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LITERATURE REVIEW

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Nanostructured tubular materials and their composites: a review of applications in tissue repair and dentistry

Materiais tubulares nanoestruturados e seus compósitos: uma revisão das aplicações em reparo tecidual e odontologia

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ABSTRACT

Biomaterials have been explored as promising tools in restorative medicine and dentistry because of their versatility and unique properties, such as biocompatibility and high surface area. Nanotubes (NTs) are a type of material that displays intriguing features for biomedical applications, including biocompatibility, excellent mechanical and chemical stability, antimicrobial activity, and distinctive physico-chemical characteristics. The properties of NTs vary depending on the chemical elements in their structure, such as carbon, boron, or titanium. This review discusses the synthesis, characterization, and *in vitro* and *in vivo* testing of NTs to assess their biological performance. Although NTs show significant potential for many biological uses, challenges remain in their purification and establishing biological safety for implanted biomedical materials. Additionally, the scarcity of *in vivo* studies slows down their clinical application. This review highlights the latest advances in NTs for these purposes and emphasizes how this innovative material can enhance restorative medicine and dentistry.

KEYWORDS

Biological applications; Biomaterials; Dentistry; Nanotubes; Tissue repair.

RESUMO

Os biomateriais têm sido amplamente explorados como ferramentas promissoras na medicina restauradora e na odontologia devido à sua versatilidade e propriedades únicas, como biocompatibilidade e maior área de superfície. Os nanotubos (NTs) fazem parte de um tipo de material que apresenta características notáveis para aplicações biomédicas, incluindo biocompatibilidade, excelente estabilidade mecânica e química, atividade antimicrobiana e propriedades físico-químicas distintas. As propriedades dos NTs variam de acordo com os elementos químicos presentes em sua estrutura, como carbono, boro ou titânio. Esta revisão aborda a síntese, caracterização e os estudos ***in vitro*** e ***in vivo*** realizados para avaliar o desempenho biológico dos NTs. Embora apresentem grande potencial para diversas aplicações biológicas, ainda existem desafios relacionados à sua purificação e comprovação da segurança biológica para aplicação como materiais biomédicos implantáveis. Além disso, a escassez de estudos ***in vivo*** tem limitado sua aplicação clínica. Esta revisão destaca os avanços mais recentes no uso de NTs com essas finalidades e enfatiza como esse material inovador pode impulsionar o desenvolvimento da medicina e da odontologia restauradora.

PALAVRAS-CHAVE

Aplicações biológicas; Biomateriais; Odontologia; Nanotubos; Reparo tecidual.

INTRODUCTION

Biomaterials have been widely studied as a promising alternative in restorative medicine. In this sense, these materials are designed to provide better conditions for restoring the damaged tissue's structures and functions [1]. Natural or synthetic polymers, ceramics, metals, and nanocomposites are the most commonly used materials. The interaction between biomaterials and the biological environment is categorized into two types: bioactive and bioinert. The first concept encompasses biomaterials that can positively alter biological behavior when in contact with this environment, such as stimulating the surrounding tissue to regenerate [2,3]. In contrast, bioinert materials have minimal interaction when in contact with living tissues, but may facilitate the rehabilitation of specific biological functions. Some examples of bioinert materials are silicone rubber and metallic prostheses or implants [4,5].

In dentistry, these biomaterials are commonly used during many procedures. Due to their applications in the oral environment, they must have desirable properties such as biocompatibility, chemical and mechanical resistance, and antimicrobial activity [6]. Once different materials have different properties that are generally interesting for many applications, the development of composites has increased [7-10]. Thus, some studies have combined the properties of these materials to overcome some of the limitations of biomaterials. This way, they can improve biomaterial's performance and provide better conditions for tissue repair, thereby enhancing the available therapeutic tools in medicine and dentistry.

Nanomaterials are among the most extensively studied alternatives for use as biomaterials and for enhancing existing biomaterials [11,12]. These nanomaterials are generally incorporated into matrices or carriers chosen according to their properties and the final applications. Nanotubes (NTs) are a promising group of nanomaterials in this scenario. NTs are part of one-dimensional (1D) materials, i.e., with one dimension between 1 and 100 nm [13]. These materials are interesting due to their unique properties, including biocompatibility, excellent mechanical behavior, antimicrobial activity, and intriguing physicochemical properties [14]. Figure 1 presents the most studied NTs and their most relevant biological applications.

In this sense, this review will address the recent outcomes from the materials science community concerning the most relevant nanotubes (NTs)

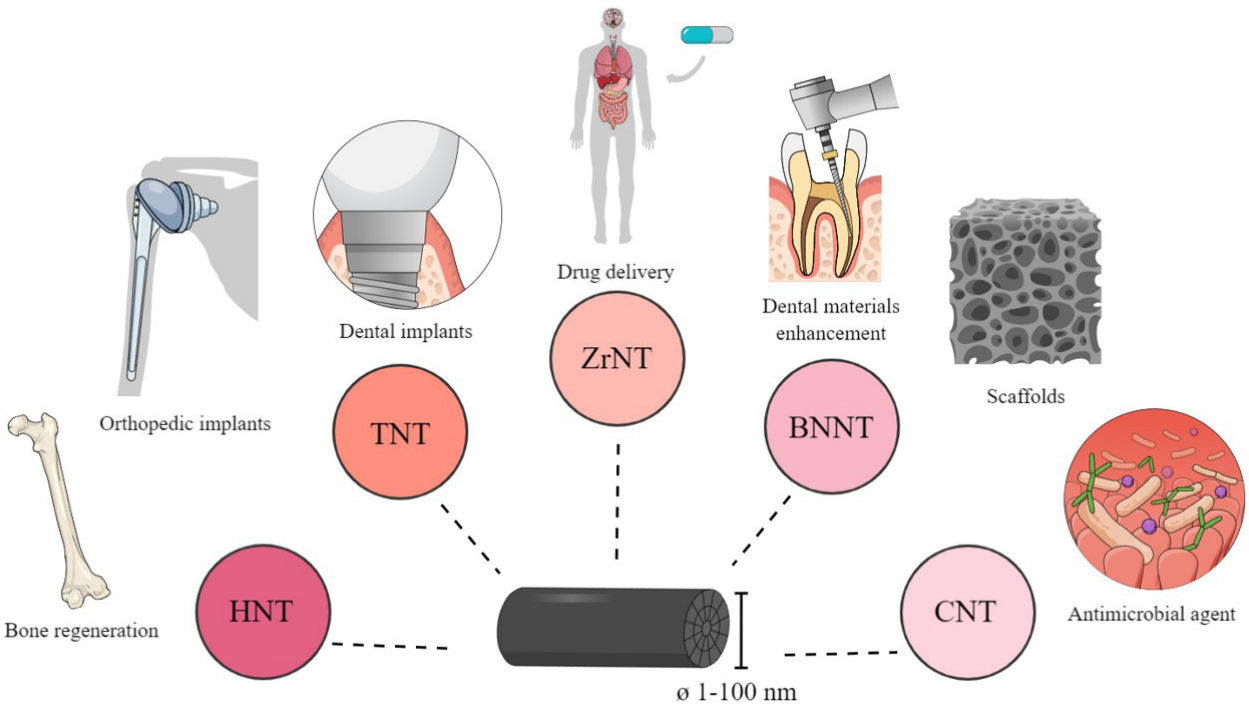

Figure 1 - The most studied nanotubes and their biological applications: halloysite nanotube (HNT), titanium dioxide nanotube (TNT), zirconium oxide nanotube (ZrNT), boron nitride nanotube (BNNT), and carbon nanotube (CNT). Original illustration by the authors. Created in BioRender [15] by Silva, D.M. (2025).

designed for biological applications. Specifically, the following topics address the properties, relevance, potential, and limitations of the NTs applications in tissue repair and dentistry. The selection of the NTs presented in this review (halloysite nanotube (HNT), titanium dioxide nanotube (TNT), zirconium oxide nanotube (ZrNT), boron nitride nanotube (BNNT), and carbon nanotube (CNT)) was strategically based on their prevalence and the number of recent publications related to their applications in biomedical and dental fields. In this case, structural representativity and chemistry are well represented by these NTs. They encompass ceramic materials, semiconductors, and carbon-based structures. Furthermore, the text is organized by each NT studied, followed by some perspectives and conclusions. The properties and applications of the NTs are presented, establishing promising and diverse alternatives for their use as biomaterials with great potential for tissue repair.

