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Review

Development of nanocomposites for bone grafting

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Abstract

This article reviews nanocomposites focusing on their impact and recent trends in the field of bone grafting. Although autogenous- and allogeneic-bone grafts have been used for a long time in bone therapies, there is still a donor shortage and infection risk. As an alternative, synthetic biomaterials have been developed and clinically used as bone grafts, but most of them differ substantially from natural bone either compositionally or structurally. It remains a great challenge to design an ideal bone graft that emulates nature's own structure. Owing to the composition and structural similarity to natural bone, most of the current investigations involve the use of nanocomposites, particularly hydroxyapatite/collagen system, as promising bone grafts, but it is surprising that none of the reports review the rationale and design strategy of such nanocomposites in detail for the benefit of researchers. Accordingly, this article addresses the state-of-the-art of those nanocomposites and provides suggestions for future research and development. This review provides an overview of the nanocomposite strategy of bone, bone grafting, synthetic approaches to bone structure, development of nanocomposites from the conventional monolithic biomaterials, and recently developed processing conditions for making nanocomposites. The review is expected to be useful for readers to gain an in-sight on the state-of-the-art of nanocomposites as a new class of synthetic bone grafts.

Keywords: Hydroxyapatite; Collagen; Bone; Nanocomposites; Biomechanics; Biomimetics; Tissue engineering; Bone grafting

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1. Introduction

Bone is an amazing and a true nanocomposite. It is a complex and a highly specialized form of connective tissue pertaining to the formation of the skeleton of the body. Bone, not only provides mechanical support but also elegantly serves as a reservoir for minerals, particularly calcium and phosphate. It is a good example of a dynamic tissue, since it has a unique capability of self-regenerating or self-remodeling to a certain extent throughout the life without leaving a scar [1]. However, many circumstances call for bone grafting owing to bone defects either from traumatic or from non-traumatic destruction. With reference to statistical reports [2–4], about 6.3 million fractures occur every year in the United States of America (USA) itself, of which about 550,000 cases require some kind of bone grafting. It was also noticed that the fractures occur at an annual rate of 2.4 per 100 population in which men seem to experience more fractures (2.8 per 100 population) than women (2.0 per 100 population). The most frequently occurring fractures are, in decreasing order, hip, ankle, tibia, and fibula fractures. It is reported that the total number of hip replacements was about 152,000 in the year 2000, which is an increase of about 33% compared to the year 1990 in the USA alone and it is expected to increase to about 272,000 by the year 2030 [5], indicating that there is still a great need for synthetic bone grafts. According to a market survey conducted by Medtech Insight [6], bone grafts sales was found to exceed US$980 million in 2001 in the USA and about US$1.16 billion in 2002, which is also expected to double by 2006. In Europe, the number of bone grafting procedures was reported to be 287,300 in 2000 and it is expected that it could be increased to about 479,079 in 2005 [7]. In 2000, the worldwide use of bone grafts was estimated to be about 1 million, of which about 15% of the surgery had used synthetic bone grafts. It was also suggested that the future growth largely attributes to tissue-engineered composites, i.e., composites containing osteogenic cells and growth factors.

The need for synthetic bone grafts depends on the complication of the bone defects. For example, if the defect is minor, bone has its own capability to self-regenerate within a few weeks. Therefore, surgery is not required. In the case of severe defects and loss of volume, bone would not heal by itself and grafting is required to restore function without damaging living tissues. There are multiple methods available for the treatment of bone defects, which includes the traditional methods of autografting and allografting. Although autografting and allografting are clinically considered as good therapies, they have limitations. For example, supply of autograft is limited and there is a possibility of pathogen transfer from allograft. Accordingly, there is a great need for the use of synthetic bone grafts. Nowadays, numerous synthetic bone graft materials, both single- and multi-phases, are available which are capable of alleviating some of the practical complications associated with the autogenous or allogeneic bones. Although there is good progress in bone grafting using synthetic bone grafts, the way in which they execute their functions in vivo is quite different and most of them differ from natural bone either compositionally or structurally. Further, a single-phase material (also called monolithic) does not always provide all the essential features required for bone growth, which leads to incessant investigation in search of an ideal bone graft. There is, therefore, a great need for engineering multi-phase materials (also called composite) with structure and composition similar to natural bone. Recently, nanocomposites, particularly hydroxyapatite (HA)- and collagen-based, have gained much recognition as bone grafts not only due to their composition and structural similarity with natural bone but also because of their unique functional properties such as larger surface area and superior mechanical strength than their single-phase constituents. Further, natural bone itself is a nanocomposite matrix composed mainly of HA nanocrystallites in the collagen-rich organic matrix [8,9]; thereby choosing a HA/collagen nanocomposite as a bone graft material is an added advantage. An extensive and informative review on HA-based biomaterials has suggested that the HA/collagen composite is probably the most suitable system for bone replacement or regenerative therapy [10].

This article emphasizes the importance of HA/collagen nanocomposites in bone grafting. It also discusses some of the critical issues and scientific challenges that might be needed for further research and development.

The authors are not suggesting that this is the only material of promise for bone grafting applications, but the key intention is to stimulate research on nanocomposite bone graft materials and to formulate them as promising bone grafts owing to their sophisticated functionalities.

2. Basics of bone science

2.1. Bone as a nanocomposite

The design strategy of an ideal bone graft, probably nanocomposite, is not straightforward without understanding at least the fundamentals of bone composition, architecture, and the way in which it is organized. The bone matrix is precisely composed of two major phases at the nanoscale level namely, organic (protein) and inorganic (mineral); and may be considered as a good example for a nanocomposite. These phases have multiple components which consist of, in decreasing proportions, minerals, collagen, water, non-collagenous proteins, lipids, vascular elements, and cells. An overall composition of the bone is given in Table 1 [11–13]. The bone mineral is mainly composed of HA and the bone protein is mainly composed of collagen. Here, collagen acts as a structural framework in which plate-like tiny crystals of HA are embedded to strengthen the bone [14]. The bone collagen has a typical fibrous structure, whose diameter varies from 100 to 2000 nm. Similarly, HA in the bone mineral is in the form of nanocrystals, with dimensions of about 4 nm by 50 nm by 50 nm [15,16]. The structural and compositional strategies, by which they are precisely built, make bone an amazing and a true nanocomposite. The bone minerals are also enriched with a few trace elements for various metabolic functions, which include carbonate, citrate, sodium, magnesium, fluoride, chloride, and potassium. The prime role of minerals is to provide toughness and rigidity to the bone, whereas collagen provides tensile strength and flexibility. Nature has built extremely hard and tough bone using such soft (collagen) and brittle (HA) ingredients. A complete biological mechanism involved in the bone building strategy is still unclear and thus research progresses in this direction significantly around the world. It is believed that key to the strength of the bone is the complex structural hierarchy into which it is organized in a self-assembling mode. It is important to note that the minerals are not directly bound to collagen, but bound through non-collagenous proteins. The non-collagenous proteins make up approximately 3–5% of the bone, which provide active sites for biomineralization and for cellular attachment. Water is also found in sufficient quantity in all the bones. It is one of the most essential substances of the body because no cells survive without water. The amount of water present in the bone is an important determinant of its mechanical behavior as well. Compiled biomechanical properties of the bone are given in Table 2 [13,17–21]. Lipids are also necessary for the cellular functions, which account to about 2% of the bone. They play an important role in the process of initial biomineralization [22]. The biomineralization typically begins only 10 days after the organic matrix is laid-down. The degree of biomineralization is the most important factor to determine the biomechanical competence of the bone. Nevertheless, the actual mechanism involved in the biomineralization remains poorly understood even with the advances of biosciences.

Table 2
Biomechanical properties of bone[a]

Properties	Measurements	
	Cortical bone	Cancellous bone
Young's modulus (GPa)	14–20	0.05–0.5
Tensile strength (MPa)	50–150	10–20
Compressive strength (MPa)	170–193	7–10
Fracture toughness (MPa $m^{1/2}$)	2–12	0.1
Strain to failure	1–3	5–7
Density (g/cm^3)	18–22	0.1–1.0
Apparent density (g/cm^3)	1.8–2.0	0.1–1.0
Surface/bone volume (mm^2/mm^3)	2.5	20
Total bone volume (mm^3)	1.4×10^6	0.35×10^6
Total internal surface	3.5×10^6	7.0×10^6

[a] Compiled from Refs. [13,17–21].

Table 1
The composition of bone[a]

Inorganic phase	wt%	Organic phase	wt%
Hydroxyapatite	~60	Collagen	~20
Carbonate	~4	Water	~9
Citrate	~0.9	Non-collagenous proteins (osteocalcin, osteonectin, osteopontin, thrombospondin, morphogenetic proteins, sialoprotein, serum proteins)	~3
Sodium	~0.7		
Magnesium	~0.5		
Other traces: Cl^-, F^-, K^+ Sr^{2+}, Pb^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}		Other traces: Polysaccharides, lipids, cytokines Primary bone cells: osteoblasts, osteocytes, osteoclasts.	

[a] Compiled from Refs. [11–13]. Composition slightly be varied from species to species and from bone to bone.

2.2. Cellular functions of bone tissue

The cellular components of bone are the essential factors for activation and control of bone metabolism. Bone formation is accomplished by synchronized multicellular actions. There are five distinct types of cells associated with the bone tissue with regard to their functions; osteoprogenitor cells, osteoblasts, osteocytes, osteoclasts, and bone-lining cells [22,23]. Bone, like other connective tissues in the embryo, is derived from mesenchymal cells. These cells have the ability to divide and differentiate into bone cells, which are known as osteoprogenitor cells. They are also called bone-precursor cells. Osteoblasts are responsible for the formation of new bone. They start by secreting collagen and then coat with non-collagenous proteins which have the ability to hold minerals, mostly calcium and phosphate, from the bloodstream, leading to new bone formation. Osteocytes are matured cells derived from osteoblasts that are responsible for the maintenance of bone. They function as transporting agents of minerals between bone and blood. Osteoclasts are the large cells that are found at the surface of the bone mineral next to the resorbing bone and are responsible for bone resorption. They use acids or enzymes to dissolve the minerals as well as collagen from the matured bone. The dissolved minerals then re-enter the bloodstream and are carried to different parts of the body. Bone-lining cells are found along the surface of the matured bone, which are responsible for regulating the transportation of minerals in and out of the bone tissue. They also respond to hormones by making some exclusive proteins that activate osteoclasts. These five types of cells are together responsible for building the bone matrix with hierarchical self-assembly, maintenance, and remodeling as required. All these processes must be in equilibrium to ensure a healthy bone.

