Treatment of osteonecrosis of the femoral head using prevascularized bone tissues constructed with human umbilical cord mesenchymal stem cells and human umbilical vein endothelial cells

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Research Article

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Abstract

Background

Studies have shown that osteonecrosis of the femoral head (ONFH) is related to bone marrow mesenchymal stem cell injury and microvascular injury. Early ONFH patients can choose core decompression and other treatments, but the clinical effect is poor. In recent years, tissue engineering technology based on mesenchymal stem cells is a new method to treat ONFH. In addition, whether the transplantation area is fully vascularized also affects whether the mesenchymal stem cells can play a repair role. In this study, we constructed prevascularized bone tissue based on human umbilical cord mesenchymal stem cells and human umbilical vein endothelial cells to evaluate the therapeutic effect of prevascularized bone tissue on ONFH.

Objective:

This paper is to investigate the effect of pre-vascularized bone tissue constructed by human umbilical cord mesenchymal stem cells and human umbilical vein endothelial cells on bone repair of necrotic femoral head, so as to provide theoretical basis for clinical treatment of necrotic femoral head.

Methods:

The rabbits were randomly divided into 5 groups, of which, 1 group was not given any intervention, while rabbit femoral head necrosis models were prepared for the other 4 groups by microwave heating method and they are respectively named mould-making group, static bone tissue group, dynamic bone tissue group and pre-vascularized bone tissue group according to the different substances injected into the femoral head. Various tests, including MRI, Micro-CT, HE staining and immunofluorescence staining, were performed after the animals were executed 8 weeks after treatment to assess the osteogenic ability of the implant and the repair effect of necrotic femoral head.

Results:

Various tests showed that the pre-vascularized bone tissue group had better ability to promote necrotic femoral head repair, and significant new bone formation and bone quality improvement occurred 8 weeks after surgery. The static bone tissue group and dynamic bone tissue group were similar in their ability to promote femoral head repair, with all the three groups outperforming the modeled group.

Conclusions:

The pre-vascularized bone tissue has a good effect on promoting the reconstruction of necrotic femoral head and shows superior osteogenesis, angiogenesis and bone repair effect. It is thus a good graft material for repairing necrotic femoral head and is of great value in the treatment of necrotic femoral head.
Introduction

Osteonecrosis of the femoral head (ONFH) is a common progressive bone disease associated with necrosis of bone cells and bone marrow tissue of the femoral head, structural changes and collapse of the femoral head leading to joint dysfunction. Buttock and hip pain is the main clinical manifestations of ONFH, and the quality of life of patients is greatly reduced. The pathogenesis of ONFH has not been fully clarified, and relevant hypotheses include bone marrow mesenchymal stem cell injury, microvascular injury, fat embolism, intramedullary pressure change, and abnormal lipid metabolism, etc. Currently, the widely recognized pathogenesis of ONFH is related to insufficient number of mesenchymal stem cells, low activity, and decreased osteogenic differentiation potential. As a result, bone reconstruction of necrotic femoral head is insufficient to compensate for the osteonecrosis, which progressively worsens and eventually leads to the development of ONFH [1]. At present, the treatment of femoral head necrosis is mainly determined according to the stage of ARCO in clinical practice. Total hip replacement (THA) is often used in patients with advanced ONFH [2], but THA has complications such as limited service life, dislocation and infection, and its application in patients is still controversial. Early ONFH patients can be treated with drug intervention, core decompression, autologous and allogeneic bone transplantation and other hip preservation therapies to delay the progression of femoral head necrosis. However, the clinical effect of the above treatment is often unsatisfactory, and long-term treatment also brings great suffering to the patients.

