Review

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Applications of nanobioceramics to healthcare technology

Abstract: The development of functional, biological solutions to repair or replace damaged tissues and organs is the goal of tissue engineering. This involves an interplay of cells, scaffolds and biomolecules that would generate a favourable response when implanted into patients, thus restoring functions lost or impaired due to injuries or diseases. Advances in nanotechnology have enabled the design and fabrication of novel materials at the nanometre scale. Hailed as the next generation of advanced materials, nanomaterials possess advantages of being biochemically and nanostructurally similar to that of physiological tissues. Moreover, nanotopological cues are incorporated, ensuring appropriate cellular responses, thereby enhancing the success of tissue regeneration. Nanobioceramics play a crucial role in bone tissue engineering due to its close chemical similarity to physiological bone and excellent biocompatibility. In addition, nanoscale engineering of these materials has the ability to enhance mechanical and biological properties. This review will begin with an introduction to nanomaterials and its associated considerations that should be taken into account. Next, the role of nanobioceramics achieving these considerations will be discussed. An overview of the current form of nanobioceramics being developed will be provided, concluding with an outlook of nanobioceramics for the healthcare industry.

Keywords: biomaterials; calcium phosphates; ceramics; nanomaterials; tissue engineering.

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1 Introduction

The type of material chosen for any biomedical implants or devices will determine its success. Synthetic materials such as metals, ceramics, polymers and composites have been developed to meet the diverse needs of the healthcare industry. In recent decades, there has been much allure and excitement with regard to the use of nanomaterials because they are being portrayed as breakthrough materials with the potential to overcome issues facing current biomaterials.

Nanomaterials are defined as materials with dimensions or features in the nanometre range of 1–100 nm [1]. Nanomaterials can be divided into three major forms according to their geometry: equiaxed, one-dimensional (fibrous) and two-dimensional (lamellar) [2]. Some of the typical applications of nanomaterials used in biomedical applications are highlighted in Table 1. There has also been growing evidence that biomaterial substrates with nanoscale features support favourable responses with biological entities for a range of applications [3–9]. For a more in-depth review on nanomaterials and their applications in the healthcare industry, readers may refer to reviews by Liu and Webster [2], Raffa et al. [10], Zhang and Webster [11] and Dvir et al. [12].

Before delving into the current progress of nanobioceramics, it is first necessary to justify the need for nanoscale engineering for tissue engineering applications. At the core of every tissue, the cell represents the basic unit, which constitutes the starting point of tissue engineering. Surrounding the cell is a vast network of extracellular matrix (ECM), secreted by the cell itself, which plays the role of securing other cells to it, performing the necessary physiological functions such as maintaining structural rigidity in bones and being elastic in muscles. In addition, the ECM provides a viable environment for cells to live in and forms the immediate medium in which the cell senses changes [13, 14].

When a scaffold is first introduced to the biological environment, proteins from the surrounding serum are adsorbed onto the surface. It is this protein layer that the cell interacts with rather than the actual biomaterial. If the surface physiochemical properties are appropriate, cells will then adhere and function properly. Otherwise, they remain rounded, exhibiting little or no adhesion to

Table 1 Selected nanomaterials and its associated biomedical applications [2].

Nanomaterials	Biomedical applications and associated attractive nanomaterial property
Equiaxed forms	
Gold nanoparticles	Cancer diagnostics and cancer therapy due to their strongly enhanced surface plasmon absorption and scattering
Platinum nanoparticles	Attractive catalysts due to a large surface-to-volume ratio and high surface energy compared with bulk catalytic materials
Titania nanoparticles	Pigments, transparent UV-scattering sun creams orthopaedic coatings
Dendrimers	Efficient multi-drug delivery system
Quantum dots	Diode lasers, amplifiers and biological sensors due to their superior transport and optical properties
Fibrous forms (one-dimensional)	
Carbon nanotubes/nanofibres	Transparent electronic conductors when combined with polymers, field emission electron guns and cathodes, and novel drug carriers
Alumina nanofibres	High efficient air filters and water filters
Polyaniline nanofibres	Chemical vapour sensors
Lamellar forms (two-dimensional)	
Graphite nanoplatelet	Reinforcing agents in polymers and enhancing mechanical and electrical properties in two directions
Nanoclay	Improving properties of plastics such as lighter weight, better scratch resistance, better barrier qualities (to keep freshness in and foreign gases out)
Nano-hydroxyapatite	Orthopaedic implants, bone/cartilage tissue engineering and drug carriers for various bone diseases

the surface. This behaviour can be explained by the selective adsorption of specific ECM proteins onto regions of the biomaterial (e.g., fibronectin, vitronectin, bone sialoprotein, type I collagen, laminin) [15]. These proteins contain the cell-binding peptide sequence arg-gly-asp acid (RGD) or tyr-ile-gly-ser-arg (YIGSR) [16] (Figure 1). These motifs are preferentially recognised by integrins (i.e., transmembranal receptors found on cells that tether the cell cytoskeleton to the adhesion proteins). Following integrin-ligand bindings, various intracellular biomolecules

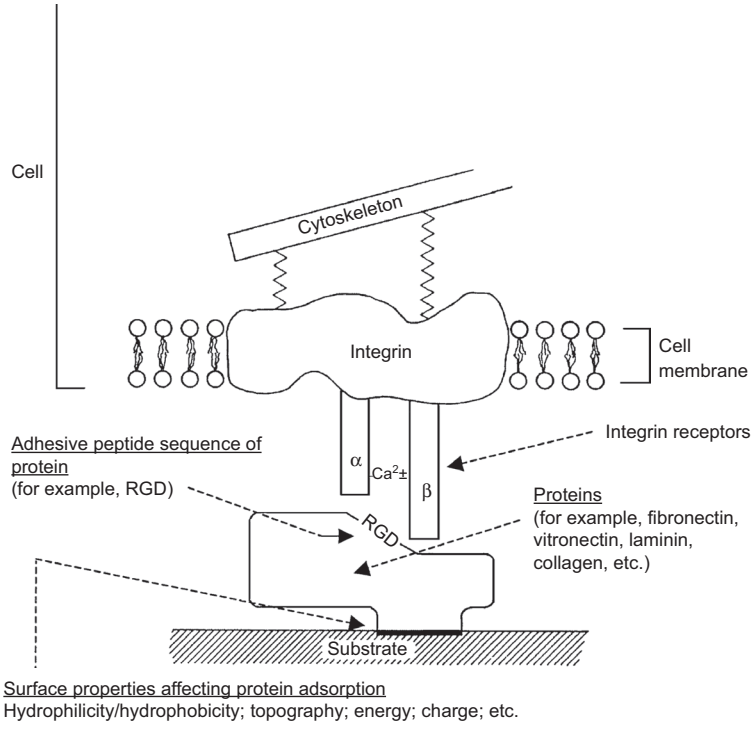

Figure 1 Initial protein interactions leading to cell recognition of implants [17].
(Reprinted from *Adv. Chem. Eng.*, Webster TJ. Nanophase ceramics: the future orthopaedic and dental implant material. 125–166, Copyright 2001, with permission from Elsevier.)

(talin, vinculin, paxillin, α-actinin) aggregate near the focal adhesion site to form a focal adhesion complex that links to cytoskeletal actin filaments. This in turn triggers a cascade of signalling pathways that regulates cell behaviour. Integrin-mediated cell attachment is responsible for cellular migration, growth, differentiation and apoptosis [18]. Focal adhesions function at the nanometre range, thus establishing the need for biomaterials to be structured at the nanoscale.