2.3. Hierarchical structure of bone

Bone can be considered as an assemblage of various levels of hierarchical structural units elegantly designed on many scales, macro to nano, to meet multiple functions. When bone is initially laid down, it is structurally weak and unorganized. But within a few days, the primary bone remodels to become lamellar bone. At the macrostructural level, the matured lamellar bone can be distinguished into two types, namely, spongy bone and compact bone. As their names imply, they radically vary in density. They are organized with multi-level pores, macro to nano, for the establishment of multiple functions, including transportation of nutrients, oxygen, and body fluids. The dimension-dependent hierarchical structure of the bone is shown in Fig. 1 [24]. The spongy bone occupies about 20% of the total bone. It is also often called trabecular or cancellous bone. It is lighter and less dense than compact bone (see Fig. 1). It has high porosity and higher concentration of blood vessels compared to compact bone. The porous architecture is easily visible under lower power microscopes and/or even to the naked eye if the pores are very large. The diameter of the pores may be from few micrometers to millimeters. On the other hand, compact bone is much denser than spongy bone. It is also called cortical or dense bone. It occupies about 80% of the total bone. It has less porosity and thus less concentration of blood vessels. Its porous architecture is not visible to naked eye. The pores may be 10–20 μm in diameter and mostly separated by intervals of 200–300 μm. The compact bone functions mechanically in tension, compression, and torsion, whereas spongy bone functions mainly in compression. At the microstructural level, the repeated structural unit of compact bone is mostly of osteon or Harversian system, which act as weight-bearing pillars. In contrast, spongy bone contains no such osteon units,

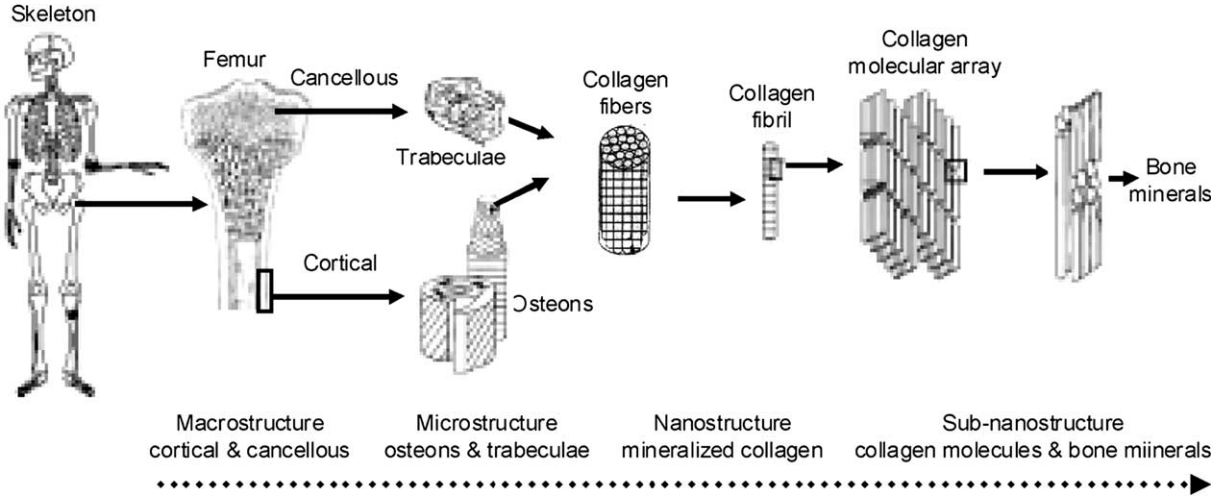


Fig. 1. The hierarchical structure of bone, from macro- to nano-assembly. (Adapted and re-drawn from Ref. [24].)

but they are made of an interconnecting framework of trabeculae. The trabeculae have three types of cellular structures: plate/plate-like, plate/bar-like, and bar/bar-like. At the nanostructural level, the bone is comprised mainly of collagen fibers and nanocrystals of bone minerals, particularly HA. Although several structural levels of bone have been identified, a complete understanding of how the mineral–matrix interactions are related to their mechanical reliability at the so far identified seven hierarchical levels of bone tissue is still needed. The rationale of cell–matrix, and cell–cell interactions are also important aspects.

3. Current scenarios of bone grafting

Bone grafts provide mechanical or structural support, fill defective gaps, and enhance bone tissue formation. They are widely used in orthopedic surgery, plastic surgery, oral and maxillofacial surgery, and dental surgery. It should be noted that bone is the second most transplanted tissue in humans. The graft materials not only replace missing bone but also help the body to regenerate its own lost bone. By this method, bone healing time is reduced and new bone formation strengthens the defective area by bridging grafted materials with host bone. There are a variety of bone grafting methodologies available, which include autografting, allografting, xenografting, and alloplastic or synthetic bone grafting, but each with their own advantages and disadvantages [25–30]. Autografting is a method in which tissue or organ is transplanted from one site to another site of the same individual. Allografting can be defined as tissue transplantation between individuals of the same species but of non-identical genetic composition. Xenografting is a process of transplanting tissue from one species to another (e.g., bone from animal to human). As an alternative to the above three types of bone grafts, synthetic substances are gaining much interest for use as bone graft materials. A surgical method that uses synthetic substances to repair or regenerate defective bone tissue is known as alloplastic or synthetic bone grafting. The benefits of synthetic grafts include availability, sterility, cost-effectiveness, and reduced morbidity. The synthetic grafts eliminate some of the shortcomings of autografts or allografts associated with donor shortage and the chance for rejection or transmission of infectious disease. However, the selection of grafting to use is purely dependent on the nature and complication of the bone defects as well as choice of available bone grafts.

4. Bone graft materials

Over the past four decades, several biomaterials have been developed and successfully used as bone grafts. Evolution of biomaterials in bone grafting is schematically illustrated in Fig. 2. Bone and joint substitutes are commonly made of metals, ceramics, polymers, and their composites (see Table 3) [26,31–36]. In most of the cases, metals and ceramics are used in hard tissue applications, whilst polymers in soft tissue applications due to their mechanical properties. Composites are widely used in both the applications. The mechanical properties of the most commonly used metals, ceramics, and polymers are given in Tables 4–6, respectively.

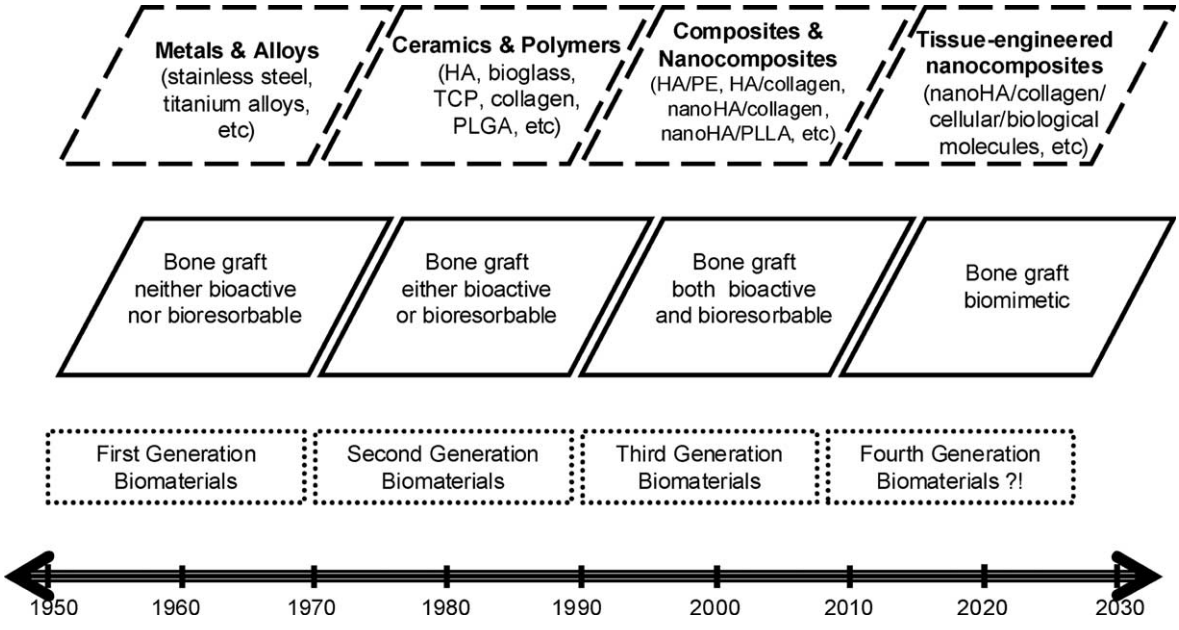


Fig. 2. Evolution of biomaterials in bone grafting.

