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Nanostructured bioceramics for maxillofacial applications

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Abstract Biomaterials science and technology have been expanding tremendously the recent years. The results of this evolution are obvious in maxillofacial applications especially with the contemporary development of Nanotechnology. Among biomaterials, bioceramics possess a specific field due to various interactions with the biological tissues. The combination of bioceramics and nanotechnology has resulted in enhanced skeletal interactions in maxillofacial applications. Nanotechnology secures better mechanical properties and more effective biological interactions with jaws. The main production methods for the synthesis of nanostructured materials include plasma arcing, chemical vapour deposition, sol–gel and precipitation. The bioceramics in Dentistry comprise inert, bioactive, resorbable and composite systems. The purpose of the present article is to describe the available nanotechnology methods and how these could be addressed to synthesise maxillofacial bioceramics with advanced properties for better biological applications. Additionally, it describes specific clinical applications in maxillofacial surgery of these biomaterials—either by themselves or in combination with others—that can be promising candidates for bone tissue engineering. Such applications include replacement of lost teeth, filling of jaws defects or reconstruction of mandible and temporomandibular joint.

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Introduction

There is a high demand for biomaterials to assist or replace organs and their functions, and to eventually enhance patients' quality of life. Interaction between biomaterials and natural tissues is a significant subject for biomaterials science. Information originating from this interaction is essential to aid the design and fabrication of new biocompatible and bioactive materials. The latest decades, 'biomaterials' is a great developing research area in the interdisciplinary field of materials-related sciences with various applications in the medical and dental treatment. The development and manufacture of biomaterials demand high standards of production process, due to the combination of both high-level mechanical and biological properties. Nevertheless, the objective has been either to synthesise novel materials or to develop new technologies for the enhancement of the properties of the already known ones.

The replacement of lost teeth or parts of the human jaws requires the development of new biomaterials with advanced properties to achieve the optimum interaction with the skeletal tissues. Such an example is to advance the nowadays osseointegrated (mechanical retention) titanium (Ti) implants within the jaws into the biointegrated (chemical interaction) ones, which are covered with bioactive nanostructured ceramic materials [1]. The revolutionary evolution of nanotechnology has brought new prospectives in the applications of biomaterials in dentistry,

by tailoring their properties. Among biomaterials in general, the bioceramics play a primary role in these applications. Bioceramics, which are synthesised by nanotechnology and applied in maxillofacial surgery, are basically the subject of the present review article.

Nanotechnology

The speed, at which advances are realised in science, has catapulted nanotechnology from its theoretical fields straight into real applications. There are now various examples of commercially available products demonstrating that nanotechnology does work, especially in healthcare field. Dentistry, as an individual healthcare discipline, is not an exempt, having already been targetted directly with novel nanomaterials at the same time as indirectly enjoying the benefits of nanorelated advances in the electronics industry through the ongoing computerisation of the modern practice [2].

Nanotechnology—also known as molecular nanotechnology or molecular engineering—is the synthesis of functional materials with structural features in the range of 0.1–100 nm (nanomaterials) by various physical or chemical methods [3]. For perspective, the size of one hydrogen atom is 0.1–0.2 nm and of a small bacterium about 1 μm (=1.000 nm). Every property has a critical length scale and by using building blocks smaller than the critical length, such as nanoparticles, this will have an impact on its 'regular' behaviour. Such an example is the light scattering; when a particle shrinks to a fraction of the wavelength of visible light (i.e., 0.4–0.8 μm), it would not scatter that particular light, resulting in the human eye's inability to detect the particles. This phenomenon has tremendous implications on the optical properties of the material [4].

Novel materials, whose microstructure can be engineered to contain desire features with nanometre scale dimensions, have become popular enough, especially during the last two decades. These 'nanostructured' materials can exhibit enhanced mechanical, electrical, magnetic, chemical, optical and biological properties compared with their conventional (micron-scale or larger) counterparts. Nanostructured materials exhibit large specific surface area and contain a large volume fraction—greater than 50%—of defects, e.g., grain or interphase boundaries, dislocations, which strongly influences their chemical and physical properties. The synthesis and control of materials in nanometre scale can provide access to new levels of properties and features that were previously unattainable. Thus, work is rapidly expanding worldwide in attempts to exploit the opportunities offered through nanostructuring [5]. Nanostructured materials can take the form of powders, dispersions, coatings or bulk materials.

Production methods

Ball milling

Ball milling, which is also better described as mechanical crushing, has been a traditional method of fabricating fine powders. This method was first used to produce nanomaterials, although today is very confined since new more effective methods were developed. Ball milling breaks down the material into nanocrystallites and can be used to synthesise a variety of new types of materials. In this process, small balls are allowed to rotate around the inside of a drum and drop under gravitational forces onto a solid, enclosed in the drum. Its significant advantage is that it can readily be implemented commercially. Notwithstanding, ball milling can hardly reduce the filler particle size below 100 nm. To circumvent this roadblock, chemical processes are used instead to produce building blocks on a molecular scale [6].

