AN OVERVIEW OF BONE REPLACEMENT MATERIALS – BIOLOGICAL MECHANISMS AND TRANSLATIONAL RESEARCH

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PREGLED MATERIJALA ZA ZAMENU KOŠTANOG TKIVA – BILOŠKI MEHANIZMI I TRANSLACIONA ISTRAŽIVANJA

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ABSTRACT

Bone defects might develop as a result of various pathological entities. Bone grafting is a widely used procedure that involves replacement of the missing tissue with natural or artificial substitute. The idea for artificial replacement of the missing bone tissue has been known for centuries and the evidence for these treatments has been found ever since prehistoric period. Bone grafting has been practiced for centuries with various non-osseous natural materials. The skeletal system plays a crucial role in the structural support, body movement and physical protection of the inner organs. Regeneration of bone defects is crucial for reestablishing of the form and function of the skeletal system. While most bone defects can heal spontaneously under suitable conditions, bone grafts or substitute biomaterials are commonly used therapeutic strategies for reconstruction of large bone segments or moderate bone defect. An ideal bone grafting material should provide mechanical strength, be both osteoinductive and osteoconductive and should provide space for vascularization. In order to overcome limitations associated with the standard treatment of bone grafts, there is an increasing interest in studying substitute biomaterials, made of naturally derived or synthetic materials. Bone substitutes can be derived from biological products or from synthetic materials. Prior to testing in human subjects, the bone substitute materials should be tested in vitro and in vivo using animal models. Establishing of a suitable animal model is an essential step in the investigation and evaluation of the bone graft materials.

Keywords: bone grafting, biomaterials, dentistry, orthopedics.

SAŽETAK

Kao rezultat različitih patoloških entiteta, mogu se razviti oštećenja kostiju. Ugradnja ili kalemljenje kostiju (eng. bone grafting) je široko korišćena procedura koja podrazumeva zamenu nedostajućeg tkiva prirodnom ili veštačkom zamenom. Ideja za veštačko nadoknađivanje nedostajućeg koštanog tkiva poznata je vekovima, a dokazi za ovaj način lečenja pronađeni su još od praistorije. Za zamenu koštanog tkiva vekovima su korišćeni prirodni, nekoštani materijali. Skeletni sistem igra presudnu ulogu u strukturnoj podršci, kretanju tela i fizičkoj zaštiti unutrašnjih organa. Za ponovno uspostavljanje oblika i funkcije koštanog sistema, obnova koštanih defekata je od presudnog značaja. Iako većina koštanih defekata može spontano zaceliti pod odgovarajućim fiziološkim uslovima, za rekonstrukciju velikih koštanih segmenata ili umereni koštani defekt, koriste se koštani graftovi ili supstitutivni biomaterijali. Idealan materijal za kalemljenje kostiju treba da obezbedi mehaničku čvrstoću, da bude i osteoinduktivan i osteokonduktivan, a takođe treba i da obezbedi prostor za vaskularizaciju. Da bi se prevazišla ograničenja koja su povezana sa standardnim tretmanom koštanim transplantatima, postoji sve veće interesovanje za proučavanje zamenskih biomaterijala, napravljenih od prirodno izvedenih ili sintetičkih materijala. Zamene kosti mogu se dobiti iz bioloških proizvoda ili iz sintetičkih materijala. Pre ispitivanja na ljudskim subjektima, biomaterijal za zamenu koštanog tkiva, je prvo potrebno testirati in vitro i in vivo na animalnim modelima. Uspostavljanje odgovarajućeg životinjskog modela je ključni korak u istraživanju i proceni materijala za koštane kaleme.

Ključne reči: graftovi koštanog tkiva, biomaterijali, stomatologija, ortopedija.

