH in rabbits, yielded a relatively higher success rate (36.2% vs. 30%) [29]. Nonetheless, the success rate of horse serum(10 ml/kg) in combination with MPS (45 mg/kg) induced ONFH in rabbits was 54%. Using MPLS (20 mg/kg), the success rate of the rabbit model was 70% and 75% [65]. Thus, it can be concluded that ONFH models induced by higher doses of steroids like MPS (20/40 mg/kg), in combination with lipopolysaccharide, or horse serum, can achieve higher success rates.

4.2. Rats

Rats have long been used as animal models of ONFH. It was found that the success rate of inducing ONFH in rats by sterile human serum (10 ml/kg) in combination with MPS (40 mg/kg/day) was significantly higher than with MPS alone (40 mg/kg/day) (90% vs. 15%). In a rat model induced by prednisolone acetate (12.5 mg/kg) alone, the success rate of bone necrosis was 71.43% [69]. Otherwise, the success rate of an established rat model induced by LPS (2 mg/kg) in combination with MPS (20 mg/kg) was 66.7% [71]. In addition, it is widely thought that the success rate differs according to the drug dose used [73]. The success rate of LPS (4 mg/kg) combined with MPS (60 mg/kg) was 73.3%, while Dong et al. developed a rat model induced by LPS (2 mg/kg) in combination with MPS (40 mg/kg), yielding a success rate of 83.3%. Overall, the higher the dose of steroids and lipopolysaccharide used in an ONFH-induced experiment, the higher the success rate of the ONFH model in rats.

4.3. Mice

Mice represent a commonly used small animal model with many applications. L. Yang et al. reported that four-week-old male BALB/CJ mice were treated with oral Dexamethasone (Dex) for up to 12 weeks. The induction success rate by continuous dosing without asparaginase was higher than discontinuous dosing without asparaginase (45% vs. 8%) [12]. With continuous doses of Dex combined with asparaginase, the success rate of induced osteonecrosis in mice was 50%. Regarding other modeling methods, S. Jin et al. reported a mouse model intraperitoneally (IP) injected with lipopolysaccharide for two consecutive days, followed by intramuscular injection (IM.) injection with methylprednisolone (MPS; 40 mg/kg/d) beginning on the third day 24–25. The results indicated an exciting ONFH rate of up to 80% [37]. Moreover, N. Kamiya et al. and C. Okuma et al. obtained ONFH mouse models by vascular dissection, with ON rates of 100% [24,25].

Commonly used small animal modeling methods can be divided into drug-induced models, surgical models, drugs combined with surgical deprivation-induced models, etc. Steroids-induced ONFH animal models are the most common among drug models, whose success rates vary widely, from 15% to 75% [7]. In the steroid-induced models, the success rates increased with increasing steroid dosage by single injection [7,29]. Moreover, the frequency of administration affected the success rate, which was higher with continuous administration than with intermittent

administration [12]. In addition, two types of drugs, such as steroids in combination with lipopolysaccharide or with serum, yield a significantly higher success rate and are therefore widely used in the laboratory [7, 30]. Overall, surgical deprivation-induced animal models have made great progress, with high success rates documented in the literature. However, the results observed in animal models are complex and difficult to interpret. Therefore, it is imperative to establish a cell model to further explore the mechanism of the occurrence and development of femoral head necrosis at the microscopic level.

5. Cell models for osteonecrosis of the femoral head

Unlike animal models, cell models offer the possibility of simultaneously testing many manipulations, a short duration of experiments,