At the forefront of nanostructured biomaterials is ceramics, which have been used extensively in orthopaedic and cranio-maxillofacial applications [19–21]. In the past, orthopaedic implants have been mainly focused on restoring the physical and macrostructural role of bones. Various implants such as hip and knee prostheses feature models made of metals and alloys with high density and tensile strength. However, it was soon apparent that such materials were far from ideal as these implants developed long-term complications such as aseptic loosening, corrosion, osteolysis, stress shielding and chronic inflammation [22–25].

To develop design considerations for the bone tissue engineered construct, it is necessary to understand the hierarchy of natural bone. There are three different scales of organisation (Figure 2), namely (i) a macrostructure consisting of the cancellous and cortical bone; (ii) a microstructure featuring the Haversian system (osteon), which comprises concentric layers of compact bone (lamellae) surrounding a Haversian canal that contains nerves and blood vessels of the bone; and (iii) a lamella consisting of bundles of organic collagen fibrils interspaced with inorganic nano-hydroxyapatite crystals [26]. Collectively, these structures make up a highly defined and specialised tissue that gives bone its unique mechanical properties whilst providing a microenvironment that is conducive for continued cell growth, migration and differentiation.

In addition to providing structural and mechanical integrity, the bone tissue engineered nanomaterial should also provide the necessary requirements for osteointegration. Once a bone fracture occurs, blood fills the area, and is followed by an initial inflammatory response to prevent infection to the fracture site. At the same time, osteoinductive growth factors are released to stimulate proliferation, migration and differentiation of mesenchymal stem cells (MSCs) and fibroblasts [27]. A granulation tissue consisting of thromocytes, leukocytes, macrophages, MSCs and fibroblasts fill the area forming a fracture callus. Next, MSCs differentiate into preosteoblasts and begin to synthesise the ECM. At the centre of the callus, fibroblasts differentiate into chondrocytes and synthesise cartilage. Once the callus is filled, endochondral ossification begins, following a sequence of events that involves cartilage maturation and degradation, vasculogenesis and osteogenesis. The ossification process continues until all cartilage has been mineralised, in which the new bone may be termed as woven bone, due to its non-structured orientation. The final stage involves bone remodelling where the woven bone is gradually converted to lamellar bone such that high-density bone is laid in the direction of applied stress. The remodelling process is long-term (1–2 months) until the bone approaches its original geometry, strength and stiffness, and is dependent on external mechanical stimuli [28].

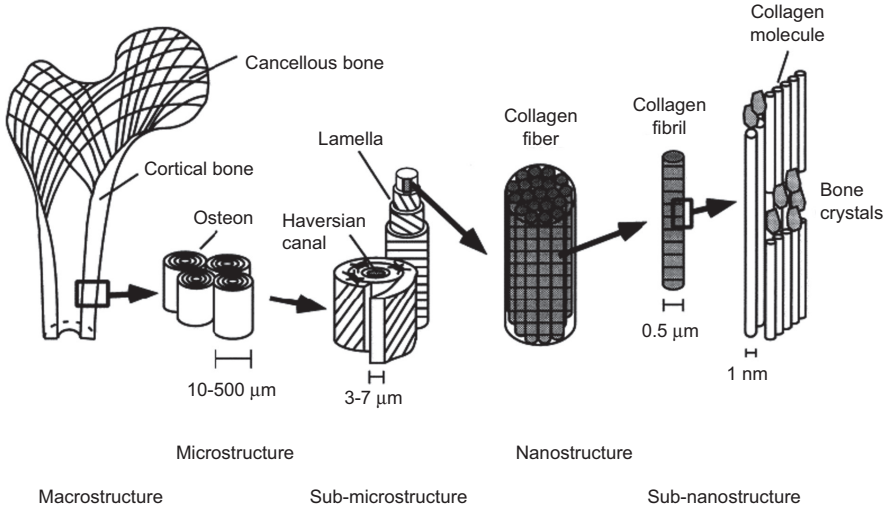

Figure 2 Hierarchical nature of cortical bone [26].
(Reprinted from *Med. Eng. Phys.*, Rho JY, Kuhn-Spearing L, Zioupos P. Mechanical properties and the hierarchical structure of bone. 92–102, Copyright 1998, with permission from Elsevier.)

Clearly, there needs to be thorough considerations for a bone tissue engineered scaffold to function as intended. Firstly, properties of osteoinduction must be incorporated such that the initial response of signalling fibroblasts and MSCs to migrate, proliferate and differentiate at the scaffold can be stimulated. Subsequently, sufficient osteoconduction must be allowed throughout the scaffold, such that vasculogenesis and angiogenesis can occur, while facilitating the mineralisation of the fibrocartilage. Finally, osteogenesis must be supported in a spatially controlled manner, thus enabling the integration of native bone to scaffold. This can be achieved by allowing mechanical stresses to be transmitted throughout the scaffold for proper remodelling to occur, such that sufficient strength between the scaffold-bone interface can be developed, thus avoiding the effects of stress shielding and reducing the risks of implant loosening [29]. To date, there have been several strategies employed to fulfil these requirements. For example, porous scaffolds have been developed to facilitate blood vessel formation [30], incorporation of growth factors to induce osteogenesis [31], as well as the use of bioceramics to promote osteogenesis [32].

With the advent of nanotechnology, various methods have been developed to synthesise existing bioceramics in the nanometre scale [33]. Following the classification offered by Gleiter [34], nanobioceramics can be defined as bioceramics composed of nanometre-sized microstructures, which include structures such as crystallites, grains, phases or features in which one of its dimensions lie within the nanometre range [35].

Nanobioceramics address the limitations of conventional biomaterials because it is now possible to direct and control cellular behaviour at the nanoscale, allowing for interesting concepts and hypotheses to be tested, thus furthering our knowledge and understanding in bone regeneration. Ultimately, the development of a bone tissue engineered scaffold using nanobioceramics would enable one to consciously incorporate properties to influence the physiological response at each stage of the bone healing process, and as such the desired outcome could be obtained with greater consistency.

2 The role of nanoscale engineering in bone tissue engineering

As previously mentioned, tissue engineering employing nanoscale strategies will bring about significant benefits towards the success of implants. Primarily, mechanical properties such as yield strength and fracture toughness can be enhanced modifying features at the nanoscale level. In bone tissue specific applications, osteointegration is crucial towards long-term implant success. Nanoscale engineering achieves this through promoting cell adhesion and preventing fibrous capsule formation. At the cellular level, a favourable response can be obtained by creating nanostructures. This would be important towards maintaining and regulating the osteogenic expression of cells growing on the surface of implants. The following sections will describe these roles in further detail.

2.1 Enhancing mechanical properties

The most commonly used materials for articulating implant surfaces are usually metal-on-polymer. However, these surfaces have high wear rates that can result in foreign body response. To reduce the wear rate, it is necessary to increase the hardness of the moving surfaces, to decrease wear rate. This can be achieved through the use of ceramics. Alumina has been widely considered for such a purpose due to its high hardness, chemical inertness, and relative high strength. In fact, alumina-alumina surfaces can achieve friction coefficients as low as 0.05 as compared with cobalt chromium-ultra high-molecular weight polyethylene (CoCr-UHMWPE), which measured 0.094 [36]. However, due to its low ductility, alumina can be brittle and is prone to crack initiation and fracture. To overcome this, it is proposed that by decreasing grain sizes below 20 nm, strength, hardness and plasticity of the material is increased according to the Hall-Petch equation [37].

$$\sigma_y = \sigma_0 + \frac{k}{\sqrt{a^3}}$$

In theory, the Hall-Petch equation postulates that yield strength (σ_y) increases as grain size (d) decreases. Webster et al. reported an increase in bending strength when grain size of the alumina was reduced from 177 to 23 nm [38]. However, it should be noted that below 10 nm, the Hall-Petch effect does not apply as flaws and defects would compromise the properties of the nanomaterial [39]. Keeping this limitation in mind, it is therefore possible to fabricate nanobioceramics with high fracture toughness whilst maintaining high hardness for a low wear rate.