ABBREVIATIONS

TGF- β - transforming growth factor beta
DBM - demineralized bone matrix
PRP - platelet-rich plasma
HA - hydroxyapatite
CaP - calcium phosphate
TCP- tricalcium phosphate

INTRODUCTION

Bone grafting is a commonly performed surgical procedure in orthopedics, dentistry and neurosurgery, it is also the second most common transplantation tissue (1). Bone defects might develop as a result of various pathological entities like trauma, infection, tumor and surgery as well (2). Bone grafting is a procedure that involves replacement of the missing tissue with natural or artificial substitute. The idea for replacement of the missing bone tissue has been known for centuries and the evidence for these orthopedic treatments has been found in the Egyptian civilization and even earlier, in prehistoric times (3). The first successful autogenic and allogenic grafts were reported in the 19th century by Van Merren (4). Bone grafting has been practiced for centuries with various non-osseous materials of the natural origin (marble, wood etc.) but with little success. In 1892, Dreesmann from Trendelenburg's Clinic in Bonn, first reported the successful use of plaster of Paris (calcium sulfate) as bone replacement in eight patients (5). Shortly thereafter, this material was investigated in experiments with animals (6).

Bone is a connective tissue with the complex hierarchical architecture. In fact, the bone is a multifaceted organ consisting of several tissues, the cortical bone, the cancellous bone with the marrow compartment and the periosteum (7). Bone remodeling is a permanent process during which bone is permanently rebuilt. The purpose of this process is to assure the bone mineral homeostasis, to regenerate micro fractures, or to adapt the bone to new mechanical load (8). The fine balance between osteoblasts, the cells that form the bone tissue, and osteoclasts, degrading bone macrophages, is essential for the bone metabolism and physiology. The skeletal system plays a crucial role in the structural support, movement and physical protection of the inner organs. Moreover, in addition to the mineral storage, the skeletal system is the site of blood cell generation and differentiation and has important hematopoietic and immunological functions (7). Considering various roles of the bone tissue, regeneration of bone defects is crucial to re-establish the form and function in the body (7).

The healing of bone defect is a time-consuming process of reconstruction of the bone tissue. Due to the regeneration ability of the bone tissue, most bone defects can heal spontaneously under suitable physiological conditions. On the other hand, large defects, also known as critical bone defects, may not heal spontaneously (9, 10). The repair of critical bone defects is still clinically challenging, despite the remarkable regenerative capacity of the bone. For reconstruction of large bone segments, or for filling of moderate bone defect, bone grafts or substitute biomaterials are commonly used therapeutic strategies.

BIOLOGIC MECHANISMS OF BONE REGENERATION

There are four essential mechanisms of bone regeneration underlying bone grafting: osteogenesis, osteoinduction, osteoconduction and osteointegration (11). Osteogenesis or bone

formation presents transformation of a preexisting mesenchymal tissue into the bone tissue (12). In the context of bone grafting, it presents the "osteogenic" potential of the graft or the ability of donor graft osteoprogenitor cells to proliferate and differentiate to osteoblasts and to contribute to the growth of a new bone (11, 13, 14). Osteoinduction presents the stimulation and activation of host mesenchymal stem cells or osteoprogenitor cells from the surrounding tissue which differentiate into osteoblasts (11). The process of osteoinduction is conducted and mediated by various signals and activation of cellular receptors particularly from TGF- β super family (14).

Osteoconduction presents the ability of bone cells, osteoblasts and osteoclasts in the grafting area, to move across a scaffold and slowly replace it with a new bone (15). This property occurs when the bone graft material serves as a scaffold for the new bone growth, which is perpetuated by the native bone (13). An osteoconductive material is one that permits generation of the bone tissue on its surface or down into its structures (16). The material with both features, osteoconductive and osteoinductive, besides its role as a scaffold for currently existing osteoblasts, will also stimulate differentiation of new osteoblasts, promoting faster integration of the graft (13).

The final result in bonding between the host bone and graft is referred as osteointegration. This process is defined as the direct anchorage of an implant by formation of the bony tissue around the implant without growth of the fibrous tissue at the bone-implant interface (16). Recent definition states that it is a "phenomenon where an implant becomes so fused with bone that they cannot be separated without fracture" (17).