HALLOYSITE NANOTUBES

Halloysite nanotubes (HNTs) are clay minerals of the kaolin group [16,17]. The crystalline structure and the morphology of HNTs ($Al_2Si_2O_5(OH)_4 \cdot nH_2O$) are represented in Figure 2. Besides the tubular form, halloysites may also be presented as spheroidal or platy-like structures. These different morphologies have been related to their geological occurrence, crystallization conditions, and extraction site [16-18]. Tubular structures of halloysite, as HNTs, are the most common in nature [16]. Their multilayer appearance originated from rolling individual layers, mainly due to the dynamic balance of intrinsic crystallographic or structural forces [19]. The tubes are generally 0.2 to 1.5 μm in length. The internal diameter ranges between 10 and 30 nm, and the external diameter ranges from 40 to 70 nm [17].

HNTs present excellent mechanical properties and good biocompatibility [18]. They exhibit controllable surface reactivity, featuring a negatively charged external surface that binds to positive particles and a positively charged internal side that can react with negatively charged molecules [17,18]. Other relevant properties are the structural stability and the phase transformation of halloysite under thermal or acid/alkaline treatments. HNTs are resistant to high temperatures, which is particularly interesting for specific techniques, such as their application in ceramics [20]. They also exhibit macro porosity, which enables the encapsulation of substances in the lumen of the tubes, allowing for their subsequent release into the surrounding environment [18]. For all these reasons, in addition to their use in ceramics, HNTs have also been functionalized as fillers to reinforce polymeric matrices and as drug carriers to deliver biologically active species [17].

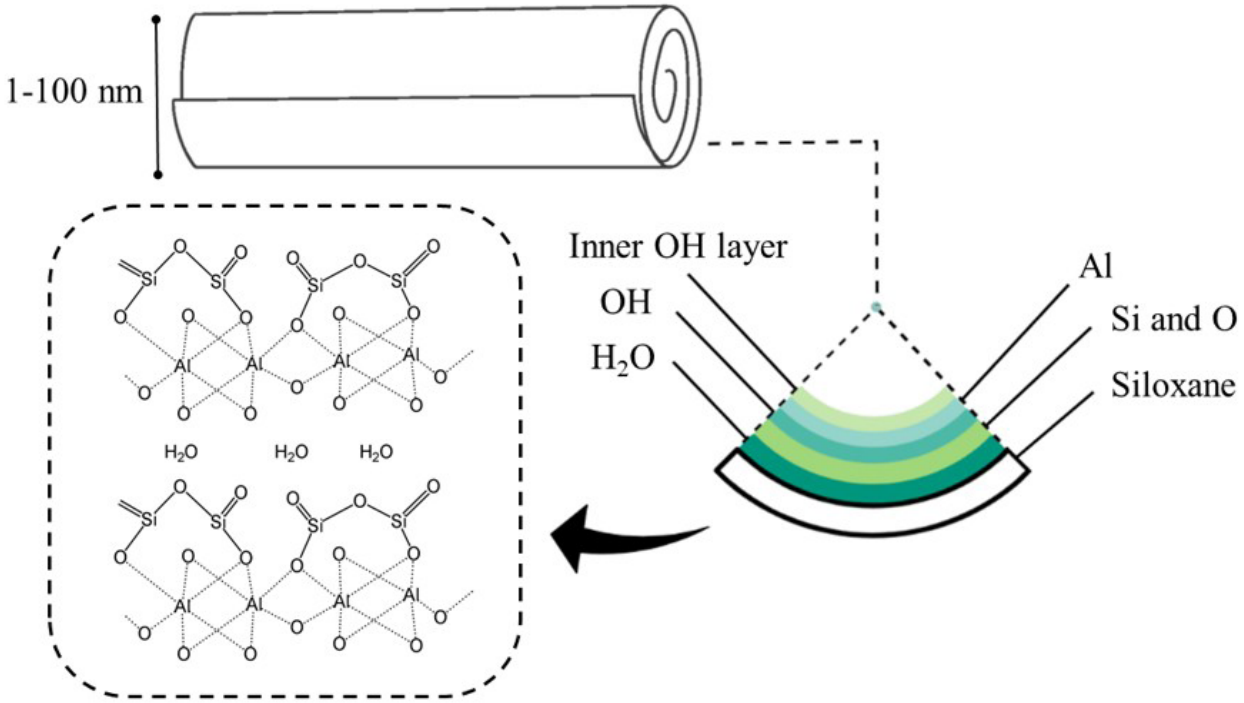

Figure 2 - The general HNT particle chemical structure with the attribution of each layer's primary composition. Original illustration by the authors.

In dentistry, HNTs have already been investigated as drug carriers for use in endodontic sealers [21]. For this purpose, they were doped with the antimicrobial agent alkyl trimethyl ammonium bromide (ATAB). Three different ratios of the materials were mixed with resin-based materials, and the proportions of ATAB/HNTs consisting of 1:1, 1:2, and 2:1 were analyzed. Control samples were formed only with resin-based materials. It was possible to see brighter circles of ATAB in the lumen of the nanotubes. The findings indicated that the chemo-mechanical properties were appropriate [21]. Significant changes were observed immediately in the degree of conversion only in the group with the same proportions of ATAB and HNTs, which may indicate that the other groups probably presented delayed polymerization. However, the polymerization process could continue, as all the groups reached similar and reliable values after 24 hours. Additionally, although the control group had presented the highest initial Knoop hardness, no difference between the groups was observed after solvent immersion, and all materials softened after solvent storage [21].

The radiopacity, which is crucial for dental materials, has not changed significantly with the addition of ATAB and HNTs in the resins. The flow of all the groups ranged from 18.26 to 22.02, but these values were considered suitable to allow the materials to reach the apical foramen and tight spaces, such as accessory canals. Antibacterial activity was observed against *E. faecalis* in direct contact inhibition of planktonic cells [21]. In both cases, the group with the highest ATAB concentration was more effective, as expected, given that ATAB exhibits antibacterial properties. Regarding biocompatibility, no group was cytotoxic to human pulpal cells [21]. Thus, the association of HNTs and ATAB in resin-based materials appears to be a promising alternative in root canal treatment, as HNTs may function as a drug delivery system for antimicrobial substances without compromising the sealer's chemo-mechanical properties and biocompatibility [21].

The functionalization of HNTs with 3-aminopropyltriethoxysilane (APTES), an organosilane, was recently performed in a resin-based dental material to enhance the loading capacity and extend the drug release time of the nanotubes, which would be particularly beneficial for populations at high risk of dental caries [22]. For this purpose, the resin-based material was synthesized, and optimal concentrations of functionalized nanotubes were determined with 10, 15, and 20 wt.% of HNTs and 0, 2, and 4 vol% of APTES. Reduced flexural strength, but a higher flexural modulus was observed in the group with 20 wt.% HNT–4%APTES, while the groups with 15 wt.% and 20 wt.% HNT with or without APTES presented increased values of ultimate tensile strength. Considering these findings, the group 15wt.% HNT–4%APTES was selected for subsequent biological tests [22].