2.2 Osteointegration

Often, the cause for orthopaedic implant failure is due to the insufficient bonding of the implant to the juxtaposed

bone. Osteointergration (also known as osseointegration) plays an important role in securing the implant in place, preventing micromotion from damaging the surrounding tissues, forming a direct structural and functional bond between the bone and implant that serves to distribute load and stress evenly throughout the organ. Insufficient bonding may thus result in a host of complications ranging from aseptic loosening due to excessive micromotion to complete migration of the entire implant. The cause for insufficient osteointegration can be attributed to the biological response of the biomaterial illicit. A bioinert material would result in no cells attaching to the surfaces, and integration to the host body thus cannot be initiated. In addition, the formation of a fibrous capsule will occur as a result of excessive secretion by inflammatory cells that have deemed the material as a foreign body. By contrast, a material that degrades too quickly *in vivo* will not only be detrimental to the structural integrity but can also result in the release of microparticles which illicit a cytokine response by macrophages that accelerate the bone resorption process. This phenomenon, known as osteolysis, is the major cause for bone loss after surgery. It is possible to prevent these undesirable responses by promoting osteointegration through nanostructuring materials that have topological features that encourage cellular attachment and proliferation whilst discouraging the formation of fibrous tissue. One method is through surface modifications such as coatings where thin layers of nanobioceramics can be deposited onto the surface of implants to improve roughness, wettability and cytocompatibility. This is achieved as bioceramics deposited at the nano level will have superior surface area compared with microscale particles, and this increases the surface energy available for surface protein adsorption, which is crucial in initiating cellular attachment.

2.3 Cellular response

It has been well established that cellular biomechanics function at the nanoscale level. Several biochemical pathways involving cellular proliferation, differentiation and regulation are due to functional changes in the immediate micro/nano environment surrounding the cells. If a group of integrins are engaged in a particular sequence or arrangement, different cellular signalling pathways can be promoted or inhibited. These integrins function at the nanoscale level, and as such if biomaterials are to be designed to prompt a certain response, they must be engineered at the nanoscale too.

The effect of nanotopological features was demonstrated in a study involving an array of nanoscale geometries ranging from nanogroove, nanopost and nanopit arrays, to study the effects of cellular contact guidance. In the study, it was observed that epithelial cells elongated and aligned along the patterns of grooves and ridges with feature dimensions as small as 70 nm, whereas cells were mostly rounded on smooth surfaces [40]. Certainly, such topological cues would present an opportunity for nanoscale engineering of a bone scaffold where scaffold constructs comprising nanobioceramic crystals can be made to align to the *c*-axis of the bone, thus allowing for the adhesion of osteoblast cells along this axis, and produce ECM which would further enhance strength and integrity of the implanted scaffold. Already such a concept has been proposed by Kim et al., where they have demonstrated that cellular attachment and proliferation was improved through the use of a nanocomposite of hydroxyapatite (HA) nanocrystals embedded in a nanofibre composed of gelatine [41]. Osteoblast adhesion is dependent on surface topology, and it has been shown that nanophase surfaces promoted better adhesion than microscale materials [42]. The experiments showed a 30% and 46% increase in osteoblast adhesion on nanophase titania (grain size 32 nm) and alumina (grain size 23 nm), respectively, when compared with conventional bioceramics [43]. In fact, nanotopology alone may account for 48–51% of total osteoblast adhesion as shown in an experiment by Palin et al. who showed that poly(lactic-co-glycolic acid) (PLGA) modelled after nanophase titania showed a similar percentage increase in osteoblast adhesion as compared with nanophase titania [44]. Moreover, alkaline phosphatase (ALP) and ECM calcium content were significantly increased when osteoblasts were cultured on nanophase bioceramics, compared with conventional microscale ceramics (Table 2) [45].

3 Relating nanoscale manipulation to performance of bioceramics

In the previous section, we have seen how features at the nanometre scale enhance various physical and biological properties of bioceramics. The application of nanotechnology on bioceramics has allowed for greater control over various features at the nanoscale level, and this has led to greater structure-function relationship elucidation. Most current tissue engineering approaches tend towards a biomimetic strategy of reconstructing the nanoscale environment of the bone. HA crystals found in

Table 2 Percent increase in ALP and ECM calcium content for osteoblasts cultured on nanoscale compared with microscale bioceramics after 28 days [45].

Bioceramic	Grain size, nm	ALP synthesis, nmol p-npp/min/mg protein	% Increase in ALP	ECM calcium content, µg calcium/mg protein	Increase in ECM calcium content
Alumina	Conventional: 167	0.056	36	1.59	4 times
	Nanophase: 24	0.076		6.38	
Titania	Conventional: 4250	0.087	22	0.72	6 times
	Nanophase: 39	0.106		4.21	
HA	Conventional: 179	0.050	37	0.26	2 times
	Nanophase: 67	0.069		0.51	

native bone are made of a continuous phase of nanocrystallites measuring approximately 40–60 nm long, 20 nm wide and 1.5–5 nm thick. They are deposited parallel to collagen fibres whose diameters range from 100 to 2000 nm. This gives the resultant nanocomposite excellent strength and flexibility. Strategies attempting to recreate such a structure will no doubt offer tremendous benefits. Not only does the material have mechanical properties similar to that of the bone but also nanostructural features tend to mimic that of the natural microenvironment of the host cell, thus ensuring enhanced cellular behaviour. Indeed, nanostructured biomaterials promote osteoblast adhesion and proliferation, osteointegration and deposition of calcium minerals [46]. It is also possible to enhance both mechanical and biological performance of calcium phosphates by controlling the characteristic features of powders such as particle size and shape, particle distribution and agglomeration [47]. As such, several nanotechnological methods have been developed. Kim [48] reported that through sol-gel derivation of bioactive ceramic-polymer nanohybrid, and textured deposition of nanostructured calcium phosphate on polymer templates, biomineralisation of calcium phosphate nanocrystals with specific composition and structure was possible. This is key towards enhancing bioactivity of the material. By controlling the Ca/P ratio, various calcium phosphates can be obtained. Liu et al. [49] used a mixture of triethyl phosphite and calcium nitrate in a stoichiometric ratio of 1:1.67 to synthesise nano-HA via the template mediated sol-gel technique, to achieve amorphous gels of diameter 8–10 nm. Sintering the primary particles at 300°C yielded crystalline apatite of diameter 20–50 nm. Other researchers have attempted various methods to reduce the size of the nano-HA powder. Shih et al. [50] used the hydrolysis method to synthesise nano-HA of diameter 20 nm before annealing. Xu et al. [46] reported obtaining nano-HA in the range of 10–100 nm by using the radio frequency plasma spraying process.