Plasma arcing

Plasma is an ionised gas and is achieved by making a gas conduct electricity, providing a potential difference across two electrodes, so that the gas yields up its electrons and thereby ionises. Plasma arcing is basically used to synthesise deposits on surfaces rather than new solid structures. As a surface deposit, the nanomaterial can be as little as few atoms layers and is not characterised as a nanomaterial, unless at least one dimension of the bulk particle is of the nanometre scale. Otherwise, it is characterised as a thin film and not a nanomaterial. A variation on plasma arcing is flame ionisation; if a material is sprayed into a flame, ions are produced which can also be collected and deposited in nanocrystallite form [6, 7].

Plasma spray coatings on maxillofacial implants exhibit several limitations—e.g., unpredictable chemistry, porosity, inherent fractures. The coatings are subjected to fracture or fragmentation during insertion and service, and have unpredictable rates of dissolution. One of the primary reasons for the lack of control with the use of this process is that extremely high temperature must be employed in melting the initial powder. That renders it difficult to obtain the proper chemistry and structure of the resulting coating [5].

Chemical vapour deposition

Chemical Vapour Deposition (CVD) involves depositing nanoparticulate material from its gas phase. The material is heated to form a gas that is afterwards allowed to deposit as a solid coating on a surface, usually under vacuum. Thereby, CVD resembles to an extent to plasma arcing

except that the involved species are vapour in the former, whilst ionised in the latter. There may be direct deposition or deposition by chemical reaction to form a new product, which differs from the initial volatilised material.

CVD can also be used to grow surfaces; the object to be coated is allowed to stand in the presence of the chemical vapour. The first layer of atoms or molecules deposited may react with the surface. Nevertheless, these first formed depositional species can act as a template on which material can grow. The structures of these materials are often aligned, because the way in which atoms and molecules are deposited, is influenced by their neighbours. This works best if the host surface is extremely flat. During deposition, a site for crystallisation may form in the depositional axis, so that aligned structures grow vertically; therefore, this is an example of self-assembly, which gives the surface unique characteristics. Additionally, CVD can be utilised to form partial surface coatings [6].

Pulsed laser deposition

In the Pulsed Laser Deposition (PLD) technique, there is a vacuum chamber containing the coating material in a sintered/pressed form which is bombarded with laser beam. In PLD there can also be a blender containing solution where a solid disk rotates. The solid disk is subjected to laser beam pulses creating hot spots on its surface. The size of the nanoparticles can be controlled by the energy of the laser and the rotation speed of the disk [7]. A similar to the PLD technique is also the ion beam sputtering process, where argon beam is usually used for the bombardment [5].

Electrodeposition

Electrodeposition has been used for a long time to synthesise electroplated materials. In nanotechnology, the aim is to place only a single molecular layer of coverage on a surface in a highly controlled way. Electrodeposition can be addressed to fill holes to synthesise dispersed nanomaterials. Nanoholes have strategically been placed in membranes. Filling nanosized holes in polymer membranes with various combinations of metals produces nanocomposites, which have different uses. For instance, if some holes are filled with a conducting metal—like gold—they can be charged and this can influence the nature of ions that will go through the unfilled holes. If there is a device at the other end that responds to charge, the device becomes a specific ion detector. Other nanocomposites, if compound-specific, can be used as the active sensing units in so-called intelligent biomaterials. The most important development is the manufacture of multipurpose chips, which will be able to sense a host of substances at once and so provide very specific and effective diagnoses [6].

Sol–gel

Sol–gel is a useful process of self-assembly for the synthesis of nanoparticles. Colloids are suspensions with molecules of 20–100 μm in diameter in a solvent. The colloid that is suspended in a liquid is the ‘sol’, and the suspension that keeps its shape is the ‘gel’. Thus, ‘sol–gels’ are suspensions of colloids in liquids that keep their shape. The sol–gel process involves the evolution of networks through the formation of a colloidal suspension and gelation of the sol to form a network in continuous liquid phase. The precursors for synthesising these colloids normally consist of ions of a metal, but also sometimes of other elements surrounded by various reactive species, -i.e., the ‘ligands’.

The sol–gel formation occurs in four stages: (a) hydrolysis, (b) condensation and polymerisation of monomers to form particles, (c) growth of the particles, (d) agglomeration of the particles followed by the formation of networks that extend throughout the liquid medium resulting in thickening, which forms a gel. Upon drying, trapped volatiles are driven off and the network shrinks as further condensation may occur. These processes are basically affected by the initial reaction conditions. By controlling these factors, it is possible to vary the structure and the properties of the sol–gel derived inorganic network. For instance, with hydrolysis under controlled conditions, dispersed spherical nanoparticles can be synthesised [6].