The nanotubes, both with and without silanization, were encapsulated with 10% chlorhexidine (CHX) to assess their antimicrobial efficacy and drug release properties. Different combinations were evaluated, including pure resin, CHX, HNTs with and without silane, and HNTs with and without CHX [22]. The inhibition against *Streptococcus mutans* was analyzed, and it was observed that the control group treated only with 0.2 vol.% CHX had a higher inhibition zone than the other groups. However, the sample HNTsil-CHX also exhibited antimicrobial efficacy, which differed from the others (without silane or CHX) and did not result in an inhibition zone [22]. The highest inhibition zone of HNTsil-CHX was observed on day 1, likely due to the initial release of CHX, which had a higher concentration on the first day but decreased over time. The findings of this study suggest that silanizing HNT with CHX may enhance the antimicrobial properties of resin-based dental materials without compromising their mechanical properties. However, this process did not sustain the release of CHX over time [22].

HNTs have already been associated with the antimicrobial agent chitosan (CS) to develop multipurpose restorative dental composites. An approach to enhance the dispersion of HNTs using a sonication-supported CS integration technique was employed to prevent the tendency of HNTs to agglomerate [23]. Additionally, modified short S-glass fibers (5 wt.%) and strontium-doped glass particles (45 wt.%) were added to CS-HNTs-reinforced composites to enhance the material's mechanical properties. The findings showed that the length of HNTs ranged from 0.4 μm to 2.8 μm. In addition, the outer diameter varied between 40 and 200 nm. The agglomerates were reduced to a size of 100 to 30 μm after high-powered sonication for 90 minutes, and some of them separated into individual nanotubes [23]. The HNTs covered with low-molecular-weight CS

achieved total dispersion. The group with more chitosan-integrated HNTs (2 wt.% CS-HNTs) presented increased flexural strength and modulus compared to pure resin. These levels have increased even further with the addition of 45 wt.% glass particles and 5 wt.% S-glass fibers, as in this group, the flexural strength, flexural modulus, and breaking energy were enhanced (up to 8.1%, 17.2%, and 9.8%, respectively) [23].

In addition to improving the nanocomposite's mechanical properties, CS-HNTs in the samples induced good biological responses. The number of *S. mutans* was reduced with the increase of CS-HNTs; moreover, group 2 wt.CS-HNTs showed the lowest bacterial viability, indicating that they were the most effective antimicrobial concentration of this nanocomposite[23]. It was suggested that the increased dispersion state of CS-HNTs achieved in this study could have enhanced the antibacterial activity and the interaction of protonated amine ($-NH_3^+$) groups of CS with the negatively charged surface membranes of S. mutans, which may lead to metabolic dysfunction. Thus, the association of chitosan-integrated halloysite nanotubes and S-glass fibers seems promising for dental composites [23].

Besides their applicability in dental nanocomposites, HNTs have also been shown to be an alternative for bone regeneration. The encapsulation of HNTs into hydrogels was previously performed to evaluate the mechanical properties and biological responses *in vitro* and *in vivo* [24]. The samples were fabricated using the photopolymerization method with gelatin methacrylate (GelMA) and hydroxyapatite nanotubes (HNTs). The characterization of the materials revealed that all the HNTs/GelMA hydrogels exhibited a porous microstructure with a mean pore diameter of 100 μm, which was considered suitable for promoting cell migration, adhesion, growth, and proliferation. They observed that the enhancement of HNTs increased the number of clumps attached to the crosslinked network of GelMA [24]. On the other hand, adding HNTs did not meaningfully influence the hygroscopicity. A similar trend was observed in the degradation rate of the hydrogels, although the scaffolds composed of HNTs/GelMA degraded slightly more slowly than those composed only of GelMA. Regarding the mechanical properties, the compressive modulus increased with the enhancement of the HNTs in the samples [24]. The confocal microscopy and SEM showed that dental pulp stem cells (hDPSCs) adhered and spread appropriately on the surface of all tested samples (GelMA hydrogels and HNTs/GelMA with 3%, 5%, 7%, and 10% of hydrogels). Cell viability was measured in the presence of the hydrogels, and no difference was observed between the groups after 24 hours. However, on the fourth day, the samples of HNTs/GelMA with concentrations of 3%, 5%, and 7% exhibited a higher proliferation rate than the scaffolds in the control group, the GelMA hydrogel, and HNTs/GelMA with a concentration of 10% [24]. Moreover, this last sample presented fewer cells than the other groups after 7 days. These results suggest a good and dose-dependent potential of HNTs/GelMA hydrogels as scaffolds for bone regeneration. An alkaline phosphatase assay (ALP) evaluated the induction of osteogenic differentiation, demonstrating higher levels in the group with 7% HNTs/GelMA after 7 days. Additionally, the analysis of gene expression and protein production of the osteogenic markers Col-1, BMP, RUNX2, and BSP indicated again that the samples with 7% HNTs/GelMA presented the best effect on osteogenic differentiation [24]. Posteriorly, the bone formation in these scaffolds was analyzed *in vivo* in rat calvarial defects. The histological evaluation confirmed that the association of HNTs and GelMA hydrogels could induce bone formation, with newly formed bone observed after 2 months and a continuous bone structure after 3 months of placement. Moreover, micro-CT images revealed that HNTs/GelMA with concentrations of 5% and 7% induced the highest mineral density, bone volume, and trabecular thickness, corroborating the in vitro findings and providing a promising perspective for clinical trials [24].

Another strategy evaluated for bone regeneration, including HNTs and hydrogels, is immobilizing ALP into these nanotubes [25,26]. Considering the biomineralization role of ALP, the complex HNTs/ALP was tested in CS-based hydrogel scaffolds in the presence and absence of collagen (COL) [26]. For this purpose, different associations of the materials were evaluated. The swelling of the groups' CS/HNTs and CS/HNTs/ALP did not differ statistically; however, samples with 30% HNTs had a slightly higher value and were selected for further testing. The optimal COL/CS ratio (20/80) was also selected based on observations of gelling time. The association

of COL and HNTs in CS samples significantly increased the swelling ratio, particularly in the presence of ALP [26]. SEM analysis demonstrated proper scaffold morphology with high porosity. The scaffold pores of the group CS/HNTs/COL were slightly more prominent, with elongated shapes, which were probably associated with the fibrous collagen appearance. ALP could be seen entrapped in the nanotubes. The addition of COL was positive, as it enhanced the porosity of the samples from 52.55% to 89.59%, a desirable property for bone regeneration. ALP-modified hydrogels with and without COL exhibited similar contact angle values, indicating high hydrophobicity —a property essential for cell adhesion and proliferation [26]. The analysis of mechanical properties showed that the storage modulus values of the groups with COL were the lowest, probably due to their higher porosity. On the other hand, adding HNTs to CS hydrogels slightly raised the storage modulus, demonstrating that the nanotubes did not compromise the mechanical properties. The degradation tests showed that less than 50% of the hydrogels degraded after one month. This slow degradation rate is advantageous because it allows the regenerated tissue to replace the scaffold gradually [26].

A biomineralization study demonstrated the ability of all samples to promote the formation of mineralized crystals in the presence of calcium and phosphorus. As expected, after biomineralization, the storage modulus of the group CS/HNTs/COL/ALP was higher compared to CS/HNTs/ALP, indicating the strengthening of the first hydrogels, likely due to accelerated mineralization in scaffolds with a higher collagen content. For the biological evaluation, the proliferation of osteoblast-like cells (MG-63) was measured after contact with each hydrogel for 24 and 72 h. Samples with 10% or 30% of HAL/ALP were tested. Half of these scaffolds were incubated for 7 days in calcium glycerophosphate solution to promote biomineralization. The cells could proliferate in all the hydrogels, and the 10% HNTs/ALP concentration showed the best results in the previously mineralized scaffolds [26]. It was suggested that, although more osteoblasts were present on non-mineralized scaffolds with 30% HNTs/ALP, the increased mineral formation on these scaffolds after biomineralization could lead to reduced porosity of the hydrogel, thereby decreasing cell adhesion and subsequently proliferation. The previous mineralization of the samples with 10% HNTs/ALP induced higher cell proliferation on COL content scaffolds. The SEM images also confirmed that MG-63 cells grew better on these samples, where the cells were well spread and interconnected, in contrast to adhesion on pure CS, which showed sparse and less connected cells. *In vivo* studies are needed to confirm that these hydrogels reinforced with HNTs/ALP/COL may promote bone regeneration [26].

