Biomaterials Applied to Bone Regeneration

Subjects: Pathology & Pathobiology

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Definition

Bone loss raises great concern in numerous situations, such as ageing and many diseases and in both orthopedic and dentistry fields of application, with an extensive impact on health care. Therefore, it is crucial to understand the mechanisms and the determinants that can regulate osteogenesis and ensure bone balance. In particular, new strategies for the improvement of the ability of biomaterials to trigger and sustain osteogenesis need to be developed. Autophagy is a well conserved lysosomal degradation pathway, which is known to be highly active during differentiation and development. An extensive revision of the literature on biomaterials, both for orthopedic and dentistry applications, enhancing osteogenesis by modulation of the autophagic process is here presented. Already investigated conditions regulating bone regeneration via autophagy need to be better understood for designing novel biomaterials with improved bioactivity.

1. Introduction

A biomaterial is any material (e.g., polymer, ceramic, metal, or composite) that has been engineered to interact with biological systems for a medical purpose, either a therapeutic (treat, augment, repair, or replace a tissue function of the body) or a diagnostic one. Biomaterials are used every day in dental and orthopedic applications, surgery, and drug delivery. They can be derived either from nature or synthesized in the laboratory using a variety of chemical approaches and materials. Biomaterials can be broadly categorized in metals, polymers, ceramics, and composite materials. This classification is followed in this section to discuss biomaterials promoting bone regeneration by modulating the autophagic process. The research in the field of biomaterials applied to bone regeneration is actually focused on the modifications of their surfaces in order to improve their bioactivity. In this perspective two strategies can be used: the functionalization of the biomaterial/cell interface by linking to its surface osteoinductive/osteoconductive molecules; and the modification of surface topography to make them more suitable for cell growth and differentiation. In the following paragraphs many examples of these strategies are given, relating them to the biomaterial used.

Table 1 provides an overview of the biomaterials discussed in this section along with the experimental model they were tested and the signaling pathway involved (where applicable).

Table 1: Biomaterials, experimental models, and signaling pathways.

Biomaterial	Model	Pathway	Reference(s)
Silicon, Orthosilic acid	Murine preosteoblast MC3T3-E1	BMP2/RUNX2 Col1	[1][2][3][4]
Silica NPs	Murine preosteoblast MC3T3-E1	ERK1/2, LC3, p62	[5][6][7][8]
Chitosan	Primary hMSCs	mTOR/S6K/S6/4E-BP1	[9][10][11]
TiAl6V4 particles	Osteocytic cell line MLO-Y4	IFN-β	[12][13][14]

Titanium	hBMSCs		[15][16]
Titanium	Human osteoblasts	PI3K/Akt	[17]
Titanium	Murine preosteoblast MC3T3-E1		[18]
Titanium	Murine preosteoblast MC3T3-E1	β-catenin/YAP	[19]
Alumina	rBMSCs	Wnt BMP	[20][21][22]
Silver NPs			[23][24][25][26][27][28]
Silver NPs	Mouse		[29]
Silver NPs	hMSCs		[30][31]
Hydroxyapatite	DPSCs		[32]
Hydroxyapatite	DPSCs	IL-6	[33]
Hydroxyapatite	Murine preosteoblast MC3T3-E1	mTOr	[34]
Hydroxyapatite	PDLSCs	AMPK mTOR	[35][36]
Fluorapatite	hASCs		[37][38]

2. Polymers

Silicon based materials have long been studied for their application in regenerative medicine either for their proangiogenic role[1] or their use in scaffolds that mimic the structure and composition of bone tissue[2]. In this field, the synthesis of silicate-containing hybrids by the sol–gel method is a new route to preparing bioactive implants with improved mechanical properties. These materials can be degraded by the physiological environment, which involves the eventual bone colonization and full tissue restoring. Actually, the research is focused on tailoring the hybrid implants for bone tissue regeneration rather than bone substitution. Silicate-containing hybrids must promote the osteogenic performance of the osteoblast-like cells[3].

Interestingly, orthosilic acid, a unique soluble form of silicon, enhanced the bone morphogenetic protein2 (BMP-2)/RUNX2 and collagen I protein expression in preosteoblastic cells, promoting differentiation and mineralization of osteoblasts through the activation of the autophagic pathway[4]. Moreover, an engineered bioactive silica-based nanoparticle formulation (NPs) was found able to stimulate in vitro differentiation and mineralization of osteoblasts and increased bone mineral density in young mice in vivo[5][6]. In the search of the mechanisms underlying such results, Ha and collaborators[7] found that the stimulation of autophagy and associated signaling suggests a cellular mechanism for

the stimulatory effects of silica nanoparticles on osteoblast differentiation and mineralization. They notably suggested that it is the size of the nanoparticles (50 nm) that stimulates autophagy rather than the materials they are made of. These considerations are remarkably in line with what was found about gold nanoparticles: the 45 nm AuNPS were the most effective in promoting both autophagy and osteogenesis[8].

