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Review

Nanomaterials in Craniofacial Tissue Regeneration: A Review

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Abstract: Nanotechnology is an exciting and innovative field when combined with tissue engineering, as it offers greater versatility in scaffold design for promoting cell adhesion, proliferation, and differentiation. The use of nanomaterials in craniofacial tissue regeneration is a newly developing field that holds great potential for treating craniofacial defects. This review presents an overview of the nanomaterials used for craniofacial tissue regeneration as well as their clinical applications for periodontal, vascular (endodontics), cartilage (temporomandibular joint), and bone tissue regeneration (dental implants and mandibular defects). To enhance periodontal tissue regeneration, nanohydroxyapatite was used in conjunction with other scaffold materials, such as polylactic acid, poly (lactic-co-glycolic acid), polyamide, chitosan, and polycaprolactone. To facilitate pulp regeneration along with the revascularization of the periapical tissue, polymeric nanofibers were used to simulate extracellular matrix formation. For temporomandibular joint (cartilage) engineering, nanofibrous-type and nanocomposite-based scaffolds improved tissue growth, cell differentiation, adhesion, and synthesis of cartilaginous extracellular matrix. To enhance bone regeneration for dental implants and mandibular bone defects, nanomaterials such as nanohydroxyapatite composite scaffolds, nanomodified mineral trioxide aggregate, and graphene were tested. Although the scientific knowledge in nanomaterials is rapidly advancing, there remain many unexplored data regarding their standardization, safety, and interactions with the nanoenvironment.

Keywords: dentistry; nanofibers; nanomaterials tissue engineering; nanoparticles; nanotechnology scaffolds engineering; oral and maxillofacial regions; oral tissue; tissue regeneration

1. Introduction

The interest of nanomaterials in dentistry is growing rapidly and has allowed for improvements in various biomedical applications, such as that of tissue regeneration [1]. Nanomaterials are materials whose components, in at least one dimension, measure 100 nm or less [2]. Sheets are nanomaterials in one dimension, nanowires and nanotubes are those in two dimensions, and quantum dots in three dimensions [2]. Nanomaterials can be synthesized either top–down (to scale down an existing structure to a nanoscale level) or bottom–up (to create by manipulating atoms and molecules) [3]. The enhanced utility of nanomaterials can be attributed to the physical and chemical properties of nanomaterials, such as strength, conductivity, color, and toxicity, which differ at the nanoscale size due to an increase in surface area and quantum effects [3].

The application of a nanofiller material for restorations, in 2002, marked the first time that nanomaterials were used in dentistry [4]. By integrating the three principal elements of tissue engineering (stem cells, scaffolds, and signaling molecules) with the prospect of nanotechnology,

nanostructured products that could provide mechanical support and promote biointegration for osteoblastic cell precursors were created [5]. Bone engineering scaffolds have been designed based on the following criteria: (1) The ability to mediate cell attachment, differentiation, and proliferation; (2) strong osteoconductivity with the host bone; (3) biodegradability; (4) mechanical strength; (5) porosity; (6) fabrication into irregular shapes; and (7) suitability for commercialization and clinical procedures [3]. As an example, scaffolds designed for the bone are to be rigid, while those designed for the pulp–dentin complex are to be soft and injectable [4].

The incorporation of nanomaterials into dentistry has been associated with many potential benefits [6]. In areas of preventative health care, nanomaterials can exhibit antimicrobial and restorative properties, while in restorative dentistry, nanofillers can enhance the mechanical and bioactive properties of restoration materials [6]. Stem cell treatments or dental fillings, to contrast, do not provide protection against future dental diseases or restore the native tooth structure, respectively [1]. Additionally, the rejection rate of dental implants may be improved by the use of nanomaterials to increase osseointegration, infection control, and biocompatibility [6]. Although some studies have shown benefits of nanomaterials, others have shown no significant findings [6]. A drawback of nanomaterials, however, is the presence of low amounts of oral toxicity and of potential systemic disturbances caused by their translocation to the gut [6]. As nanomaterial-based studies become more prevalent for dentistry, the potential benefits and risks of nanomaterials as an alternative or complementary treatment should be considered and further examined.