In recent years, with the development of biomedical technology, tissue engineering technology based on mesenchymal stem cells (MSCs) is a new method for the treatment of femoral head necrosis, which has gradually replaced autologous bone and allogeneic bone implants in the repair of necrotic femoral head through basic experiments and clinical practice [3–6]. Since abnormal osteogenic differentiation of MSCs and defective bone repair, microcirculatory damage and endothelial dysfunction are the main pathogenic mechanisms of ONFH, the treatment of early ONFH can focus on providing viable MSCs in order to enhance osteogenesis and angiogenesis in the necrotic area of the femoral head. MSCs have a strong capacity for self-renewal and the potential to differentiate into multiple cell types for bone repair and angiogenesis by differentiating into osteoblasts and endothelial cells. In addition, MSCs can secrete various cytokines to promote blood supply in necrotic areas [7–10]. Studies have shown that biomimetic mechanical stimulation is considered to be the key to the formation of bone tissue during the construction of tissue engineered bone in vitro. Fluid shear stress is the main factor affecting the osteogenic differentiation process of MSCs. Therefore, applying fluid shear stress may be an effective way to promote the osteogenic ability of MSCs.

The necrotic area of the femoral head is often accompanied by microcirculation damage, which not only inhibits the proliferation and differentiation of MSCs, but also leads to the progression of ONFH. Therefore, it is also important to promote vascularization of the transplanted area on the basis of transplanted MSCs. In this study, we constructed the prevascularization bone tissue based on human umbilical cord mesenchymal stem cells(hUCMSCs) and human umbilical vein endothelial cells(HUVECs), and assessed the therapeutic effect of the prevascularized bone tissues on ONFH by testing the
osteogenic and angiogenic functions of the femoral head, thus providing experimental basis for the clinical treatment of ONFH.

1 Materials and methods

1.1 The constructed tissue preparation

The constructed bone tissue and prevascularized bone tissue used in this experiment were provided by East China University of Science and Technology. The tissue was constructed with modular tissue construction strategy, which meant that the tissue to be built was assembled from microtissues. The specific preparation process was as follows:

1.1.1 Cell Culture

Human umbilical cord mesenchymal stem cells (hUCMSCs) (Cyagen) were cultured in α-MEM (Gibco) supplemented with 5% human platelet lysate (Helios bioscience), at 37 °C in a 5% CO₂ humidified atmosphere. Human umbilical vein endothelial cells (HUVECs) (Sciencell) were cultured in endothelial cell growth medium (Sciencell).

1.1.2 Preparation of bone tissue

The constructed bone tissue consisted of a large number of osteogenic microtissues. Osteogenic microtissues are the co-cultures of hUCMSCs and HUVECs in the ratio of 1:2 on demineralized bone matrix (DBM) microcarriers (Beijing xinkangchen medical technology development co., ltd), which contain a large number of bone morphogenetic proteins, promoting the differentiation of MSCs into osteoblasts and bone formation [11]. In spinner flask bioreactor, hUCMSCs and HUVECs were inoculated into DBM microcarrier in osteogenic induced medium (OIM) inducing osteogenic microtissue formation for one week. Among them, osteogenic microtissues were classified into dynamic osteogenic microtissues cultured in 45 rpm (with fluid shear stress) and static osteogenic microtissues cultured in 0 rpm (without fluid shear stress), to form dynamic bone tissue and static bone tissue respectively.

1.1.3 Preparation of angiogenic microtissue

Angiogenic microtissues are the co-cultures of hUCMSCs and HUVECs in the ratio of 1:1 on cultispher microcarriers (Sigma) for one week. The mixed growth medium formed by the growth medium of hUCMSCs and HUVECs in the ratio of 1:1, was used to construct angiogenic microtissues in spinner flask bioreactor in 45 rpm.

1.1.4 Preparation of prevascularized bone tissue

The prevascularized bone tissue was formed by mixing of the dynamic osteogenic microtissues and angiogenic microtissues in the ratio of 1:1 in hydrogel. The prevascularized bone tissue was injected into the necrotic area of rabbit femoral head.
1.2 Materials

Main reagents: CD31 antibody (santa Cruz, USA), OCN antibody (santa Cruz, USA), all secondary antibodies (abbkine, USA), PBS buffer solution (meilunbio, China).