In addition to the manipulation of the size of nanobioceramics, nanosurface morphology of bioceramics can be controlled. Divya Rani et al. [51] conducted a study on the effect of different TiO_2 nanostructures on the surface of Ti. By using the hydrothermal method, various surface nanomorphologies were obtained: mesoporous nanoscaffolds, nanoflowers, nanoneedles, nanorods and octahedral bipyramids. This was achieved by altering the hydrothermal conditions such as reaction medium composition, concentration, temperature and time duration. All Ti plates containing nanostructures exhibited higher fibronectin and vitronectin adsorption as compared with the polished Ti plates, with the octahedral bipyramid adsorbing the most adhesion proteins. Subsequently, a follow-up study was conducted to investigate *in vitro* and *in vivo* osteoblast viability and proliferation characteristics of such nanomorphologies on the surface of Ti screws. Nanoleaves surface morphology resulted in the greatest alkaline phosphatase activity, collagen synthesis and osteoblast cell proliferation as compared with nanotubes, nanoscaffolds or nanoneedles. The nanoleaf morphology also revealed favourable osteoblast intracellular signalling expression of actin, vinculin and focal adhesion kinases, which was distinctly different from those responses observed on other surfaces [52].

A popular nanotechnological method is the use of electrospinning to produce uniform nanoscale fibres. This process involves the use of an electrical charge to draw very fine fibres from a liquid solution. Owing to its simplicity and ease of operation, electrospinning has been used for various tissue engineering applications [53]. Moreover, structural features such as fibre diameter and orientation could be controlled during the electrospinning process. The structural-function relationship between nanofibrous structures and cellular response has been reported by Ma et al. [54] that osteoblast adhesion, proliferation, alkaline phosphatase activity and ECM secretion on carbon nanofibres increased with decreasing fibre diameter in the range 60–200 nm, whereas the arrangement of these fibres

(ordered vs. random) had a profound effect on cellular adhesion and alignment. Nanoporosity is also a property that can be engineered into the bioceramic material. By using anodisation, a study conducted by Karlsson et al. [55] reported depositing highly controlled nanoporous alumina layers to create nanopores of 20–200 nm. This material had been shown to promote cell adhesion due to higher local adsorption at the pores, which might be beneficial for biomineralisation [55].

In summary, there exist various nanotechnological methods to manipulate the properties of bioceramics, and these properties have the potential to influence cellular response. By developing better methods to manipulate these parameters, a greater understanding of various cellular responses towards nanomorphological cues can be gathered, which will certainly advance the development of nanobioceramics.

4 Nanobioceramics

The use of ceramics has been one of the most widely used materials in medical practice, particularly in orthopaedic applications. These inorganic compounds have been selected due to their excellent cytocompatibility and *in vivo* biological responses that mirror innate physiological characteristics. These materials known as bioceramics, can be categorised as bioinert or bioactive, and bioactive ceramics may be resorbable or non-resorbable [19]. However, these bioceramics share a common disadvantage of being brittle, and as such cannot be used to replace functions where extensive mechanical loading exists. By structuring these bioceramics in the nanoscale, it is possible to overcome this limitation.

4.1 α-Alumina

Alumina has a chemical formula of Al_2O_3. At physiological conditions, it exists as α-alumina and is a bioinert ceramic. Since 1975, it has been chosen due to its high hardness and resistance to wear degradation. This is due to high surface energy, which is able to provide hydrodynamic lubrication in articulating joints, and high surface smoothness, which greatly improves wear resistance of the material. In a hip simulator test conducted by Oonishi et al., wear on Al_2O_3/UHMWPE total hip arthroplasties was decreased by 25–30% when compared with that of a metal/UHMWPE. Wear rate of the alumina-alumina was observed to be near zero [21]. Several *in vitro* and *in vivo* studies using larger than 28 mm femoral heads demonstrated the advantage of using alumina-alumina pairing in young patients or patients with high demand bodily functions [56, 57]. Despite being considered bioinert, nanoscale modifications have made it possible to circumvent the issue of fibroblast attachment and fibrous capsule formation by decreasing grain size and thus promoting surface protein adsorption, which would allow for osteoblast adhesion. This is shown through a study which reported that as the grain size of alumina decreased from 167 to 24 nm, a 51% increase in osteoblast adhesion and a 235% decrease in fibroblast adhesion were observed [42].

The *in vivo* performance of alumina ceramic components has been poor due to its low fracture toughness and inability to arrest crack growth. Some improved techniques for the densification of powder compacts of nano-alumina have been reported such as the transformation-assisted consolidation (TAC) process and plasma spraying. By employing the TAC method, single-phase ceramics have been fabricated with densities of more than 99% and grain sizes of <18 nm [58]. A variant of the TAC method called plasma spraying can be used to prepare alumina-titania multiphase nanoceramics.

4.2 Zirconia

Zirconia (ZrO_2) is in the same bioceramic class as alumina. It has the advantages of having a higher Weibull modulus and hence better reliability, higher flexural strength and fracture toughness, lower Young's modulus and the ability to be polished to a superior surface finish [59, 60]. ZrO_2 can undergo a process known as transformation toughening to improve its mechanical properties [61]. In such a process, the metastable tetragonal phase of zirconia is finely dispersed in a matrix of cubic zirconia. Tensile stresses applied to the material are amplified at the tip of an advancing crack, which induces phase changes to the tetragonal zirconia grains just ahead of the crack tip. This phase change involves the transformation of the grains from their tetragonal phase to a monoclinic phase which is accompanied by volume expansion. The result is an opposing stress field generated by the transformed zirconia grains which arrests incoming crack propagation and thus toughens the material [61, 62] (Figure 3).

The most commonly used method to induce the transformation toughening effect is through the addition of 3 mol% Y_2O_3, which serves to stabilise and lower the transformation temperature. Yttria-stabilised tetragonal zirconia polycrystalline (Y-TZP) offers the best mechanical properties due to its ultrafine grain size of <100 nm. Use

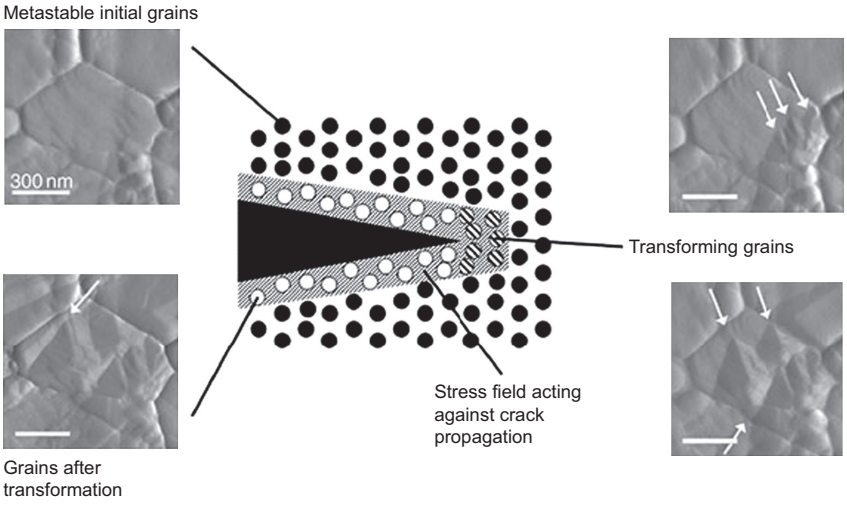

Figure 3 Schematic illustration of the transformation-toughening mechanism in tetragonal zirconia polycrystalline (TZP) ceramics, as visualised by atomic force microscopy [63].
(From Deville S, Chevalier J. *J. Am. Ceram. Soc.*, 2003, 86, 2225.)

of Y-TZP for femoral heads has gained much popularity from 1985 to 1997 where over 300,000 TZP ball heads were implanted [64]. However, a high incident of Y-TZP implant failure were soon reported [65], and this was attributed to the aging process, which affected the metastability of the Y-TZP ceramic. It was discovered that Y-TZP stability was greatly affected by moisture and temperature in which the implant was subjected to during autoclave sterilisation treatment. This degradation led to a drastic decrease in strength and toughness of the material.