Based on these findings, HNTs are a type of nanotube with promising features for application in dentistry. They have shown applicability in dental composites and scaffolds for bone regeneration. Considering their natural occurrence, HNTs tend to present a low cost compared to other nanotubes, which may increase their indication [17].

TITANIUM DIOXIDE NANOTUBES

In the biomedical field, particularly in dental and orthopedic applications, implants based on medical-grade alloys (e.g., titanium) are frequently used due to their excellent biocompatibility [27-30]. However, these materials lack antibacterial activity, which can lead to microbial infections associated with slow osteogenesis, potentially resulting in implant failure. Hence, to increase the implant's antibacterial activity and osteogenic properties, nanotubular TiO_2, i.e., TiO_2 nanotubes/titania nanotubes (TNT), have attracted particular attention owing to their enhanced osteo-differentiation [27] and antibacterial characteristics [31]. This section discusses the applications of TNT in bone and dental fields, as well as their modifications to achieve desirable characteristics, including osteogenesis and antibacterial activity.

TNT are widely used in dental implants due to their excellent biocompatibility; however, their surface is susceptible to bacterial attachment, which can be prevented by treatments such as depositing nanosized antibiotic-loaded particles (tetracycline, TC) encapsulated in poly(lactic-co-glycolic acid) (PLGA) [32]. After TNT synthesis through Ti disc electrochemical anodization, TC loading into TNT was achieved by electrospray deposition (ESD), using 0.1 wt.% TC and 0.1 wt.% PLGA dispersed in dichloromethane. Then, the assessments of antibacterial activity, cytotoxicity, osteogenic gene expression/RT-PCR, and extracellular matrix mineralization were conducted in control groups MA (Ti discs),

T0 (TNT without treatment), and TNT samples subjected to 2, 4, 8, 16, 30, and 60 minutes of the ESD treatment, to assess their suitability as a biomaterial [32]. As mentioned by the authors, the nanotubular surface is susceptible to bacterial attachment; therefore, sample T0 presented a higher number of live bacteria than the MA sample in the antibacterial test using *S. aureus*. Nevertheless, after ESD, the number of live bacteria was reduced in samples treated for 2 and 4 minutes owing to the TC antibacterial property. Moreover, no viable bacteria were isolated in groups treated for at least 8 minutes. However, a TC overdose is toxic to cells; thus, the cell viability was assessed. There was no change in cell distribution and viability in samples treated with ESD for 2, 4, and 8 minutes compared to the control sample (T0) [32]. However, thinner and dead cells were noted in samples sprayed with TC for up to 16 minutes. The control groups and the 8-minute TC-treated sample (T8) had their bioactivity evaluated through RT-PCR and gene expression of OCN and OPN (osteogenesis markers). The RT-PCR analysis revealed an increase in cell density of osteogenic differentiation by osteoblast cells in samples with a tubular structure, specifically in the T0 and T8 samples [32]. Additionally, there was an upregulation of OCN in the T0 and T8 groups, indicating induced differentiation into osteoblasts. Moreover, the OPN expression exhibited the same pattern as the cells in T0 and T8, confirming that the TNT applied tension to the cells through stretching, initiating osteogenic signaling. Finally, the extracellular matrix mineralization, as measured by ARS staining, showed an increased calcium content in the tubular samples, with a similar calcium crystallization rate in groups T0 and T8. Hence, this study indicates that the TC treatment did not affect the TNT's osseointegration ability, with the nanotubes now exhibiting antibacterial activity due to antibiotic-controlled release by the PLGA matrix [32].

In dental applications, TiO_2 nanotubes are used as reinforcement in resins for improving the matrix physicochemical and biological properties; still, the nanotubes' lack of antibacterial activity requires another component to enhance the nanocomposite durability, which a triazinemethacrylate monomer (TAT) functionalization could provide due to this positively charged monomer's antibacterial property and organic solvent stability [33]. In this sense, TiO_2 nanotubes synthesized by the hydrothermal method were mixed with TAT and incorporated into an adhesive resin (66.66 wt.% bisphenol A glycolate methacrylate and 33.33 wt.% 2-hydroxyethyl methacrylate). The nanocomposite was subjected to mechanical testing, antibacterial activity study, and cytotoxicity evaluation. In the antibacterial activity analysis using *S. mutans*, samples modified with TAT exhibited lower antibiofilm effect, particularly in groups reinforced with 5 wt.% TiO_2/TAT (3.64 log CFU/mL) and 2.5 wt.% TiO_2/TAT (4.10 log CFU/mL) [33]. As the antibacterial properties of TNT are not fully understood, the control group (resin/TiO_2 nanocomposite) was also tested and did not exhibit potential antibacterial activity. Hence, the improved antibacterial property after nanotube modification with TAT resulted in reduced bacterial viability, likely due to the electrostatic interaction between the bacteria and TAT that disrupted the bacterial membrane [33]. Moreover, the cytotoxicity of the nanocomposite was evaluated using a colorimetric assay and SRB human pulp cells, which displayed increased cell viability (up to 70%) and no signs of cytotoxicity, indicating that this reinforced adhesive is suitable for biological use. Furthermore, the mechanical properties of biomaterials must be reliable, especially in areas of restored teeth. The immediate micro tensile bond strength, which evaluates the adhesive bond strength (a fundamental property for dental adhesives), exhibited a slightly lower value for the 2.5 wt.% TiO_2/TAT nanocomposite compared to the control group. Nonetheless, for higher TiO_2/TAT concentrations, a statistical difference was not observed between the control groups—the 2.5 wt.% TiO_2/TAT-reinforced sample showed the best long-term bond strength after 1 year of storage in distilled water, exhibiting improved mechanical performance due to TNT and their modification with TAT, suggesting that this is a promising adhesive with antibacterial activity for dental applications[33].

Titanium alloys are commonly used in bone regeneration and joint replacement. Nevertheless, bacterial adhesion and proliferation on the prosthesis surface are also frequent. Coating TNT with antibacterial polymers such as polyhexamethylene guanidine (PHMG) can prevent the material's antibacterial activity [34]. TNT prepared via electrochemical anodization of a pure titanium (cp-Ti) foil or rod were used to form composites with a 25% PHMG aqueous solution. Antibacterial activity against *S. aureus*

was tested, showing that the PHMG-TNT sample had lower bacterial adhesion after 4 h in culture compared to the cp-Ti and TNT groups. However, the viable bacteria in the samples were attributed to the high fluorine content in the anodized TNT and the remaining inorganic residue that increases bacterial adhesion. In addition to the in vitro study, bone implantation in rabbits was employed to assess implant infection. Blood agar plate cultures suggested that the implanted PHMG-TNT animals did not exhibit signs of *S. aureus* infection, compared to 100% of the rabbits injected with cp-Ti bacterial infection and 87.5% of the subjects implanted with TNT [34]. The cause of disease was bacterial adhesion on the material surface, which was lower in the PHMG-TNT groups due to the polymer-enhanced antibacterial activity. Regarding histopathological analysis, the control groups (cp-Ti and TNT) induced fibrogenesis, multinucleated osteoclasts, and apparent osteonecrosis in the tissue. Nonetheless, the PHMG-TNT nanocomposite exhibited a higher differentiation level than in the control groups and a lower degree of pathological changes, with more cells and denser reticular connective tissues [34]. Moreover, gene expression of osteogenic genes, as determined by qRT-PCR, indicated that the studied genes were upregulated in the PHMG-TNT sample. Thus, the results suggest that this material is suitable for bone implants, exhibiting great antibacterial activity and promoting bone regeneration and osseointegration.