Precipitation

Precipitation of a solid from a solution is a common method for the fabrication of nanoparticles. In the precipitation process, the salts of various elements are taken in the required proportion and are dissolved in water or together with suitable solvents to acquire complete mixing on an atomic scale. A precipitating reagent is added, which results in the precipitation of the components at the required ratio. The precipitate is dried and manipulated in the same way as powders, except that normally there is no further need for finer grinding.

Particles size and morphology can be controlled by changing different reaction parameters. For obtaining the precipitate of well-defined stoichiometry, the factors that have to be taken into consideration are: (a) the chemical conditions, -i.e., pH and anion concentration-, (b) the hydrodynamic conditions, -i.e., vigorous mixing- and (c) the counter ions. Precipitation technique can provide

uniform nucleation, growth and aging of the nanoparticles throughout the solution [8].

Bioceramics in maxillofacial surgery

Bioceramics, as a category of biomaterials, are generally used for the rehabilitation or replacement of skeletal tissues. Their use depends upon how a stable interface with the adjacent tissues can be achieved and how their mechanical behaviour can satisfactorily replace the lost tissues.

Classification

The most appropriate classification of implant bioceramics is related to their interactions with the biological tissues as (a) inert and (b) bioactive. Bioactive bioceramics are further classified as resorbable and non-resorbable dependant upon the level they are adsorbed by the living tissues [9–11].

Inert

Inert bioceramics are biocompatible materials, exhibiting a morphological fixation with the surrounding tissues without any biochemical bonding. In maxillofacial surgery, the most significant representatives are alumina (Al_2O_3), zirconia (ZrO_2) and carbons (C), which have also been used in other medical fields, e.g., orthopaedics, angeology [10, 12, 13]. During the last decades, Ti and its alloys have replaced these inert bioceramics to a great extent. However nowadays, with the advance of nanotechnology these inert bioceramics have gained again active role. Via nanostructuring their mechanical properties, biocompatibility and chemical homogeneity are enhanced [5].

Bioactive

Non-resorbable: The bioactive materials elicit a specific biological response at the interface, which results in the formation of a biological bond between the adjacent tissues and the material itself [14]. They include Calcium Phosphate Ceramics (CPC), bioactive glasses, bioactive glass–ceramics and Mineral Trioxide Aggregate (MTA). The rapid healing, the thickness and the strength of the bonding zone depend upon the various materials [15]. The common characteristic of all the known bioactive implant materials is that in order to have a bond with tissues, a layer of biologically active hydroxylcarbonate apatite must form at the interface [10, 15]. The formation of this apatite, which resembles to bone apatite, is mostly due to the calcium and phosphorous ions coming out from the biomaterial surface [10]. The apatite layer is the bridge connecting the ionically bonded bioceramic to the organically bonded bone. Some bioactive ceramics bond to soft tissues, as well as bone.

The main type of the CPC family is hydroxyapatite. Hydroxyapatite has nominal chemical composition $Ca_{10}(PO_4)_6(OH)_2$ with ratio of Ca/P = 1.67. Hydroxyapatite is the mineral constituent of bone and when fired as a ceramic is named hydroxylapatite (HA) [16]. The initial reason for its use as an implant material is that it forms direct bond with living bone [14]. Furthermore, HA causes a substantial enhancement of the early-stage interfacial bond development of implants [10, 17]. When HA presents high crystallinity it is classified as a non-resorbable bioactive ceramic, whilst in the case of low crystallinity it is classified as resorbable [10].

CPC have been used in maxillofacial rehabilitation for almost 25 years. Applications include dental implants, periodontal treatment, alveolar ridge augmentation and maxillofacial surgery [10, 18]. Due to process difficulties and the poor mechanical properties of conventional HA, its applications are currently confined to powders for filling bone cavities, coatings, porous bodies/scaffolds and non-load-bearing implants. In particular, HA is used as a filling material in case of bone loss or as a coating material on Ti dental implants technique in order to promote a stronger and faster bonding with bone [19]. In case HA is used as a filling material, HA may be implanted in the form of particles or porous blocks in bone [14]. In such a case, new lamellar cancellous bone forms within 4–8 weeks [20]. A cellular bone matrix from differentiated osteoblasts appear on the biomaterial surface, producing a narrow (i.e., 0.05–2.00 μm) amorphous electron dense zone. Bone mineral crystals have been identified in this zone. Between this area and the cells, collagen bundles are found. The result is normal lamellar bone attached through a thin bonding layer to the bulk implant [10, 21]. Sinus augmentation is one of the most common cases in maxillofacial surgery of using HA or natural bone, for the creation of adequate bone basement for the application of dental implants. Another application of high crystallinity HA is as 'ridge retainer'. In such a case, special cones of this material are implanted in extraction sockets in order to maintain the height of the maxillary/mandibular alveolar ridge to support later a complete denture.