Table 3
Classification of biomaterials for bone grafting[a]

Biomaterials	Advantages[b]	Disadvantages	Applications	Examples
Metal and alloy	Too strong, tough, ductile	Dense, may corrode	Bone plates, load-bearing bone implants, dental arch wire, and dental brackets	Titanium, stainless steel, Co–Cr alloys, and Ti alloys
Ceramic	Bioinert	Brittle, poor tensile, low toughness, lack of resilience	Hip joints and load-bearing bone implants	Alumina, zirconia
	Bioactive Bioresorbable High resistance to wear		Bone filler, coatings on bio-implants, orbital implant, alveolar ridge augmentation, maxillofacial reconstruction, and bone tissue engineering	HA, bioglass TCP
Polymer	Flexible, resilient, surface modifiable, selection of chemical functional groups	Not strong, toxic of a few degraded products	Bone tissue scaffolds, bone screws, pins, bone plates, bone and dental filler, and bone drug delivery	Collagen, gelatin, chitosan, alginate, PLA, PGA, PLGA, PCL, PMMA, PE
Composite	Strong, design flexibility, enhanced mechanical reliability than monolithic	Properties might be varied with respect to fabrication methodology	Bone graft substitutes, middle ear implants, bone tissue scaffolds, guided bone regenerative membranes, and bone drug delivery	HA/collagen, HA/gelatin, HA/chitosan, HA/alginate, HA/PLGA, HA/PLLA, HA/PE
Nanocomposite	Larger surface area, high surface reactivity, relatively strong interfacial-bonding, design flexibility, enhanced mechanical reliability than monolithic and/or microcomposite	No optimized technique for material processing	Major areas of orthopedics, tissue engineering, and drug delivery	Nano-HA/collagen, Nano-HA/gelatin, Nano-HA/chitosan, Nano-HA/PLLA

[a] Compiled from Refs. [26,31–36].
[b] Common characteristics of biomaterials like biocompatibility are not highlighted.

Table 4
Mechanical properties of metals[a]

Biometals	Young's modulus (GPa)	Tensile strength (MPa)	Compressive strength (MPa)	Hardness (Vickers, kg/mm)	Fatigue strength (MPa)
Ti	110	300–740	550	120–200	240
Ti–6Al–4V alloy	120	860–1140	860	310	280–600
Stainless steel	190	500–950	600	130–180	260–280
Co–Cr alloy	210	665–1277	655	300–400	200–300

[a] Compiled from Refs. [36–38,47].

Table 5
Mechanical properties of ceramics[a]

Bioceramics	Young's modulus (GPa)	Tensile strength (GPa)	Compressive strength (GPa)	Fracture toughness (MPa $m^{1/2}$)	Hardness (HV)	Flexural strength (MPa)	Density (g/cm^3)	Bond strength (GPa)
Alumina	390	0.31	3.9	5.2	2000	390	3.9	300–400
Zirconia[b]	205	0.42	3	12	1150	1300	6	200–500
HA	80–110	0.05	0.4–0.9	0.7–1.2	600	37	3.16	120

[a] Compiled from Refs. [13,41,49].
[b] Partially stabilized zirconia.

Metals have been used in clinical orthopedics since the early 1900s. Titanium, stainless steel, Co–Cr, and titanium alloys are notable examples, which are mostly used at load-bearing sites [36–39]. The elastic modulus of stainless steel and Co–Cr alloys is higher than that of natural bone, i.e., about 10 times greater (see Table 4), which gives complications of mechanical incompatibility. Nowadays, titanium and its alloys (e.g., Ti–6Al–4V) are widely used in load-bearing orthopedic applications. The elastic modulus of these materials is found to be about 5 times greater than natural bone (see Table 4). According to Wolff's law, if a stiffer implant material is placed into bone, the bone will be subjected to reduced mechanical stress that gradually leads to bone resorp-

Table 6
Mechanical properties of polymers[a]

Polymers	Young's modulus (GPa)	Tensile strength (MPa)
Biodegradable		
Poly(L-lactic acid)	2.7	50
Poly(D,L-lactic acid)	1.9	29
Poly(caprolactone)	0.4	16
Poly(β-hydroxybutyrate)	2.5	36
Non-biodegradable		
Poly(ethylene)	0.88	35
Poly(urethane)	0.02	35
Poly(tetrafluoroethylene)	0.5	27.5
Poly(methyl methacrylate)	2.55	59
Poly(ethylene terephthalate)	2.85	61

[a] Compiled from Refs. [13,47,100].

tion. This phenomenon is known as stress-shielding. A typical stress–strain relationship of different biomaterials used in orthopedics is illustrated in Fig. 3 in comparison with a natural bone. It has been recognized that matching the stiffness of the implant with that of the host tissue limits the stress-shielding effect. Owing to insufficient interfacial bonding between metal implant and host tissue, there is limited osteointegration. In order to improve tissue bonding, HA-coated titanium alloys are widely and successfully used in orthopedic surgery.

Ceramics were introduced to orthopedics during the 1960s. They have high compressive strength and hardness (see Table 5); they are also highly biocompatible and tissue responsive. They are also called bioceramics. According to their tissue response, they can be categorized into three types; (i) nearly bioinert (e.g., alumina and zirconia), (ii) bioactive (e.g., HA and bioglass), and (iii) bioresorbable (tri-calcium phosphate (TCP)). Alumina was the first clinically used bioceramic material in 1970 owing to its excellent biocompatibility, hardness, strength to resist fatigue, and corrosion resistance. Zirconia has been in use in orthopedics since 1985 in either the pure form or partially yittria-stabilized form. It exhibits fracture toughness greater than alumina. Alumina and zirconia are predominantly used as femoral heads of total hip joints [40,41]. They do not cause a response from host tissue because they do not chemically or biologically react with surrounding tissues due to their thermodynamic stability. Due to exceptional bioactivity, HA and bioglasses are frequently used as bone graft substitute and as coating-agent on biometallic or biocomposite implants [41]. Prototypes of such HA-based bone graft materials are shown in Fig. 4. They elicit a strong interfacial interaction with host tissue due to their bioactivity; thereby they are considered to provide osteointegrative stimuli. However, they are very less bioresorbable. TCP is widely used as a bioresorbable bone graft [41]. The resorbable ceramics provide a

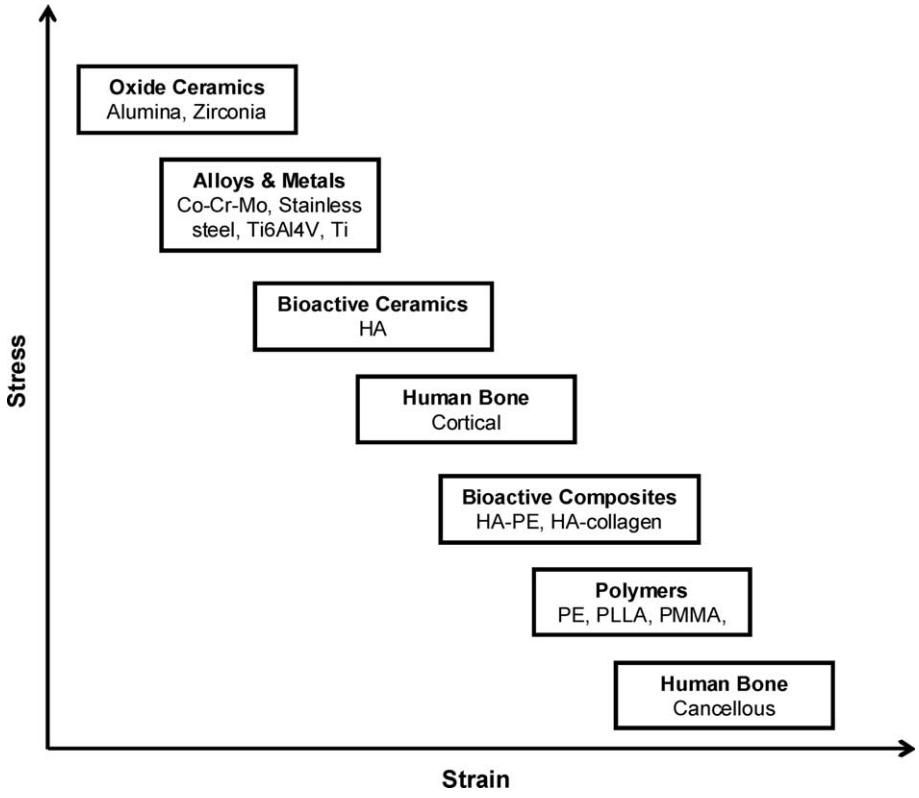


Fig. 3. A typical stress–strain relationship of a variety of bone implants.

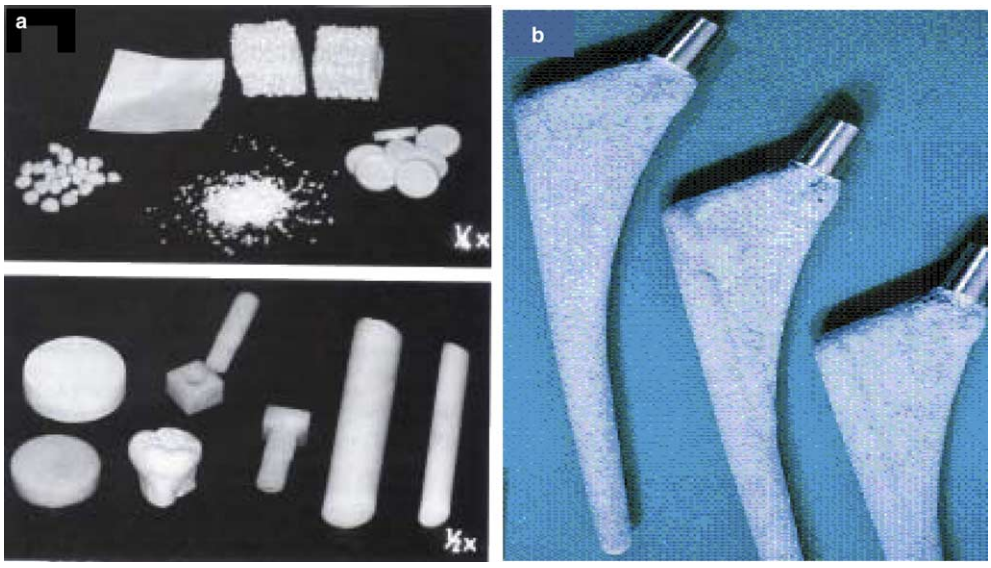


Fig. 4. Prototypes of HA-based bone graft materials. (a) Different shapes and sizes of HA. (Courtesy: Prof. H. Aoki, Tokyo Denki University, Japan.) (b) HA-coated bioimplants. (Reprinted with permission from Evans SL, Gregson PJ. Biomaterials 1998: 19: 1329–1342. © 1998, Elsevier.)

framework for new bone tissue to grow while being resorbed, leaving only the new bone behind after complete resorption. However, the rate of bioresorption of TCP is unpredictable and they have certain drawbacks, which include poor mechanical properties (e.g., brittleness and low toughness). Therefore, they are used only in low-weight bearing orthopedic applications [42]. Overall, the ceramics have many advantages that include biocompatibility, easy availability, shapeability, non-toxic, and non-immunogenic.

