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A new strategy for repairing large bone defects using an interventional micro-circulation system.

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Abstract

Repairing large bone defects remains a difficult clinical problem because of the variability in the defects. The traditional methods, such as autograft, allograft bone and biological filler material transplantation are still facing difficulties in clinical application nowadays. Tissue engineering technology has the potential to solve this problem, and it has thus become a popular research topic. However, ideal solutions for engineering large pieces of bone tissue with vascularization and other key technical problems have not been found to date. Using conventional repair approaches for large bone defects also faces enormous challenges. In order to circumvent these difficulties, we proposed and established a new method called the interventional micro-circulatory system (IMCS) for repairing large-segment bone defects in situ. On the one hand, the system provides nutrition and removes inflammatory cytokines, oxygen free radicals, and toxic metabolites to improve the ischemic injury microenvironment; on the other hand, seed cells are supplied dynamically, and their biological behavior ability such as the migration, proliferation, differentiation, and directional distribution are promoted. We demonstrate the repair of large bone defects in an animal model using this system. Compared with conventional reconstruction methods, this strategy has the potential to provide a new approach to clinical stem cell transplantation for the treatment of large bone defects.

Keywords: SDF-1/CXCR4 axis, BMSCs, Segmental defects, Intervention microcirculation system, Stem cell transplantation.

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Introduction

Large-segment bone defects are commonly seen in clinical practice. They are caused by different types of trauma, infections, congenital malformations, and cancers, and their restoration remains a challenge. In recent years, some progress has been made in the approach to treat large-segment bone defects by using seed cells implanted into a scaffold containing a complex of bone morphogenetic proteins to construct a tissue-engineered bone graft [1,2]. However, the defect area is characterized by ischemia and hypoxia, the presence of inflammatory cytokines, oxygen free radical accumulation, and a scar-forming microenvironment, which greatly limits seed cell survival, homing, proliferation, and differentiation and ultimately limits bone regeneration. To aid in finding clinical solutions, large animals can be used as segmental bone defect models and to develop clinical treatment targets. Unfortunately, after implanting a large volume of tissue-engineered bone (TEB) *in vivo*, the implanted cells rely mainly on the host tissue fluid and blood to provide nutrients, and their survival rate is low even when the thickness of the new tissue is less than 0.5 mm, suggesting that implantation of a large segment of TEB seed *in vivo* makes it difficult for cells to obtain nutrition by diffusion [3,4].

A serious issue in tissue engineering is the inability to maintain large grafts of living cells upon transfer from *in vitro* to *in vivo* conditions. Most cells more than few hundred micrometers from the nearest capillary will die, mainly because of diffusion limitations. Engineering bone constructs *in vitro* with a pre-existing vascular component is a major challenge [5]. One study found that [6,7] autologous bone with a vascular network of blood vessels possessing physiological functions and containing the seed cells could promote bone formation and improve function when transplanted into the bone defect region. However, the occurrence of secondary damage has limited its application. Zhang et al. [8] reported that seed cells carrying pro-angiogenic factors can promote large bone vascularization, while a prerequisite for the survival of seed cells is early nutrition and blood supply, namely, applying TEB to the damaged area to repair large bone defects have no choice but to overcome the problem of the lack of blood supply and

provided a nutritional microenvironment early in the repair process. In addition, the lack of nutrition in the damage zone and accumulation of inflammatory cytokines, oxygen free radicals, and high concentrations of potassium ions generates traumatic hematoma products that are toxic to cells, negatively affecting cell survival [9] and limiting seed cell survival and bone regeneration. Therefore, there is a need to develop an approach that will improve the nutrition network in the ischemic injury microenvironment, increase the number of seed cells for transplantation, and promote their survival, proliferation, and differentiation to achieve a regenerative effect in large bone defects.

Hypothesis

The interventional micro-circulatory system consists of a (1) perfusion discharge device, (2) membrane-guided bone regeneration system, and (3) portable circulating pump (Figure 1) and perfusion discharge device consisting of an *in vivo* part and an *in vitro* part. The *in vivo* part is a subcutaneous implantable drug delivery device, and the *in vitro* part consists of catheter with needle and micro-circulation pumpnamely perfusion devicecirculation pump and discharge device as show in the Figure 1. The perfusion device is connected to the discharge device to form a circulation loop that pumps nutrients, seed cells, and inducible factors into the bone defect, where the local membrane-guided bone regeneration system is located, and removes locally generated metabolic waste. After the skin is disinfected, a needle is inserted subcutaneously, and the needle is connected to the infusion tube, which is connected to the circulation pump, establishing the system as follows: (1) metabolic wastes and harmful factors are removed to improve the injury microenvironment; (2) bone regeneration is guided by the membrane to prevent fibrous tissue invasion and the loss of bone morphogenetic protein-2 (BMP-2) into the surrounding tissue, which could cause heterotopic ossification or stiff joints; the retention of BMP-2 improves the efficiency of BMP-2, and reduce side effects, thus not only preventing scar formation but also inducing safe bone regeneration process in membrane guided bone regeneration room/system; (3) through the circulation channel, C-X-C chemokine receptor 4 (CXCR4)-overexpressing seed cells and the adult bone morphogenetic protein BMP-2 are pumped into the damaged area, promoting seed cell proliferation and directed differentiation; and (4) using a biodegradable guide film and controlling the slow release of stromal cell-derived factor-1 (SDF-1) will help to continuously attract CXCR4+-BMSC to locate at the site of bone formation.

