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# Research progress on effects of 3D-printed antler powder scaffolds on biological behavior of macrophages

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***Abstract:*** *Macrophages are important cells in the regulation of host immune and inflammatory response. Host inflammatory response is the normal response of tissue to injury and foreign body. The intensity and persistence of inflammation affect the biocompatibility and stability of biomaterials in vivo. Therefore, its responsiveness to biomaterials plays an important role in the cognitive material-host response. The effect of scaffold material on the biological behavior of macrophages plays an important role in the process of bone tissue regeneration. Calcined deer horn cancellous bone is a new type of bone graft material. By studying the influence of deer horn powder /PVA scaffold material on the biological behavior of macrophages, the relationship between subtypes of macrophages and bone regeneration is understood, and the advantages of deer horn powder as a new bone graft material are understood.*

***Keywords:*** *red deer horn powder, macrophage, polarization, bone tissue regeneration*

## 1. Introduction

The use of biomaterials to repair bone defects is now widely used in orthopedics and dentistry, and antler is one of the only fully regenerated organs in mammals. Fast growth rate, annual natural shedding, renewable, structural composition close to human bone. Red deer horn powder is a kind of natural bone material, which has better biocompatibility than other inorganic materials. Because of its wide source, it can avoid the ethical problems involved in the slaughter of animals, and can be used as a large number of raw materials for xenogeneic bone scaffold materials. [1] Among them, velvet antler polypeptide can stimulate the growth of cartilage and osteoblast-like cells, directly promote the bone and cartilage division and fracture repair. Calcined antler cancellous bone CACB, as a new type of bone graft material, has been confirmed by Deng Xuliang's group that calcined antellous bone cacb can promote bone defect repair [2] At the same time, it can avoid animal ethical problems caused by animal slaughter. Therefore, deer antler has become a new direction in the research of xenograft materials. Host inflammatory response is a normal tissue response to injury and foreign body. The intensity and persistence of inflammation affect the biocompatibility and stability of biomaterials in vivo. Macrophages are important cells in the regulation of host immune and inflammatory responses [3] Therefore, its responsiveness to biomaterials plays an important role in the response to cognitive biomaterials -- the host response. There is an interaction between scaffold materials and macrophages.

## 2. Regeneration mechanism of bone tissue

As a complex and highly dynamic organ, when bone tissue is damaged, the surrounding environment initiates the coordination of various cell types, and finally achieves bone healing[4]Blood vessels rupture, platelets are activated, a variety of coagulation factors, cytokines and chemokines are released, triggering hemostatic effect, a variety of cell types are recruited and migrated to the injured site, contributing to the initial stage of bone tissue repair[5]. In clinical practice, autologous bone transplantation or allograft bone is mainly used, but autologous bone transplantation is the gold standard for treatment. However, large segmental bone loss requires a large amount of autologous bone, and autologous bone not only has a

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small amount of bone, but also a high incidence of complications at the bone extraction site[6] While allograft is prone to immune rejection, therefore, artificial bone implant materials become a new choice for the treatment of bone defect repair, and have good research and application prospects. The ideal artificial bone implant materials should have both good biocompatibility and resorbability, and have a good promoting effect on bone regeneration[6].Bone homeostasis is maintained by a dynamic balance between the activity of osteoblasts and osteoclasts, and is a guarantee for achieving bone development and repair.Osteoblasts, which are mainly responsible for bone formation, originate from mesenchymal stem cells and bone stem cells in bone marrow, and eventually differentiate into bone cells.The main function of osteoclasts is bone resorption, which originates from the bone immunological effect of hematopoietic stem cells macrophages[7].In addition to the above bone cells, inflammatory cells, endothelial cells and Schwann cells in the microenvironment around bone are also involved in bone development and repair[8-10]As an important part of the innate immune system, macrophages play a crucial role in host defense and inflammatory response. According to the different sources of macrophages, they can be divided into tissue-domiciliary macrophages which mainly perform phagocytosis function under homeostasis and inflammatory macrophages derived from bone marrow monocytes under inflammatory stimulation.Tissue domiciliary macrophages are found in almost all tissues and are involved in tissue repair, immune monitoring and homeostasis maintenance.Inflammatory macrophages arrive at the site of inflammation through the blood circulation and are stimulated by the microenvironment to differentiate into different functional types of inflammatory macrophages: M1 macrophages and M2 macrophages.M1-type macrophages have pro-inflammatory effects and are involved in host defense against pathogen infection.M2-type macrophages contribute to tissue repair and inflammation resolution[11].