Polymers are widely used in bone grafting owing to their biocompatibility, design flexibility, functional groups availability, surface modifiability, light weight, and ductile nature [43,44]. Although they have many desirable characteristics, they exhibit low stiffness (see Table 6). The substantial interest in polymers for various biomedical applications is mainly due to their design flexibility and the biodegradation of certain polymers at body pH. Polymers can include chemical bonds that undergo hydrolysis upon exposure to the body's aqueous environment, and they can also degrade by cellular or enzymatic pathways. The rate of biodegradation can also be controlled by manipulating the polymer properties such as hydrophobicity and crystallinity. Poly(methyl methacrylate) (PMMA) was the first synthetic polymer used in clinical practice in 1937. Since then, numerous polymers are developed and used in a variety of orthopedic and other medical applications. They can be categorized into two types; (i) biodegradable polymers and (ii) non-biodegradable polymers. Collagen, gelatin, poly(lactic acid) (PLA), and poly(lactic-co-glycolic acid) (PLGA) are a few notable examples of biodegradable polymers. Poly(ethylene) (PE), poly(ethylene terephthalate) (PET), and PMMA are examples of non-biodegradable polymers.

4.1. Key factors of an ideal bone graft

Restoration of bone defects by the regeneration of living tissue is the ultimate goal of bone grafting. Engineering a bone graft equivalent to autogenous bone is a prime objective. The main role of bone graft is to provide a framework for regeneration of new bone tissue, soft tissue, vascular-, and other metabolic-components. In the subsequent sections, we provide detailed information on bone grafts with regard to their osteogenic characteristics, the most critical characteristic of an ideal bone graft. Bone grafts can be classified into three types depending on their bone tissue response in vivo. They are: (i) osteoconductive grafts, (ii) osteoinductive grafts, and (iii) osteogenic grafts.

4.1.1. Osteoconductive bone grafts

The main role of osteoconductive grafts is to serve as a structural framework through which the host bone infiltrate and regenerate a new bone tissue. Autogenous bone, allogeneic bone, HA, and collagen are best examples of osteoconductive bone grafts. Some of the commercially available synthetic bone grafts are listed in Table 7. Since HA and collagen are widely used not only in bone grafting but also in other medical applications; we describe their impact in detail here.

4.1.1.1. Hydroxyapatite. Hydroxyapatite is a class of calcium phosphate-based bioceramic, frequently used as a bone graft substitute owing to its chemical and structural similarity with natural bone mineral [45,46]. The stoichiometric HA has a chemical composition of $Ca_{10}(PO_4)_6(OH)_2$ with Ca/P ratio of 1.67. The HA derived either from natural sources or from synthetic

Table 7
Commercially available bone graft materials[a]

Materials	Product names/suppliers
Synthetic HA	Endobon (Merck KGaA), Osteomin (Pacific Coast Tissue Bank), G-bone (SurgiWear), SynHA (Bioland), Periograf (Cooke-Waite Lab), Osprovit (Cerasiv), Ceros 80 (Straumann), Cerapatite (Ceraver Osteal)
Coralline HA	ProOsteon (Interpore)
Animal-derived apatites	Kiel bone (Surgical University Clinic of Kiel), Pyrost (Stryker Howmedica Osteonics), Bio-Gen & Osteoplant (BioTeck)
Coral	Biocoral (Inoteb)
TCP	Synthograft (Johnson & Johnson), Orthograft (DePuy), Augmen (Milter), VitoOss (Orthovita), Ceros 80 & Ceros 82 (Mathys Medical Ltd Osteosynthesis), Calciresorb (Ceraver Osteal),
Calcium sulphate	Osteoset & Osteoset-T (Wright Med Tech)
Bioactive glasses	Bioglass, PerioGlas, NovaBone, & NovaBone-C/M (NovaBone, USBiomaterials)
DBM	DynaGraft, Accell DBM100, Accell Connexus & OrthoBlast (IsoTis), Renegaform (Regen. Tech.), Grafton (Osteotech), Osteofil (Sofamor Danek), Opteform & Optefil (Exactech), DBX (Synthes)
Growth factors (e.g., BMP)	rhBMP-2 (Genetic Institute), rhBMP-7 (Creative Biomolecules), InFuse (Medtronic Somafor Danek), Neosteo (Intermedics Orthop.)
Calcium phosphate-based cements/composites	SRS (Norian), BoneSource (Leibinger), α-BSM (E-TEX), Biobon (Biomet Merck), Vitagraft, Orthocomp, & Biogran (Orthovita), Triosite (Zimmer), Ostilit (Stryker Howmedica Osteonics), BoneSave (Stryker Howmedica Osteonics), OsSatura (IsoTis), HAPEX (Smit & Nephew)
Collagen & Collagen-based composites	Biocollagen (Bioteck), Collagraft (Zimmer Inc. & Collagen Corp.), Collapat (Ostobalag) Bio-Oss (Geistlich Biomaterials, Inc), OsteoGraf (Ceremed Dental, Inc), Healos (CE Mark & Orquest), Lyostypt (Braun-Melsungen AG), Surgibone (Unilab), Cerapatite-Collagen (Ceraver Osteal), Biofibre (Norian)

[a] Compiled from the web search of respective suppliers.

sources is regarded as bioactive substance, since it forms a strong chemical bond with host bone tissue, and hence it is recognized as a good bone graft material. HA is not only bioactive but also osteoconductive, non-toxic, non-immunogenic, and its structure is crystallographically similar to that of bone mineral with adequate amount of carbonate substitution. A compilation of physiochemical, mechanical, and biological properties of HA are given in Table 8 [13,26,47–51], which makes HA an appropriate bone graft material. Possible clinical uses of HA range from augmenting atrophic alveolar ridges to repairing long bone defects, ununited bone fractures, middle ear prostheses, spinal fusions, cranioplasty, craniofacial repair, and vertebral fusions. On the other hand, it has also been used in dental surgery, biomolecular delivery, and drug delivery. As per the literature survey, HA has a long history of use as a biomaterial. Daubree [52] carried out the first synthesis of apatite in 1851 by a process in which phosphorous trichloride vapor was passed over red-hot lime. In 1951, a synthetic HA was prepared by Ray and Ward [53], suitable for bone defects. They implanted HA into surgically created defects in the long bones and iliac wings of dogs and the skulls of cats and monkeys and obtained affirmative results. Since then many investigations on HA were carried out and tested both in animals and in humans. In the 1970s, Aoki and Kato [54], De Groot [55], and Jarcho [56] pioneered multi-shapeable HA suitable for clinical orthopedics. Since then, a number of fabrication

Table 8
Physicochemical, mechanical, and biological properties of HA[a]

Properties	Experimental data
Chemical composition	$Ca_{10}(PO_4)_6(OH)_2$
Ca/P molar	1.67
Crystal system	Hexagonal
Space group	$P6_3/m$
Cell dimensions (Å)	$a = b = 9.42$, $c = 6.88$
Young's modulus (GPa)	80–110
Elastic modulus (GPa)	114
Compressive strength (MPa)	400–900
Bending strength (MPa)	115–200
Density (g/cm^3)	3.16
Relative density (%)	95–99.5
Fracture toughness (MPa m$^{1/2}$)	0.7–1.2
Hardness (HV)	600
Decomposition temperature (°C)	>1000
Melting point (°C)	1614
Dielectric constant	7.40–10.47
Thermal conductivity (W/cm K)	0.013
Biocompatibility	High
Bioactivity	High
Biodegradation	Low
Cellular-compatibility	High
Osteoinduction	Nil
Osteoconduction	High

[a] Compiled from Refs. [13,26,47–51].

methodologies of HA have been reported. These studies concentrated on microscale HA (>1 μm).

Recently, nanoscale HA (~10–100 nm) has received much attention owing to its superior functional properties over its microscale counterpart, particularly surface reactivity and ultra-fine structure, which are the most imperative properties for tissue–graft interaction upon implantation. During the past few years, significant research effort has been devoted to nanostructure processing of HA and its composites in order to obtain ultra-fine structures with physical, mechanical, chemical, and biological properties better than their microscale counterparts and, at the same time, similar to natural bone mineral. It has also been proven that the nano-HA, compared to conventional micro HA, promotes osteoblast adhesion, differentiation and proliferation, osteointegration, and deposition of calcium-containing minerals on its surface, which leads to enhanced formation of new bone tissue within a short period [57]. The impact of nano-HA has also been extensively reviewed with regard to recently developed manufacturing techniques [26,13]. Some of the prominent processing methods for manufacturing nano-HA include solid state [58], wet chemical [59,60], hydrothermal [61,62], mechanochemical [63], pH shock wave [64], and microwave processing [65] (see Table 9). HA can also be processed from animal bone [66,67] and coral exo-skeleton [68,69], but on a microscale. Many companies have commercially developed and sold HA for clinical use. Endobon [70], Cerapatite [71], and ProOsteon [72] are a few examples of commercially available HA-based bone grafts. HA has a unique capability of binding to the natural bone through biochemical bonding, which promotes the interaction between host bone and grafted material. Although nano-HA is an excellent bone graft material, its inherent low fracture toughness has limited its use in certain orthopedic applications, in particular heavy load-bearing implantation [73]. The fracture toughness of HA is about 1.0 MPa $m^{1/2}$, which is very low compared to natural bone (2–12 MPa $m^{1/2}$) and the Weibull modulus is also sufficiently low that depromotes the reliability of HA for heavily loaded implants. The Weibull modulus of HA is in the range between 5 and 18, which means that HA behaves as a typical brittle material; thereby it is used only in low weight-bearing orthopedic applications, e.g., as a bone defect filler, coating-agent on metallic bioimplants, biomolecular delivery, and drug delivery. In order to improve reliability, it is necessary to introduce some biocompatible reinforcement agents or matrix materials. However, introduction of foreign materials may lead to a decrease of reliability of HA; thereby choosing the reinforcement agents or matrix materials is of great importance in making composites. Intensive research is ongoing to produce HA-based composites with improved fracture toughness. Recently, composites of nano-HA with natural polymers, in particular collagen, are preferred to improve the reliability of nano-HA. Collagen is a natural extracellular matrix (ECM) material available in human bone tissue; thereby choosing collagen as a matrix material could enhance the composite's reliability.