To overcome the aging process, nanocomposite ceramics featuring nanosized TZP phases dispersed in a microcrystalline alumina matrix were developed and have been demonstrated to have less aging degradation, with improved long-term *in vitro* wear [66, 67]. More recently, zirconia toughened alumina (ZTA) featuring nanograins of zirconia distributed within a nanocrystalline matrix of alumina has been developed and proposed as the next zirconia-based nanobioceramic [68]. From a nanotechnological view standpoint, ZTA would be an ideal material for tissue engineering applications involving high loading stresses due to their high hardness and fracture toughness, good elasticity and resistance to aging degradation.

4.3 Calcium phosphates

One of the requirements for biomaterials to be used for tissue engineering is the need for host tissue integration into the implant/scaffold. As such, the bioactivity of the material with the surrounding tissue is favoured over a bioinertness of the material. Calcium phosphates are a class of bioactive ceramics that have been extensively used in biomedical engineering applications involving bone. This includes spinal fusion, cranio-maxillofacial reconstruction, treatment of bone defects, total joint replacements and also as coatings for various orthopaedic implants. Depending on the ratio of calcium to phosphorous, various forms of calcium phosphates may be obtained.

HA is one of the most extensively used synthetic calcium phosphate ceramics. This is attributed to its chemical similarity to the inorganic phase of bone tissues. HA [$Ca_{10}(PO_4)_6(OH)_2$] has a theoretical composition of 39.68 wt% Ca, 18.45 wt% P; Ca/P weight ratio of 2.151 and Ca/P molar ratio of 1.667. Compared with other calcium phosphates, HA has the highest stability in aqueous media within a pH range of 4.2–8.0. Owing to these properties, HA has been chosen in several applications involving nanobioceramics [69–75]. Currently, Ostim (Ostaris GmbH, Hanau, Germany) is a commercially available injectable cement made of nanocrystalline HA. It has shown clinical success in several applications including the management of human intrabony periodontal defects [76], metaphyseal radius fractures [77] and maxillary sinus floor augmentation [78].

Furthermore, HA has the unique property of undergoing either cationic or anionic substitutions, which can result in enhanced bioactivity and osteointegration. For example, the use of nanocrystalline silicon-substituted HA thin films has demonstrated improved cell adhesion and increased cell spreading and ECM production [79]. Zinc-substituted nano-HA was found to enhance proliferation of

adipose-derived MSCs and support osteogenic differentiation whilst inhibiting microbial activity of *Staphylococcus aureus* [80]. Zinc and carbonate co-substituted nano-HA were also studied, and the balance of substitution ions was found to have effects on *in vitro* apatite formation [81].

Attention should be focussed on the crystallinity of nanobioceramics. In a study conducted by Hu et al., highly crystallised thin nano-HA films resulted in higher cell proliferation than amorphous calcium phosphate (ACP) [82]. Although a more detailed study has to be conducted to investigate this effect, it is important to achieve greater control over the phase and size of nanobioceramics to ensure appropriate cellular response.

Tricalcium phosphate (TCP) is a bioresorbable ceramic due to its ease of dissolution in physiological media. It has the chemical formula of $Ca_3(PO_4)_2$ and has four polymorphs namely α, super-α, β and γ. Because the super-α form is only stable at high temperatures and the γ form only exists at high pressure conditions, α and β-TCP are the most commonly encountered phases in the biological context [83]. α-TCP is not regarded as a good biomaterial due to its quick degradability [84]. By contrast, β-TCP degrades at a rate which is similar to bone growth [85]. This is ideal because structural integrity needs to be maintained while bone growth occurs.

The degradable nature of β-TCP makes it possible to develop nanostructured scaffolds that are resorbed into the body once healing has completed. This makes it easier for bone remodelling in the scaffold, thus fostering osteointegration [86]. Indeed, BoneSaves (Stryker, Newbury, UK) is a commercially available bone cement made of nano β-TCP and biphasic HA which has shown successful clinical outcomes in a majority of orthopaedic cases involving spinal fusion [87]. In addition, nano-β-TCP particles may serve as drug delivery systems for a variety of remedies such as antibiotics, anti-tumour and anti-inflammatory drugs [88].

In the foreseeable future, it is possible to envision nano-calcium phosphate coatings on existing orthopaedic implants, offering the benefits of osteoconductivity and at the same time incorporating drug delivery capabilities such that proteins and growth factors can be adsorbed onto the surface of the biomaterial. These coatings will then promote and augment the healing process, enabling faster recovery times and ensuring implant longevity.

5 Outlook of nanobioceramics

The nanobioceramics featured thus far mainly describe single ceramic materials for use as coatings on existing implants or as minor bone defect fillers. In critical-sized bone defects, it is necessary to construct a scaffold that incorporates a combination of materials that gives the scaffold better flexural strength and fracture toughness. Looking forward, future biomaterials are those featuring nanobioceramics that incorporate polymeric materials. These nanocomposites not only impart better physical characteristics, but also simulate the mineral and organic phase of physiological bone. This creates a tissue-engineered scaffold that better mimics the *in vivo* microenvironment, thus enabling better cellular response.

On another front, nanobioceramics play a prominent role in drug release systems. These systems feature mesoporous materials made from nanobioceramics. The surfaces of the pore walls can be functionalised to alter the affinity of the material to specific drugs, thus allowing drugs to be released *in vivo* in a controlled and sustained manner. These systems open up new possibilities in the field of pharmacology, as it is now possible to influence cellular behaviour in a spatiotemporal manner.

5.1 Nanocomposites featuring nanobioceramics

There have been several recent developments of nanocomposite scaffolds comprising two or more types of materials. This not only gives the scaffold the required mechanical properties but also provides appropriate micro- and nanoscale cues necessary for proper bone cell survival and function. It is this reason that current solutions involving nanobioceramics are increasingly being developed together with other types of biomaterials to produce nanocomposites. These novel biomaterials usually feature reinforcing phases embedded within a matrix phase so as to mimic the chemical and biological structure of bone [13]. Nanocomposites featuring nanobioceramics often come in two forms, namely ceramic matrix nanocomposites (CMCs) or polymer matrix ceramic nanocomposites (PMCs).

In CMCs, the nanobioceramic matrix is reinforced with other ceramics or metal inclusions, which provide the material with better ductility, fracture toughness and stiffness almost similar to bone. One promising development of biomaterials involving CMCs is a bioactive bone filler called SYNTHETBONE. The composite consists of a mixture of HA, TCP, biphasic HA-TCP, bioglass (45S5) and bioactive glass-ceramics [89]. *In vivo* studies show that SYNTHETBONE enhanced implant resorption and osteogenesis, and clinical study involving the use of the material for repairing bone defects demonstrated excellent

osteointegration and bone remodelling, and was even used in surgical treatment of the biggest skull defect without implant rejection.

Another CMC worth mentioning is zirconia toughened alumina (ZTA), developed for articulating surfaces of prosthetic implants [90]. The nanocomposite features zirconia nanoparticles dispersed within an alumina matrix. The material exhibited excellent cytocompatibility and lower wear debris as compared with alumina after 8 million cycles [91].