Experimental animals: The study adhered to the ARRIVE guidelines and the ethical guidelines of the Laboratory Animal Center of Binzhou Medical University. All animal experiments were carefully performed in all possible steps to avoid animal suffering. The experimental animals were kept in cages in animal houses with appropriate temperature, humidity and light, and sufficient food and water. We established the humane endpoints for the study including weight loss, loss of appetite, hypothermia, self-harming behavior, terminal disease, etc and the monitoring frequency is once every two days. According to the guidelines for sample size calculations of Boston University, a total of 40 26-week-old New Zealand male white rabbits (weight: 2.6-4.0kg) were used to establish the ONFH model induced by microwave heating, which were all from Jinan Xiling Jiao Biotechnology Co., LTD. Animal feeding, surgical operation and specimen collection are all conducted in the Animal Laboratory of the Binzhou Medical University Hospital. Animal experiments were conducted in strict compliance with animal ethical requirements and approved by the Experimental Animal Ethics Committee (Approved Number: 20211008-12, date: October 8, 2021).

Experimental instruments: Scanning electron microscope (Hitachi, Japan), inverted microscope (Olympus, Japan), Micro-CT (Brock, Germany), universal table centrifuge (Eppendorf, Germany).

1.3 Experimental method

1.3.1 Grouping of laboratory animals

According to the random number table method, we randomly divided the forty New Zealand white rabbits into the following five groups (n = 8/group): (1) blank group, (2) modeled group, (3) static bone tissue group, (4) dynamic bone tissue group, and (5) pre-vascularized group. Meanwhile, we were unaware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis). In the blank group, no intervention was performed on the femoral head. However, the experimental animals in the modeled group, static bone tissue group, dynamic bone tissue group and pre-vascularized group were respectively injected with saline, static bone tissues, dynamic bone tissues and pre-vascularized bone tissues into the necrotic femoral head after constructing the ONFH model via microwave heating method. The rabbits were fed in random cages to minimise potential confounders. No death or lethargy were found in the experimental animals, and all the experimental animals were eventually included in the study.

1.3.2 Experimental model construction

The rabbit ONFH model was constructed by microwave heating molding method [13], and the model was started after 1 week of adaptive feeding of New Zealand white rabbits in random cages. The heating temperature and heating time of microwave heating molding method were controlled accurately, and this
method has the advantages of low animal mortality, high success rate of necrosis and simple operation. The anesthesia was performed by intraperitoneal injection of pentobarbital sodium 25-30mg/kg. After anesthesia with femoral trochanter as the center, the hair was moistened with normal saline and then cut with curved tissue scissors close to the skin with a radius of approximately 5cm, and the skin was disinfected and prepared with iodophor. Under conventional aseptic operation, a posterolateral hip incision was made and a 1cm vertical incision was made at the lower margin of the greater trochanter. Most of the joint capsule was dissected and the femoral head was exposed. A 1mm diameter Kirschner wire was used. With the lateral aspect of the inferior border of the greater trochanter as the entry point, a hole was drilled along the central femoral neck towards the center of the femoral head until the tip reached approximately 0.5cm below the articular surface. After the electric drill was pulled out, the guide needle of the microwave therapy instrument was inserted into the drill hole of the femoral head. The temperature of the microwave guide needle was set at 55°C and the heating time was 10 min. The ONFH model was prepared by microwave heating to inactivate the femoral head. After the femoral head necrosis was formed by 10-min high temperature heating, the hip incision of rabbits was sutured layer by layer and separated into cages. After the femoral head of the rabbit was inactivated, penicillin was intramuscularly administered 40,000 U/each daily for 3 days to prevent infection. After the New Zealand rabbit femur head necrosis model was constructed, HE staining and MRI examination were performed to determine whether the model was successfully constructed.

1.3.3 The constructed tissue transplantation

After successful microwave heating molding of the three experimental groups, the guide needle of the microwave therapy instrument was pulled out, the blood in the bone marrow pore was drained with a sterile syringe, and the static bone tissue, dynamic bone tissue and pre-vascularization bone tissue were slowly injected into the femoral head drill. Then the bone marrow aperture was plugged and sealed with bone wax, and the incision was sutured layer by layer.