TYPES OF BONE SUBSTITUTES

The bone grafting material should provide mechanical strength, be both osteoinductive and osteoconductive and should provide space for vascularization and tissue infiltration (18). In selection of a graft, some aspects need to be assessed: biologic acceptability, predictability, clinical feasibility, and minimal postoperative sequelae (19). Utilizing bone obtained from the same individual receiving the graft is autologous bone grafting (20). For bone defect restoration, the gold standard is autologous grafting, due to its histocompatibility and non-immunogenic properties (21). Also, autografts provide optimal osteoconductive, osteoconductive, and osteogenic properties (14). However, disadvantages are also numerous. These grafts are associated with a high rate of postoperative complications, such as chronic pain on the donor site or cosmetic disadvantages (22, 23). The most frequently chosen bone substitute is allograft, derived from a human, but from an individual other than the one receiving the graft. Allografts are often derived from cadavers and from tissue banks. The use of this type of material is also associated with certain restrictions. Eliciting of the immune response and transmitting various diseases are the most significant limitations (24). Therefore, allografts are prepared using various techniques designed to minimize the host's immune response

and to reduce the risk of transmission of a disease. However, the preparation methods can significantly weaken the biologic and mechanical properties initially present in the bone tissue (25, 26).

There is an increasing interest in studying substitute biomaterials, made of naturally derived or synthetic materials in order to overcome limitations associated with the standard treatment of bone grafts (27). Bone substitutes can be derived from biological products or synthetic materials. Demineralized bone matrix (DBM) is derived from a human bone by acid removal of the mineral matrix, while maintaining the organic matrix and growth factors. DBM is mostly used for filling purposes (28). Platelet-rich plasma (PRP), obtained from a patient's blood is generally used as a supplement to other materials (29).

IN VIVO ANIMAL MODELS IN TRANSLATIONAL RESEARCH

The bone substitute materials, prior to testing in human subjects, should be tested in vitro and in vivo using animal models. Establishing of a suitable animal model is an essential step in the investigation and evaluation of the bone substitute materials. It should be kept in mind that results obtained from in vivo animal studies often differ in their translatability to the clinical condition (33).

Hydroxyapatite (HA) is the primary mineral component of teeth and bone tissue. It has slow resorption and very good mechanical properties. This material is available in natural and synthetic form and it can be used alone or as a part of some composite materials (28). Considering synthetic bone substitutes, calcium sulfate, as mentioned before, was introduced in 1892 (5). Nevertheless, this material has only recently been accepted for general use (30). Although its use has some advantages such as its similarity to the bone and osteoconductiveness, it also shows some disadvantages, fast resorption and absence of osteoinductivity and osteogenicity, as well as relatively common local complications (31). Synthetic scaffolds made from calcium phosphate are ceramics and they have been widely used in dentistry and in orthopedics for a few decades (32).

For the selection of a specific animal species as a testing model, various factors have to be considered. Most importantly, the chosen animal model should clearly demonstrate analogies in comparison to humans, both physiological and pathophysiological. Small animals, such as rodents, are suitable for the investigation of pathophysiology and pathogenesis, and large animals are often used to study long-term diseases processes and treatment options (Table 1) (8).

Table 1. Animal models characteristics in bone defect studies

Animal species	Bone defect topography	Advantages	Disadvantages
Rodents	Femur Calvaria	<ul style="list-style-type: none">• small size• easy to handle• suitable life span• cost	<ul style="list-style-type: none">• small-sized long bones• thin and fragile cortices• absent Haversian-type remodelling in the cortex
Pig	Craniofacial	<ul style="list-style-type: none">• bone anatomy• healing capacity,• remodelling similar to humans;• similar bone mineral density and concentration• lamellar bone structure	<ul style="list-style-type: none">• denser trabecular network• difficult to handle• shorter tibiae and femur• large growth rates• very high body weight
Sheep	Tibiae	<ul style="list-style-type: none">• body weight similar to adult humans• easy to handle	<ul style="list-style-type: none">• significantly higher trabecular bone density• greater bone strength• larger amount of bone ingrowth
Rabbit	Tibiae Femur	<ul style="list-style-type: none">• easy to handle• small size• early skeletal maturity	<ul style="list-style-type: none">• small size• differences in bone anatomy,• faster skeletal change and bone turnover