This review presents updates on the current state of nanomaterials used in craniofacial tissue regeneration: We examine the various types of nanomaterials, followed by a focus on specific clinical applications.

2. Overview of Nanomaterials in Craniofacial Tissue Engineering

Nanomaterials have allowed tissue engineering scaffolds to display greater mechanical strength and to promote greater cell growth, differentiation, and stability of bone formation-related growth factors [7]. Dependent on the direction of tissue engineering, unique combinations of nanomaterials can be generated or used to create unique scaffolds with key properties. The main nanomaterials used in craniofacial tissue engineering include metallic, polymeric, carbon-based, ceramic, and composite nanomaterials.

2.1. Metallic Nanomaterials

Metal-based nanomaterials are used for their high mechanical strength and include metals such as gold, silver, and titanium [8,9]. In contrast to bulk metals, metallic nanoparticles, due to quantum effects, can promote osseointegration in addition to promoting many other factors, such as osteoconductivity, mineralization, proliferation, osteoblast and chondrocyte cell adhesion, increased mechanical strength, stimulation of collagen production, stimulation of alkaline phosphatase activity, and stimulation of calcium deposition [8–10]. For instance, Tran and Webster found that iron oxide nanoparticles with a hydroxyapatite coating could increase osteoblast functions [11]. Due to their antibacterial properties, metallic nanoparticles such as zinc and copper have been incorporated into scaffolds, while silver nanoparticles have been investigated as a replacement for certain dental filling agents [3,12]. Unfortunately, metallic nanomaterials can accumulate in organs due to their small size and cause potential health effects [7]. These health effects have been hypothesized to be due to oxidative stress and inflammatory reactions [7].

2.2. Polymeric Nanomaterials

Polymeric nanomaterials have nanoparticles composed of compounds such as carbon, hydrogen, oxygen, and nitrogen. These compounds form monomeric chemical structures, which repeat to form longer chains or nanoparticles [13]. Polymeric nanomaterials have vast uses and potential in medicine due to their unique properties; these potential uses include drug delivery, surface

coatings, and filtration [14]. Some examples of polymeric nanomaterials used, particularly in dentistry, include polyethylene glycol, solid lipids, nanogels, dendrimers, chitosan, peptides, polylactic acids, and gelatin [13,15–17]. Polymeric particles have the ability to encapsulate various molecules, including vaccine antigens, proteins, drugs, various growth factors, and even cells, making polymeric nanomaterials extremely versatile and a powerful tool for transportation and delivery [18]. Additionally, certain polymeric nanomaterials have nanofibers and structural compositions that resemble a human extracellular matrix (ECM) and thus can be used as a scaffold to nurture and facilitate tissue regeneration [15]. The versatility in polymeric nanomaterials highlights its potential to overcome many challenges faced in the field of medicine, specifically in dentistry today.

2.3. Carbon-Based Nanomaterials

Carbon-based nanomaterials have unique physical, chemical, and biological properties, such as remarkable mechanical strength, electrical conductivity, and thermal stability [19]. These properties allow carbon-based nanomaterials to exhibit features similar to those of a biological ECM, in addition to promoting adhesion, proliferation, and osteogenic differentiation of bone marrow-derived stem cells (BMSCs) [20]. Graphene and carbon nanotubes are commonly used examples of carbon-based nanomaterials.

2.4. Ceramic Nanomaterials

Ceramic nanomaterials share characteristics of both metallic and nonmetallic elements. These nanomaterials are hard and brittle, have electrical insulation properties, and can tolerate harsher chemical environments than metallic and polymeric nanomaterials [21]. Ceramic nanomaterials display good biocompatibility, osteoconductivity, osteoinductivity, biodegradability, restorability, and hydrophilicity [21]. Examples of ceramic nanomaterials include bioactive glass, nanohydroxyapatite (nHA), and calcium phosphate, among many other oxides, phosphates, nitrides, and carbides. Because ceramic nanomaterials are similar in composition to inorganic components found in human bone, they have great potential in tissue engineering and are often used as scaffolds [21].