1.3.4 Laboratory animal sampling

After 8 weeks, the animals were euthanized by air embolization of auricular vein. New Zealand rabbit lower limbs were severed and soft tissue was separated along the femur. Then, with the greater trochanter as the incision, the muscles and ligament tissues around the femoral head were separated from the outside inwards and from the bottom upwards. The joint capsule was then opened, and the femoral head was cut off at the neck of the femur with the bone masseur forceps to remove femoral head and the attached tissues around the femoral head. The removed femoral head was examined by Micro-CT, HE staining and immunofluorescence staining.

1.3.5 MRI

After 8 weeks, the experimental animals were examined by MRI. The New Zealand white rabbits were placed on the MRI bed in prone position. Keeping the bilateral medullary joints abducted and slightly posteriorly extended, and bilateral hip joints were scanned and images were collected to understand the
necrosis and repair of femoral head in rabbits. The main MRI indicators of femoral head necrosis in rabbits include focal signal changes in the subchondral weight bearing area, such as low signal zone, double line sign and cystic low signal area [14,15].

1.3.6 Micro-CT

The femoral head and trochanter were removed with bone rongeur. The longitudinal axis of the femur was parallel to the moving axis of the slide rail of the micro-CT examination table for micro-CT scanning. The trabecular repair of the femoral head and the improvement of the microscopic bone structure were observed.

1.3.7 HE staining and immunofluorescence staining

After the femoral head was removed and decalcified, the femoral head was divided into two sections along the coronal plane with a thickness of 5 μm. The sections were stained with hematoxylin-eosin (HE) and immunofluorescence, respectively. For immunofluorescence staining, OCN and CD31 primary antibodies were added dropwise and left overnight at 4°C, followed by the addition of the relevant secondary antibodies and DAPI solution to stain the nuclei of the cells. Finally, each section was observed under confocal microscope.

1.4 Statistical analysis

SPSS22.0 statistical analysis software was used for data processing. The normality of the data from each group was checked, and all experimental data results were expressed as mean ± standard deviation. T-test was used for inter-group comparison, and P<0.05 was considered statistically significant.

2 Results

2.1 Construction of osteogenic microtissue and vasogenic microtissue

After constructing osteogenic microtissues and angiogenic microtissues, the cell viability and death staining were performed on the two tissues to determine the cell activity of the osteogenic microtissues and angiogenic microtissues, and alkaline phosphatase staining and fibrinogen gel migration experiments were performed on the osteogenic microtissues and angiogenic microtissues, respectively, to determine the osteogenic ability and angiogenic ability of the osteogenic microtissues and angiogenic microtissues. The results of cell survival and death staining showed that a large number of new cells were generated on the microcarrier, and the microcarriers aggregated and formed clusters, without obvious dead cells, indicating that the cells maintained high activity during the preparation of osteogenic microtissues and angiogenic microtissues (Figure 1, 2). Compared with no-load DBM medium, alkaline phosphatase staining was more obvious in the medium cultured osteogenic microtissues, which proved that osteogenic microtissues had certain osteogenic ability (Figure 3). In addition, the fibrinogen gel migration experiment showed that a large number of hUCMSCs and HUVECs migrated out of the vasoblastic microtissues and increased with time (Figure 4).
2.2 Establishment of the rabbit model of osteonecrosis of the femoral head

After the animal model of femoral head necrosis was constructed by microwave heating method, histological and imaging MRI examinations were performed on the experimental animals after the modeling to verify the success of the model. The criteria of femoral head necrosis were as follows: HE staining: Sparse, thin and disorganised bone trabeculae, some of which were fractured, with dead trabeculae visible and no surrounding osteoblasts or bone marrow present; MRI examination: Focal signal changes such as hypo-signal bands, bilinear signs, edematous high-signal areas and cystic hyposignal areas were seen in the subchondral weight-bearing area. The results showed that compared with the blank group, the trabeculae of the femoral head of rabbits in the modeled group, the static bone tissue group, the dynamic bone tissue group and the prevascularization bone tissue group were sparly broken, and the dead bone trabeculae were visible, and there were focal signal changes such as hypo-signal area, which indicated that the rabbit femoral head necrosis model was successfully constructed, providing accurate and reliable guarantee for further experiments (Figure 5).