Chitosan is a polysaccharide copolymer of glucosamine and N-acetylglucosamine derived by partial deacetylation of chitin from crustacean shells. Recently, many studies have investigated the effects of chitosan film or membrane on the morphology, stemness, and multi-differentiation abilities of mesenchymal stemc cells (MSCs). It has been demonstrated that MSCs cultured on chitosan film formed spheres and the expression of stemness marker genes increased significantly when MSCs were cultured using chitosan film compared with 2D monolayer culture systems[9]. More importantly, culture on chitosan film resulted in an increased differentiation potential of MSCs into mesenchymal lineages, such as osteoblasts[10]. In the same experimental model, mTOR signaling was activated especially in senescent cells, whereas its suppression or knockdown selected more primitive MSCs that are enriched in gene expression of pluripotency, in vitro osteogenesis, and in vivo bone formation[11].

3. Metals

3.1. Titanium and Nanostructure

Most of the recent research on biomaterials is actually focused on titanium, the most often used material, due to its biocompatibility and mechanical properties, both for orthopedic and dentistry applications, in substitution of ceramics, polymers, and other metals[12][13].

In a lately published paper, an osteocyte-conditioned medium proved to inhibit osteoclast differentiation from bone marrow monocytes (BMMs) to osteoclasts. However, TiAl6V4 alloy particles (TiPs) attenuated this inhibitory effect by markedly decreasing the expression of interferon-β (IFN-β), an osteoclastogenesis-associated factor. Additional evidence suggested that TiPs decreased the expression of IFN-β in osteocytes via stimulation of autophagy[14].

Among the others, one distinctive strategy used to improve the bio-functionality for titanium implants, was the use of exosomes derived by macrophage stimulated with BMP2, that were already known for their beneficial effects on osteogenic differentiation[15]. The incorporation of BMP2/macrophage derived exosomes dramatically increased the expression of osteoblastic differentiation markers in MSCs. Remarkably, the pro-osteogenic role of the titanium nanotubes incorporated with BMP2/macrophage-derived exosomes is mediated by autophagy[16].

In the biomaterial field of research, it is already known that biomaterials with varied surface topography have more biocompatible features and better interactions with the surrounding living tissues. Rough surfaces caused osteoblast differentiation via the autophagic-dependent PI3/Akt signaling pathway. One surface provoked the development of a third population of small, granular cells, responsible for cell cluster formation, which were important for the formation of bone noduli and mineralization. When autophagy was inhibited, both mature osteoblasts and small cells were absent, and the cell cluster formation was also prevented. Autophagy therefore has to play an essential role in the osteoblast differentiation on titanium-based surfaces with rough topography[17].

The nanosized surface is well known for its ability to interfere with intracellular procedures and a nanotube structure was found able to enhance mTOR-independent autophagy in osteoblasts compared to a flat surface. Further analysis revealed that autophagy was temporally promoted by nanotubes in the initial day contact, and cell membrane stretching appeared to be the central regulation factor. The process was also reversible by exchanging the substrate nanotopographies in different cell lines. In summary, the nanotopographic surface is able to induce temporal and reversible autophagy, which may be used as a versatile method to control cell differentiation[18].

Implant topography is associated with the functionality of osteogenic transcription factors directed by β-catenin in the nucleus. This protein can be degraded by YAP (Yes-associated protein) which is susceptible to autophagic flux. Nanotopography, in comparison with smooth surfaces, was associated with higher β-catenin nuclear translocation, osteogenic differentiation, and autophagy, and less cytoplasmic YAP in MC3T3-E1 cells. These results demonstrated an

involvement of this pathway in the osteogenesis observed in response to titanium implants[19].

3.2. Alumina

The osteoimmune environment plays indispensable roles in bone regeneration because the early immune environment that exists during the regenerative process promotes the recruitment and differentiation of osteoblastic lineage cells[20]. Nanoporous anodic alumina with different sized pores had modulatory effects on macrophage responses and consequently on the osteogenic differentiation of bone marrow stem cells (BMSCs). The role of macrophages in osteogenesis was already suggested to be indispensable[21]. The effect of the 50 nm nanoporous alumina structures on macrophage spreading and shape resulted in osteogenic differentiation of BMSCs, improving the osteogenic capacity of bone biomaterials with a mechanism related to autophagy activation[22].