2.5. Composite Nanomaterials

Composite-based nanomaterials are created by combining a minimum of two nanomaterials together, usually containing a matrix material and nanoscale particles. The use of such a technique allows researchers to synthesize novel nanomaterials with unique characteristics, or to harness desired physical and mechanical properties from multiple types of nanomaterials [22]. For example, in some studies, nanomaterials have been combined to create novel composite nanomaterials with similar properties to that of human bone. When creating a composite-based nanomaterial, various properties, including biocompatibility and biofunctionality, modulus of elasticity, surface hardness, degree of polymerization shrinkage, compressive strength, tensile strength, and material flow, should be considered [22–24]. In dentistry, composite-based nanomaterials are often the result of combining biocompatible and biofunctional ceramics and polymers [22].

3. Clinical Applications

3.1. Periodontal Tissue Regeneration

3.1.1. Membranes for Periodontal Tissue Regeneration

Periodontitis is the inflammation of the periodontium and causes damage to the surrounding tissues, such as the alveolar bone and periodontal ligament. Without treatment, loss of the supporting structures around the tooth eventually results in tooth loss. To reconstruct periodontal tissue, the development of membranes for guided tissue regeneration (GTR) and guided bone regeneration (GBR) with adequate mechanical and biological properties have been explored [7].

Membranes must satisfy several important criteria, such as biocompatibility, space maintenance, cell exclusion, tissue integration, and clinical manageability. On one hand, nonresorbable membranes require additional surgery for their removal and increase the risks of post-operative infection. On the other hand, resorbable membranes have unpredictable degradation rates and often lack sufficient strength. Synthetic membranes combine strong mechanical properties to prevent collapse within the bony defect and biological properties to deliver biomolecules and cells to promote tissue regeneration. Polycaprolactone (PCL) membranes synthesized using the electrospinning technique can be loaded with anti-inflammatory drugs (such as ibuprofen) and growth factors (such as bone morphogenetic protein-2 (BMP-2)) to enhance periodontal regeneration [25]. Biomimetic fish collagen/ bioactive glass/ chitosan composite nanofiber membranes (Col/BG/CS) have good hydrophilicity, higher porosity and surface area promoting cell–cell and cell–matrix interaction, adequate tensile strength, and limited antibacterial properties [26]. Clinically available collagen membranes may be functionalized by electrospinning poly-DL-lactic acid (PDLLA) polymers to enhance periodontal regeneration. In fact, membranes functionalized with antibiotics and growth factor coated PDLLA nanofibers can facilitate healing and regenerative processes while reducing the risk of bacterial infection [27].

3.1.2. Scaffolds for Periodontal Tissue Regeneration

Nanostructured biomimetic materials, for periodontal regeneration, may offer higher performance due to a greater surface area to volume ratio and to chemical/ electrical synergistic effects. However, these materials may be eliminated from periodontal defects by phagocytosis [28].

Nanohydroxyapatite (nHA) is an alloplastic material that is chemically similar to the inorganic building blocks of a bone matrix. It is widely used as a bone repair material because it is biocompatible, osteoconductive, and resorbable. There are some limitations when it comes to the use of nHA, including insufficient toughness and poor mechanical properties. To overcome these limitations, material scientists have developed composite materials containing nHA and synthetic polymers, such as polylactic acid (PLA), poly lactic-co-glycolic acid (PLGA), polyamide, chitosan, and PCL [7]. One group showed that a composite graft of nHA and microsized beta-tricalcium phosphate (B-TCP) helped to enhance the binding and retention of nHA to periodontal defects by minimizing its dissemination and phagocytosis [29]. Nanomaterials such as nHA may be used in combination with biologics such as enamel matrix derivative (EMD) to enhance regenerative effects [30]. In fact, amelogenin, the main active ingredient of EMD, induces the formation of acellular cementum and stimulates the proliferation and differentiation of periodontal fibroblasts and osteoblasts [31].