## 3. Feasibility of 3D printing technology to assist jaw defect repair

For a long time, autologous bone transplantation has been used to treat the patients with jaw defect repair[12]Although this method can effectively reduce the occurrence of immune rejection, it inevitably causes additional trauma to patients, which increases the difficulty of postoperative rehabilitation of patients, and also increases the probability of postoperative infection.Not only the operation time is long, intraoperative bleeding, but also in the reconstruction of the jaw and other operations should be operated will cause the impact on the normal occlusal relations[13].Since the advent of 3D printing technology, its application in the medical field has gradually become prominent.For oral and maxillofacial surgery with bone defects beyond the limit, and for pre-implantation preparation due to insufficient alveolar bone mass[14], Compared with autogenous bone, xenogeneic bone and artificial bone, due to the problems of availability, healing time, donor site lesions and immune response, tissue engineered bone composed of 3D printed medical biological scaffold materials, stem cells and cytokines can better solve this problem in clinical practice[15].And after clinical trials[16]Compared with traditional surgical treatment, 3D printing technology has shown significant advantages in terms of surgical time, surgical trauma, intraoperative blood loss and recurrence rate. At the same time, 3D printing technology can achieve a certain accuracy of jaw repair through computer calculation, achieving both aesthetic and functional effects, which confirms the feasibility of using 3D printing technology in jaw repair.Xiao Chenliang[17], when know[18]Schepers[19]The same conclusion was also reached through clinical comparative experiments, indicating that 3D printing technology can effectively reduce the damage to patients in the application of jaw repair, and can better meet the aesthetic and functional needs of patients after maxillofacial injury.Printing consumables are particularly important in the 3D printing bone tissue engineering scaffold. The bone tissue engineering scaffold printed with a single material often has limitations in performance, while the composite material composed of a variety of materials can give full play to the complementary role between materials, and gradually become the preferred printing consumables for bone tissue engineering scaffold[20].The ideal bone tissue engineering scaffold should have the following characteristics[21]① It is beneficial to seed cell attachment, growth and differentiation, has good biocompatibility, and will not have harmful effects on surrounding tissues;② It is beneficial to vascular reconstruction, can integrate with host bone through bone conduction and bone induction, can repair irregular shape defects and bear mechanical load, and has the potential of biodegradation, and can be gradually replaced by new bone;③ It can be stored and disinfected.Because a single printing material has limitations in performance, and if the use of two or more materials composed of composite materials this can make up for the advantages and disadvantages of each material, the performance of the scaffold material can be effectively improved, conducive to cell colonization and blood vessel growth, and has good biomechanical properties, can meet the needs of bone tissue engineering scaffold to a greater extent[22].

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## 4. Influence of culture environment on biological behavior of macrophages

Macrophage is one of the important innate immune cells in human body. Its cell phenotype is highly plastic, and its activation is a highly active process.Undifferentiated macrophages (M0) can be polarized into two opposite functional phenotypes under different microenvironmental stimuli: the classical activated type (M1) and the alternative activated type (M2).The M1/M2 phenotype of macrophages is associated with the pathological process of various diseases, but the specific mechanism is not clear. Currently, the major pathways involved are known to be JAK/STATX signaling pathway, IRF signaling pathway, Notch signaling pathway, and PI3K/Akt signaling pathway[23].After implantation of biological scaffolds as foreign implants, the biological scaffolds can cause inflammation in the body. In the early stage of inflammation after implantation of biological scaffolds, M1-type macrophages express high levels of pro-inflammatory cytokines after activation, and are responsible for angiogenesis and clearance of necrotic tissues[24].In advanced stages of inflammation, M2 macrophages express high levels of arginase 1 and anti-inflammatory molecules after activation, responsible for immune regulation, matrix deposition, and tissue remodeling[25].