Furthermore, doping of HA with Mg^{2+}, Zn^{2+}, Cd^{2+} and Y^{3+} enhances biocompatibility [22, 23]. These ions presumably substitute for Ca^{2+} ions in the HA crystal structure to provide sites for protein adsorption, and subsequent adhesion of cells. Compared amongst the tested dopants, osteoblast adhesion was significantly greater on HA doped with Y, maybe due to its increased porosity. Furthermore, doped HA with various metal cations (Mg^{2+}, Zn^{2+}, La^{3+},

Y^{3+}, In^{3+} and Bi^{3+}) has been synthesised [24]. HA doped with trivalent cations had a slower dissolution rate than either undoped HA or HA doped with bivalent cations, and among them Bi-doped HA had the slowest dissolution rate. Thus, doping with Bi offers the potential benefits according to criteria, critical for bone augmentation clinical success.

In case HA is used as a coating on Ti dental implants, there was an initial enthusiasm when such implants were applied in an area of reduced quality of bone -e.g., in IV type of bone [25–27]. Two additional applications of HA-coated implants are their direct placement in alveolar ridge immediately after extraction, as well as for the rehabilitation of the posterior region of maxillae in case of surgical sinus lifting procedures, where they have been successfully used [28–30]. Nevertheless, the use of HA-coated Ti dental implants exhibited eventually unsatisfactory metalloceramic bonding, dissolution at areas of low pH -i.e., inflammation- and lack of quality reproducibility, leading to inevitable failure [31, 32]. Thus, although in the early clinical studies the success percentage had been found higher than 90%, during the last years their use has been confined tremendously [33–35]. In a recent study, the sol–gel processing has been addressed to coat Ti substrates with HA. The coatings had a high degree of crystallinity, satisfactory resistance to cracking and micron surface roughness with islands. Cytocompatibility tests of this novel coating revealed advanced cell adhesion compared to plasma-sprayed HA-coating [36].

Nevertheless, during the recent years, tracing the development of technology, the idea of constructing biocompatible scaffolds, where osteoblasts and other human cells could proliferate and differentiate, has increased. In an attempt to rehabilitate severe bone loss in a patient's mandible, a computer-generated three-dimension (3D) scaffold made of high crystallinity HA was fabricated [37]. The porous structure of the scaffold allows bone to grow into it, providing the future basis for the growth of new bone in the patient.

Bioactive glasses and glass-ceramics can bond to living tissues [10, 38, 39]. Bioactive glass-ceramics have acquired enhanced mechanical properties via controlled crystallisation compared to bioactive glasses. Both of them are used to fill the tooth socket immediately after the extraction in order to maintain the bone level and the contours for prosthetic reasons [40]. Nanostructuring process of both of them is expected to increase their biocompatibility and mechanical properties. Another special material is MTA, which is used as a root-end filling material in endodontically treated teeth and consists of Portland cement, Bi_2O_3 and $CaSO_4 \cdot H_2O$ [41].

Resorbable: Resorbable bioceramics degrade gradually over a period of time and are replaced by the natural host tissues. This can become the optimal solution if the requirements of strength and short-term performance can be fulfilled [14]. Complications in the development of resorbable bioceramics are the maintenance of the strength and stability of the interface during the degradation and replacement period by the natural host tissue [10], and the matching resorption rates to the repair rates of the body tissues [10, 14].

It is essential that a resorbable biomaterial consists only of metabolically acceptable substances. Almost all resorbable ceramics are variations of $Ca \cdot PO_4$ [10]. Porous or particulate CPC are successful materials for resorbable hard tissue replacements when low loads are applied to the biomaterial—e.g., a multicrystalline porous form of β-tricalciumphosphate (β-TCP) has successfully been used to correct periodontal defects and to augment bony contours. When TCP and tetracalcium phosphate are implanted, they gradually degrade, being totally replaced by the host tissue and therefore are classified as resorbable bioceramics [14]. Another application of resorbable bioceramics is the drug-delivery devices. Resorption or biodegradation of CPC is caused by physicochemical dissolution, physical disintegration or phagocytosis of the materials [20].

Furthermore, corals belong to the category of resorbable bioceramics. Corals are a natural substance made by marine invertebrates, and those used as bone-implants are selected on the basis of structural similarity to bone. Coral provides an excellent structure for the ingrowth of bone, and its main component—i.e., $CaCO_3$—is gradually resorbed by the body. Furthermore, corals can be converted to HA by a hydrothermal exchange process. Both pure coral and coral transformed to HA are currently used to repair traumatised bone, replace diseased bone or correct various bone defects [20].

Moreover, calcium sulphate ($CaSO_4$) is a resorbable material and can replace any other form of barrier when used in conjunction with demineralised freeze dried bone allograft. The $CaSO_4$ has been used as a regenerative material in periodontal therapy. There have been many studies assessing the osteopromoting effect of $CaSO_4$ as a barrier concluding that $CaSO_4$ barriers can exclude connective tissues, allowing bone regeneration during healing [42]. Its biocompatibility was proved in an in vitro investigation, where it was shown that the osteoblasts continued to function and flourish in the presence of $CaSO_4$ [16]. In a recent study, autogenous bone graft—harvested from the chin—was mixed approximately in a 1:3 ratio with $CaSO_4$ and was used for sinus lifting with success [43]. Moreover, it was shown that $CaSO_4$ is a valid bone substitute, which is completely resorbed and substituted by new bone [44]. Nanotechnology provides the opportunity to develop the so-called 'smart resorbable materials' with special properties by incorporating specific agents and by advancing

the integration between these materials and the natural tissues [45].