Adapted from: Li et al (9)

Rodents are one of the most commonly used animal models in testing biomaterials as bone substitutes and regeneration of the bone tissue (34). These mammals are mostly used for the age-related metabolic and regenerative studies of the bone tissue since their bone biology is strongly dependent on the age and gender (35). However, there are obvious limitations in using rodent models. Rodents have small-sized long bones and thin and fragile cortices and trabecular bone content is limited (36). Also, Haversian remodeling does not occur in these animals (34). Bearing in mind all the shortcomings, rodents are nevertheless, widely used in studies of the bone substitute materials, mostly as a model of critical-size defects, the smallest wounds established intraosseously, which cannot heal spontaneously during the lifetime of the animal (37). Preclinical testing of the bone replacement materials is also broadly performed in large skeletal animals including pigs, dogs, goats and sheep. The bone tissue structure and composition of these animals is highly similar to those of humans (38). In preclinical studies focused on the processes which are related to the body size or metabolic characteristics, like biomechanics, the species of experimental animals should be as close to human as possible (39). Sheep and goats are suitable for testing the human implants and prostheses due to their body weight and long bones (40). Although their bones have macrostructure similar to human, in sheep, bone consists predominantly of the primary bone structure unlike humans and they have significantly higher bone densities compared to the human bone (41). Regardless of the observed differences in the bone microstructure, studies reported that sheep exhibit similar bone remodeling compared to human (42). The sheep tibia defect model has been widely used to test the bone substitute materials in the bone regeneration (43, 44).

Regarding the bone anatomy, morphology, metabolism and mineral density, pigs are considered to be close representative models of the human bone investigations (45, 46). Also, histology of the bone tissue and lamellar bone structure is similar to that of humans (47). Disadvantages of this animal model refer to the length of tibiae and femora in pigs, which is relatively small and therefore unsuitable for the implant studies. Also, high body weight makes them unsuitable for the orthopedic research (9). On the other hand, the craniofacial bone defect model is used widely for testing the bone substitute biomaterials in these animals (9).

ADVERSE EFFECTS OF BONE REPLACEMENT MATERIALS

The application of the bone replacement biomaterials causes a cascade of reactions in the biological environment. The biocompatibility and longevity of the grafts are determined by the host tissue, together with the physical and chemical properties of biomaterials. Adverse reactions to the graft in the body may be diverse and include foreign body reactions, chronic inflammation, infections, hypersensitivity reactions etc. (48). Also, biomaterials can potentially induce a toxic effect to the host tissues, in terms of the acute toxicity of the material itself or to the chronic toxicity due to the

material wear over time. The replacement biomaterials may release over time, fine debris particles and the chronic effects of these particles, in terms of the local and systemic toxicity become the major criteria in the toxicity evaluation (49). Pre-clinical studies, prior to the clinical use, should investigate various aspects of the long term impact of graft materials on the host tissue, such as cytotoxicity, genotoxicity, systemic acute, sub acute and chronic toxicity and carcinogenicity. For instance, in vivo studies of calcium phosphate (CaP) compounds, the material widely used in the regenerative clinical treatments, demonstrated that release of nanosized CaP may have a chronic hepatotoxic effect (50). Furthermore, nano-TCP particles incorporated into rabbit tibial defects elicited an excessive and prolonged inflammatory response associated with the decreased bone regeneration (51). A recent study demonstrated that the long term administration of hydroxyapatite or tricalcium phosphate nano particles had a significant prodepressant effect in rodents. Also, a prolonged treatment with amorphous calcium phosphate also resulted in a significant decline in cognitive functions, and these adverse effects were accompanied by the increased oxidative damage and apoptosis in the prefrontal cortex (52).

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