2.3 MRI results

2 months after implantation, hypo-signal changes, cystic changes, bone marrow edema and joint effusion were observed in the femoral head of the modeled group, which were signs of femoral head necrosis. In the static and dynamic bone tissue groups, small flake low-signal areas were observed in the femoral head, and most of the bone tissue signals were normal, suggesting that the femoral head necrosis was repaired well, and the repair effect of the femoral head was similar between the two groups. The signal in the femoral head of the pre-vascularized bone tissue group was uniform, and no obvious hypo-signal appeared, suggesting that the pre-vascularized bone tissue had the strongest effect in promoting necrotic femoral head repair (Figure 6).

2.4 Micro-CT results

2 months after surgery, the modeled group showed osteonecrosis with disorganized and sparse trabeculae, widespread fractures and sparse numbers of osteonecrosis, and all three groups of implants had different degrees of repair effect on the osteonecrosis of the femoral head, and thick and dense new bone trabeculae were formed, especially in the prevascular bone tissue group. However, there was no significant difference in the bone trabecular microstructure between the static bone tissue group and the dynamic bone tissue group (Figure 7, Figure 8).

2.5 HE staining results

At 3 months after implantation, trabeculae and bone marrow regeneration were observed in the osteonecrosis area of femoral head in the three groups. A large number of fragments of trabeculae and fractured trabeculae were visible in the modeled group, with dead trabeculae and no surrounding osteoblasts or bone marrow present. Compared with the control group, the bone trabeculae in the static stage and the dynamic stage were robust but still had fracture areas, with fewer osteoblasts attached on
the surface of the trabeculae and a few bone marrow structures around them. There was no significant
difference in HE staining results between the two groups. The prevascularized bone tissue group had the
most obvious bone tissue regeneration, complete bone trabecular structure, a large number of
osteoblasts attached to the surface, and abundant bone marrow structures around it, indicating that this
group had better bone repair effect (Figure 9).

2.6 Immunofluorescence staining results

2 months after implantation, the expressions of CD31 angiogenic protein (red) and OCN osteoblast
protein (green) were most obvious in the prevascularized bone tissue group while OCN osteoblast protein
was highly expressed and CD31 angiogenic protein was slightly expressed in bone tissues of the static
and dynamic bone tissue groups, and the expressions of the two proteins were similar. However, the two
fluorescent proteins were significantly less expressed in the modeled group (Figure 10).

3 Discussion

The ideal bone tissue engineering implant should have good osteogenic activity to facilitate the repair of
ONFH. We found that three kinds of bone tissues exhibited excellent bone repair activity in static stage,
dynamic stage and pre-vascularization stage. As the basis of tissue engineering transplantation, MSCs
can promote the formation of new bone in necrotic areas by promoting osteogenic differentiation of stem
cells and inhibiting lipogenic differentiation, and participate in bone repair in necrotic bone tissue areas
[16–18]. There are many kinds of MSCs involved in bone tissue engineering transplantation, including
bone marrow-derived, umbilical cord-derived, adipose-derived and peripheral blood-derived mesenchymal
stem cells, etc. hUCMSCs were extracted from Wharton gel prepared from infant umbilical cord, which
has high proliferation and differentiation ability and low immunogenicity, making it relatively non-
invasive, painless, easy and safe to obtain. It is easy to be amplified in vitro and has high osteogenic
activity [19, 20]. Wang et al. [21] studied the therapeutic effect of hUCMSCs on ONFH, and found that
osteoblasts proliferated and differentiated significantly in the hUCMSCs group, and bone trabeculae were
thick and dense compared with the control group, indicating that hUCMSCs had a repairing effect on the
necrotic femoral head by promoting bone reconstruction. This is consistent with our findings. In our
study, hUCMSCs were used to construct bone tissues. The results showed that, compared with the
modeled group, the necrotic femoral head transplanted with bone tissue showed bone repair with thicker
trabeculae, increased attachment of osteoblasts on the trabecular surface, and regeneration of the
surrounding bone marrow structure, and immunofluorescence showed high expression of OCN osteoblast
protein.