Discussion—recent developments

Nanophase materials are promising materials for various bioapplications, since human being tissues are composed of nanometre components (i.e., proteins, inorganics). Natural bone is comprised of nanostructured HA and collagen fibres, which continuously provide an extracellular matrix surface to osteoblasts with a high degree of nanometre roughness. Despite this fact, materials currently utilised for implants, whether metallic or ceramic, have constituent grain sizes in the micron regime, which is non-biologically inspired [46]. Nanophase biomaterials hold great promise in tissue engineering and pave the way to new opportunities in various maxillofacial applications. The state-of-the-art of such nanostructured bioceramics is summarised herewith, and are compared concerning their adhesion, differentiation, growth proliferation and viability of osteoblasts with the conventional biomaterials [47]. These parameters are crucial prerequisites for successful apposition of satisfactory bone quality.

Nanophase HA

Select various functions—adhesion, proliferation, occupancy—of osteoblast, fibroblast and endothelial cells on nanophase and conventional HA have been investigated using in vitro cellular models [48, 49]. The adhesion and proliferation of osteoblasts was significantly greater on nanophase HA than on conventional. Contrarily, the adhesion of endothelial and fibroblast cells, and the surface occupancy of osteoblast colonies was less on the nanophase ceramic. Select enhanced osteoblast adhesion was independent upon the surface chemistry or mineral phase, but on the surface topography of nanophase HA. Thereby, nanophase HA clearly represents a unique and promising class of maxillofacial implant formulations with improved osseointegrative properties**.** Apart from nanostructured HA, both nanophase Al_2O_3 and titania (TiO_2) demonstrated the same properties.

In another study [50], enhanced osteoclast-like cells (i.e., the bone-resorbing cells) function on HA surfaces with nanometre-size surface topography has been demonstrated. Compared to conventional HA, osteoclast-like cells cultures were significantly greater on the nanophase one. Since bone resorption by osteoclasts is accompanied by subsequent deposition of Ca-containing mineral by osteoblasts in vivo, the results of the present study imply that enhanced coordinated functions of osteoclasts and osteoblasts may occur on nanophase HA. Such enhanced corresponding events between osteoclasts and osteoblasts may lead to improved osseointegration of maxillofacial implants into juxtaposed bone. Similar research work has been performed on Al_2O_3 with nanometre size surface topography, where it exhibited same behaviour [47].

One of the prime biomedical applications of HA in maxillofacial surgery is as a coating material. In a study [51], nanoapatite coating, closely mimicking bone mineral, was grown directly on Ti alloy—Ti6Al4V—soaked in blood plasma at a physiological-related temperature, with composition and structure equivalent to those of bone mineral. The biomimetic nanoapatite was demonstrated to be capable of conducting bone formation and promoting direct bone apposition. This bioactive coating also affected the behaviour of human osteoblasts as indicated by their morphologies, observed in cell culture.

Biomimetic processes have attracted huge attention in recent years due to their significant applications in biomedical areas, such as bone tissue engineering. In another biomimetic process [52], a thin bone-like nanocrystallite HA coating on Ti was formed via an alkali pretreatment. This was followed by immersion in a simulated body fluid. Analysis of the coating has shown that the HA layer, grown in this way, exhibits similar stoichiometry to that of natural bone and is firmly adhered on Ti. Besides, its thickness has increased as the immersion period increased. The adhesion of the HA layer on the Ti substrate was further confirmed by a shear test. The bioactivity of the coating was finally examined by cell culturing experiments. The results have manifested that the nanophase HA prepared using the present method possesses enhanced mechanical properties and bioactivity.

It has been revealed [53–55] the formation of a nanometre rough $CaTiO_3$ layer as a consequence of interactions between HA and Ti during coating processes. Results from cytocompatibility tests showed increased osteoblast adhesion on materials that contained $CaTiO_3$ compared to both pure HA and uncoated Ti [47]. The greatest osteoblast adhesion was observed on Ti-coated HA annealed under air conditions. This implies that coatings, which form $CaTiO_3$, could increase osseointegration with juxtaposed bone needed for increased implant efficacy.

Nanotechnology has been addressed to fabricate nano HA scaffolds. Transplantation of osteogenic cells with a suitable matrix is one strategy for engineering bone tissue. Three-dimension distribution and growth of cells within the porous scaffold are of clinical significance for the repair of large bony defects. In a study [56], 3D porous nano HA has been fabricated as scaffold, where rat bone marrow stromal cells were seeded in vitro. The cells adhered, proliferated and differentiated well.