Several polymers may be used for periodontal regeneration, including natural and synthetic materials, such as collagen, chitosan, dextran, alginate, PLA, poly-glycolic acid (PGA), and polyethylene glycol (PEG). In addition to acting as scaffolds, hydrogels, micro/nanoparticles, and membranes may also be used as drug/cell delivery systems to improve periodontal regeneration [32,33].

3.2. Vascular Tissue Regeneration (Endodontics)

3.2.1. Dental Pulp

Underneath the exterior hard tissues of a tooth is an unmineralized soft tissue core called dental pulp. The pulp is composed of connective tissue, immune cells, odontoblasts, nerve fibers, and blood vessels [15,34]. This unique composition enables the pulp to produce and maintain dentin, and provides immunity, nutrition, tooth vitality, and sensation. However, the pulp is prone to caries, trauma, and infections, which lead to pulpitis—inflammation of the pulp, which is generally painful and irreversible. This ultimately results in pulp necrosis, which ceases tooth functionality and vitality [15,16,35]. There is an ongoing challenge in overcoming these pulpal injuries; to date, there is little to no success in the restoration of the pulp tissue once diseased or damaged. A major challenge that hinders the success of restoring pulp tissue is due to difficulties in revascularization of the tissue caused by the unique and miniscule source of blood flow at the apex of the tooth root [17]. The lack of a

vasculature system leads to a loss of nutrients diffusion. As a result, patients will often have to undergo a root canal procedure to salvage the remaining hard tissues of the tooth, which leaves the tooth in a weakened, nonvital state [15,17]. The crucial role of vascular tissue, in addition to the insufficient repair protocols for dental pulp, highlights the need for research in alternative methods for treating dental pulp injury. This section aims to examine recent studies exploring the uses of nanomaterials as a potential alternative solution to address pulp injuries.

There is increasing evidence on the feasibility of using nanomaterials in tissue engineering in recent literature. In general, using nanomaterials as a scaffold offers improved controlled allocation of active molecules and, thus, better promotion of niche formations, mirroring the complexity found in soft tissues [35]. Polymeric nanomaterials, more specifically, are explored for drug delivery, tissue engineering scaffolds, the creation of medical devices, and for diagnosis due to their advantages, as previously discussed in Section 2.2. [36].

In a study by Galler et al., they addressed pulp injuries by exploring pulp tissue regeneration via a biomaterial scaffold composed of self-assembling peptide nanofibers [15]. The small 6-nm-diameter property of these nanofibers enabled the synthetic scaffold to mimic a structural mesh that was similar to a naturally-occurring ECM, which was optimal for cellular activities such as cell-cell and cell-matrix interactions. Additionally, the nanofiber material was also able to encapsulate dental pulp stem cells (DPSCs) and the various growth factors needed to foster angiogenesis (the formation of new blood vessels) and cell growth, differentiation, and morphogenesis, such as the fibroblast growth factor 2 (FGF2), transforming growth factor β1, and vascular endothelial growth factor (VEGF) [15]. The encapsulation of such growth factors allows for the sustained and unmonitored, yet controlled release of these factors over a period of time. Galler et al. were able to induce in vivo angiogenesis in artificial teeth implanted in mice using these self-assembling peptide nanofibers.

To investigate the potential of graphene in tissue engineering, Xie et al. compared the effect of graphene to glass. They found that graphene could induce greater mineralization, and osteogenic, but not odontoblastic, differentiation of DPSCs [37].

Similarly, the concept of using polymeric nanomaterials to encapsulate drugs, particles, cells, and/or growth factors to facilitate tissue engineering was reinforced in other studies, such as the ones by Kuang et al. and Li et al. [16,17]. However, while both studies also took advantage of polymeric nanofibers to simulate natural ECM to promote pulp formation and vascularization, different types of polymers were used to form the nanofibers; Kuang et al. used a novel polymer, star-shaped poly (L-lactic acid)-block-polylysine (SS-PLLA-b-PLYS), while Li et al. used a gelatin polymer [16,17]. In the study by Li et al., specifically, they were able to regenerate pulp-like tissue containing blood vessels, albeit only in the lower third (3-4 mm) of the human molar root, in an in vivo mice study over nine weeks [17]. Additionally, the start and maintenance of angiogenesis was also shown to be possible through another polymeric nanomaterial, bioactive glass 45S5, as shown in a study by El-Glandy et al.; however, their study observed vascularization in bone grafts instead of in dental pulp tissue regeneration [38].