Surface modification of titania with natural biopolymers and TNT has attracted attention because it promotes faster bone healing and osseointegration in orthopedic implants. Fabricating TNT by anodization is a well-established technique that can be improved through nanotube surface modification, such as with polyelectrolyte multilayers based on Tanfloc (TA, a tannin derivative) and glycosaminoglycans (heparin, HP; and hyaluronic acid) for enhanced osteogenic differentiation [35]. The TNTs were fabricated by anodization on titanium sheets and treated with TA, HP, and hyaluronic acid deposition. Cell adhesion, proliferation, and differentiation were conducted using human adipose-derived stem cells (ADSCs) isolated from adipose tissue. This cell type was chosen because it can differentiate into various cell lineages, such as osteoblasts. The *in vitro* study showed higher cell viability in the sample TNT-TA-HP, indicating superior metabolic activity to the TNT-TA-hyaluronic acid sample. Regarding the adhesion and proliferation test, the TNT group displayed a higher number of ADSCs than both TNT-polymer-modified groups, suggesting that TNT positively affects stem cell proliferation, as the nanotubes have higher protein adsorption [35]. Nevertheless, ADSCs appear to be well spread in all samples. Moreover, initially, there was no significant difference in ALP enzyme activity. After three weeks, samples TNT-TA-HP and TNT-TA-hyaluronic acid exhibited superior ALP activity compared to the TNT sample, indicating that the TNT-polymer modification enhanced osteoblast differentiation. Furthermore, osteocalcin expression and mineral deposits were more significant in the TNT-TA-HP group, supporting the ALP activity result. This result indicates that the sample presented more substantial osteoblast differentiation, even with lower adhesion of ADSCs, than the unmodified TNT group. Thus, the TNT-TA-HP nanocomposite is a promising biomaterial for bone regeneration, exhibiting enhanced osteoblast differentiation and supporting suitable cell proliferation and adhesion [35].

Another combination of the biocompatible titanium alloy modified with TNT showed a promising osteogenic potential for application in bone implants after the nanotube coating with silk fibroin (SF) isolated from *B. mori* cocoons [36]. Ti-6Al-4V alloy coated with TNT through anodic oxidation was covered with SF by electrophoretic deposition. *In vitro* studies were conducted to investigate the nanocomposite biomineralization, cell adhesion, and cell proliferation. For bone-bonding purposes, apatite formation is a factor of interest; hence, results showed that samples coated with SF and TNT-SF had dense calcium agglomerates deposited, suggesting the composites were covered with apatite [36]. Furthermore, the cell adhesion study revealed that MG63 cells were attached to all samples, with SF- and TNT-SF-coated samples displaying elongated cells that were anchored in the substrate. The morphology of adhered cells in the TNT-SF group was well spread, with the formation of filopodia extensions on the nanotubular surface. Additionally, the cytoskeletal organization and cell morphology in all groups were evaluated, with the apparent formation of F-actin stress fibers (a good indicator of cell spreading) in the TNT-SF sample. Regarding the cell proliferation

assay, the proliferation of MG63 cells was higher in the TNT-SF sample; however, the uncoated TNT group exhibited a higher proliferation rate than the SF-coated sample [36]. Finally, a gene expression assay confirmed the material's potential for bone regeneration. The TNT-SF sample displayed a higher Runx2 expression level and upregulation of Osterix protein, indicating osteoblast formation. Notably, the presence of nanotubes and SF enhanced osteocalcin expression, a crucial marker of osteogenic regeneration, suggesting that combining TNT and SF with Ti alloy is a suitable approach for biomaterials in bone implantation [36].

3D bioprinting combines versatile scaffold design with facile manufacturing, transforming standard titanium alloys into enhanced implant options. However, this alloy presents no osteogenic and antibacterial activity. This drawback could be bypassed with scaffold modification, such as reinforcement with TNT loaded with a mesoporous bioactive glass (MBG) to enhance Ti implant cell adhesion and proliferation [37]. Medical-grade Ti-6Al-4V alloy powder was used to fabricate the porous Titanium implants in a selective laser melting machine. Furthermore, the Ti implants were subjected to a two-step anodization process to form TiO_2 nanotubes on the scaffold surface, followed by immersion in an MBG solution and a 5-hour heat treatment [37]. The resulting material was characterized by mechanical testing, bioactive ion release, and cytotoxicity/cell proliferation study. Regarding the mechanical performance of the nanocomposite, neither the addition of TiO_2 nanotubes nor TiO_2/MGB presented a significant difference in the mechanical strength of the Ti alloy, indicating that all samples met the criteria for cancellous bone replacement. For assessing the release of calcium and silicon ions, the TNT/MGB/Ti nanocomposite was immersed in a human mesenchymal stem cell medium (MSCBM), indicating that the ion release was continuous, thereby providing nutrients for cell proliferation and osteogenesis [37]. This ongoing calcium and silicon discharge was attributed to the TNT as carriers that enabled the controlled ion release, which is fundamental for long-term bone regeneration. Moreover, the cell adhesion and proliferation study conducted on human bone mesenchymal stem cells (hBMSCs) showed that cells spread more extensively on the surface of the nanotubes and even more so on the TNT/MBG surface. Cell adhesion is associated with the hydrophilic nature of TiO_2 nanotubes, which is conducive to protein adsorption.

Biofilm formation is a significant concern in implants, as infections and inadequate bone healing are common challenges in dental and orthopedic applications within the surgical field. As discussed previously, TNT exhibits antibacterial and osteogenic properties; moreover, TNT nanofilms associated with alkaline earth metals and lanthanides can induce faster healing, as seen in nanotube modifications with strontium (Sr) and samarium (Sm), due to properties such as osteoinductive collagen and antimicrobial properties [38]. In this work, the TNT was first prepared by anodizing a titanium plate and undergoing hydrothermal treatment for nanotube modification with Sr, followed by immersion in polydopamine (PDA) and Sm solutions. Then, the nanofilm was evaluated through *in vitro* antibacterial, osteogenesis-related gene expression, and biocompatibility assays. The antibacterial evaluation was carried out using *E. coli* and *S. aureus* strains, as both bacteria are associated with infections following bone repair surgery [38]. The results showed better antibacterial performance in samples doped with Sm ions, reaching up to 80% inhibition in both bacterial strains. Regarding cell adhesion, MC3T3-E1 cells exhibited higher adhesion on the TNT/Sr/PDA/Sm sample due to its larger surface area, which covered the entire nanofilm surface with well-spread preosteoblasts. Furthermore, an osteogenesis gene expression assay revealed that osteogenic gene expression on nanotube surfaces doped with Sr was enhanced; however, the TNT/Sr/PDA/Sm group exhibited lower gene expression than the TNT/Sr sample [38]. Therefore, although the TNT/Sr/PDA/Sm nanofilm had a slightly lower osteo-differentiation potential, the presence of Sm does not exhibit cytotoxicity, and its addition could be beneficial, as the antibacterial activity was better in this system, indicating the suitability of this nanocomposite as an implant coating.

ZIRCONIUM OXIDE NANOTUBES

Zirconia (ZrO_2) is a metal oxide with numerous applications because of its excellent mechanical properties, resistance to physical corrosion, and biocompatibility. It has been applied in prostheses, especially in dental, oral,

and orthopedic implants [39]. Zirconia (ZrO_2) has garnered attention in recent years due to its high mechanical strength and thermal stability, making it a fascinating material from a technological perspective. Nanostructures of ZrO_2 are primarily used in dentistry, but their production also extends their applications in photocatalysis, gas sensing, and wastewater treatment [40]. Nanostructured ZrO_2 can be produced using chemical methods, including hydrothermal synthesis, sol−gel processes, and anodization. [41,42].

Anodic oxidation, also known as anodization, is a strategy to redesign the zirconium surface regarding its roughness and wettability. To biomaterials, surface roughness is essential for cell attachment and process differentiation, as higher surface roughness increases the available area for cells to interact with implants. The anodization process involves applying a voltage to electrodes immersed in an electrolyte, where an oxidation reaction occurs at the metal substrate, designated as the anode. While an electrical current passes through the electrolyte containing fluoride ions, an oxide film forms at the surface of the substrate, and a nanotubular structure develops. Similar to the growth mechanism of nanotubes on titanium, ZrO_2 nanotube (ZrNT) formation in electrolytes that contain fluoride is a competition between oxide layer formation and its chemical dissolution by fluoride ions [43].