In another study [57], a nano HA/collagen porous composite that mimics the natural bone both in composition and microstructure was produced and employed as a matrix for the tissue engineering of bone. Osteogenic cells and the 3D nano HA/collagen composite constructs were developed in vitro. Spindle-shaped cells migrating out of bone fragments continuously proliferated and migrated throughout the network of the coil. Finally, new bone matrix was synthesised at the interface of bone fragments and the composite.

Similarly, porous nano HA/collagen/alginate composite, containing nano HA/collagen and Ca-crosslinked alginate, has been synthesised biomimetically [58]. This composite shows a significant enhancement in mechanical properties compared with nano HA/collagen composite. Primary biocompatibility experiments in vitro, including fibroblasts and osteoblasts co-culture, indicated its high biocompatibility. Likewise, bone scaffold material, made of nano HA/collagen/poly(lactic acid) (PLA) composite, has been developed by biomimetic synthesis [59]. The nanocomposite exhibits some features of natural bone both in main composition and hierarchical microstructure. The 3D porous nanocomposite mimics the microstructure of cancellous bone. Cell culture and animal model tests demonstrated that the scaffold nanocomposite is bioactive; neonatal rat calvaria osteoblasts adhered, spread and proliferated throughout its pores. Both scaffolds have promise for the clinical repair of large bony defects, according to the principles of bone tissue engineering.

Fluorapatite/collagen composites have been synthesised via a biomimetic coprecipitation method in order to improve the structural stability and cellular responses. The precipitated composites were freeze-dried and isostatically pressed in a dense body. The added fluorine was incorporated nearly fully into the apatite structure (fluoridation) and a near stoichiometric fluorapatite/collagen composite was obtained with complete fluoridation. The freeze-dried composites had a typical biomimetic network, consisting of collagen fibres and precipitates of nanosized apatite crystals. The human osteoblast-like cells on the fluorapatite/collagen composites exhibited significantly higher proliferation and differentiation than those on the HA/collagen composite. These enhanced osteoblastic cell responses were attributed to the fluorine release and the reduced dissolution rate [60].

Self-hardening CPC sets to form HA with high osteoconductivity, but its brittleness and low strength limit its use to only non-stress bearing locations. Previous studies developed bioactive composites containing HA fillers in bisphenol-a-glycidyl methacrylate based composites for bone repair applications, and they possessed higher strength values. However, these strengths were still lower than the strength of cortical bone. Strong and bioactive composites have been developed by combining CPC fillers with nanosized silica (SiO_2) fused whiskers in a resin matrix. Their mechanical properties and cell response have been characterised. SiO_2 particles were fused to CSi whiskers to roughen the whisker surfaces for enhanced retention in the matrix. The mechanical properties of the CPC-whisker composites nearly matched those of cortical and trabecular bone. The observed osteoblast-like cell adhesion, proliferation and viability suggest that the new CPC-whisker composite was noncytotoxic [61].

Several other applications of nanostructured HA are in progress, some of which are here summarised. There has been an attempt to modify the surface of nano HA particles by coating them with SiO_2 in order to influence its colloid stability, prevent its dissolution in case of low pH -e.g., inflammation-, serve as an intermediate layer to allow strong bond formation between HA-polymer matrices and potentially enhance its bioactivity [62].

In another study [63], nanosized, rod-like HA crystals have been synthesised and electrosprayed onto glass substrates via a novel processing routine. There was no significant evidence of either cytotoxicity or inflammatory response, and so the nano HA sprayed substrates were able to support the attachment and the growth of human osteoblast cells. Thus, nano HA composites maybe suitable for intraosseous implantation and offer the potential to formulate enhanced composites for biomedical applications. Moreover, the formation of nanosized needle-like HA crystals have been revealed in premixed calcium phosphate cements, promoting rapid setting, when immersed in a physiological solution. These cements had strengths matching those of cancellous bone and noncytotoxicity, rendering more effective in bone repair surgery [64].

Nanophase Al_2O_3—nanophase TiO_2

Al_2O_3 of varying particulate size, chemistry and crystalline phase has been tested in order to determine what formulation might be the most beneficial for bone regeneration. Specifically, in vitro osteoblast adhesion, proliferation, intracellular alkaline phosphatase activity and Ca deposition was observed on (1) nanospherical δ-Al_2O_3, (2) conventional spherical α-Al_2O_3 and (3) boehmite Al_2O_3 nanofibre. Results showed increased osteoblast functions on the nanofibre Al_2O_3. Some of the possible explanations for such an enhanced osteoblast behaviour may be attributed to crystalline phase and topography. Increased osteoblast function on boehmite Al_2O_3 nanofibres suggests that it may be an ideal material for use in maxillofacial applications [65]. The same scientific group investigated various crystalline forms of nanofibre Al_2O_3—(1) boehmite, (2) γ-Al_2O_3, (3) $\gamma + \delta$ Al_2O_3, (4) $\theta + \delta$ Al_2O_3 and (5) α-

Al_2O_3—which were compared with reference substrate—a borosilicate glass—regarding osteoblasts deposition and adhesion. Compared to any other Al_2O_3 formulation, osteoblast functions were the greatest on $\theta + \delta$ Al_2O_3 nanofibre. The ability of such Al_2O_3 fibres to approximate the nanometre dimensions of constituent components of bone offers exciting possibilities in the design of maxillofacial implants with greater efficacy [46].