Testing the Hypothesis

The *in vitro* system components include the perfusion delivery and discharge devices consisting of two subcutaneous implantable drug delivery systems, discharge tubes, and needles. This device has good long-term biocompatibility when implanted in the body to form a "guided dosing channel," and the circulating pump delivers nutrition to the bone microenvironment and removes metabolic waste. The circulation pump is composed of micro-electromechanical system (MEMS). The MEMS is portable because of its small size, and it can be adjusted to re-administer a drug or to adjust the infusion rate [10]; the use of an MEMS in an *in vitro* cell culture system ensures suitable culture conditions for BMSCs and the long-term maintenance of the proliferation and differentiation of the cultured seed cells [11]. These characteristics of the MEMS make possible the fine control of the infusion time and dose of nutrient solution through the circulating pump. In addition, the MEMS can control the waste that is discharged from the established microcirculation membrane-guided bone regeneration system. The circulation pump infuses the nutrient solution into the body through the entry injection site, and the metabolic wastes are removed from the body through the exit site. The nutrient perfusion liquid circulates through the system at a pressure greater than the ambient pressure, causing the liquid nutrients to move from the area of higher pressure in the tubing to the area of lower pressure in the pores of the scaffold; on the contrary, the negative pressure within the circulation tubing causes the hydraulic pressure in the pores of the film-guided scaffold of the bone metabolic regeneration system to be higher than the pressure in the circulation tubing, causing the metabolic waste fluid to move into the circulation tubing namely discharged device to be removed from the body. This continuous and dynamic action of the circulating pump system can not only fully deliver nutrients into scaffolds but also remove metabolic waste products from scaffold, thereby improving the regenerated TEB microenvironment and promoting seed cell survival (Figure 1).

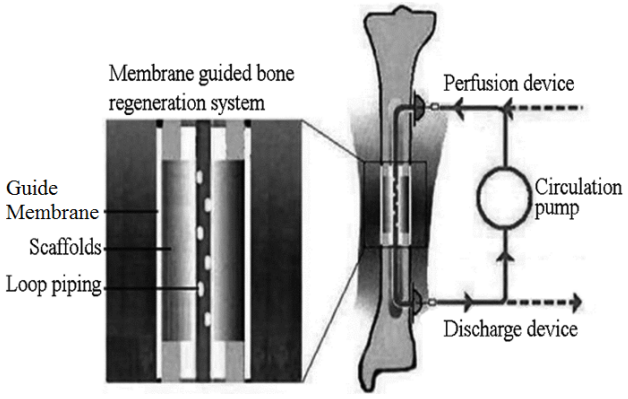

Figure 1. *IMCS situ remediation tibial large segmental defects.* ***first step:*** *perfusion nutrition, waste discharge;* ***second step:*** *seed cells containing bone, osteogenic factor infusion of nutrient solution into the membrane guided bone regeneration system;* ***third step:*** *start Cycle.*

Seed Cells

CXCR4 is a seven-transmembrane domain G protein-coupled membrane receptor and is widely expressed on the surface of mononuclear cells, stromal cells, and CD34+ cells. SDF-1 and CXCR4 constitute the SDF-1/CXCR4 axis, which plays an important regulatory role in the tissue injury and bone repair process. During bone regeneration and repair, the secretion of SDF-1 is increased; CXCR4+ stem cells, which are involved in

bone repair and reconstruction, move along the SDF-1 concentration gradient to reach the site of the injury. Primary BMSCs expressing a high level CXCR4 will be used as seed cells. Along with the continuously releasing of SDF-1 factorCXCR4+-BMSC can be attracted to home to the region of bone defectand along the SDF-1 concentration gradientgradually distributed to the bone formation site [12,13]. Studies have shown that the repair of large bone defects requires transplants that can ensure the long-term migration and colonization ability of seed cells. CXCR4-expressing BMSCs can meet this requirement, which is a key factor in the success of the repair of the bone defect [14].

Membrane-Guided Bone Regeneration System

The structure of the membrane-guided bone regeneration system (Figure 1) consists of the circulation loop tubing, scaffold, and directing film. The circulation tubing is located in the central tunnel of columnar coral porite scaffold with interconnected three-dimensional pore, creating a pathway to deliver nutrients and remove waste products as well as a BMSC migration channel. The scaffold is made from natural coral porites because this material has a similar porous structure as cancellous bone, is composed of calcium carbonate, and all of the pores are interconnected. The scaffold is highly porous and permeable, which favors nutrient and metabolite exchange, and has high biocompatibility; in addition, the scaffold is more easily degraded than ceramic stents, and the degradation rate can be matched with the bone tissue regeneration rate. Therefore, this scaffold material favors nutrition delivery and metabolic waste removal and provides an ideal place for BMSCs to migrate, proliferate, and differentiate [15-17].