Through the above studies, we confirmed that the bone tissues constructed by hUCMSCs have good
osteogenic activity. However, through further literature review, we found that MSCs are primarily
stimulated by fluid shear stress in the process of osteogenic differentiation, and this mechanical
stimulation is considered to be critical for bone tissue formation. This suggests that fluid shear stress
plays an important role in osteogenic differentiation of MSCs. In order to verify this hypothesis, in our
study, we constructed dynamic and static bone tissues respectively depending on whether the fluid shear stress was applied during the osteogenesis induction of hUCMScs, so as to explore whether there was any difference in the repair of femoral head necrotic bone tissue between the two. Our results showed that after transplanting static or dynamic bone tissue, bone trabecular increase and bone improvement were observed in the femoral head, and osteoblast OCN expression was up-regulated, which proved that both of them had certain osteogenic activity and bone repair ability. However, their repair effects were similar, indicating that for repairing osteonecrosis, there was no significant difference between static and dynamic stages. We hypothesized that this might be related to the different responses of MSCs at different stages of osteogenic differentiation to fluid shear stress, which inhibited the expression of osteogenic genes during rapid proliferation, promoted the osteogenic differentiation of MSCs during matrix maturation, and reduced the mineralization of nodules during mineralization.

This study further explored the treatment strategy of ONFH by combining HUVECs. In order to solve the problem that the osteogenic effect of MSCs was reduced due to the incomplete vascular system in the transplantation area, HUVECs were co-transplanted to promote angiogenesis, improve the local hypoxic microenvironment, promote the survival, proliferation and differentiation of MSCs, and thus improve the ability of stem cells to repair necrotic bone tissue. Studies have shown that MSCs and venous endothelial cells promote each other in differentiation and proliferation through the coupled mechanism of angiogenesis and osteogenesis, so as to achieve remodeling of the femoral head [22–27]. Xu et al. [28] found that bone trabeculae and angiogenesis increased after co-culture of MSCs and endothelial progenitor cells, showing enhanced osteogenesis and angiogenesis, with high expression of osteogenesis factors and angiogenesis factors, and low expression of lipogenesis related factors. In addition, it was found in vivo that necrosis of femoral head was significantly improved after transplantation. In our study, imaging results of 2 months after transplantation showed that compared with bone tissue graft, the femoral head grafted with pre-vascularized bone tissue had a stronger trabecular reconstruction effect, with a large number of thick and dense new bone trabeculae visible and a better osteogenic effect than the bone tissue. In addition, HE and immunofluorescence staining showed a large number of neovascularization and high expression of angiogenic protein CD31 in the prevascularized bone tissue group, indicating that active angiogenesis could promote bone tissue regeneration, and the combination of venous endothelial cells to promote angiogenesis is an effective strategy to enhance the osteogenic ability of MSCs. Although the femoral head grafted with bone tissue also showed some bone tissue repair performance, the repair effect was far less obvious than that of pre-vascularized bone tissue due to the relative lack of osteoblasts and angiogenesis cells. No obvious bone repair effect was found in the necrotic femoral head without any treatment, which was still a large area of osteonecrosis, indicating that the number and function of the MSCs in the femoral head decreased significantly after necrosis, so it is necessary to transplant abundant and active exogenous MSCs.

The type of cell transplantation is closely related to the repair effect of necrotic area. In this study, the transplanted cells were amplified using microcarrier. The microcarrier could provide a suitable microenvironment for cell proliferation, increase the surface area for cell attachment and growth, and ensure efficient harvest of a large number of active MSCs, venous endothelial cells and related
extracellular matrix to meet the number and density of cells required in the area of femoral head necrosis [29–31]. The prevascularized bone tissue is a mixture of osteogenic microtissue and angiogenic microtissue. Different from transplantation of hUCMSCs and HUVECs alone, the prevascularized bone tissue simulates the microenvironment of bone tissue and vascularization, which is conducive to the survival, differentiation and proliferation of the transplanted cells in the femoral head and the formation of new bone and blood vessels after transplantation, further improving the repair efficiency of necrotic femoral head. During tissue construction, MSCs in osteogenic tissue were induced to differentiate to osteogenic direction by osteogenic induction medium, and the potential of osteogenic differentiation was demonstrated by ALP staining. At the same time, we evaluated the angiogenic ability of venous endothelial cells in angiogenic tissue by fibrinogen migration assay, and the results showed that they have angiogenic potential. After confirming the osteogenic differentiation potential and angiogenic potential of the two, we mixed osteogenic tissue and angiogenic tissue in a gel to prepare prevascularized bone tissue, which were able to acquire osteogenic and angiogenic properties. Our experimental results showed that all three groups, i.e., static bone tissue, dynamic bone tissue and prevascularized bone tissue, could promote bone repair, which proved that bone tissue is a viable and effective way for mesenchymal stem cell transplantation.