These studies provided evidence for the promising use of polymer-based nanomaterials to facilitate pulp regeneration along with the revascularization of the soft tissue. The regeneration or stimulation of angiogenesis is crucial for the development of a functional and vital pulp, which can be induced using various polymer-based nanomaterials, as shown in these studies. However, future work should be done in the innervation of the pulp; to date, no studies have explored the innervation of nanomaterial-based regeneration of the dental pulp [35].

3.2.2. Root Canal Therapy

An alternative direction involving the use of nanomaterials in endodontics is to improve root canal therapy itself by improving the armamentarium, specifically the obturating material. The obturating material is the filling used in root canal therapy to replace the pulp tissue within the cementum of the injured tooth. The most commonly used obturating material to date is gutta-percha, as its

bioinert properties make it a good choice. While it is the most widely used material for root canal therapy, it has many mechanical and functional disadvantages, including its pliable, non-adhesive, and minimal antimicrobial property, and can easily be displaced in the presence of pressure [39]. Furthermore, the use of gutta-percha can lead to microleakage post-therapy, as its thermoplastic property can lead to shrinkage, creating gaps and microleakages [39,40]. Current studies look to overcome these disadvantages by exploring the use of nanomaterials. Because nanomaterials are composed of nanoparticle structures, there is an increase in contact surface area, which ultimately reduces gaps and microleakages, in addition to increasing the antimicrobial property and superficial dentin flexural strength of the obturating materials [34,39].

One material that can be used as such is bioactive glass 45S5. Bioactive glass 45S5, in addition to its nanoparticle feature, has a chemical composition that is similar to that of human bone and dentin, allowing it to bond to bone and making it an excellent choice as an alternative obturating material [40]. However, it should be noted that due to the chemical composition of bioactive glass 45S5—that is it is composed of calcium silicate—retreating protocols cannot be done for root canals that are obturated with only bioactive glass 45S5 [40]. However, this problem can be overcome by using bioactive glass 45S5 (or any other calcium silicate-based materials) in conjunction with a matrix polymer such as that of gutta-percha [40]. Marending et al. explored the use of bioactive glass and gutta-percha and showed the adherence of the obturating material to dentin without the use of a sealer as used in the conventional root canal therapy protocol [41]. In a more recent experiment by Alhashimi et al., they thoroughly explored the properties of bioactive glass in combination with gutta-percha as an obturating material compared to gutta-percha only [42]. Their study revealed that the former had enhanced thermal properties as it had lower melting points, which was optimal in the case of a retreatment procedure [42]. Alhashimi et al. provided further evidence for the promising use of bioactive glass to improve endodontic procedures by showing that the incorporation of bioactive glass to gutta-percha enhanced its antimicrobial properties due to its high alkalinity property and had excellent radiopacity results [42]. The use of nanomaterials in endodontics extends far beyond bioactive glass; other nanoparticles than can be used as a filling in conjunction with gutta-percha also include silver nanoparticles, chitosan nanoparticles, and zinc oxide nanoparticles [43].

3.3. Cartilage Tissue Regeneration (Temporomandibular Joint)

Up to 70% of temporomandibular joint disorder (TMD) cases are associated with displacement of the temporomandibular joint (TMJ) disc [44]. The difficulty in restoring the damaged TMJ disc lies in its limited intrinsic regenerative capacity. Current treatments of a severely damaged or displaced TMJ disc often results in a discectomy when the TMJ disc becomes too difficult to repair or to reposition [45,46]. Research in TMJ disc tissue engineering has explored methods based on scaffolds, cell sources, and biological/biomechanical stimuli [44,47]. Potential applications for TMJ disc regeneration include the incorporation of nanomaterial scaffolds (nanosurfaces, nanofibers, and nanocomposites) used for cartilage tissue engineering [48–50].