The ZrNTs' properties, such as antibacterial activity, are of great interest to Dentistry. This property is primarily attributed to the widened surface area-to-volume ratio, which enables greater exposure of surface atoms. ZrNTs can be obtained with varying inner diameters and lengths by changing anodization process parameters such as time, applied voltage, and electrolyte concentration [44-46]. Pre-anodization of zirconium is reported to be beneficial for forming highly ordered ZrNT arrays [47]. Furthermore, pre-treatments on Zr substrates (electropolishing and dip etching) have also been proposed to attain self-organized nanotubular arrays [40,41].

ZrO_2 has been widely used as a ceramic material for dental caries treatment. A study investigated the capacity of ZrNTs to inhibit the growth of cariogenic bacteria such as *Streptococcus mutans*, the most prevalent species in dental caries [46]. ZrNTs are bioengineered-qualified implants as long as they do not cause cell death, chronic inflammation, or other impairment of cellular or tissue functions. ZrNTs show appropriate effects on L929 proliferation once they show behavior similar to the control. In addition, ZrNTs exhibit higher adhesion activity for cells than ZrO_2 nanoparticles due to higher wettability. The material could be considered safe once the implant was shown to cause no positive chromosome aberrations in rats' bone marrow. Therefore, ZrNTs can inhibit the growth of *Streptococcus mutans*, inducing cell death and suppressing bacterial adhesion [46].

Nanostructures of ZrO_2 serve as reinforcing elements in composites used for dental restorative procedures and dental implants, thereby enhancing their mechanical properties [48]. For example, to increase the mechanical properties of HA and β-tricalcium phosphate (β-TCP) for clinical applications, ZrNTs can be added as a second phase. Particle or fiber reinforcement can benefit composites through the crack-bridging mechanism, where fibers can delay or prevent crack propagation [49]. A numerical investigation using the finite element method with representative volume element models was conducted to analyze the elastic properties of random and ordered ZrNT-reinforced HA and β-TCP biocomposites. Different distribution types (longitudinal, transversal, and random) were studied with various volume fractions and aspect ratios of ZrNTs. Under the same volume fraction and aspect ratio of reinforcements, the best enhancement for ordered reinforced nanocomposites is achieved, showing promising results for bone repair and substitute applications [50]. The elastic modulus for nanocomposites with 5.5 vol.% ZrNT-reinforced HA was 115.4 GPa, similar to the elastic modulus reported for boron nitride-reinforced hydroxyapatite (129.1 GPa) [51]. However, ZrNTs provide an enhanced osteoblast response compared to boron nitride nanotubes, making ZrNTs a better candidate as a reinforcement agent. Therefore, ZrNTs reinforce phosphate composites that combine the advantageous properties of both biomaterials, which were previously applied separately [52].

On the other hand, zirconium (Zr) has been investigated for forming binary and ternary alloys with other metals, such as titanium, for use in orthopedic and dental surgery implants. Zirconium (Zr) combined with titanium (Ti) is commonly investigated as an alternative to pure titanium (Ti) in oral cavity restorative applications, as it is more resistant and has excellent osseointegration properties. Among TiZr alloys compared to Ti grade IV, the TiZr alloy with 50% Zr was

recently reported [49] to feature self-organized nanotubes prepared via a two-step anodizing process, resulting in a structure with nanocavities. The surface texturing on Ti50Zr alloys exhibited better performance, including an antibacterial effect, compared to polished surfaces. This improvement was achieved by decreasing the contact angle from 71° to 29° through the formation of a nanotubular layer, thereby enhancing hydrophilicity. Surfaces with low contact angles repel attaching microorganisms, keeping the surfaces free of bacteria. The MG63 osteoblast-like cells exhibited a well-developed cytoskeleton on nanotubular Ti50Zr samples, tending to grow in a multilayered manner, which resulted in higher viability values compared to the control. The nanotubes with the larger mean diameter had the highest antibacterial effect against the Gram-positive bacterium (*S. aureus*). Once the negative charge of the hydrophilic nanotube attaches to cells, such as osteoblasts, the same surface repels the negatively charged microbes, resulting in a reduction of biofilm extension [45].

The material surface characteristics are critical for the substrate-tissue interaction, significantly influencing cell behavior. Several concepts for implant coating and local drug delivery have been developed to enhance therapeutic efficiency for dental implants [53]. In this way, the fabrication of nanotubes on Ti50Zr alloy was investigated as a potential drug delivery system to enhance bone response, thereby promoting a treatment for implant-associated osteomyelitis. The elaboration of TiZr hybrid nanostructures (nanopores and nanotubes) brings the possibility of serving as a drug reservoir [47]. TiZr alloys have a protective passive stratum of mixed oxides, effective in the controlled release of gentamicin [54]. Using nanotubes as local delivery systems has essential advantages due to the possibility of producing structures with controlled dimensions and uniformity. A comparative study using nanotubular and nanoporous Ti50Zr alloy investigated gentamicin loading and release as a function of dimensions and hydrophilic/hydrophobic balance. Gentamicin sulfate (GS) was directly loaded onto the nanostructures and coated with a chitosan polymer solution. FT-IR analysis revealed that on the nanoporous surface, a mixed layer of GS and chitosan formed, while on the nanotubular surface, the drug was adsorbed more deeply within the sample, with the polymer covering the tops of the nanotubes. The combination of chitosan and GS is hydrophilic, indicating a better chance of biomineralization. The GS release mechanism was studied using three mathematical models: Korsmeyer, Peppas, and Lindner-Lippold. The best approximation for the release mechanism was obtained with the Lindner-Lippold model. A longer-term release for such deeper nano architectures was observed. By selecting the chitosan film thickness appropriately, it is possible to control the release of drugs from nanotubes for specific applications in therapies for inflammation, bacterial infections, and bone cancer [47].

Several studies have reported that self-organized nanotube oxide layers grown on pure Ti and its alloys have potential benefits in improving the biocompatibility of the substrate [45,47,55]. This way, a nanotubular layer of $TiO_2-Nb_2O_5-ZrO_2$ was developed via anodization on a β-type Ti35Zr28Nb alloy surface to improve implant fixation between the bone and the implant. Mixed nanotubular oxide structures exhibit improved biocompatibility, characterized by reduced adverse reactions resulting from decreased macrophage adhesion to the implant's surface. Morphological analysis of the anodized samples revealed that the inner diameter and wall thickness increased with an increase in the water content of the electrolyte and the applied voltage during anodization. The difference in surface roughness and surface energies affects the biocompatibility of the base alloy. The cell viability, assessed with a human sarcoma osteogenic cell line (Saos-2), exhibited the highest value (108.55%) for nanotubes with the largest inner diameter (75.9 nm) due to the increased surface energies, which led to high adsorption of proteins on the top surface of the nanotubes [44].

ZrO_2 and Zr alloys have been proposed as promising materials for use in biomedical implants due to their biocompatibility, corrosion resistance, high bending strength, and fracture toughness. Besides, the mixed TiO_2–ZrO_2–$ZrTiO_4$ nanotubes formed on Ti50Zr are promising for biomedical applications. Morphological characteristics of ZrNTs, such as hydrophilicity and the potential for drug loading, are the most promising features for ZrO_2-based materials in terms of rapid osseointegration. However, drug loading and release from Ti50Zr are not well investigated, and more extensive research needs to be done on this material.

BORON NITRIDE NANOTUBES

Boron nitride nanotube (BNNT) is structurally similar to CNTs, where boron and nitrogen atoms are organized in a hexagonal lattice structure [56,57]. When first synthesized in 1995, BNNTs did not exhibit a high yield and quality compared to CNTs, which limited their application [58]. BNNT also has some interesting characteristics, such as high thermal conductivity, mechanical strength, and chemical stability [59], and hydrogen storage capacity [60]. Thus, in recent years, with the advancement of research on this topic, BNNTs have been synthesized through various methods, including arc-discharge, ball-milling, chemical vapor deposition (CVD), and laser ablation [58].

