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Research progress and application prospect of tissue engineering for periosteal materials

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Abstract: *For a long time, how to repair the bone defects caused by bone trauma, bone tuberculosis and other bone diseases, is one of the difficult problems faced by surgeons. In the process of bone formation and repair, the periosteum, as an important "place" for blood supply and bone formation and regeneration, its importance is self-evident. However, the number of healthy periosteum cannot meet the repair of large bone defects, and tissue engineering periosteum emerged at the historic moment. At the same time, the selection of biocompatible and prepared biomaterials to promote bone healing and bone repair has gradually attracted great attention from clinicians and researchers. Based on the material, design and preparation methods of tissue engineering periosteum, this paper reviews the research progress and application prospect of tissue engineering periosteum materials, which provides valuable reference for further profound research, and also provides basic ideas and methods.*

Keywords: *bone tissue engineering; tissue engineering periosteum; oral repair; bone defect*

1. Introduction

The repair of bone defects caused by surgical treatment such as bone tumors is a major problem facing orthopedics, and the long-term loss of total dentition caused by severe periodontitis also makes the treatment of clinical dentists difficult. Autologous bone transplantation, the "gold standard" called bone repair, is to transplant bone from other parts of the body into the defect. It has excellent vascularization and osteogenesis properties and good repair effect. However, due to the limited source of autologous bone reconstruction, it cannot completely meet the needs of bone defect area. In addition, allogeneic bone graft, xenograft and artificial bone graft also have their own advantages and disadvantages. Allogeneic bone grafts often produce immune rejection due to body intolerance, which limits their clinical application to some extent. In addition, the treatment of large-segment bone defect reconstruction with tissue-engineering bone stent reconstruction also has limitations such as insufficient vascularization, slow osteogenesis, and difficulty in combining with bone formation. Therefore, how to obtain efficient and well biocompatible tissue engineering periosteal membrane has become an urgent problem to be solved by clinical researchers.

In recent years, tissue engineering and clinical medicine promote each other together, and domestic and foreign scholars have explored tissue engineering bone transplantation as a treatment option for bone defects, while the research on periosteum applications is limited. During bone formation, the periosteum promotes the biomineralization of bone by coordinating various types of cells, such as periosteal cells, bone marrow mesenchymal stem cells, and osteoblasts. In the process of bone formation and regeneration, the current mainstream research believes that the above-mentioned cell-mediated mineralization of the bone formation is essential. Meanwhile, the preparation of biomaterials to promote bone healing and repair has been a hot topic in bone regeneration and repair. Because the periosteum plays an extremely critical role in osteogenesis and bone repair processes, the preparation and application of the tissue engineering periosteal materials are increasingly valued by surgeons and researchers. Based on this, this paper summarizes the material selection, application and research progress of tissue engineering periosteum.

2. Role of the periosteum

In the process of bone defect healing, the importance of bone is self-evident, and the periosteum also plays an irreplaceable role. Biomineralization of bone is a critical stage of bone regeneration and repair into vascularization and osteogenesis. With vascular properties of periosteum on the surface of cortical bone, it through the periosteal cells, bone marrow mesenchymal stem cells, osteoblasts and other cells to promote bone biomineralization, osteoblasts and chondrogenic progenitors and other related biological activity factors provide blood circulation for bone, the loss of periosteum can lead to bone nutrition supply, leading to poor bone healing and failure.

Studies at home and abroad have shown that removal of osteoprogenitor cells from bone marrow has almost no effect on bone tissue regeneration, while removal of periosteum reduces new bone formation by 73%[1]. HUH class[2]in the experimental study of canine mandibular defect, it was found that the 5 cm defect area could be repaired by self-osteogenesis, while the bone defect segment lacking the periosteum could only repair the 1.5 cm defect area,This suggests that the periosteum can function in induced osteogenesis.

3. Characteristics of the biomimetic periosteum

Natural periosteum is composed of multilayer structure, and its outer layer contains rich fibers of osteoblastic fibroblasts, collagen and elastic fibers, as well as rich blood vessels and nerve networks, which have laid the foundation for the mechanical strength of the periosteum. The inner periosteal layer contains a variety of cells that can provide an abundance of seed cells[3]growth factors and minerals needed for bone mineralization make the periosteum accelerate the formation of new bone and promote fracture healing in the treatment of bone defects. Therefore, biomimetic periosteal materials should have strong proliferation ability and differentiation potential. In the process of new bone tissue generation, it should provide sufficient nutrients for bone defect repair, rich growth factors and necessary cell support, and can synthesize, secrete and mineralize bone matrix to promote fracture healing.