Nanosurface-type scaffolds have geometrically defined nanopatterns on their surfaces [50]. Balasundaram et al. modified polyurethane and PCL surfaces by casting these surfaces over a spiky titanium surface, which resulted in an increase of chondrocyte numbers, intracellular chondrocyte protein production, and chondrocyte collagen secretion [51]. Surface modifications were also performed by Park et al., who modified PLGA scaffolds with sodium hydroxide, resulting in an increase of chondrocyte numbers, and intracellular/extracellular protein contents [52].

Nanofibrous-type scaffolds consist of proteins such as collagen and elastin and can be created through electrospinning or thermally induced phase separation [53]. Erisken et al. found that a nanofibrous osteochondral scaffold could selectively differentiate human adipose-derived stromal cells resulting in the regeneration of osteochondral tissue [54]. In areas of increased insulin and β-glycerophosphate, they found that chondrogenic differentiation and mineralization increased, respectively [54]. Nanofibrous scaffolds were also made from poly(β-caprolactone)-block-

poly(L-lactide) copolymers and were found to increase collagen type II expression in chondrocytes [50]. Electrospun PLA nanofibers with surface carboxylate groups were created by Chen and Su and were found to enhance the viability, proliferation, and differentiation of rabbit articular chondrocytes [55]. Similarly, electrospun poly(ε-caprolactone) matrices with a nanofiber network also showed an increase in osteochondral regeneration [56].

Nanocomposite-based scaffolds have been shown to be useful in this field as well. Electrospun PLA with hydroxyapatite nanoparticles have been found to increase chondrogenic differentiation of mesenchymal stem cells, while gold nanoparticles incorporated into porcine collagen hydrogels increased the mechanical properties of the hydrogel and increased chondrocyte proliferation [50]. Similarly, PLGA/titanium dioxide composite scaffolds with modified surfaces were created by treatment with various concentrations of sodium hydroxide and resulted in an increase in osteoblast and chondrocyte adhesion [57].

Other studies, such as the one by Chahine et al., assessed the biocompatibility of single-wall carbon nanotubes for articular cartilage tissue engineering and found that these nanotubes promoted the expression of a chondrogenic ECM [58].

TMJ disc cartilage tissue engineering is a relatively new and underexplored field [48]. The use of nanomaterials in cartilage tissue engineering has improved the interaction between cells and scaffolds to allow for greater tissue growth, cell differentiation, adhesion, and synthesis of a cartilaginous ECM [50]. Many cartilage tissue engineering studies, however, focus on the articular cartilage of the knee joint, and the data supporting TMJ disc regeneration is still lacking [7]. As research in this field further develops, it is expected that research involving the use of nanomaterials will play a greater role in addressing TMJ cartilage tissue engineering through engineered TMJ cartilage constructs that functionally resemble its natural cartilage tissue counterpart.

3.4. Bone Regeneration for Dental Implants and Mandibular Defects

With the wide use of dental implants as an option to replace missing teeth, bone regeneration is becoming an integral part of oral rehabilitation. Prior to the placement of a dental implant, an adequate amount of alveolar bone is essential. Similarly, segmental bone defects in the oral maxillofacial region—a condition that arises through trauma, infection, tumor resection or osteoradionecrosis—can also be addressed, given a sufficient amount of alveolar process. To facilitate such repairs, autogenous bone grafts (autografts) have been the gold standard in bone regeneration [59]. While serving as a scaffold for bone formation, autografts also contain bone-forming cells and bioactive substances that induce new bone formation. However, several disadvantages are associated with autograft use, including endogenous donor site morbidity and limited availability. A potential need for general anesthesia motivates clinicians and researchers to develop substitute bone graft materials that have excellent bioactivity and osteoconductivity [60].