4.1.1.2. Collagen. Collagen is a natural polymer (protein) being used as a good biomaterial in various biomedical applications and approved by the United States of Food and Drug Administration. It is present in the organic phase of bone and primarily serves as a structural protein of native ECM. It has many desirable functional properties for cellular growth. Purified collagen has excellent biocompatibility, biodegradability, non-toxicity, and non-antigenecity that make collagen a prime and safe source of materials for use in a variety of biomedical applications, particularly bone tissue engineering [74]. Collagen is also a haemostatic and osteoconductive agent [74–79]. There are many types of collagen available based on their molecular sequences. So far, 27 distinct types with at least 42 distinct polypeptide chains of collagen have been identified. Although many types of collagen exist in a living organism, the most abundant form of collagen in connective tissue is

Table 9
Methods involved in the synthesis of nano-HA

Methods	Grain size (nm)	General remarks	References
Solid state	500	Inhomogeneous, large grain size (micro to nano), irregular shapes, reaction condition 900–1300 °C	[58]
Wet chemical	20–200	Nanograin size, low crystallinity, homogeneous, reaction condition: room temperature to 100 °C	[59,60]
Precipitation/hydrothermal	10–25	Homogeneous, ultra-fine particles, low crystallinity, reaction condition: room temperature to 200 °C (1–2 MPa)	[61]
Hydrothermal	10–80	Homogeneous, fine crystals, high temperature, and high-pressure atmosphere	[62]
Mechanochemical	<20	Easy production, semi-crystallinity, ultra-fine crystals, room temperature process	[63]
pH shock wave	20–100	High-energy dispersing, nonporous, monocrystalline particles with Ca/P molar ratio 1.43–1.66	[64]
Microwave	100–300	Uniformity, nanosize particles, time and energy saving	[65]

type I. Type I collagen is composed of two α_1 (I) chains and one α_2 (I) chain in the form of a triple helix pattern with a fiber diameter of about 50 nm [78,79]. All the collagens are composed of three polypeptide chains (α-chains) that are each coiled into a left-handed helix. These three chains are then wrapped around each other into a right-handed triple helix; thereby the final structure is in a triple helical rope-like fashion. The triple-helical domain has a characteristic primary structure, where glycine in every third amino acid generates repeating $(\text{Gly X–Y})_n$ units; X is alanine or proline and Y is hydroxyproline [78,79]. In general, collagen extracted from natural tissues is capable of eliciting an immunogenic response upon implantation; thereby direct use of this type of collagen is limited. Nowadays, a purified form of collagen known as reconstituted collagen is processed by various biochemical methods and commercially available. The reconstituted collagen has relatively lower immunogenicity than native collagen. Collagen can also be chemically modified by various methods (succinylation, for example) to increase its surface reactivity through inducing negative charges, which in turn causes the collagen to dissolve in neutral pH; thereby widening its usage in biomedicine. However, it does not have significant strength or stiffness, which further leads to the advantage of composites. There is a possibility of enhancing the functionality of collagen by incorporating other bone graft materials (e.g., HA, BMP, and osteoprogenitor precursors) in the form of a composite. Collagraft, Bio-Oss, and Healos are examples of commercially available collagen-based bone grafts for clinical use [80–82]. Collagraft, which is a biocomposite made of calcium phosphates and collagen (Zimmer, IN and Collagen Corporation, CA), is used for low-weight bearing applications. It is available in the form of paste or soft strips. Bio-Oss is a craniofacial graft, which is made of bovine collagen and de-organified bovine bone. Healos (CE Mark and Orquest, CA) is a sponge-like mineralized collagen fiber, mainly used as an osteoconductive matrix. The demineralized form of bone collagen was extensively used as a bone graft in the treatment of acquired and congenital bone defects in combination with HA [83]. The results confirmed the ability of osteoconduction and osteoinduction of the graft. Another study suggested that addition of retinoic acid to collagen could enhance bone regeneration [84]. Collagen was also used in combination with rhBMP-2 as a bone substitute [85,86]. Accordingly, collagen can be considered as an accomplished biomaterial for bone grafting and, in fact, can be well utilized as a precursor while making nanocomposite bone grafts.

4.1.2. Osteoinductive bone grafts

Osteoinductive grafts are capable of inducing differentiation of undifferentiated stem cells into osteogenic cells or to induce stem cells to proliferate. In 1965, Urist [87,88] discovered that the DBM was able to promote new bone formation in bone defective sites. It was found, after systematic investigations, that the proteins intact within the matrix were responsible for bone formation owing to their osteoinductive ability. These proteins were later named bone morphogenetic proteins (BMPs). They are considered as one of the most promising groups of biological substances and have a wide range of potential for bone grafting. BMPs have been extensively studied in native and recombinant forms. The experimental evidence show that native BMPs are immunogenic and recombinant BMPs (rhBMPs) are non-immunogenic [89,90]. Among a variety of rhBMPs, rhBMP-2 has been widely used for bone grafting and clinically proven to exhibit very high osteoinductivity [91]. However, the mode of delivery of BMPs into the defective site is still under investigation since they need a carrier matrix in order to function effectively. Nanocomposites could help for this purpose. Ono et al. [92] used HA as an osteoconductive carrier matrix for the delivery of rhBMP-2. They hypothesized that the combinations of HA and rhBMP-2 could provide a potent alternative to autogenous bone grafts. They implanted HA in the form of rods treated with 1.7 and 5.7 g of rhBMP-2 into the cranial bone of rabbits and found that bone formation was inclined to be greater at the higher dose of rhBMP-2 (5.7 g). The induction of new bone is dose-dependent. Even with a small dose, considerable increases in the strength of HA were observed compared to pure HA. As a result, early bone ingrowth in the pores of HA was noticed, which is a good sign of the efficiency of the carrier system. Recently, Jung et al. [93] clinically studied 11 patients to evaluate whether or not the addition of rhBMP-2 to a commercial bone graft product of Bio-Oss (a bioengineered graft from bovine source) will improve guided bone regeneration therapy regarding bone volume, density, and maturation. They found that the combination of Bio-Oss with rhBMP-2 could enhance the maturation process of bone regeneration and could increase osteointegration between graft and host bone. These studies clearly demonstrated that rhBMP-2 has the osteoinductive potential to improve and accelerate bone regeneration.

4.1.3. Osteogenic bone grafts

Great interest is shown in BMA in bone grafting owing to its osteogenic potential, similar to autogenous bone [94]. However, the effectiveness of BMA depends on the density and activity of stem cells intact within the aspirate. The advantage of BMA over autogenous bone is that it can be harvested from the patient without complicated surgery and could be delivered at the bone defect. This type of transplantation is termed minimally invasive bone grafting. The efficiency of BMA depends on its carrier system. It can be mixed with other bone graft materials, in particular osteoconductive grafts,

and can be transplanted at defective sites. It has been reported that there is a possibility of increasing the rate of differentiation and proliferation of bone marrow stem cells by delivering in conjunction with DBM [95], collagen [80], and HA [96] in the form of composite bone grafts. It is also believed that nanocomposites, particularly HA/collagen, can be used more effectively than their constituents alone as a bone graft.

4.2. Composite bone grafts

Single-phase materials do not always provide all the properties necessary for bone grafting and are very far from the characteristics of a true autogenous bone graft. Our ultimate aim is to make a bone graft equivalent to autogenous bone by integrating all the factors associated with osteoconduction, osteoinduction, and osteogenicity. The term composite can be defined as a heterogeneous combination of two or more materials, differing in morphology or composition on a microscale, in other words microcomposite [97]. Using the composite approach, it is possible to manipulate the mechanical properties such as strength and modulus of the composites closer to natural bone with help of secondary substitution phases. For example, HA-polymer composites have an elastic modulus near to that of bone and are more mechanically reliable than their monolithic constituents. A graphical representation of mechanical consistency of various HA-based composites is given in Fig. 5 in comparison with a natural bone. It is also well known that composites implanted in human body tend to elicit a response by the host tissue depending on characteristics, such as surface reactivity. According to their interaction with host tissue, the composite bone grafts

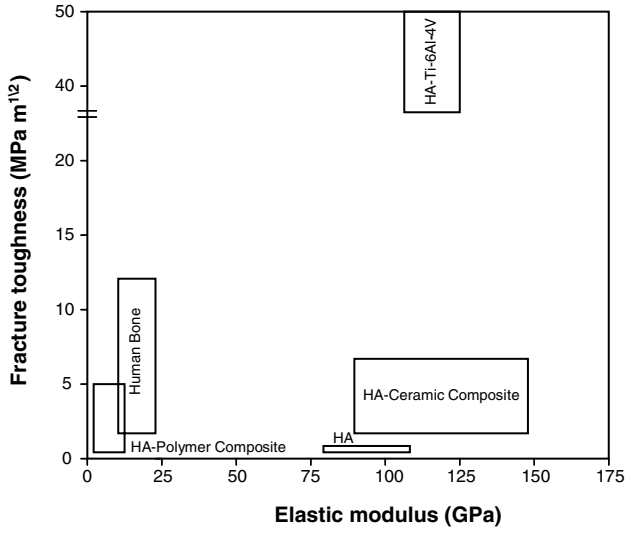
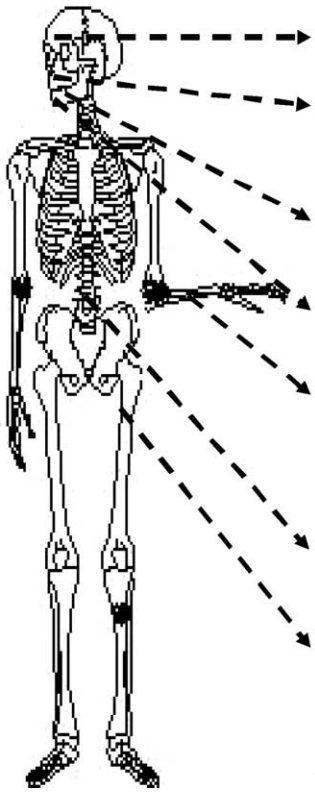


Fig. 5. A graphical representation of relationship between toughness and modulus of various HA-based composite materials. (Complied from Refs. [10,13].)

can be classified into three types; (i) nearly bioinert, (ii) bioactive, and (iii) bioresorbable. The interface of the bioinert grafts is neither chemically nor biologically bonded to living tissue. Alumina-coated biometals, carbon/carbon, and carbon/PEEK are examples of this kind of graft. The bioactive composite grafts are designed to essentially achieve interfacial bonding between the graft and the host tissues. HA/collagen, HA/PE, and HA/Ti–6Al–4V are a notable examples of bioactive composite grafts. The bioresorbable composite grafts are designed to biodegrade over time and are gradually replaced with new bone tissue. TCP/collagen, TCP/PLA, and TCP/PCL are examples of this kind of graft.