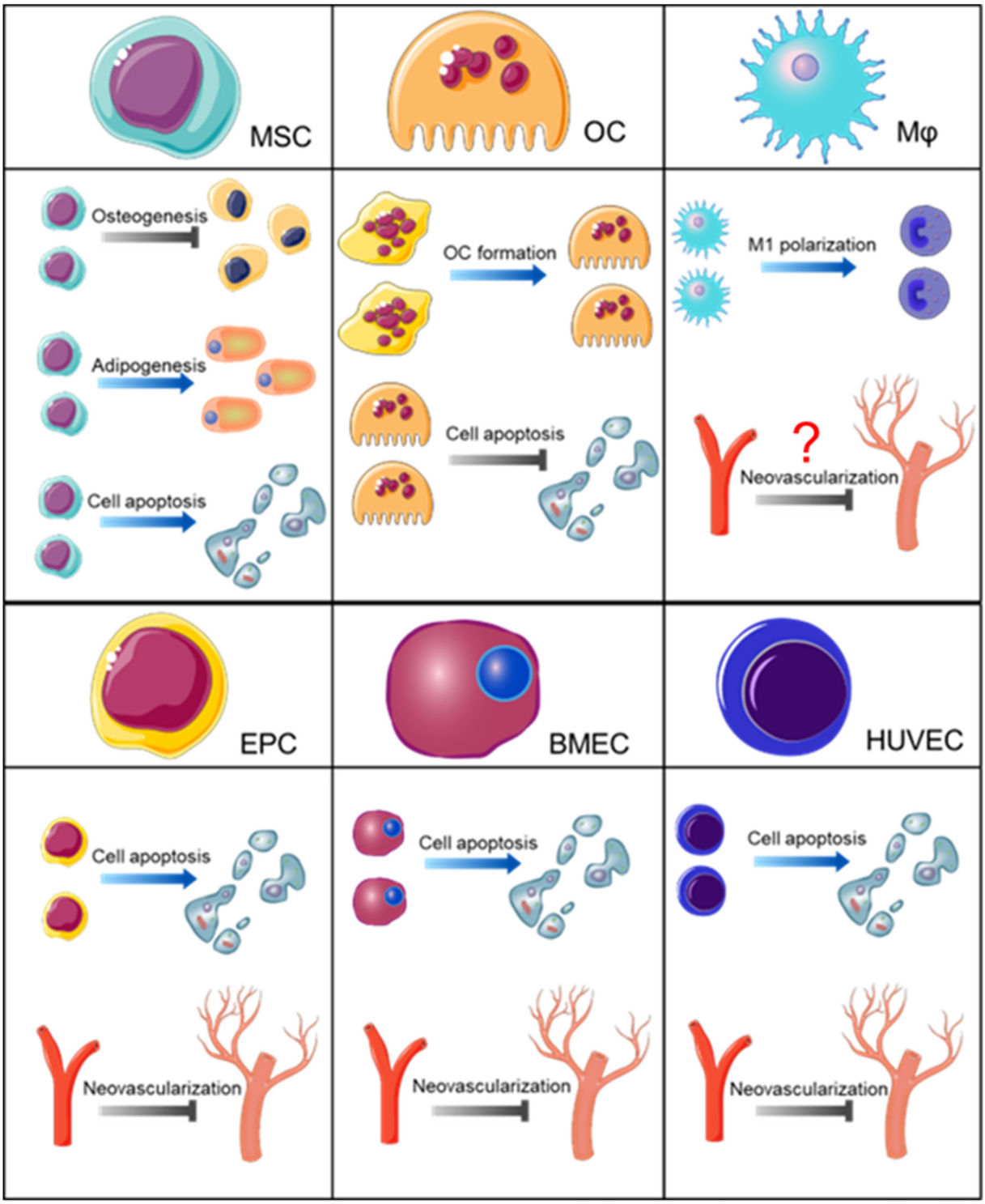

Fig. 3. Cell Models to study ONFH. The cell types used include MSC, OC, Mφ, EPC, BMEC, and HUVEC. A high dose of dexamethasone inhibits the osteogenesis of MSCs and promotes adipogenesis and cell apoptosis. For OC, cell formation and cell activity are promoted when ONFH develops. As for Mφ, M1 macrophage polarization is activated in the process of ONFH, thus inhibiting neovascularization. For EPC, BMEC, and HUVEC, 10^{-6} mol/L dexamethasone inhibits angiogenesis and promotes cell apoptosis.

few ethical and regulatory issues, easier genetic and pharmacological intervention, and available biochemical analyses. In the meantime, cultured cells are reduced systems that allow us to answer specific questions quickly, clarify signaling pathways and resolve mechanistic details. Still, being reduced systems means that they do not represent all aspects of ONFH and that results obtained in cultured cells must be confirmed in vivo [74–76].