3.3. Silver

Silver is used in a variety of medical and general devices for its antimicrobial properties. It is, therefore, widely used in the form of nanoparticles in medicine, in order to retard and avoid bacterial infection[23][24]. Despite their antimicrobial action, silver nanoparticles (AgNPs) lack toxicity towards eukaryotic cells, because of the induction of the autophagic process[25]. Interestingly, linking the silver nanoparticles to thermosets made of materials commonly used in the dental practice resulted in further reduced cytotoxicity[26], confirming that the adsorption of molecules on biomaterial surfaces can improve their biocompatibility.

Many results were recently achieved regarding effects of AgNPs on osteogenesis of stem cells[27][28][29]. Again, the linking of AgNPs, whose potential toxicity raises serious concerns, on titanium surfaces proved to be a successful strategy[30]. Moreover, AgNPs activated autophagy and osteogenesis. The administration of the autophagy inhibitor 3-methyladenine could reverse both processes, binding the occurrence of osteogenesis to the autophagic activity in human MSCs[31].

4. Ceramics

Hydroxyapatite (HA) is a natural occurring mineral present in the human skeleton. In biomaterial applications it can be used in combination with alginate to study the improved osteoblast differentiation of dental pulp cells (DPSCs)[32][33].

HA-nanoparticles (HANPs) promoted osteoblast differentiation in a dose-dependent manner the osteoblast cell line MC3T3E1. In addition, the internalized HANPs were located in typical autophagic vacuoles and increased the ratio of LC3II/LC3I, indicating HANPs induced cell autophagy. Moreover, the induction of autophagy was via the mTOR signaling pathway also in a concentration dependent manner. Collectively, these results revealed that HANPs modulates osteoblast differentiation by mediating autophagy in a dose-dependent manner[34].

Polydopamine-templated hydroxyapatite (tHA) is a type of nano-biomaterial, designed as an alternative to the traditional hydroyapatite (HA,) that can promote osteogenesis in bone tissue engineering. The reinforcement of polycaprolactone (PCL) matrix with tHA enhanced cell adhesion, spreading, and proliferation of human mesenchymal stem cells. More importantly, tHA nanoparticles exposed on the surface of composite nanofibers could further promote osteogenesis of human MSCs in vitro[35]. However, as already seen in other experimental systems, the concentration is crucial. Indeed, high concentrations of tHA stimulated reactive oxygen species (ROS) production, resulting in cell injury and apoptosis in PDLSCs. Nevertheless, the triggering of the AMPK/mTOR signaling pathway when tHA is in combination with metformin, led to autophagy activation and consequent increased viability of human PDLSCs with a further improvement of the osteogenic effect[36].

Interestingly, also the incorporation of fluorapatite (FA) crystals within the three-dimensional PCL nanofiber scaffolds provided a favorable extracellular matrix microenvironment for the growth, differentiation, and mineralization of human DPSCs[37]. In a different cellular model, the inhibition of autophagy at earlier stages (days 1 to 3) could affect human adipose stem cell (hASCs) osteogenic capability and mineralization when grown on PCL+FA scaffolds. These results suggested that autophagy was indispensable during the early stage of osteogenic differentiation in this model[38].

Concluding, the health of the bone tissue is strictly related to the differentiation of osteoblasts, the cell responsible for the deposition of organic osteoid and matrix mineralization, which leads to osteogenesis. Autophagy is thoroughly involved in

the development of these cells, contributing therefore to bone homeostasis. Impairment of autophagic activity leads to disruption of the bone-remodeling balance, which leads to pathological state and failure of biomaterial implants. Autophagy modulation has been shown to have an intriguing potential as target for ageing, biomaterial design, and the therapy of various pathological conditions. Here the role of autophagy in osteogenesis promoted by different types of biomaterials is largely discussed. The knowledge of the conditions improving biomaterial bioactivity will help future research to design new biomaterial solutions.

Abbreviations

ASCs	adipose stem cells
BMMs	bone marrow monocytes
BMP	bone morphogenetic protein
BMSCs	bone marrow mesenchymal stem cells
DPSCs	dental pulp stem cells
FA	fluorapatite
HA	hydroxyapatite
MSCs	mesenchymal stem cells
NPs	nanoparticles
PDLSCs	periodontal ligament stem cells
PCL	polycaprolactone
ROS	reactive oxygen species

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Keywords

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