PMCs feature polymer matrices reinforced with nanobioceramics. These composites offer tremendous potential in bone tissue engineering due to their biomimetic strategy, which include polymers substituting for collagen fibrils, and nanobioceramic substitution for the inorganic phase of bone. In recent years, several PMC nanocomposites are being developed, and Table 3 summarises some of the current developments of nanocomposites featuring nanobioceramics being applied in bone tissue engineering.

Jose et al. proposed an aligned nanofibrous PLGA/collagen/nano-HA blend to simulate the nano- and microstructure of bone, providing mechanical strength and allowing MSCs to bind to polymers [92]. To improve the stability of collagen fibrils, Maas et al. and Ou et al. used calcium phosphate and ACP to reinforce the material whilst ensuring that proliferation and osteogenic differentiation of MSCs are supported [93, 94]. Similarly, Liuyun et al., Polini et al., and Chen and Chang have developed scaffolds with nano-HA to improve the structural integrity of the polymer and at the same time promoting osteogenic differentiation among stem cells [6, 95, 96].

Chitin- or chitosan-based biomaterials have been intensively studied for wound healing [102], tissue engineering [103] and drug delivery [104] applications due to their ease of handling, biodegradability and versatility. Adding an additional nanobioceramic phase to this polymer would certainly increase its efficacy in bone tissue engineering due to enhancement of the bioactivity of the material. Thein-Han and Misra investigated the effect of chitosan/nano-HA on pre-osteoblasts (MC3T3-E1). Addition of nano-HA increased compression modulus, and the scaffold exhibited better cell attachment, proliferation and morphology, suggesting that such nanocomposites can be used for applications requiring high bioactivity and biodegradability [98].

Many *in vivo* studies have also been carried out to investigate the efficacy of nanocomposites in bone tissue engineering. While it was demonstrated that grafting poly(lactic acid) (PLA)/nanosized demineralised bone powder (DBP) scaffolds generated repair in rat skull defects [97], a more efficient method for bone regeneration *in vivo* can be achieved with the use of stem cells [105, 106].

The use of stem cells to initiate and augment the bone healing process is a well-documented strategy [107–109], and the use of nanocomposites with nanotopological and biochemical cues similar to that of natural bone could be sufficient to stimulate stem cells to differentiate into osteoblasts. Lock and Liu reported that nano-PLGA-HA composite alone was able to promote osteogenic differentiation of human MSCs similar to that of direct injection of BMP-7 derived short peptide (DIF-7c). Moreover, Polini et al. reported that human MSCs upregulated several genes (Runx-2, BSP) that were crucial to the osteogenic differentiation process when cultured on polycaprolactone (PCL)/TCP nanofibrous scaffolds in the absence of bone induction media. These nanocomposites would pave new ways for strategies that direct stem cell differentiation without the use of growth factors, which are difficult to control *in vivo*.

Table 3 Nanocomposites featuring nanobioceramics applied in bone tissue engineering.

Scaffolds	Components
Aligned nanofibrous multi-component scaffolds [92]	PLGA/collagen/nano-HA
Mineralised nanofibres [93]	Calcium phosphate contained collagen fibrils
Nanocomposites [94]	Collagen/nano-HA/nano-ACP
Double membrane [5]	ACP/collagen/PLGA
Biodegradable composite scaffolds [95]	Nano-HA/chitosan/CMC
Nanofibrous scaffolds [6]	PCL/nano-HA or PCL/TCP
Nanofibrous membranes [96]	PCL/nano-HA
Nanofibrous composite scaffolds [97]	Nano-sized demineralised bone powders (DBPs)/PLA
Composite scaffolds [98]	Chitosan/nano-HA
Solid casted composite film [99]	Nano-PLGA-HA
Nanostructured mesoporous silicon fibrous scaffolds [100]	Silicon (PSi)/PCL fibres
Electrospun fibres calcium phosphate cements [101]	PLGA/CPCs (tetracalcium phosphate, dicalciumphosphate anhydrous and chitosan lactate)

Ultimately, the role of nanobioceramics in bone tissue engineering is gaining prominence, and continued research and development of these materials in nanocomposites would definitely bring about significant changes in the way tissue engineering is being approached.

5.2 Nanobioceramics used in drug delivery

In addition to constructing novel scaffolds, nanobioceramics have been featured as drug release systems. The development of drug release systems has experienced a remarkable growth and is now an important market for the industrial sector. The introduction of nanotechnology has made it possible to design nanoscale drug delivery systems. Specifically, the development of these systems will enable a strategy whereby drugs are directed to their targeted sites, and functioning in tandem with scaffolds, can offer a multifaceted approach whereby scaffolds incorporating nanobioceramics allow for cellular adhesion and proliferation, while the localised biologically active molecule enhances the regenerative process.

Currently, these systems come in the form of liposomes, nanocapsules or nanospheres with the use of materials such as polymers, surfactants or lipids. Another approach involves the use of nanotechnology to structure new materials from the "bottom-up" approach, forming microspheres and other macrostructures. New functionalities can be obtained by controlling their nanostructures, thereby changing the delivery mechanism and release profiles, and may be used in a myriad of applications such as tissue engineering, cancer therapy, cardiovascular and infectious diseases, vaccines and imaging.

The advantages of using nanobioceramics in drug delivery are discussed. Firstly, when compared to using polymeric materials, bioceramics have extended dissolution rates, which will be essential in designing drugs with gradual, diffusion controlled release profiles. Secondly, bioceramics are stable under physiological conditions. For example, they do not encounter problems of swelling associated with hydrogel drug delivery systems [110]. Thirdly, bioceramics can possibly exhibit chemical similarity to that of the native tissue with high biocompatibility and bioactivity. Calcium phosphates have already been used extensively as delivery carriers for a wide array of drugs (antibiotics, anti-inflammatory, analgesic and anticancer) as well as growth factors, proteins and genes [111, 112].

In this particular area, the concept of mesoporous materials may offer promising possibilities. Mesoporous materials are materials with pores of diameter between 2 and 50 nm, with high surface area and porosity. Nanobioceramics such as hydroxyapatite, silica and bioglass are commonly used for the formation of mesoporous materials. Incorporation of such a nanobioceramic not only delivers high drug loading capacity, it also ensures implant osteointegration and excellent cytocompatibility. The functionalisation of nanobioceramics within the pore walls aims to increase drug loading and sustain drug release characteristics. This occurs through replacing the hydrogen atom in silanol by an organic group R that can be linked to the oxygen atom by a covalent bond. It is thus possible to graft certain biomolecules such as peptides, proteins or growth factors that act as signals to enhance or stimulate the desired *in vivo* response. Affinity to certain molecules can also be increased through such functionalisation, thereby changing the drug release profile of the mesoporous material. When affinity is high, a low, sustained rate of drug release can be obtained, compared with non-functionalised materials where drug release follows an initial burst of high dosage, which may not be an appropriate response. Indeed, aminopropyl functionalisation of mesoporous silica resulted in a three times reduction of bovine serum albumin (BSA) in the initial burst effect as compared with its unmodified counterpart [113]. This can be explained through the change in mechanisms of protein affinity from weak hydrogen interactions between BSA and unmodified silanol of mesoporous silica to the stronger electrostatic attraction between the negative $-COO^-$ of BSA with the positive $-NH_3^+$ of aminopropyl functionalised mesoporous silica (Figure 4) [114].