In recent years, cell therapy has been used in various fields and plays an important role in treating diseases, representing a promising treatment strategy [77–81]. For example, stem cell transplantation strategies significantly affect bone tissue repair and regeneration engineering [82–84]. An increasing body of evidence suggests that stem cell-derived exosomes can target and regulate various factors and thus play an important role in maintaining bone marrow microenvironment homeostasis [85–89]. To harness stem cell therapy to treat human ONFH, we must first establish a cell model to study ONFH in vitro.

Importantly, cell models can illustrate the mechanisms underlying the occurrence and development of ONFH [90–97] (Fig. 3). The pathogenesis of glucocorticoid (GC)-induced ONFH remains largely unclear. Furthermore, the findings from cell models may further complete the comprehensive network of ONFH and provide targetable or druggable nodes, additive to the current treatment modalities. However, as the most common cause of non-traumatic ONFH, long-term or high-dose steroids inevitably cause disorders of the body's circulation and stem cell function [98–100]. Therefore, it is imperative to work on the cell models to study GC-induced ONFH. The cell types used included bone marrow mesenchymal stem cells (BMMSCs), osteoclasts, macrophages, bone marrow endothelial progenitor cells (BMEPCs), bone microvascular endothelial cells (BMECs), Human umbilical vein endothelial cells (HUVECs) [100] (Table 3).

5.1. Mesenchymal stem cells (MSCs)

MSC is active in bone metabolism, which plays the very important role in the pathogenesis of the ONFH. Its cell activity and function are largely influenced by GCs. Numerous studies have been done to verify the influence of GCs on MSCs. Therefore, MSCs are usually induced by GCs to simulate ONFH in vitro. The effect of Dex was investigated on the mitochondrial function of human mesenchymal stem cells, treated with Dex at 10^{-9}, 10^{-8}, and 10^{-7} mol/L, respectively, from day 1 to day 5 [101]. Results showed that high concentrations of dexamethasone (Dex, 10^{-6} mol/L) decreased cell activity, promoted apoptosis, elevated levels of reactive oxygen species, and disrupted mitochondrial dynamics. Another study investigated the influence of different concentrations (0, 10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} mol/L) of Dex added to BMSCs and kept for 4 days, finding that high concentrations (10^{-5}, 10^{-6} mol/L) of Dex significantly inhibited the proliferation and osteogenic differentiation of BMSCs [106]. Overwhelming evidence suggests that the lineage commitment of MSCs is modulated by transcription factors or post-transcriptional regulators, which can directly or indirectly act on target genes of various signaling pathways, thus initiating and promoting osteogenic or adipogenic differentiation of MSCs [109–113]. Glucocorticoids can positively or negatively regulate the expression of multiple transcription factors (e.g., PPARγ, Runx2) and post-transcriptional regulators (e.g., miRNAs, lncRNAs), thereby affecting the differentiation of MSCs into osteoblasts or adipocytes. For example, researchers have reported that previously unexplained effects of glucocorticoid on bone loss may be mediated in part by suppression of CBFα1, resulting in a decrease in the expression and activity of the TGF-beta type I receptor on matrix-producing bone cells [114]. It was found that GCs could rapidly inhibit the expression of functional Runx2 in nuclear extracts from the GC-treated primary osteoblast cultures of fetal rat.

5.2. Osteoclasts (OCs)

Excessive osteoclast activity is important in bone integrity loss and subsequent subchondral bone fracture. A growing literature suggests the presence of hyperactive osteoclasts in vivo ONFH models [115–118]. However, exogenous glucocorticoids have been proved to directly impair osteoclast function in vitro [119]. For example, Liu et al. treated osteoclast cells with 10^{-6} mol/L MPS and found that GC treatment suppresses osteoclast formation. They figured out that GC treatment inhibited angiogenin (ANG) production by suppressing osteoclast formation in the metaphysis, resulting in endothelial cell senescence and bone loss [120]. Nonetheless, enhanced reactive oxygen species and pro-inflammatory cytokines (IL-15, IL-34 etc.) were found in steroids-induced ONFH animal models, which may mediate osteoclast activation [121–123]. Thus, further study is needed to clarify the mechanisms underlying the regulation of osteoclast differentiation in vitro.