The success of new bone regeneration can be greatly improved when the osteoconductive scaffold in use contains macropores (>200 μm). Such a structure allows for adequate flow and distribution of biofactors such as oxygen and nutrients, rapid vascularization, osteoblast proliferation and differentiation, bone remodeling, and osseointegration throughout the healing process [61,62]. New bone deposition can be furthered by enhancing these scaffolds with bactericidal properties, which ultimately control infection post-treatment [62]. This section will explore the feasibility of using various nanomaterials in dental implants and mandibular bone defects, such as nHA composite scaffolds, nanomodified mineral trioxide aggregate, and graphene.

Previously, a multitude of synthetic bone graft substitutes have been developed to function as scaffolds, including hydroxyapatite (HA), biphasic calcium phosphate ceramics, and tricalcium phosphate. The field of nanotechnology can be applied to scaffolds such as HA to create nHA, which was shown to enhance bone regeneration and to accelerate healing after sinus augmentation [60]. In fact, nHA displayed similar effects in bone regeneration compared to freeze dried bone allografts in a rabbit model [63]. In addition, nHA used in the augmentation of extraction sockets was

shown to reduce the degree of gingival invaginations [64]. While nHA was promising for bone regeneration, there were some drawbacks, such as its brittleness and low mechanical properties. These limitations consequently prevented nHA from bearing larger loads or forces. However, using nHA in combination with other high molecular weight polymers could improve its mechanical strength as well as its biocompatibility. nHA was combined with other materials, including collagen, PLA, PLGA, polyamide, coralline, chitosan, and PCL [7]. For example, a nanostructured carbonate hydroxyapatite/calcium alginate (CHA) microsphere was developed by substituting the phosphate group (PO_4) with a carbonate group (CO_3). The microspheres produced at low temperatures showed lower crystallinity and higher solubility, which favored an earlier resorption time and improved bone regeneration [65,66]. When combined with collagen, nHA/collagen composite scaffolds aimed to mimic the architecture of native bone. In a study by Wang et al., they showed that as the ratio of collagen to nHA increased, the mechanical property of the scaffold decreased but could increase the rate of bone tissue formation [67]. Another group, Wang et al., studied the effects of nHA/collagen/PLA composite scaffolds with human alveolar BMSCs and determined that they could be used to successfully correct mandibular bone defects, while Zhou et al. improved the osteogenesis activity of nHA/recombinant human-like collagen/PLA scaffolds with the addition of a polydopamine-assisted BMP-2-derived peptide [59,68].

An alternative to nHA for bone regeneration, with a well-established history in endodontics, is the use of mineral trioxide aggregate (MTA)—a silicate-based inorganic material containing tricalcium silicate, dicalcium silicate, calcium sulfate dihydrate, and bismuth oxide [69,70]. While many scaffolds are osteoconductive (used as the framework for bone growth on the surface), the unique composition of MTA enables this material to also possess osteoinductive properties (the recruitment and stimulation of immature cells into preosteoblasts). It is hypothesized that MTA functions through an increase in pH, and in calcium and hydroxide ions, which induce mineralization [69]. Despite its history of use, some limitations, such as a long settling time, weak handling properties, and discoloration over time, remain, though they may be overcome through the incorporation of nanostructures [69]. Nanoparticles have an increased surface area and can thus release more calcium and hydroxide ions and set more quickly. Nanomodified MTA (nMTA) is a novel concept explored by Saghiri et al. nMTA contains additional compounds, such as gypsum, strontium carbonate, zeolite, and di-sodium hydrogen phosphates of sizes 40 to 100 nm [69]. In an in vivo white rabbit study, they showed that nMTA was superior in comparison to the standard MTA in various aspects, including reduced inflammatory reaction, reduced immunogenicity, increased calcium and hydroxide ion release, and increased bone regeneration [69]. Additionally, in a prior study, they also showed that nMTA set significantly more quickly than MTA as well as having a greater push-out bond strength—that is, the force required to displace the material post-setting [71]. While these studies show evidence for a promising alternative to nHA for bone regeneration, further studies are needed to reinforce the evidence found by Saghiri et al., as they are the only group exploring this material to date.