A lint-reinforced plaster was the first composite used in clinical orthopedics as an external immobilizer (bandage) in the treatment of bone fracture by Mathijsen in 1852 [98], followed by Dreesman in 1892 [99]. Some of the composite grafts, both cellular and acellular grafts, used in the last few decades for clinical bone therapy are given in Fig. 6. Recently, Ramakrishna et al. [100] reviewed the recent trends and potential applications of polymer composites in various biomedical applications, including bone reconstruction. Considerable attention has been paid in the past two decades to bioactive composite grafts that consists of bioactive ceramic filler in a polymeric matrix. In the 1980s, Bonfield et al. [101,102] developed a bioactive composite based on HA and PE, which has a capability of promoting extensive bone-bonding function upon implantation. This has been commercialized in the name of HAPAX™. Unfortunately, it is not a good biodegradable graft, which limits its wider usage in clinical medicine. It is primarily used as a middle ear implant, which can be readily shaped during surgery. Bioresorbable composites have been developed and investigated as bone grafts. For example, Laurencin et al. [103] developed a composite containing HA and PLGA and demonstrated its cellular-compatibility suitable for bone tissue regeneration. It was found that the composite highly supported osteoblasts proliferation, differentiation, and deposition of calcium phosphate minerals. Cells proliferated for up to 21 days and formed a mineralized layer on the composite. However, some studies report that the physical properties of the composite are not completely favorable upon implantation because of its conventional mode of processing [104]. We have also explored the possibility of utilizing HA, both at micro- and nano-level, in conjunction with natural and synthetic polymers in the form of composites suitable for bone grafting and bone drug delivery [68,105–108]. As compared to monolithic biomaterials, the composites, in particular HA-polymer composites, are consistent with the mechanical strength of human cortical bone (see Figs. 3 and 5).

Of particular interest is the combination of HA with collagen as a bioactive composite, which appears a natural choice for bone grafting, i.e., it mimics the bone



Clinical use	Acellular biomaterials	Cellular biomaterials
Cranial bone defects	Bioactive glass, alumina, HA, HA-collagen	Auotogenous bone, allogenous bone, DBM
Maxillofacial reconstruction	HA, bioactive glass, alumina, zirconia, HA-PE, Bioglass-PE, PTFE-carbon	Auotogenous bone, allogenous bone
Alveolar ride augmentation	HA, TCP, bioglass, Alumina, HA-PLA, HA-collagen, HA-PLGA	Auotogenous bone, allogenous bone, HA-autogenous bone composite
Periodontal defects	HA, TCP, bioactive glass, HA-PLA, HA-PLGA, HA-collagen	Auotogenous bone, allogenous bone, DBM
Bone void fillers	HA, TCP, biocoral, calcium sulfate, PMMA, HA-collagen, bioactive glass-ceramic composite	Auotogenous bone, demineralized allograft, demineralized xenograft, patient-derived osteoblasts in conjunction with HA matrix
Spinal surgery	HA, bioactive glass, HA-collagen, PET-silicon, bioglass-PU	Autogenous bone, DBM, tissue-engineered HA, HA-BMA composite
Orthopedic prostheses	Alumina, zirconia, stainless steel, Ti, Ti-6Al-4V, Co-Cr-Mo-Ni, A-W glass ceramics, HA-PE, HA-collagen, bioactive glass-coated biometals, HA-coated biometals, Carbon fibers-PE	Osteogenic cells intact HA- and bioactive glass-coated porous surfaces

Fig. 6. Cellular/acellular grafts in clinical bone therapy.

components [109]. The unique characteristic of this composite is the spatial orientation between HA and collagen macromolecules, which seems to be the source of the mechanical strength of the composite. Therefore, considerable effort should be paid on the spatial orientation of the bone grafts. Further, the composite is highly biocompatible, osteoconductive, and there is a suggestion that it might be osteoinductive as well. However, the performance of this composite depends on the source of collagen from which it was processed. Chapmen et al. [110] demonstrated the effectiveness of this type of composite in conjunction with BMA in the treatment of long bone fracture. They found that it had a performance equivalent to autogenous bone graft. The overall results suggest that HA/collagen could act as a good bioactive composite for bone grafting.

However, most of the HA/collagen microcomposites are conventionally processed by anchoring HA particulates into the matrix of collagen [111,112], which makes it quite difficult to obtain a uniform or a homogeneous composite graft. Further, most of the HA used in this process are sintered and large-size crystallites, which is in contrast to the natural bone apatite; thereby it may take a longer time to remodel into bone tissue upon implantation. In addition, some of the composites exhibit very poor mechanical properties [113,114], probably due to the lack of strong interfacial-bonding between the constituents. There is a chance of improving osteointegration by reducing the grain size of the reinforcing agent or by activating the nucleation of ultra-fine apatite growth onto the matrix [115,116]. This may lead to enhanced mechanical strength and osteointegration with improved biological and biochemical affinity to the host bone. It is also anticipated that high fracture toughness could be conferred by controlling the extent of interfacial-bonding between their constituents. Recently, with advances in nanoscience and nanotechnology, nanocomposites have gained much interest and are perceived to be beneficial in many aspects as bone grafts over microcomposites, which open a new arena in the field of bone grafting. Since this field is still at infancy, there is no standardized method for making such nanocomposite grafts. The following sections describe their impact and recently developed processing methodology with suitable illustrations.

5. Bone grafting using nanocomposites – a new approach

5.1. Rationale and benefits of nanocomposites

Nanocomposites could play a pivotal role in bone grafting as a new class of bone graft material, which uses a combination of several nanoscale bone graft materials

and/or in conjunction with osteoinductive growth factors and osteogenic cellular components. The term nanocomposite can be defined as a heterogeneous combination of two or more materials in which at least one of those materials should be on a nanometer-scale. Nanomaterial is considered as a new class of material as it possesses superior properties over its microscale counterpart. Nanocrystalline HA promotes osteoblast cells adhesion, differentiation, and proliferation, osteointegration and deposition of calcium containing minerals on its surface better than microcrystalline HA; thus enhancing the formation of new bone tissue within a short period [57,117,118]. Since bone is a typical example of a nanocomposite, designing bone graft in the form of nanocomposite is perceived to be beneficial over monolithic and microcomposite materials. Nanocomposite bone graft made of nano-HA and collagen facilitates greater osteoconduction and related functions than conventional bone grafts [119]. This system exhibits some features of natural bone in composition and structure to a certain extent. Current trends in HA-based nanocomposites for bone grafting applications are summarized in Table 10 [120–135]. Innovations in the processing of nanocomposites are raising the possibility of realizing bone grafts with improved performance.

5.2. Design and performance of nanocomposites

As nanocomposites are gaining much interest in bone grafting owing to their sophisticated functional properties as discussed in the previous sections, it is useful to review their processing methodology and performance in vitro and in vivo. They can generally be processed through three distinct ways: (i) conventional methods, (ii) biomimetic self-assembly, and (iii) tissue engineering, which are descried in detail below.

5.2.1. Conventional nanocomposites

Nanocomposites can be made, conventionally, by blending or mixing a heterogeneous combination of two or more materials, differing in morphology or composition in which at least one of the materials should be on nanoscale. Blending is a technique used to produce a composite product with specific characteristics by combining at least two materials. Although it is not a new concept, it has gained a considerable interest in the past few years. It is well known that blending of multiple materials with different characteristics leads to composites with tailor-made properties, but it is quite difficult to control homogeneity and uniformity of the secondary or reinforcing phases. The procedure begins with a macroscopic material and incorporates nanoscale components to form the nanocomposite. Although, there is a possibility of direct mixing of nanoscale components, controlling their size and structure is quite intricate. Very few studies have reported the processing of nanocomposites by blending nano HA with a collagen phase at required compositions.