5.3. Endothelial progenitor cells (EPCs) and bone microvascular endothelial cells (BMECs)

Similarly, endothelial progenitor cells were also examined in patients with glucocorticoid-induced ONFH [94,124]. Chao Chen et al. analyzed 33 patients with glucocorticoid-induced ONFH and 33 age- and sex-matched control subjects [125] for abnormalities in early EPCs and endothelial colony forming cells (ECFCs) and compared their functions in

Table 3
Cell models of osteonecrosis of the femoral head.

Cell type	Drug	Dosage(mol/L)	Dosing time	Function of cell model	Ref.
BMSC of human	DEX	10^{-9}, 10^{-8}, 10^{-7}	5 days	DEX inhibits cell activity and osteogenesis of HBMSCs, and promotes cell apoptosis and adipogenesis.	[101]
HBMEC	HC	$5.5*10^{-5}$, $1.1*10^{-4}$, $2.2*10^{-4}$, $2.76*10^{-4}$, $5.5*10^{-4}$	24 h	GC inhibits angiogenesis and proliferation of BMECs.	[102]
HUVEC	HC	2.76*10-3	24 h	GC induces cell damage and apoptosis.	[100]
EPC	PS/MPS	10^{-6}, 10^{-5}, 10^{-4}, 10^{-3}, 10^{-2}	3 days	Steroids inhibit neovascularization by EPCs in vitro. High concentration of GCs suppresses cell growth.	[103]
BMSC of rabbit	DEX	10^{-7}	24 h	DEX inhibits osteogenic differentiation and promote adipogenesis.	[104]
BM-EPC of rats	COR/ DEX	10^{-3}	48 h	GC induces apoptosis of BM-EPCs.	[105]
BMSC of rat	DEX	10^{-9}, 10^{-8}, 10^{-7}, 10^{-6}, 10^{-5}	4 days	DEX inhibits the proliferation and osteogenic differentiation of BMSCs.	[106]
BMSC of mouse	DEX	10^{-8}, 10^{-7}, 10^{-6}	3 days	GCs impairs bone formation of bone marrow stromal stem cells, decreases the viability of mouse MSCs.	[107]
		10^{-9}, 10^{-8}, 10^{-7}	48 h	DEX promotes adipogenic differentiation, suppresses osteogenic differentiation.	[108]

Abbreviation: BMSC: bone marrow mesenchymal stem cell; HBMEC: human bone microvascular endothelial cell; HUVEC: human umbilical vein endothelial cell; EPC: endothelial progenitor cell; BM-EPC: bone marrow endothelial progenitor cell; DEX: dexamethasone; HC: hydrocortisone; PS: prednisolone; MPS: methylprednisolone; COR: cortisone.

glucocorticoid (GC)-induced avascular osteonecrosis of the femoral head. It was found that early EPCs and ECFCs were impaired in GC-induced ONFH patients, and their distinct reduced capacity profiles might reflect their different roles in endothelial dysfunction of GC-induced ONFH. In addition, researchers developed a TNF-α -induced ONFH model in human BMECs in vitro [126]. They assessed the cell viability of BMECs after incubation of TNF-α proteins (200 ng/ml, 100 ng/ml). Results showed significantly reduced cell viability and increased necroptosis of BMECs. Their study also demonstrated that the TNF-α aptamer could protect BMECs from necroptosis by inhibiting the RIP1/-RIP3/MLKL signaling pathway, thus producing a protective effect on the development of ONFH.

5.4. *Macrophages(Mφ)*

Studies have substantiated the dynamic feature of macrophage M1/M2 imbalance in ONFH animal models. It is generally accepted that M1 macrophages secrete inflammatory cytokines and exert a pro-inflammatory effect, while M2 macrophages secrete anti-inflammatory cytokines, contribute to forming blood vessels and reconstructing normal bone [127–129]. In vitro, macrophages treated with 100 μg/ml necrotic bone fluid showed increased inflammatory cytokine expression and M1 macrophage polarization [130]. Interestingly, researchers repolarized macrophages from the M1-like phenotype to the M2-like phenotype, thus promoting the survival of osteocytes, decreasing inflammatory cytokines, and finally ameliorating steroid-induced osteonecrosis of the femoral [131,132]. Therefore, switching macrophages from M1 to an M2 phenotype may be a useful therapeutic strategy against ONFH.