Recent studies used graphene to modify the surface of dental implants, facilitate the development of platforms for therapeutics delivery, and to generate synthetic membranes used in bone regeneration. Graphene is a carbon-based nanomaterial consisting of a single layer of carbon atoms arranged in hexagonal lattices, thus providing exceptional mechanical strength, thermal stability, and electrical conductivity. Graphene is a versatile nanomaterial that can be incorporated into different scaffolds, used to develop nanocomposites, and functionalized with a variety of bioactive molecules [19]. In fact, implant surfaces coated with graphene can be functionalized with antibacterial substances that may help to prevent implant-associated infections [19]. Enrichment of collagen membranes with graphene provided the membrane with advantages such as higher stiffness, lower deformability, increased roughness, and decreased hydration [19].

4. Current State of Nanomaterials in Craniofacial Tissue Regeneration

4.1. Challenges in Clinical Translation

While it is evident that nanomaterials serve a promising purpose in craniofacial tissue regeneration, there are challenges that need to be addressed to further the integration, application, and translation of nanomaterial use in clinical settings. One challenge is optimizing cell viability. Nanomaterial-based hydrogels can support cell growth during implantation for bone regeneration; however, these hydrogels tend to have low mechanical strength. Various nanopolymers can be used to strengthen the hydrogel but can compromise the biofabrication and fabrication time, thus reducing cell viability [72]. To improve cell viability, novel scaffold development techniques have been suggested, such as solution electrospinning and 3D printing techniques, though these techniques greatly limit the manufacturing and production of these scaffolds. Regardless, to date, these techniques have yet to be applied to nanomaterial-based scaffolds for craniofacial tissue engineering [72]. Additionally, immunogenic properties must be considered as well: Although one material may have a superior structural resemblance to native tissue and/or have a superior cell viability, it may be more likely to elicit a severe immune response in certain individuals [72]. Another challenge, as previously mentioned, is the innervation of the tissues engineered via nanomaterials, such as dental pulp, which has not been studied to our knowledge. In general, although the results are promising, further groundwork (such as a better understanding of the biomechanisms and the impact of nanoscale spacing and orientation) is required before the application of nanomaterials in a clinical setting can be considered [73].

4.2. Long-Term Toxicity and Side Effects

Although nanomaterials have useful applications for craniofacial tissue regeneration, exposure to these materials can cause harmful effects. The small size of the nanoparticles, and their nano-intrinsic properties, allow them to interact with targets normally unreachable by their larger counterparts [74]. More specifically, nanoparticle toxicity depends on the particle size, shape, surface charge, composition, stability, and its ability to affect normal physiology through means such as oxidative stress and proinflammatory gene activation [75]. Nanoparticles that enter through the gastrointestinal route, for instance, can be endocytosed by intestinal epithelial cells, remain in the submucosa, or enter the bloodstream and accumulate in the liver and spleen [74,76]. Silver nanoparticles, which can act as a developmental neurotoxin in zebrafish, have been shown to distribute systemically and accumulate in various tissues following gastrointestinal absorption [75,77]. Accumulation of nanoparticles in the liver can result in hepatocellular injury through mechanisms such as cytochrome P450 activation and protein synthesis inhibition, while accumulation in the spleen can affect immunopathology [76]. A more detailed summary of nanoparticle toxicity can be found in Reference [75]. A common method of determining nanomaterial toxicity is through dose–response correlations combined with in vitro assays [78]. Unfortunately, as stated by Bergin and Witzmann, the estimated toxicity dosages do not always translate well into in vivo scenarios and new methods of assessing toxicity for in vivo applications are needed [78]. Therefore, more research is required to further understand the potential long-term toxicity and side effects associated with nanomaterials.

5. Conclusions

The development of nanotechnology in craniofacial tissue engineering is an exciting and innovative field. It offers greater versatility in scaffold design for promoting cell adhesion, proliferation, and differentiation. Many studies have shown the potential of implementing nanomaterials in bone, cartilage, dental pulp, and periodontal tissue regeneration. Despite a need for further understanding of its theoretical and practical aspects, such as the toxicity and the metabolic in vivo effects of nanomaterials in craniofacial tissue engineering, it is hoped that this field will benefit from future technological advances to successfully aid in the treatment of craniofacial defects.

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