Meanwhile, increasing studies have shown that successful bone grafting depends on adequate vascularization of the host[4].In the case of reduced blood supply at the bone defect site, the induction of vascularization is a necessary factor for the survival, integration and function of tissue-engineered bone, and the degree of vascularization is directly proportional to the degree of prognosis in bone repair.

Secondly, once the biomimetic periosteum is implanted into the body, it must meet the conditions of non-toxic biological materials, good biocompatibility, and can reduce the adverse reactions such as immune rejection caused by inflammation. Various types of polymers are being used for tissue engineering. The deeper research is polyvinylidene fluoride (PVDF). PVDF has three crystal types: α, β and γ, and the complex crystal structure corresponds to the different properties of the material.M. Wang class[5]PVDF / PVP composite nanofiber membranes with a core-shell structure were developed. The survival rate of mouse fibroblasts was 94% after 3h, indicating that PVDF / PVP had good biocompatibility and can effectively promote wound healing. Many studies have shown that the good biocompatibility of PVDF and its nanofibers offer a broader prospect for the selection of clinical materials.

One of the inherent properties of bone is the piezoelectric properties[6]. At present, researchers have focused on improving the biological activity of tissue-engineered bone to promote its bony integration with the tissue, and few scholars have paid attention to the electrophysiological microenvironment in which bone growth is located. In fact, the electrical microenvironment is one of the most important microenvironments where the bone tissue is located. Piezoelectric coefficient of bone tissue (0.7 pC / N)[7]can stimulate skeletal growth because of bone activation by biological stress composed of body movements. Piezoelectric materials have particularly great potential as active biomaterials for bone tissue regeneration[8]. The β phase has an all-trans conformation that can provide a net dipole moment and thus is therefore piezoically suitable for the development of electroactive structures. Deng[9]the P (VDF-PTrFE) composite polydopamine and BaTiO3 nanoparticles were used to mimic the potential of bone defect repair. In vitro and in vivo experiments show that these membranes continuously maintain the electrical microenvironment, improve MS dissemination and osteogenic differentiation, and even rapid bone regeneration.

4. Preparation of the biomimetic periosteum

Preparation methods generally used for bone tissue scaffolds include foam replication method, electrospinning, freeze-drying, gas foaming, solvent casting / particle leaching, phase separation, and molecular self-assembly. The 3D printing technology is a new dimension in the BTE field of —— 3 D bioprinting.3D printing technology can customize personalized bone stents according to the size and shape of the bone defect for immediate repair. Among these different fabrication methods described above, electrospinning is the most effective one-step method for developing scaffolds with both fibrous structure and piezoelectric properties. The comparative advantage of electrospinning is that piezoelectricity can be achieved in situ during nanofiber network formation, which is beneficial for tissue engineering.

4.1 Electrospinning method

With the progress of tissue engineering, the manufacturing technology of bionic periosteum has also been developed. Electrospinning is one of the most effective devices to prepare fiber structure and piezoelectric stent composed of high voltage power supply, injection pump and receiving device. By adjusting the process parameters such as the inner diameter, flow rate, voltage and receiving distance of the spinning nozzle, the best parameters for preparing nanofibers can be determined, so that the bionic periosteum meets the optimal porosity suitable for cell growth and can effectively promote the growth of osteocytes and shorten the time period of bone healing.

PU / tio 2 / HA based bone integrated membranes were prepared by electrospinning method by Nattawat et al. It was found that the PU electrospinning membranes containing Ti 2 and HA were smaller than those containing Ti 2 and HA. PU membranes with TiO 2 and HA accumulate on the fiber surface and exhibit higher stiffness and mechanical strength than PU membranes without TiO 2 and HA. The electrospin PU membrane with a ratio of 50:50 (TiO2: HA) has a good biological performance for enhancing osseointegration. This membrane is promising in oral and maxillofacial surgery.

According to the purpose of the solution, electrospinning is divided into solution electrospinning and melt electrospinning.

4.1.1 Solution was electrospun

The solvent used in solution electrospinning is usually designed to evaporate as the material passes through the air gap between the spray plate and the collector plate. However, when this evaporation process is unstable, it causes the residual solvent to accumulate on the collected fibers. The residual charger associated with the residual solvent on the collected grid creates repulsive forces between successive layers, weakening the interfibrous bonds. Therefore, this process is limited to the production of soft fiber pads with a thickness of up to 4 mm. Another major issue associated with residual solvents is their potentially deleterious toxic effects on cells and tissues when used for TE.Venugoupal[10]and et al. used electrospinning to create a highly porous scaffold from a mixture of PCL, nHA and biocomposite (PCL / nHA / col) of collagen. Using this method, scaffolds with pore sizes of up to 2 – 35 μ m were prepared to form nanofiber structures with porosity over 80%. The incoherence and stochasticity of fiber deposition in this approach yields a surface roughness suitable for cell attachment in bone scaffolds.