The guide film is a composite derived from the stromal cell factor-1 chitosan/collagen carrier membrane. As reported in the literature [18-20], the G protein-coupled transmembrane receptors of SDF-1 are activated to induce CXCR4+-BMSCs to migrate to the site of bone formation. The chitosan/collagen carrier has controllable biocompatibility and biodegradability and can be used as an SDF-1-releasing carrier, which can delay the removal of SDF-1 and extend the directed migration of CXCR4+-BMSCs [21]. Therefore, the SDF-1 complex, chitosan, and collagen are combined to construct the SDF-1/chitosan/collagen composite film, which has the ability for the slow controlled release of SDF-1 to ensure the long-term directed migration of CXCR4+-BMSCs. However, under normal circumstances, this kind of membrane was only used to form a mechanical barrier that can prevent the rapid ingrowth of tissue cells (such as fibroblasts), fibrosis from scarring while provide a space for guided bone regeneration by ensuring seed cell differentiation and proliferation [22], the Off-the shelf membrane does not attract CXCR4+-BMSCs through directional migration and distribution. Thus, the developed SDF-1/chitosan/collagen composite film has both a barrier function and, through the use of the SDF-1/CXCR4 axis to recruit stem cells, the ability to actively regulate CXCR4+-BMSC directional distribution in the bone formation site.

Osteogenic Factors

After implantation, the directed differentiation of bone seed cells requires inducing factors in the microenvironment, for example BMP-2 is a member of the transforming growth factor superfamily and plays a significant role in bone formation. BMP-2 binds to type I and type II transmembrane serines as a dimer or to hydroxybutyrate isomers of the threonine kinase receptor protein as a tetramer to trigger the nuclear and osteoblast-specific transcription factor Runx2/Cbfa1/Osf2/AML3 interaction and positive regulation of gene expression in bone cells. The BMP-2 protein plays a vital role in the migration of bone progenitor cells, mesenchymal cell proliferation, differentiation of cartilage- and bone-derived cells, vascular ingrowth, and remodeling.

The initial reaction is the release of BMP-2 from the matrix after the fracture and the homing of primitive mesenchymal cells that also secrete BMP-2. BMP-2 is expressed in the early cartilage stage of bone formation and continues to induce mesenchymal differentiation into cartilage cells and osteogenic differentiation until woven bone formation. Tsuji et al. [23] stated that in the absence of BMP-2, the bone fracture healing process is blocked, and although other osteogenic stimuli exist, they cannot compensate for the function of BMP-2. BMP-2 is essential to start the process of bone repair. Hosogane et al. [24] demonstrated that BMP-2-induced BMSC osteogenic differentiation through the SDF-1/CXCR4 axis also played an important role, as it promoted the osteogenic potential of BMSCs. Otsuru et al. [25] found that the SDF-1/CXCR4 axis caused bone marrow-derived precursor cells to migrate to the bone formation site, with the full ability to differentiate into bone following the induction by BMP-2. Therefore, an interventional micro-circulatory system (IMCS) is used to pump BMP-2 into the bone formation site. The SDF-1/CXCR4 axis can further enhance BMP-2 to induce BMSC to differentiate into bone ability to differentiate into bone.

The Relationship between the IMCS and Large Bone Regeneration

SDF-1 can induce many endogenous stem cells to migrate to the damaged area and regenerate the damaged bone tissue [27,28], and the SDF-1/CXCR4 axis of the induced stem cells plays a key role in homing to bone formation area and promoting directed differentiation. The guided bone regeneration membrane was placed in the periosteum defect site, where it controlled the slow release of SDF-1, similar to the outer membrane of embryonic cartilage, and the live bone graft membrane efficiently expressed SDF-1 to attract a sufficient number of CXCR4+-BMSCs through directed migration to the bone formation site to regenerate bone. In addition, the study demonstrated that SDF-1 and BMP-2 synergistically promote osteogenic potential of BMSCs. Thus, the use of the IMCS combined with the SDF-1/CXCR4 axis can promote stem cell to home as to recruit a sufficient number of CXCR4+-BMSCs to the bone formation site; in addition, the SDF-1/CXCR4 axis was used to promote the BMP-2-induced osteoblast action of CXCR4+-BMSCs to regenerate

and repair large bone defects. We will apply IMCS to repair goat tibial defect model *in vivo* and determine its effectiveness in inducing bone repair.

Conclusion

Based on the literature and the results of previous studies, we established an IMCS composed of the following: (1) the combined capabilities of the delivery of nutrients and the removal of waste to improve the ischemic injury microenvironment; (2) membrane-guided bone regeneration that did not cause scarring and safely created an osteoinduction microenvironment; and (3) the addition of a sufficient number of bone seed cells, inducing factors of BMP-2 and (4) the use of the SDF-1/CXCR4 axis to attract stem cells to the bone formation site and something important, under genuine stress stimulus ,the morphological repair and functional reconstruction of large bone defect form the nature of the case be promoted. Compared with conventional reconstruction methods, this strategy is a potential new approach to treating large bone defects using clinical stem cell transplantation.

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