In a study [66], osteoblast adhesion on nanophase Al_2O_3 has been investigated in vitro. The study provides evidence of the ability of nanophase Al_2O_3 to simulate material characteristics—e.g., surface grain size—of physiological bone that enhance protein interactions and subsequent osteoblast adhesion, which is critical for the clinical success of maxillofacial implants. Further investigation of the dependence of osteoblast adhesion on Al_2O_3 grain size indicated the presence of a critical grain size (between 49 and 67 nm) for osteoblast adhesion of Al_2O_3. Additionally, the factor of serum as an intermediate medium for the osteoblasts proliferation has been shown to be of prime significance. Similar properties were reported for nanostructured TiO_2, where the critical grain size was between 32–56 nm.

Osteoblast viability and cell density have also been investigated when cultured in the presence of nanophase compared to conventional Al_2O_3 particles at various concentrations of cell culture media. Results provided evidence of increased apoptotic cell death when cultured in the presence of conventional Al_2O_3 particles, compared with the nanophase ones. Moreover, since material characterisation studies revealed that the only difference between respective Al_2O_3 particles was their dimensions, these results indicated that osteoblast viability and densities were influenced solely by particle size. Such nanometre particulate wear debris may result from friction between articulating components of implants composed of novel nanophase ceramic materials. Results of a less detrimental effect of nanometre—as compared to conventional-dimensioned particles on the functions of osteoblasts—provide additional evidence that nanophase ceramics may become the next generation of bone reconstruction maxillofacial materials with increased efficacy. Such an osteoblast viability and densities were also demonstrated in the case of nanophase TiO_2 [67].

As in the case of nanophase HA, osteoblast adhesion was significantly greater on nanophase Al_2O_3 than on conventional formulations of the same ceramics, whilst fibroblast and endothelial cells adhesion was significantly less on nanophase Al_2O_3 [47]. Furthermore at another study [48], enhanced proliferation and long-term functions of osteoblasts have been observed when cultured on nanophase Al_2O_3, whereas less occupancy of osteoblast colonies has been observed. Similar results have also been reported in the case of nanostructured TiO_2 [47, 48]. As already mentioned [50], osteoclast cells cultures were significantly greater on the nanophase Al_2O_3 compared to conventional. Such enhanced corresponding events between osteoclasts and osteoblasts may lead to improved osseointegration of maxillofacial implants into juxtaposed bone.

Nanoporous Al_2O_3 membranes, developed by anodisation, have been used as a coating on Ti alloy implants. Human cell culture interactions with the nanoporous membrane of Al_2O_3 have been investigated via biochemical and morphological parameters, assessing cell viability, proliferation and phenotype. Results showed a normal osteoblastic growth pattern with increasing cell numbers. Cell adhesion has been observed on the nano porous of the material [68]. Complementary to this work, nanoporous, highly adherent layers of anodised Al formed on the surface of Ti alloy and pure Ti have been developed as coatings for metallic surgical implants [69]. The layers are formed by anodisation of a thick layer of Al, which has been deposited on substrate material by beam evaporation. The surface ceramic layer so produced is Al_2O_3 with phosphate ions and porosity, running perpendicular to the surface. Mechanical testing showed satisfactory coating's shear and tensile strength. Initial cell/material studies showed promising cellular response to the nano-porous Al_2O_3. A normal osteoblastic growth pattern with increasing cell number was shown, with slightly higher proliferative activity on the nanoporous Al_2O_3. Normal osteoblast morphology of the cells on the porous membrane was observed. Furthermore, flattened cells with filopodia attaching to the pores and good coverage were detected. The pore structure produced in these ceramic coatings is expected to be suitable for loading with bioactive material to enhance further their biological properties.

Another use of Al_2O_3 is as a constituent in nanocomposites with ZrO_2. The biocompatibility of ZrO_2/Al_2O_3 in load-bearing applications, such as maxillofacial implants, has been significantly enhanced by the addition of bioactive HA. The ZrO_2/Al_2O_3 matrix, composed of nanocomposite powder, had higher flexural strength than conventionally mixed ZrO_2/Al_2O_3 composite. From the in vitro test with osteoblastic cell-lines, the proliferation and the differentiation of the cellular response on the HA-added ZrO_2/Al_2O_3 nanocomposites gradually increased, as the amount of the added HA increased [70].

In biological applications, most of the research to date has focused on the interactions between mammalian cells and synthetic nanophase surfaces for the creation of better tissue engineering materials. Although mammalian cells have shown a definite positive response to nanophase materials, information on bacterial interactions

with nanomaterials still remain elusive. It has been designed to assess the adhesion of bacteria (Pseudomonas fluorescence) on nanophase Al_2O_3 substrates compared to conventional grain size ones. Nanostructured surface features of Al_2O_3 have shown to enhance bacterial adhesion [71].