Other properties must be considered regarding the biological applications of BNNTs. According to the literature, BNNTs are biocompatible [61,62] and nontoxic for HEK 293 cells at 100 mg/mL, with dimensions of 20-30 nm in diameter and lengths up to 10 mm [63]. Due to the low solubility of BNNTs in aqueous media, the authors argued that surface modification of BNNTs could enhance their solubility and expand their biological applications [64].

BNNTs have also been studied as fillers for resin-based materials, mainly used in dentistry. A recent study evaluated the influence of BNNTs as fillers for resin-based dental sealants (RBDs) [65]. This concept would be interesting for improving antibiofilm activity aimed at avoiding caries. Some BNNT properties were listed as attractive to this application, such as the ability to form apatite and mineral precipitation in physiological solutions. The studied RBDs consisted of 90% triethylene glycol dimethacrylate (TEGDMA), 10% bisphenol A-glycidyl methacrylate (Bis-GMA), 1 mol% camphorquinone (CQ), and 1 mol% ethyl 4-dimethylaminobenzoate (EDAB). Both 0.1 and 0.2 wt.% of BNNTs (average tube length of 200 μm) were used during the study. The BNNTs were first dispersed in Bis-GMA under sonication, followed by the addition of TEGDMA, CQ, and EDAB. A final sonication step was performed in the dark. The cytotoxicity assay was performed with keratinocytes (HaCaT) and pulp fibroblasts from a patient's third molar. The cells were incubated for 3 days, and the results showed that the viability was higher than 75% for all the cell lines. Mineral deposition was observed at both BNNTs/RDBs concentrations of 0.1 wt.% and 0.2 wt.% at 7 and 14 days, respectively. The addition of BNNTs in the RBDs did not significantly affect the sealant's degree of conversion. The addition of BNNTs did not affect the ultimate tensile strength, which does not compromise the effectiveness of the RDB.

Functionalized BNNTs were also incorporated into a hydrogel-based bioink for 3D bioprinting [66]. Once hydrophilic polymers are present in the bio-ink composition, BNNTs must be functionalized before incorporation to achieve better dispersion and subsequent performance [64]. In this way, synthesized BNNTs were functionalized with hydroxyl groups. The obtained BNNTs were incorporated into the gelatin-alginate (GA) gel, which comprised the final hydrogel-based ink solution used during printing. Four different solutions were tested, with the concentration of BNNTs varied (0, 0.05, 0.075, and 0.1 m/v%). BNNTs improved the printability and mechanical properties of the ink solutions compared to the sample without BNNTs, serving as a control. The compressive strength of the samples with BNNTs increased compared to the control at 6 kPa (0.05 m/v% BNNTs), 8 kPa (0.075 m/v% BNNTs), and 9 kPa (0.1 m/v% BNNTs), making them a great candidate for soft tissue regeneration. The cell viability against HEK293T cells showed a decrease in behavior with the increase in BNNT concentration. During the days that the cells remained in contact with the scaffolds, only the control and the scaffold without BNNTs showed higher cell viability after the second and third days compared to the first day. Despite that, the viability of all the scaffolds was above 80%. The scaffolds are a potential 3D printable biomaterial, but further studies are still necessary.

The properties of dental resin adhesives have also been a topic of interest in dentistry. Studies have been conducted to address some limitations associated with these materials [67]. Some of these limitations are bond strength reduction, degradation (interface and marginal), and biocompatibility. In this sense, BNNTs have been studied as an alternative filler. The BNNTs' attributes, including biocompatibility and enhanced physicochemical properties, are desirable for this application. The adhesives were obtained at a concentration of 66.6 wt.% BisGMA, 33.3 wt.% HEMA, and 1 mol% CQ and EDAB. The BNNTs in different concentrations (from 0 to 0.15 wt.%) were dispersed in BisGMA using sonication before the addition of HEMA [67]. The degree of conversion was studied using Fourier Transform Infrared (FT-IR) analysis equipped

with Attenuated Total Reflectance (ATR). The 0.075 and 0.1 wt.% nanocomposites achieved the highest degree of conversion, ranging from 68.9 to 70.2%. The maximum polymerization rates were observed in the same samples, showing values above 14 s^{-1}. The contact angle did not show significant changes in samples up to 0.1 wt.% compared to the control group. The opposite was observed in the sample containing 0.15 wt.% of BNNTs, increasing the adhesive hydrophobicity (more than 10°). Micro tensile bond strength (μTBS) was studied immediately and after six months. Only the samples with concentrations ranging from 0.05 to 0.1 wt.% did not show a statistical difference between 24 hours and six months of analysis. Both control and 0.15 wt.% showed a decrease ($p > 0.05$) in the μTBS values after six months. An indirect contact assay was performed to evaluate the cytotoxicity of the samples. Then, fibroblasts were obtained from the healthy human pulp of a patient. The cells were cultivated in a medium that remained in contact with the samples for 72 h before the indirect contact test for cytotoxicity evaluation. All the samples showed results around 100% cell viability ($p > 0.05$). The incorporation of BNNTs enhanced the adhesive properties, leading to improved performance in dentistry.

Calcium phosphate cements (CPCs) share the same limitations as adhesive resins in terms of mechanical properties, as the development of fractures is commonly observed. CPC scaffolds generally replace hard tissues, which demand better mechanical properties. BNNTs were studied as fillers for the CPC matrix, aiming to improve the matrix's mechanical properties. In this sense, a recent study evaluated the incorporation of BNNTs into the β-TCP matrix [68]. BNNTs were synthesized using a mechano-chemical reactor. The final powder material was obtained after crystallizing at 900°C for 1 h. BNNTs were incorporated into the β-TCP matrix using ball milling, with monocalcium phosphate monohydrate (MCPM) added in a proportion of 0.01:1:0.75, respectively. A 0.4 M citric acid solution was used to obtain the cement, aiming at a final concentration of 4.2 g/mL. SEM analysis showed that the obtained cement was not homogeneous. Biological properties were assessed using the hMSCs cell line. The method used to study cell proliferation was Alamar Blue, which quantifies metabolic activity. No statistical difference was observed during the first seven days compared to cells cultivated on culture plates, which served as the control. The results showed that at day 14, the number of cells that proliferated on the β-TCP/BNNTs was half that of the control group. An ALP expression assay was performed to analyze the differentiation of hMSCs into osteoblasts. The assay was performed over 21 days, and the results were normalized in terms of ng of ALP per μg of DNA. In contrast, the cell proliferation results showed that ALP was twice as high as the control at 14 days, measured as ng ALP/μg of DNA. The results observed at both tests indicated that the cells that remained in contact with the β-TCP/BNNTs transitioned to the new phenotype. Once no supplementation induced this cell behavior, the property can be attributed to the BNNTs. The inflammatory response was also studied using a co-culture method with osteoblasts and macrophage cells. An ELISA test was performed to analyze the expression of nitrite and IL-6 after 3 days. The increase in the expression of both nitrite and IL-6 cytokines was observed in the β-TCP/BNNTs sample, indicating a direct relationship between the degree of inflammation and stimulation of new bone. These findings suggested that this sample could be a promising material for stimulating new bone production.

CARBON NANOTUBES

Carbon nanotubes (CNTs) are a type of carbon allotrope [69], with tubular morphology [70,71], consisting of concentrically rolled graphene sheets [72], which may be open-ended or have the tube ends closed with a fullerene-like structure [73,74]. They can be single (SWCNT), double (DWCNT), or multi-walled (MWCNT), which refers to the number of carbon layers [75], with diameters on the nanometer scale and lengths of micrometers [73,75]. CNTs can be produced by many methods, but the most frequently adopted are electric-arc discharge, laser ablation, and chemical vapor deposition (CVD) [76,77]. Depending on their chirality, they can be classified as metallic or semiconductors [78]. In addition, CNTs possess attractive properties, including a high Young's modulus, tensile strength, fracture toughness, chemical stability, electrical and thermal conductivity, and a large specific surface area [79-82].