Functionalisation plays a key role in drug adsorption and release profile, and as such it is crucial to choose the appropriate type of functionalisation for the specific drug, and subsequently its strength of attraction shall be chosen in accordance to the desired release prolife. For example, the condensation route is chosen when functionalisation of both the inner and outer surfaces of the pore wall are required, but this imposes restrictions on the level of functionalisation. Conversely, the post-synthesis method leads to functionalisation of the outer walls only, resulting in a well-defined structure where high degrees of functionalisation can be achieved. Subsequently, the type of functionalisation molecules that are attached range from various amino and hydrophobic groups. For example, mesoporous materials functionalised with polar groups loaded higher amounts of ibuprofen when compared with nonpolar groups [115]. By contrast, functionalisation with hydrophobic groups will be suitable for applications where the intended drug is hydrophobic or a delayed drug release is desired. For example, the functionalisation of these matrices with hydrophobic groups such as octyl (C_8)

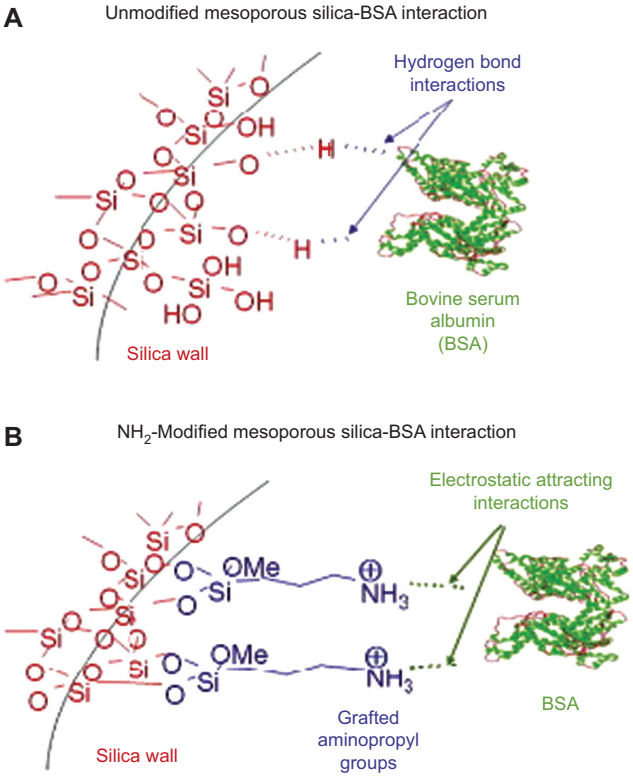

Figure 4 Schematic representation of mesoporous matrix-bovine serum albumin (BSA) host-guest interactions for (A) pure mesoporous silica and (B) aminopropyl-functionalised mesoporous silica [114].
(Reprinted from *Comprehens. Biomater.* Colilla M, Vallet-Regi M. Ordered mesoporous silica materials. 497–514. Copyright 2011, with permission from Elsevier.)

and octadecyl (C_{18}) can control the release profile of erythromycin, a hydrophobic drug [116]. Modification using trimethylsilyl groups led to a delay of ibuprofen release due to the difficultly in diffusing the delivery medium inside the mesopore channels [117].

5.3 Nanobioceramics in gene therapy

In addition to conventional drug therapy strategies, recent advances in gene therapeutics have shifted attention to a field whereby intracellular mechanisms are altered via genetic modification. The idea here is to target the fundamental cause of diseases such as cancer or other genetic disorders by modifying the genetic code. This strategy holds immense potential of subverting toxicity risks and side effects associated with conventional drug therapy as well as increasing the success and efficacy of solutions delivered to treat or even cure existing genetic disorders. However, there remains existing challenges. Among these are the instability of unpackaged DNA, risks associated with viral vectors and low transfection efficiency of synthetic carriers.

Several leading reviews have begun advocating for the application of nanobiotechnology in the area of gene therapy [118–121]. Specific attention has been called upon nanobioceramics because it is possible to manipulate parameters such as particle size, dissolution rate, loading capacity by the creation of structures and manipulation of physiochemical properties of bioceramics.

In a study conducted by Tan et al. [122], three nanobioceramic particles namely silica, HA and zirconia, were assessed. The surface charge of these nanoparticles were negative, neutral and positive, respectively. By coating them with protamine sulphate (PS), surface charge could be modulated to accept the negatively charged DNA. It was observed that nanoparticles of silica offered the best loading of DNA due to the ability of PS to modulate surface charges effectively, and complex with DNA such that the net positive charge resulting from the presence of amine groups provide protection from endosome/lysosome activity [123, 124]. Subsequently, PS-silica-DNA nanoplexes were able to target the spleen of mice when administered intraperitoneally. This study showed that physiochemical characteristics of nanobioceramics are crucial considerations in gene therapy. Bioceramics offer the unique property of allowing protein adsorption. As such, surface charge modulation by way of protein coating is possible, and this in turn will enable efficient loading of DNA. Furthermore, engineering of these particles at the nanometre level will certainly enable high loading capacity and efficient endocytosis while evading immunogenic response.

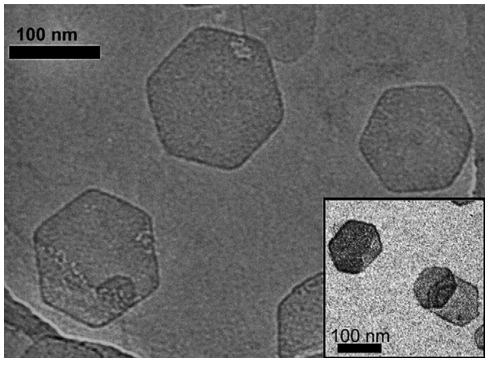

Figure 5 Transmission electron micrograph (TEM) of $Mg_2Al(OH)_6NO_3$ LDH nanoparticles [125].
(Reprinted from *Biomaterials*. Ladewig K, Niebert M, Xu ZP, Gray PP, Lu GQ. Efficient siRNA delivery to mammalian cells using layered double hydroxide nanoparticles. 31, 1821–1829. Copyright 2010, with permission from Elsevier.)

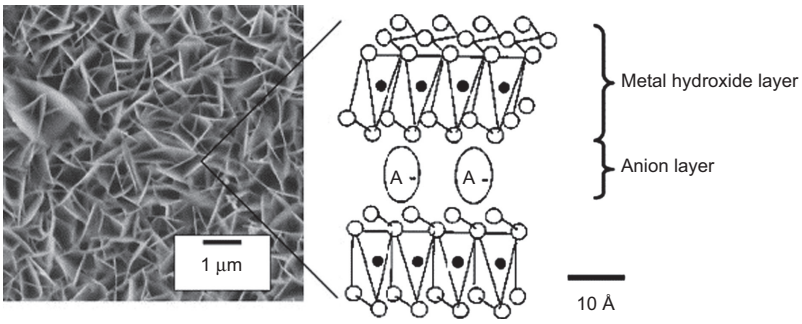

Figure 6 A schematic illustration of the structure of layered double hydroxide nanostructure.
Metal hydroxide layers are located on the top and bottom layers, whereas anion layer is located in the middle [126]. (Reprinted from *Prog. Organ. Coat.* Wong F, Buchheit R. Ultilizing the structural memory effect of layered double hydroxides for sensing water uptake in organic coatings. 51, 91–102. Copyright 2004, with permission from Elsevier.)