A method for making a nanocomposite using mixing of nano-HA with collagen is specifically given here [120]. First, the nano-HA was synthesized with a routine precipitation method using aqueous solutions of $Ca(OH)_2$ and H_3PO_4 at 40–50 °C and then it was gently mixed with collagen solution that was dissolved in acetic acid at room temperature and then freeze-dried. The nanocomposite is made up of bundles of collagen fibers embedded with nanocrystals of HA in a sponge-like structure. However, the crystallite sizes are not uniform, often aggregated and randomly distributed into the fibrous matrix; therefore there is no structural uniformity observed that is close to natural bone. Further, there is no sign of chemical interaction between HA and collagen phases, which is probably due to the lack of suitable

Table 10
Current trends in HA-based nanocomposites for bone grafting

Nanocomposite systems	Methods	Experimental studies performed			References
		Materials characterization	In vitro cell culture	In vivo animal study	
HA/collagen	Precipitation	+	–	–	[120]
HA/collagen	Biomimetics	+	+	–	[121]
HA/collagen	Biomimetics	+	–	+	[122]
HA/collagen	Biomimetics	+	+	+	[123]
HA/collagen/PLA	Biomimetics	+	+	+	[124]
HA/collagen/alginate	Biomimetics	+	+	–	[125]
HA/chitosan	Precipitation	+	–	–	[126]
HA/gelatin	Biomimetics	+	–	–	[127]
HA/silk fibroin	Mechanochemical	+	–	–	[128]
HA/PCL	Solvent-casting	+	–	–	[129]
HA/PLA	Solvent-casting	+	–	–	[130]
HA/PEG/PBT	Precipitation	+	–	–	[131]
HA/PHMA	Biomimetics	+	–	–	[132]
HA/Polyanhydride	Photo polymerization	+	–	–	[133]
HA/PHEMA	Biomimetics	+	–	–	[134]
HA/PAA	In-situ polymerization	+	–	–	[135]

interfacial-bonding, which is in contrast to the way in which native collagen is biomineralized. This type of processing method is not recommended for making high performance nanocomposites which anticipate the structural and compositional character of natural bone. In order to enhance interfacial-bonding, an advanced processing strategy is required.

5.2.2. Biomimetic nanocomposites

Reconstruction of bone tissue using nanocomposite bone grafts, having structure, composition, physicochemical, biomechanical, and biological features that mimic the natural bone is a goal to be pursued. It is well known that the natural bone consists of nano-sized blade-like crystals of HA grown in intimate contact with an organic matrix rich in collagen fibers. A novel way of fabricating nanocomposite bone grafts using strategies found in nature has recently received much attention and is perceived to be beneficial over conventional methods. The term biomimetic process can be defined as a microstructural processing technique that either mimics or inspires the biological mechanism, in part or whole [136]. It was derived from the Greek words, bios meaning life and mimesis meaning imitation. It is also called by several distinct names; bionics, biognosis, bioinspired, and biomimicry are few of them. The biological process generates highly ordered materials with hybrid composition, complex texture, and ultra-fine crystallites through hierarchical self-assembly. So, it is believed that making of nanocomposite grafts with certain features of natural bone either compositionally or structurally using biomimetic self-assembly may replicate the natural process. This method involves a bottom-up approach, which begins by designing and synthesizing molecules that have the ability to self-assemble or self-organize spontaneously into a higher order of microscale or macroscale structure [137,138].

5.2.2.1. Nucleation and growth of HA nanocrystals onto collagen. Nucleation and growth of HA crystallites onto collagen in a controlled fashion is perceived to be beneficial to enhance the properties of nanocomposites. A key step involved in the biomimetic strategy associated with the crystal growth of apatite phase onto collagen matrix is schematically illustrated in Fig. 7. This process partly mimics the biological phenomenon and provides a good system for bone regeneration with enhanced osteoconductivity than pure HA and pure collagen [139,140]. A biomimetic nanocomposite was developed by nucleating HA nanocrystals onto collagen fibers [140]. The direct nucleation of HA nanocrystals onto collagen fibers has been performed by starting from an aqueous suspension of $Ca(OH)_2$ and H_3PO_4 together with collagen solution of pH 9–10 at 25 °C. The obtained composite product was freeze-dried at −40 °C and then gradually warmed to 35 °C for 36 h.

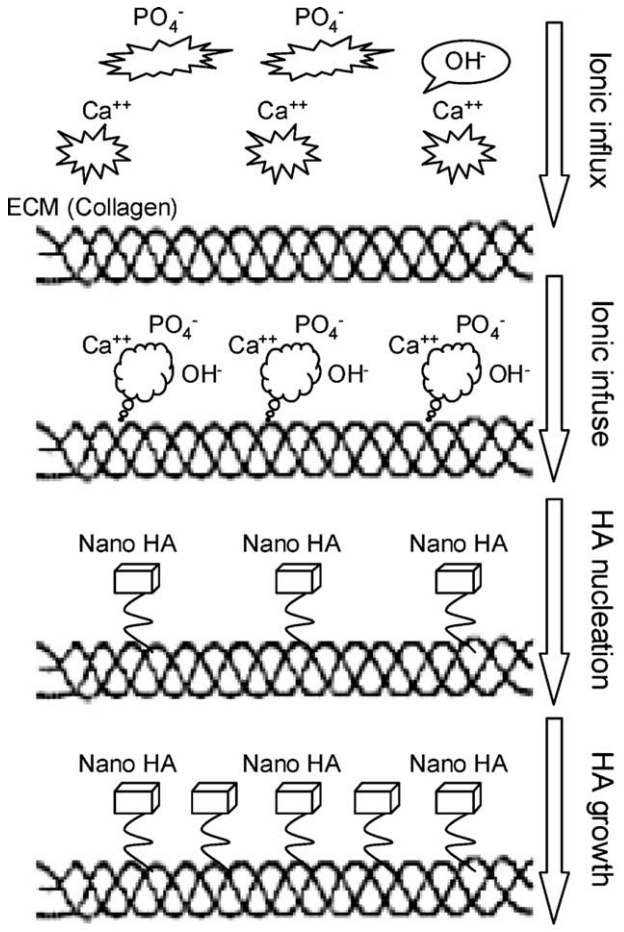


Fig. 7. A scheme for a self-assembly of HA/collagen nanocomposite graft.

It was noticed that the nanocrystals of HA elegantly aligned with their c-axis preferentially oriented along the collagen fibers, which indicates a close interaction between HA and collagen phases. As this process mimics biomineralization of the natural bone to a certain extent, it is suggested that the HA/collagen nanocomposite can be used for bone repair in orthopedic and maxillofacial surgeries. A similar bone-like nanocomposite consisting of HA and collagen, by a self-organization mechanism using $Ca(OH)_2$, H_3PO_4 and porcine atelocollagen as starting materials was developed by Kikuchi et al. [119,139]. The length of self-assembled fiber bundles was found to be 20 μm in which each bundle consisted of many collagen fibrils of 300 nm length embedded with blade-like HA nanocrystals of 50 nm in size. They found that increasing the degree of self-assembling eventually increases the bending strength of the composite, suggesting that high mechanical strength can be attained by optimizing the self-assembling mechanism between HA and collagen. The in vivo performance of this nanocomposite was evaluated in beagle dogs by creating an artificial bone defect. The results of the animal study proved the excellent biocompatibility,

osteocompatibility, and bioactivity of the composite with surrounding tissues and stimulated the formation of new bone growth with Haversian systems. Further, the nanocomposite enhanced the rate of bone-healing compared with conventional biomaterials, which might have resulted from its nanostructural and compositional similarity with natural bone tissue. This kind of composite can readily be incorporated into bone metabolism rather than being a permanent implant.

An interesting method for the self-assembly of HA coatings onto collagen membrane involved soaking the collagen membrane in stimulated body fluid (SBF) solution with and without citric acid [141]. There was no nucleation of apatite crystals on the surface of the membrane when it was soaked in SBF without citric acid. Interestingly, the membrane soaked in SBF with citric acid has gradually stimulated the nucleation of HA crystals. Later, other groups demonstrated nucleation of HA crystals onto collagen through a chemical interaction of carboxylate groups of collagen macromolecules [142–144]. The mechanical reliability of the nanocomposite does not match exactly with that of the host bone in many cases. In order to enhance the mechanical properties of the mineralized collagen, a glutaraldehyde-crosslinked porous HA/collagen nanocomposite was developed [145]. Here, $Ca(OH)_2$, H_3PO_4, and collagen were used as precursors. A homogeneous suspension of 0.1994 mol of $Ca(OH)_2$ dispersed in 2000 ml of H_2O and 59.7 mM of H_3PO_4 was gradually added to the aqueous solution of 5 g collagen. The pH of the reaction mixture was adjusted to 8.4 and the reaction was maintained at 38 °C and finally aged for 12 h by adjusting the pH to 7. In order to mimic the crosslinking process in the toughening of natural bone, 0.2% aqueous glutaraldehyde solution was slowly added into the slurry solution at the same reaction temperature. The 3 D porous nanostructure was able to support cellular growth. If crosslinking agent was added, the size of the reaction precipitate increased and seemed to be triggered by the assembly of HA nanocrystals (50 nm) around the collagen fibers (300 nm), leading to the formation of a thick composite bundle. The thickness of the mineralized collagen depends on the amount of glutaraldehyde added. Since this system has exhibited relatively good toughness, it was suggested for use as a bone graft.

Although many investigations have been carried out to reproduce bone artificially, using HA and collagen [146,147], it should be noted that bone is not a simple mixture of HA crystals and collagen fibers. In addition to collagen, bone contains bioorganics such as glycoproteins or glycosaminoglycans, which play a pivotal role in controlling the functions of bone cells [148,149]. In this regard, an attempt was made to mimic both the bioorganic composition of bone and the peculiar configurational arrays of HA nanocrystals on those bioorganics [150,151]. A design strategy for making such a nanocomposite using HA, collagen, and chondroitin sulfate (proteoglycans) was developed with an intention of using it as a biomimetic bone graft for cartilage repair, a pre-mature bone, as well as for bone repair. The hypothesis was that the cartilage is a key tissue in almost all growing bones because bone formation is initiated from the calcification of cartilage and then it would be replaced by the bone through endochondral ossification. Chondroitin sulfate is best known for its ability to promote the binding of chondronectin, which is the chondrocyte attachment factor, to collagen and thus gradually stimulates chondrocyte adhesion. Accordingly, this nanocomposite system is likely to provide specific binding sites for chondrocytes. The crystallographic studies confirmed the orientation of *c*-axis of the HA nanocrystals along the longitudinal direction of the collagen and chondroitin sulfate complex. The distribution of HA was found to be almost uniform and the composite exhibited substantial mechanical strength with fracture toughness 35–50 MPa and hardness 119–219 MPa. Further, it has a potential for bone remodeling through the process of endochondral ossification. Based on these results, the proteoglycans-immobilized HA/collagen nanocomposite may be a promising bone substitute.