4.1.2 Molten electrospinning

Melt electrospinning is the electrospun of a polymer in the molten state. In the absence of a solvent, the process does not significantly reduce the surface tension, a common problem in solution electrospinning. The nanofiber membranes produced by this method have a larger fiber diameter. Although the melt electrospinning nanofiber membrane performed poorly in the initial cell adhesion, the problem of poor cell inward growth and mineralization gradient were less obvious than the solution electrospinning. Melt electrospinning[11]of the lateral whipping motion was significantly reduced. Moreover, its potential cellular and tissue toxicity is also small in the absence of residual solvent and associated residual charge. The melt electrospinning device is more complex and less widely used than the solution electrospinning.

5. Materials of the biomimetic periosteum

To repair bone defects by artificially constructing an environment dedicated to promote natural tissue and cell regeneration and growth. In the context of bone tissue engineering, 3D (3 D) structures with controlled porosity are needed, with the aim of creating a suitable environment for the growth of bone tissue. These structures are often referred to as bone stents. Tissue engineering bone is driven by a very feasible method of materials and manufacturing process.

In the rapid repair of bone defects, tissue engineered bone replacement materials with piezoelectric effect are used to induce early hard tissue growth at the bone damage site and accelerate the healing of bone defects. As a bone replacement material, piezoelectric material can not only play the advantages of good biocompatibility of piezoelectric material, but also without additional power supply during implantation, relying on the activities of the human body or external electric field stimulation to conduct the stress state of the bone to the bone defect, so as to achieve the purpose of stimulating bone growth and shorten the non-load period.

5.1 Piezoelectric bioceramic materials

Biodegradable materials are preferred in scaffold fabrication to reduce the residual synthetic matrix at the regeneration site. One widely used material for preparing bone stents is the bioceramic material: hydroxyapatite HA ($Ca_{10}(PO_4)_6(OH)_2$. HA not only is naturally present in the bone site, but also has a high mechanical strength and can undergo cellular degradation without obvious toxic by-products. In addition to HA, other related Ca / P-based ceramics are also used to manufacture bone stents; these include brush stone (DCPD; CaHPO42H2O, Ca / P ratio 1.00), calcium phosphate (OCP (Ca 8 (HPO4) 2 (PO4), 4.4 · 5H2O, Ca / phosphorus ratio 1.33), α -calcium phosphate (α -calcium; TCP; calcium phosphate / phosphorus ratio 1.5), β -calcium phosphate (β -TCP; calcium phosphate / phosphorus ratio 1.5) and tetracalcid phosphate (Ca 4 (PO 4) 2O, Ca / P ratio 2.0)[12]. Among them, β -tricalcium phosphate is widely used. They have varying degrees of osteoinduction and osteoconduction properties, and their biodegradable rates can be modulated by careful optimization of the Ca and P molar ratios in the compounds. However, due to the insufficient mechanical properties and high brittleness of calcium phosphate materials, they can only be applied to areas with low negative weight or non-weight bearing.

To overcome these shortcomings, the researchers shifted their focus to synthetic nanohydroxyapatite (nHA) composites and added glass and other minerals as fillers[13]. This approach not only helps to improve the mechanical properties of the scaffold materials but also allows customization of materials to meet specific functions and requirements.

Barium titanate and magnesium silicate are two other piezoelectric drug candidates that have been shown to be biocompatible in vivo. There are studies have demonstrated that barium titanate promotes the canine femur[14]the osteogenic. Ma Hui[15]piezoelectric ceramic coating was prepared on the HA surface to verify its biocompatibility in vivo. The results showed that the biological piezo ceramic specimen is not hemolytic, sensitized, thermogenic and cytotoxic, which provides a new idea for bone tissue engineering materials. Jian Qing et al[16]the HA-BaTiO3 composites were fabricated and were polarized in the direction of their longitudinal axis. The composites were then implanted both vertically and horizontally into the dog's mandible. It was observed that the surface of vertically implanted specimens developed more new proteins than in parallel implanted specimens. This suggests that osteogenesis is direction-dependent and is promoted in the polarization direction (i. e., the longitudinal axis of the specimen). Furthermore, tissue formation on the HA-barium titanate composite was higher than on the control (HA) samples.