Over the years, cellular models for studying ONFH still have limitations. For example, M. Song and Y Xiao et al. revealed that 10^{-9}, 10^{-8}mol/L Dex promotes osteogenesis and inhibits fat formation, but 10^{-7}, 10^{-6}, 10^{-5}mol/L Dex inhibits osteogenesis and promotes fat formation [133,134]. Moreover, there are still some differences in different cells at the same concentration when developing steroid-induced cell models. Zha et al. cultured C57 mouse MSCs and HUVECs with dexamethasone, revealing that Dex inhibited mouse MSCs proliferation, and this effect was promoted by increases in concentration (10^{-8}, 10^{-7}, 10^{-6}mol/L) and duration of exposure [135]. Moreover, they demonstrated that Dex inhibited HUVEC viability and VEGF secretion in a dose- and time-dependent manner. Besides, HUVEC proliferation, migration and tube formation ability were inhibited by Dex. In short, targeting and regulating a variety of factors at the cellular level is promising for the treatment of osteonecrosis. Indeed, further research is needed to reveal the mechanisms at the cellular level.

Current evidence suggests that stem cells and progenitors hold the ability of inducible and multiple differentiation. Any factors that decrease the number or alter the function of differentiation and multiplication of progenitor cells can lead to an imbalance between osteocyte formation and apoptosis or necrosis, and as a result, ONFH can develop if the imbalance cannot be restored [107,136]. Consequently, we can use gradient concentration or a particular concentration of steroids to induce MSCs or EPCs, through which researchers may develop cell models to study ONFH and discuss their mechanisms.

6. Conclusion and perspectives

In summary, we recommend modeling solutions according to the needs of researchers. If researchers want to use large samples in the laboratory, it is recommended to use non-traumatic agents such as glucocorticoids in combination with LPS to induce ONFH animal models, which can guarantee a success rate of more than 60%. If the researchers want to achieve a higher success rate for ONFH animal modeling, it is recommended to combine traumatic vascular deprivation with non-traumatic steroids for the ONFH induction, with success rates ranging from 80% to 100%. However, animals that undergo vascular deprivation surgery may not survive the glucocorticoid induction process. As for cell models, we recommend 10^{-6}mol/L Dex to treat bone marrow stem cells, which is optimal for establishing cell models.

High-intensity focused ultrasound (HIFU) is widely thought to have huge prospects for ONFH animal modeling. Intriguingly, HIFU has the potential to induce osteonecrosis non-invasively by thermally inducing osteocyte damage and vascular thrombosis. This combination may closely mimic clinical osteonecrosis, characterized by ischemia and a lack of a reparative response [137]. Hyperthermia induces osteonecrosis, and the vascular insult results in ischemia and a lack of reparative response. HIFU ultrasound models have proved feasible [138], and this non-invasive method has great prospects. In addition, many reports revealed that ONFH could be induced by radiofrequency [138–142]. Martel et al. described that percutaneous radiofrequency ablation in long bone in dogs and documented ONFH and reparative reaction in trabecular bone with cortical bone and articular cartilage intact [142]. Accordingly, radiofrequency represents an effective biophysical means of creating experimental ONFH models.

As a new type of cell, H subtype vascular endothelial cells (HSVECs) have hitherto not been reported to establish models of ONFH. Experimental verification of HSVECs in the human femoral heads has been conducted in recent years [143–145]. In previous research, it was depicted that steroids influence the endothelial cells of blood vessels. Interestingly, vascular endothelial cells were reduced in patients with femur head necrosis [125,103,146,147]. Accordingly, future studies should focus on using HSVEC models to study ONFH.

Author contributions

Zilin Li and Yong Feng had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Author statement

Zilin Li; Conception and design of study. Drafting the manuscript. Yong Feng: Conception and design of study. Wenkai Shao: Conception and design of study. Drafting the manuscript. Xiao Lv: acquisition of data. Bo Wang: acquisition of data. Lizi Han: analysis and/or interpretation of data. Song Gong: analysis and/or interpretation of data. Ping Wang: analysis and/or interpretation of data.

Declaration of competing interest

The authors have no conflicts of interest to disclose in relation to this article.

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