4 Conclusion

Our study shows that prevascularized bone tissues constructed from human umbilical cord mesenchymal stem cells and human umbilical vein endothelial cells can facilitate the repair of osteonecrosis of the femoral head by promoting osteogenesis and angiogenesis. This study provides an experimental basis for the treatment of osteonecrosis of the femoral head with prevascularized bone tissue, indicating that the transplantation of prevascularized bone tissue is an effective treatment strategy for osteonecrosis of the femoral head, which provides a new scheme for the clinical treatment of osteonecrosis of the femoral head.

Abbreviations

ONFH osteonecrosis of the femoral head
ARCO association Research circulation osseous
THA total hip replacement
MSCs mesenchymal stem cells
hUCMSCs human umbilical cord mesenchymal stem cells
HUVECs human umbilical vein endothelial cells
DBM demineralized bone matrix
OIM osteogenic induced medium

OCN osteocalcin

CD31 cluster of differentiation31

ALP alkaline phosphatase

DAPI dimensional assessment of personality impairment

Declarations

Ethics approval and consent to participate

1 Title of the approved project Repair of osteonecrosis of the femoral head in rabbits with prevascularized bone microtissue; (2) Name of the institutional approval committee or unit Experimental Animal Ethics Committee of the Binzhou Medical University Hospital; (3) Approval number 20211008-12; (4) Date of approval October 8th, 2021.

Consent for publication

Not applicable.

Availability of data and material

All data generated or analysed during this study are included in this published article.

Competing interests

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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Authors’ contributions

QR: Investigation, Methodology, Data analysis. QR, MMC, KJC: Resources, Methodology. QR YZG YZ: Writing and original draft preparation. QR CXY: review and editing the manuscript. CXY NC: Conceptualization, Supervision, Funding acquisition. All authors discussed the results, read and approved the final version of the manuscript.
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Acknowledgements

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References


Figure 1

Live and Dead staining of hUCMSCs and HUVECs on DBM microcarriers (fluorescence microscope ×40/×100)

Figure 2

Live and Dead staining of hUCMSCs and HUVECs on Sigma microcarriers (fluorescence microscope ×40/×100)
Figure 3

Alkaline phosphatase staining was used to determine the osteogenic ability of osteogenic microtissues (fluorescence microscope ×100)

Figure 4

The fibrin gel migration assay was used to determine the angiogenesis of angiogenic microtissues.
**Figure 5**

MRI and HE staining results of femoral head necrosis modeling in rabbits HE×40

(a) (b) (c) (d) (e)

**Figure 6**

MRI was used to detect the repair of necrotic femoral head

Note: a is the module making group; b is the blank group; c is the bone tissue group at static stage; d is the bone tissue group at dynamic stage; e is the prevascularized bone tissue group
Figure 7

Microct was used to detect the regeneration of bone tissue

Note: a is the module making group; b is the blank group; c is the bone tissue group at static stage; d is the bone tissue group at dynamic stage; e is the prevascularized bone tissue group

Figure 8

Comparison of BV/TV values of necrotic femoral head in 4 groups
Figure 9

Biopsy of the specimen 2 months after implantation HE×200

Note: a is the module making group; b is the blank group; c is the bone tissue group at static stage; d is the bone tissue group at dynamic stage; e is the prevascularized bone tissue group
Figure 10

Immunofluorescence staining of femoral head 2 months after implantation laser confocal microscope×400

Note: a is the module making group; b is the blank group; c is the bone tissue group at static stage; d is the bone tissue group at dynamic stage; e is the prevascularized bone tissue group

Supplementary Files

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