Another application of nanophase TiO_2 is as a constituent in nanocomposites with poly-lactic/glycolic acid (PLGA). Chondrocytes (cartilage-synthesising cells) cell density and synthesis of select intracellular proteins by chondrocytes were investigated on novel nanophase PLGA/TiO_2 composites in vitro. Composites of either conventional or nanophase PLGA with either conventional or nanophase TiO_2 have also been synthesised. Results demonstrated stagnant confluent cell densities on nanostructured surfaces, whilst increased chondrocytes functions were observed on PLGA/TiO_2 nanocomposites compared to surfaces with a conventional topography [72].

In another study [73], novel nanoceramic/polymer composite formulations have been fabricated and characterised with respect to their cytocompatibility and mechanical properties, in an attempt to simulate the microstructure and mechanical properties of natural bone. The bending moduli of nanocomposite samples of either PLA or poly(methyl methacrylate) (PMMA) with nanophase Al_2O_3, TiO_2 and HA loadings were significantly greater than those of pertinent composite formulations with conventional, coarser grained ceramics. The nanocomposite bending moduli were 1–2 orders of magnitude larger than those of the homogeneous respective polymer. Osteoblast adhesion on the surfaces of the nanophase Al_2O_3/PLA composites increased as a function of the nanophase ceramic content. Most importantly, osteoblast adhesion on the nanophase Al_2O_3/PLA substrates was similar to that observed on the pure nanophase ceramic substrates. Similar trends of osteoblast adhesion were observed on the surfaces of the nanophase TiO_2/polymer and nanophase HA/polymer composites. In contrast, fibroblast adhesion on the nanophase composites was either similar or lower than that observed on the conventional composites with either PLA or PMMA, and minimum on all tested neat nanophase substrates. The Ca-content in the extracellular matrix of cultured osteoblasts was also enhanced on the nanoceramic/PLA composite substrates, tested as a function of the nanophase ceramic loading and duration of cell culture. The results of that in vitro study provided evidence that nanoceramic/polymer composites were promising alternatives to conventional materials, because they can potentially be designed to match the properties of bone tissue in order to overcome the limitations of the biomaterials currently used as bone prostheses.

Nanophase C

Novel nanocomposites consisting of blends of PLA and C nanotubes can effectively be used to expose cells to electrical stimulation. When osteoblasts cultured on the surfaces of these nanocomposites were exposed to electric stimulation, there was an increase in cell proliferation. These results have provided evidence that electrical stimulation delivered through novel, current conducting polymer/nanophase C composites promoted osteoblast functions that were responsible for the chemical composition of the organic and inorganic phases of bone. Moreover, this evidence elucidated aspects of the cellular/molecular-level mechanisms involved in new bone formation under electrical stimulation [74].

Carbon nanofibres have exceptional theoretical mechanical properties that, along with possessing nanoscale fibre dimensions similar to crystalline HA found in bone, suggest strong possibilities for use as a maxillofacial implant material. Osteoblast, fibroblast, chondrocytes and smooth muscle cells adhesion have been assessed on C nanofibres in vitro. Results provided evidence that, compared to conventional C fibres, nanometre dimension C fibres promoted select osteoblast adhesion, by contrast to the smooth muscle cells, fibroblasts and chondrocytes. To determine properties that selectively enhanced osteoblast adhesion, similar cell adhesion assays have been performed on polymer casts of C fibre compacts. Compared to PLGA casts of conventional C fibres, results provided the first evidence of enhanced select osteoblast adhesion on PLGA casts of nanophase C fibres. The summation of these results demonstrate that due to a high degree of nanometre surface roughness, C fibres with nanometre dimensions may be optimal materials to selectively increase osteoblast adhesion necessary for successful maxillofacial implant applications [75]. In a similar study, bone cell adhesion on novel C/polycarbonate urethane nanofibre composites has been investigated in vitro. Greater weight percentages of high surface energy C nanofibres composite increased osteoblast adhesion, while at the same time decreased fibroblast adhesion [76].

Conclusions—future prospectives

The capability of synthesising and processing nanomaterials with tailored structures and topographies, in an attempt to simulate the microstructure and mechanical properties of natural bone and control select subsequent cell functions, provides the possibility of designing the novel proactive bioceramics necessary for enhanced implant efficacy. These are promising alternatives to conventional materials, because they can potentially be

designed to match the properties of bone tissue in order to overcome the limitations of the biomaterials currently used as bone prostheses. These novel bioceramics can limit the curing period from the patients' side, since the life expectancy of the implants increases, whilst the economical negative consequences from repeated surgical operations decrease. The various primary positive results, regarding the biocompatibility and biomimicity of the novel nanostructured bioceramics to natural bone, merit further confirmation.

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