Th1 cytokines such as IFN-γ, tumor necrosis factor-α (TNF-α), etiological associated molecular patterns such as LPS or endogenous danger Signal 16 all induce M1 polarization[26].M1-type macrophages are highly phagocytic and can quickly clear invading pathogens, mediate tissue damage, and initiate inflammatory response to protect the host. They play an important role in the initial stage of inflammation, but excessive macrophages can also damage tissue regeneration, affect wound healing, and cause host damage[27].

M2-type macrophages activate STAT6 by IL-4 receptor alpha (IL-4Rα), which induces polarization by Th2 cytokines IL-4 and IL-13.In addition to IL-4 and IL-13, other cytokines such as IL-10 can regulate M2 polarization by activating STAT3 through the IL-10 receptor (IL-10R)[28].

## 5. The effect of biological behavior of macrophages on bone tissue regeneration

Macrophages have diversity and plasticity, and can differentiate into different phenotypes in different microenvironments, so as to play different roles. This process is called macrophage polarization.M1-type macrophages secrete high levels of pro-inflammatory factors that promote inflammation and kill microorganisms.M2-type macrophages are characterized by high expression of scavenger receptors, which play the functions of phagocytosis and immune regulation, and can promote tissue repair[29].

After bone injury, mononuclear macrophages in peripheral blood or residing in tissues will change from resting state to active state, and will be polarized into M1 type (classical activated type) or M2 type (selective activated type) macrophages[30].Bone regeneration depends on the stable and effective functioning of the immune environment, both of which are regulated by multiple signaling pathways[31].Regeneration of bone tissue is a multi-system cooperative process, and the role of immune system may be one of the key factors for the success of bone repair materials.Macrophages, as the first batch of arriving immune cells, are key mediators in the reaction with foreign bodies during inflammation and immunity, and have the ability to respond to signal polarisation into different functional phenotype cell subtypes (M1/M2) in the local microenvironment.The derived signals of these macrophage polarized subtypes are almost continuously present in the whole process of bone tissue formation[32].

M1-type macrophages can promote fracture healing in the early stage of fracture development, and the absence of M1-type macrophages may delay bone tissue regeneration.M1 macrophages not only negatively regulate bone tissue repair, but also positively regulate bone tissue repair, and can promote bone tissue repair. A large number of studies have shown that M2-type macrophages can promote the differentiation of MSC into osteoblasts and facilitate the repair and regeneration of bone tissue.M2-type macrophages play an important role in the middle and late stage of bone inflammatory repair, especially in its ability to clear cell debris and promote mineralization of osteogenic differentiation.Macrophages are important for maintaining bone homeostasis and promoting bone repair.Due to their heterogeneity, the diversity of their functions makes them an effective potential research direction for promoting bone repair.On the one hand, M0 type macrophages before polarization maintain the physiological state of bone tissue, on the other hand, M1/M2 type macrophages exist throughout the whole process of bone repair, and M1-type macrophages are mainly involved in the progression of initial inflammation, and M2-type macrophages are mainly involved in the later stage of bone repair[11].

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## 6. Study the current situation

Antlers can be regrown and shed naturally throughout a deer's life, and have a composition structure similar to that of human bone[33]Zhao Xiaoqi's group has proved that the 3D-printed deer antler /PVA scaffold can be used as a scaffold material for bone tissue engineering by testing its porosity, pore size, water absorption, compression chemical performance and biocompatibility.Shanshuai Group[34]The macrophages of antigen-free cancellous bone and unantigen-free cancellous bone were detected by contrast experiment. The results of qRT-PCR showed that the pro-inflammatory factors of TNF-α, IL-6 and IL-6 were higher than those of the control group after one day of co-culture.In ELISA, CD206 in the experimental group was higher than that in the control group. It was concluded that ARCB powder had a positive effect on inhibiting the inflammatory expression of M1-type macrophages and promoting the emotional polarization of the macrophages towards M2.In summary, the experimental research methods used in the above experiments have certain reference significance for future research.

## 7. Summary and prospect

To sum up, macrophages, as cells that play an important role in the process of bone regeneration, are characterized by diversity and plasticity. They are differentiated into different subtypes in different environments, thus playing different roles in the process of bone regeneration. By studying the influence of red deer horn PVA scaffold material on the biological behavior of macrophages, we can understand its influence on bone tissue regeneration. Red deer horn powder as a new xenograft material has important reference significance in the relevant research.

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