MgSiO3 Is biodegradable, and the released Mg2 + and Si4 + ions differentiate into osteoblasts by mesenchymal stem cells[17]to stimulate the osteogenic properties.Na-Hyun Lee[18]by introducing strontium to release nanoscale cement, thus providing dual therapeutic effects (contributing to bone and anti-osteoclogenesis), ultimately for the regeneration of osteoporotic bone defects. The experiment found that over time, the nanoscale glass particles doped by Sr can release a variety of ions, including silicate, calcium and strontium. When preosteoblasts were treated with Sr-nanoclasts, they stimulated osteogenic mRNA levels (Runx 2, Opn, Bsp, Ocn), alkaline phosphatase activity, calcium deposition, and target luciferase reporter genes.

Zinc, magnesium have been shown to be stimulating the osteoblast activity[19]important elements.Kaushik Sarka[20]et al. compared the in vitro and in vivo behaviors of magnesium phosphate (MgP) biocamic degradation, biocompatibility and the potential role of Zn (Zn) on degradation and found that the new bone formation rate of the zinc-doped MgP was 69 ± 3% and 78 ± 3%, respectively, while the new bone regeneration rate of undoped MgP ceramics was only 56 ± 3%. It indicates that zinc stimulates bone formation; in vitro experiments show that the samples add zinc to MgP, and the cell proliferation of zinc-doped samples is significantly increase

5.2 Biopolymers class

5.2.1 Synthetic polymer

Polyvinylidene fluoride is one of the few synthetic polymers with piezoelectric properties, and PVDF is widely used in bone tissue engineering due to its good biocompatibility and electrical activity. Guo Hongfeng et al. prepared PU / PVDF piezo cell scaffold by electrospinning. They verified the biocompatibility of the scaffold by subcutaneous implantation in rats, and found that the piezoelectric of the scaffold had a stimulating effect on fibroblasts. In vitro experiments confirmed that the piezo 1 properties of the scaffold could enhance the migration, adhesion, and secretion of fibroblasts. Provide new ideas for new trauma dressings. NASA[21]proposed using PVDF as the main material for the preparation of medical gauze. The tiny electric current generated can accelerate wound healing by gently touching or tapping the gauze on the victim's body. It mainly uses the piezoelectric and pyroelectric characteristics of PVDF to generate artificial current and accelerate wound healing.

5.2.2 Natural polymers

Natural polymers are characterized by their superior biocompatibility and small negative immune effects, and can be divided into two major classes, proteins and polysaccharides. The proteins exhibiting piezoelectric activity are collagen, keratin, and fibrin.

As a natural polymer material, collagen is one of the good choices for bone implants. Collagen biocompatibility, but mechanical properties and bone induction ability can not meet more clinical needs. Kakudo class[22]3D cultured hASC-honeycomb collagen composite scaffold were implanted into the subcutaneous tissue of mice for 8 weeks. Subsequently, experimental results revealed that the differentiation of hASC produced osteoblasts and subsequently accelerated the formation of hard tissues.

Chitosan is another piezoelectric natural polysaccharide polymer, produced by Martino et al[23]summarize. Studies have shown that chitosan-based polymer scaffolds have excellent bone conductivity, porosity, easy formability, antibacterial response and minimal foreign body reaction and other characteristics. Cellulose is another natural polymer of piezoelectric polysaccharide and can be a suitable candidate for bone tissue applications due to its superior biocompatibility and good mechanical strength.Zaborowska[24]et al observed that cellulose-based microporous scaffolds resulted in significantly increased proliferation of MC3T3-E1 osteoblasts compared to nanoporous 1.

5.3 Metals and their alloys

At present, the most commonly used clinical titanium and its alloy, its good biocompatibility and mechanical strength, low sensitization, light quality and so on have become the most potential bone implants. However, in the early stages of implantation, titanium-based implants still have disadvantages such as difficulty in forming sufficient chemical binding with the surrounding bone because of their bioinert nature[25]. Therefore, it is necessary to modify the titanium-based implants to appropriately promote osteogenesis, osteoconduction, and osseointegration. BMP 2 promotes osteoblast differentiation of MSCs and is the most commonly used growth factor to achieve implant bone fusion. A study led by Bae et al showed that BMP-2 and calcium phosphate combined with Ti implants coated with biodegradable polymer and extracellular matrix prolonged BMP-2 release and improved osteogenesis[26、27、28]. TiO 2 nanotube-coated titanium implants have better corrosion resistance and biocompatibility than pure titanium, with an elastic modulus closer to that of natural bone.

6. Expectation

Tissue engineering of the periosteum improves the therapeutic effect of bone defects and provides new opportunities for the development of bone tissue engineering. The results of these studies on tissue engineered periosteum suggest that further optimization of the materials, construction method and their preparation methods can promote bone regeneration. Both the selection of materials, the design concept, and the preparation method of researchers need more in-depth research and continuous clinical verification to improve the need for future clinical bone repair.

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