Recently, the development of a class of bioceramics with layered double hydroxide (LDH) nanostructure (Figure 5) has generated much interest with regard to gene delivery applications. These LDH nanoparticles can be described as materials having positively charged layers (cationic brucite-like layers) which are weakly bound and interspaced with charge balancing anions located in the interlayer region (Figure 6). The general chemical composition of LDH nanoparticles can be expressed as:

$$M(II)_{1-x}\,M(III)x\,(OH)_2\,(A^{n-})_{x/n}\,x\,y\,H_2O$$

where M(II) is divalent cation, M(III) is trivalent cation, A is interlayer anion, n is charge on interlayer ion, and x and y are fraction constants.

LDH nanoparticles can be synthesised via the co-precipitation method, whereby two metal salts are mixed in an aqueous solution and added dropwise to an aqueous solution of an anionic solution. A basic pH is maintained with NaOH to induce co-precipitation. Because incorporation of carbonate anions at the interlayer can be difficult to remove, the process is carried out in an inert atmosphere. Size of the LDH nanoparticles can be controlled via

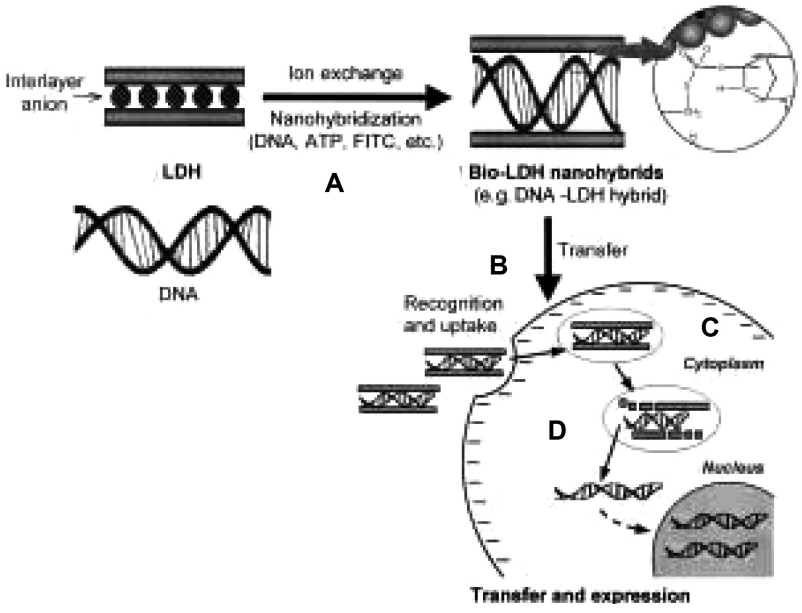

Figure 7 Hybridisation and transfer mechanism of LDH nanoparticles.
(A) Anion exchange between interlayer NO_3^- or Cl^- anions and negatively charged biomolecules, for example, DNA, leads to the formation of LDH-nanobiohybrids. (B) Uptake via receptor-mediated endocytosis. (C) Acidification of the endosome causes LDH particles to dissolve slowly, thereby buffering the endosomal pH ("proton-sponge effect") and releasing the biomolecule. (D) Further influx of H^+ into the endosome and dissolution of LDH particles leads to an increase in ionic strength inside the endosome and causes the endosomal membrane to rupture, which liberates the payload [129]. (From Choy JH, Kwak SY, Jeong YJ, Park JS. *Angew. Chem. Int. Ed.* 2000, 39, 4041.)

hydrothermal treatment or varying the temperature and treatment time. Through this method, LDH nanoparticles can be controlled in the range of 50–300 nm [127]. Organic anions such as DNA can then be intercalated to the interlayer region via ion exchange or precipitation [128].

LDH nanoparticles act as soluble inorganic non-viral vectors for DNA biomolecules. Once encapsulated, DNA is protected from degradation due to the positive bioceramic layer. This material is then adsorbed into the cell via receptor-mediated endocytosis. Once inside the cell, acidic action by endosomes degrades the layer to expose DNA (Figure 7), which is then free to migrate into the nucleus.

Dey and Sistiabudi [130] reported that due to their inherent stability in physiological conditions and surface activation characteristics, bioceramics are excellent candidates for nanoparticles with a LDH nanostructure to hybridise with DNA. Currently, research has reported success with delivery of small nucleic acids [antisense oligonucleotides and small interfering RNA (siRNA)] [125, 131, 132]. Choy et al. [131] used Mg-Al LDH nanoparticles to hybridise with c-myc antisense oligonucleotide and incubated with human promyelocytic leukaemia cells (HL-60). Cells with the c-myc LDH hybrid experienced a 65% growth inhibition as compared with untreated and naked c-myc treated cells. Ladewig et al. [125] also used Mg-Al nanoparticles, but loaded them with siRNA. Results demonstrated an uptake of 99% efficiency when siRNA LDH nanoparticles were introduced to human embryonic kidney (HEK293T) cells. A knockdown of ERK2 protein expression was also observed with HEK293T cells treated with anti-ERK2 siRNA LDH nanoparticles.

6 Conclusions

The development of tissue engineered constructs requires several thorough considerations. For example, implants intended for articulating joints should have excellent wear characteristics whilst implants intended as scaffolds to repair femur defects should have high strength to withstand high loads. These set of macrostructural requirements have been widely understood in the development of conventional implants and can be improved upon through nanoscale engineering such as decreasing grain size and introducing nanophase particles to strengthen a material.

Next is the biocompatibility of the material, entailing diverse considerations that require an in-depth understanding of cell biology, biomolecules and cell microenvironment interactions. Nanobioceramics play a key role in the development of bone tissue engineered biomaterials due to their high strength and chemical similarity to natural bone. Furthermore, it is now understood that several factors influencing cellular response occurs at the nanoscale level. The incorporation of new nanobioceramics and its incorporation into existing biomaterials will lead to better attempts at recreating a truly biomimetic microenvironment that will ensure osteointegration and long-term implant success.

However, challenges still exist before clinical implementation of a nanocomposite can be achieved. One challenge is ensuring that the appropriate biomolecular cue is presented in a spatial-temporal manner that allows for proper signalling in response to external mechanical stimuli. This process known as mechanotransduction will be key towards strengthening of the implant and maintenance of phenotypic expression. Another challenge is ensuring adequate vascularisation and angiogenesis throughout the implant such that a constant exchange of nutrients and waste between developing cells and blood is maintained. Despite these challenges, the continued development of nanobioceramics will undoubtedly bring about great medical benefits in the years ahead.

In drug delivery, nanobioceramics introduce an exciting concept of consciously engineering for features which enable the release of therapeutic agents in a controlled manner. This includes strategies such as forming mesoporous nanostructures and functionalising pore walls such that the desired drug is incorporated and a controlled, sustained release profile is ensured. As our understanding of biological processes progresses, greater attention is now being focused towards the influence of genes towards disease progression. Nanobioceramics stand to play an instrumental role in gene therapeutics by serving as gene delivery vehicles. Several novel nanostructures have been proposed, and these exploit the physiochemical nature of nanobioceramics to deliver targeted and effective gene transfection to the desired site. Undoubtedly, there exists serious and valid concerns of toxicity and metabolic fate of these nanobioceramics. Nevertheless, through continual advances of understanding metabolic pathways and nano-cytotoxicity, issues can be anticipated and engineering considerations can be made to reduce or eliminate such risks.

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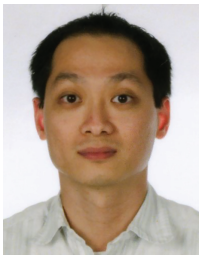

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