5.2.2.2. Nanocomposites as carrier for growth factor delivery. Growth factors can be effectively delivered to a bone defect through nanocomposites. Recently, a HA/collagen nanocomposite system was biomimetically developed and used as a carrier for the delivery of rhBMP-2 [122,152]. The mechanical reliability of this system corroborated well with the strength of autogeneous cancellous bone and the in vivo performance of the nanocomposite with rhBMP-2 is better than the nanocomposite without rhBMP-2. Early bone formation occurred with the use of rhBMP-2 treated composite, which implies its efficacy as a good bone graft. The composite has also enhanced the osteoconduction and related functions. This system can be used for anterior fusion of the cervical spine as well as inlay grafting bone defects in weight-bearing sites. Sun et al. [153] developed a nanocomposite bone graft system made of nano-HA and collagen in conjunction with rhBMP-2. It was used as a graft extender and enhancer on lumbar intertransverse fusion in rabbits. This system was found to be non-immunogenic, biodegradable, highly effective in osteoconduction and osteoinduction, ready for rapid vascularization and mesenchymal cell invasion, adaptive to various shapes of bone defects, and can provide mechanical support when needed.

5.2.3. Tissue-engineered nanocomposites

Although nanocomposites without living cells, as described above, show good performance in many bone defects, some of them fail to stimulate several complex biological functions, particularly osteogenesis. Since

only living bone cells ultimately generate new bone tissue, a unique approach is to develop nanocomposites through tissue engineering that are cell-responsive upon implantation. Tissue engineering applies the principles of bioengineering and biosciences towards the development of novel biological substitutes capable of restoring, maintaining or improving a tissue function, which fails to regenerate or heal spontaneously. The prime concept of tissue engineering is to isolate a small biopsy of specific cells from a patient, to allow them to culture on scaffold, to transplant the cell-engineered scaffold into the defective site of the patient's body that needs bone regeneration, and to guide or direct new tissue formation into the scaffold that can be biodegraded over time [154]. Three key factors have to be considered for the success of bone tissue engineering. They are cells, scaffold, and cell–matrix (scaffold) interaction. The scaffold, an artificial extracellular matrix (ECM), plays a pivotal role in accommodating the cells. These cells then undergo proliferation, migration, and differentiation, leading to the formation of a specific tissue while secreting the ECM that is required for tissue regeneration. Direct delivery of cell suspension has been used in some cases without using scaffolds [155,156], but this process encountered difficulties in having poor control over the localization of transplanted cells. It is also known that most of the cells are anchorage-dependent and will not survive if delivered without a suitable scaffold. Scaffolds loaded with growth factor have regulated cellular growth and related functions in a better way [123].

Keeping the above points in view, bone tissue engineering approach to treat bone defects must involve the use of osteoconductive scaffold with osteogenic cells and osteoinductive growth factors, which may create a true bone graft. Fig. 8 shows a design strategy for a tissue-engineered nanocomposite graft. As HA is an osteoconductive agent, it can be used as a scaffold matrix for bone tissue engineering. However, it does not possess osteoinduction ability and its biodegradability is also relatively slow. To circumvent these drawbacks, biodegradable polymers can be employed to make a composite in conjunction with HA and osteogenic potential cells, probably BMA. Some of the tissue-engineered HA and its nanocomposite grafts are listed in Table 11 [121,157–159,65,160–164,153,165] and their impacts are briefly described in this section.

A tissue-engineered HA/collagen nanocomposite matrix seems to be a very promising system for bone reconstructive or regenerative surgery. Osteogenic cells/nanocomposite scaffold structures were developed using culture techniques as well as by conventional methods and their in vitro cellular functions were investigated [121]. It was noticed that the scaffolds supported well the cellular growth and related functions, leading to new bone formation. Later, a 3D bone-resembling nanocomposite matrix using nano-HA/collagen/osteoblasts was developed in conjunction with poly(lactic acid) [157]. This system supports cellular adhesion, proliferation, and migration. Interestingly, the cells penetrated deep into the matrix to about 200–400 μm within a short period (12 days), probably owing to its composition and structural similarity to natural bone; thereby providing a promising scaffold for bone tissue engineering. Its in vivo efficacy was evaluated in a rabbit model, by implanting in a 15-mm segmental defect [158]. This system incorporated rhBMP-2 growth factor. Another study reported the importance of nano-HA/collagen system in bone tissue engineering in conjunction

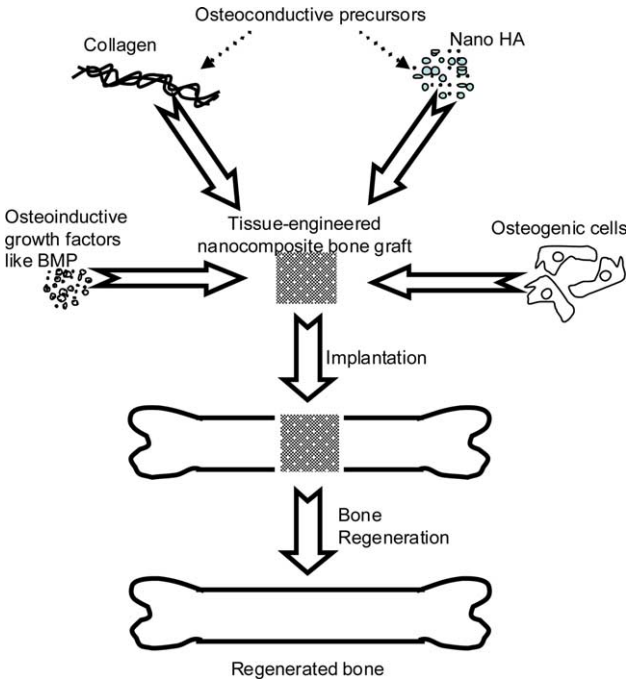


Fig. 8. Design strategy of tissue-engineered nanocomposite bone graft. (Adapted and re-drawn from Ref. [13].)

Table 11
Tissue-engineered bone scaffolds based on nano-HA and its nanocomposites

Bone tissue scaffolds	Cells/growth factors studied	References
Nano-HA	Osteoblast-like cells	[65]
Nano-HA	Mesenchymal stem cells	[160]
Nano-HA	Intended as tissue scaffold	[161,162]
Nano-HA	Cell carrier	[163]
Nano-HA	Angiogenic factors	[164]
Nano-HA/collagen	Mesenchymal cells	[121]
Nano-HA/collagen	rhBMP-2	[122,152,153]
Nano-HA/collagen	rhBMP-2/osteoblasts	[158]
Nano-HA/collagen	Chondroitin sulfate	[150,151]
Nano-HA/collagen/PLA	rhBMP-2	[165]
Nano-HA/collagen/PLA	Osteoblasts	[157,158]
Nano-HA/collagen/alginate	Fibroblasts/osteoblasts	[159]

with alginate [159]. The reason for the selection of alginate was that natural bone is composed of HA, collagen and an amount of polysaccharides; thereby using alginate (polysaccharide) is perceived to be beneficial to enhance the strength and efficiency of the composite. This system provided an adequate mechanical strength, comparable to natural bone. Furthermore, it is biocompatible, bioactive, and stimulated the cellular growth. The in vitro efficacy of this system was studied by using fibroblasts with co-cultured osteoblasts. The cells were found to attach and proliferate well on the scaffold matrix, which is a proof of the cellular-compatibility of the system and suggests that the nano-HA/collagen/alginate system could be used in bone tissue engineering.

Experimental results indicate that an effective bone graft should consist of osteoconductive matrix in conjunction with osteogenic cells and osteoinductive/growth factors with structure, composition, physicochemical, mechanical, and biological features analogous to natural bone. Bone tissue engineering using nanocomposites is at the infant stage and still growing. Although in vitro and in vivo evidence strongly supports the use of nanocomposites as bone grafts, further clinical studies are needed to confirm their promise as an effective bone graft. Most studies concentrate on the physiochemical and biological characteristics of the HA/collagen nanocomposites rather than their mechanical behavior. The probable reason for this is that they are primarily involved in the use of low-weight bearing orthopedic applications. Nowadays, a considerable amount of work is in progress around the world to analyze their mechanical properties in order to widen their use in a variety of clinical orthopedic applications.

6. Concluding remarks

The experimental examples summarized in this review represent some of the developments of nanocomposites from a variety of approaches, conventional to tissue engineering. Nanocomposites combined with osteoconductive, osteoinductive factors, and/or osteogenic cells have gained much interest as a new and versatile class of biomaterial and are perceived to be beneficial in many aspects as bone grafts. However, further substantial research efforts are required to address the following key challenges.

- Optimizing nanocomposite processing conditions.
- Optimization of interfacial-bonding and strength equivalent to natural bone.
- Matching the bioresorbability of the grafts and their biomechanical properties while forming new bone.
- To understand the molecular mechanism by which the cells and nanocomposite matrix interact with each other in vivo to promote bone regeneration.
- Lastly, the ability to improve angiogenesis within the nanocomposite graft is probably the most significant challenge in bone tissue engineering because cells will not survive without an adequate blood supply.

Despite these challenges, nanocomposites are likely to prove invaluable in the future development of bone grafting.

Acronyms

μm	micrometer
3D	three dimension
AIDS	acquired immune deficiency syndrome
BMA	bone marrow aspirate
BMP	bone morphogenetic protein
Ca/P	calcium to phosphorous ratio
ChS	chondroitin sulfate
DBM	demineralized bone matrix
ECM	extracellular matrix
HA	hydroxyapatite
nm	nanometer
PA	polyanhydride
PAA	polyacrylic acid
PBT	polybutylene terephthalate
PCL	polycaprolactone
PE	polyethylene
PEG	polyethylene glycol
PGA	polyglycolic acid
PHEMA	polyhydroxyethyl methacrylate
PHMA	polyhexamethylene adipamide
PLA	polylactic acid
PLGA	poly(lactide-co-glycolide)
PMMA	polymethyl methacrylate
rhBMP	recombinant human bone morphogenetic protein
SBF	stimulated body fluid
TCP	tri-calcium phosphate

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