Since their first report [83]CNTs have been widely studied in diverse areas, such as

micro-electronic devices [78], sensors and actuators [71,84] for mechanical [85-88] and electrical [81,89,90], reinforcement in composites, among others. The use of CNTs as biomaterials is relatively new and remains controversial due to their possible toxicity. However, several studies have shown that varying factors, including shape, size, composition, number of walls, and chirality, can mitigate the toxic effects of CNTs [91]. Functionalizing carbon nanotubes (CNTs) with biomolecules is a recently explored alternative to reduce their toxicity, including encapsulation, coating with polymers, and liposomes, among other possibilities [91,92]. It has been suggested that functionalized CNTs, less toxic than non-functionalized ones, can be excreted from the human body [93].

CNTs have shown promising results as carriers for drug delivery, promoting the proliferation and differentiation of osteogenic cells [91]. The potential use as scaffolds for bone regeneration and the production of nanocomposites opens up several possibilities for applications in biomedicine, pharmacology, and neuroscience [94]. Regarding bone regeneration and dentistry, a wide variety of matrices are available. Some of the most cited are calcium phosphates, calcium silicates, hydroxyapatite, bioglasses, and poly (methyl methacrylate) (PMMA) [95-102].

A study incorporated carboxylated (-COOH) multi-walled carbon nanotubes (MWCNT) in poly (methyl methacrylate) (PMMA) to prevent microbial adhesion without the addition of other antimicrobial compounds [103]. Composites were prepared by adding 0.25, 0.50, 1.0, and 2.0 wt.% of MWCNT to the matrix. Samples with 2% CNTs did not exhibit satisfactory mechanical resistance and, therefore, were excluded from the biological assays. The antimicrobial activity of materials immersed in artificial saliva against *C. albicans, S. aureus, and S. mutans* showed better results on smooth surfaces than on rough ones. The addition of CNT resulted in a significant reduction in microbial adhesion compared to neat PMMA. The decrease was proportional to the amount of CNT added to the samples. The anti-adhesive effect was evaluated using *C. albicans* (with 0.25% CNT excluded), and results showed a reduction of attached cells in samples with CNT. The cytotoxicity of extracts of the materials at concentrations of 50, 25, 12.5, and 6.25% against oral keratinocytes showed that viability was not significantly reduced by incorporating CNT. PMMA with 1% CNT showed optimal mechanical and anti-adhesive properties, suggesting that this material could be a promising biomaterial in dentistry [103].

Focusing on guided bone regeneration, porous poly(lactic acid) (PLA) membranes with bioglass (BG) and multi-walled carbon nanotubes (MWCNT) were developed [90]. Samples were prepared by solvent casting, with 5 wt.% of BG and varying MWCNT contents (0.5, 1.0, and 1.5 wt.%). The addition of MWCNT did not affect the bioactivity of the membranes, as evaluated by X-ray diffraction through the formation of hydroxyapatite. Samples produced without MWCNT did not exhibit inhibitory activity against *C. albicans*, *E. coli*, and *S. aureus*; however, the addition of MWCNT resulted in antimicrobial activity. The increase in MWCNT showed better results against *E. coli*; however, a concentration of 0.5 wt.% was not sufficient to promote antimicrobial activity. Cell viability using mesenchymal stem cells (MSCs) from the femur of rats was low for all samples. Higher cell viability was observed for the membranes without MWCNT and those with 0.5 and 1.0 wt%. On the other hand, the authors obtained a better result for total protein for samples with higher concentrations of MWCNTs. ALP activity was similar for all samples, indicating that the membranes facilitate cellular differentiation in addition to being an effective antimicrobial material [104].

PCL/MWCNT scaffolds were produced by extrusion additive manufacturing to be used in bone regeneration [105]. PCL/MWCNT pellets were produced by melt blending using 1 and 3 wt.% of MWCNT. The nanocomposite pellets were used for the scaffold fabrication, employing a screw-assisted additive biomanufacturing system. Protein adsorption, as studied through the bicinchoninic acid (BCA) assay, showed that after 6 hours of incubation, samples with MWCNT exhibited increased protein adsorption compared to neat PCL. Cell viability and proliferation on days 1 and 3 showed no statistically significant difference between the neat PCL and the nanocomposite scaffolds. Still, the results suggest that all scaffolds are appropriate substrates for cell proliferation. From day 7, the cell proliferation was statistically higher for samples with MWCNT. Images of confocal microscopy taken after 14 days of proliferation showed large cell attachment and spreading, with better confluence and cell distribution for PCL/MWCNT scaffolds than neat PCL. ALP activity

and calcium deposition were similar to those of PCL and PCL/MWCNT samples [105].

Polyurethane (PU), chitosan, zein, and carboxylated multi-walled carbon nanotubes (MWCNT) (0.1 mg/mL) were mixed in a solution to prepare electrospun fibrous scaffolds for bone regeneration [106]. *In vitro* biomineralization assay, which evaluates the formation of calcium phosphate on the samples, showed that a uniform mineral layer entirely covered scaffolds produced with MWCNT after 5 days. The carboxylic groups on the surface of MWCNTs provide an electron charge density, which can nucleate positively charged ions, affecting hydroxyapatite formation. The incorporation of MWCNT resulted in a calcium-to-phosphorus ratio of 1.56, which is almost similar to that of human bone. Antibacterial activity was evaluated against *E. coli, S. aureus, M. luteus, and S. epidermidis.* Pure PU membranes did not exhibit antibacterial activity; however, MWCNTs influenced the bactericidal efficacy, showing consistent results in inhibition zones. This result is likely due to the spike in MWCNTs or the oxidative stress caused by the oxygenated radical groups on the surface of the tubes. The cytocompatibility of MC3T3E1 cells was evaluated by the CCK-8 test method. On day 1, there was no significant difference in cell proliferation on the scaffolds. However, after 5 and 7 days, the scaffolds with MWCNT showed significantly improved cell proliferation. Besides affecting the porosity and surface area of the scaffolds, MWCNT also influences electrical conductivity, favoring the regulation and transfer of mineral ions and proteins (biomolecules). ALP activity was also enhanced by incorporating MWCNT into the nanofibrous membranes, as well as the differentiation of preosteoblast cells and the formation of calcium ion nodules. The membranes produced with MWCNTs showed great potential for regenerating new bone tissue [106].

Inorganic matrices have also been reinforced with CNTs, with a focus on bone regeneration. Nanocomposites of hydroxyapatite (HA) with CNT (HA/CNT) and gold nanoparticles (HA/CNT/Au) were obtained by blending them in the reaction mixture [107]. Bioactivity was evaluated after immersion in SBF for 3 and 7 days, and both neat HA and HA/CNT presented crystals of hydroxyapatite precipitates; however, the HA/CNT/Au sample had a significantly higher number of crystals. *In vitro* cytocompatibility (MTT) using adipose tissue-derived stem cells showed viability higher than 75% for all samples after 24 h incubation. Moreover, the cell viability was slightly higher for HA/CNT samples. The functionalization of CNTs with particles or molecules can be attractive for improving biological properties [107].

Considering the results discussed in the studies above, CNTs are promising for dental applications and bone regeneration, increasing mechanical properties and inducing antimicrobial activity, among other properties. However, *in vivo* studies are still necessary to evaluate the effects of these materials.

PERSPECTIVES AND CONCLUSION

In this review, we examined the different nanotubes (NTs) used in tissue repair and dental applications, providing an overview of their potential and limitations. Applications such as tissue repair, drug delivery systems, and reinforcement of polymer matrices were discussed. A significant limitation of many existing studies is the lack of in vivo analysis, as most investigations conducted so far have not included this type of assessment. Without in vivo data, concerns arise about the reliability of information obtained solely from in vitro assays for future clinical use of NTs. Also, the limitations of monolayer cell cultures and their inability to replicate whole-organism processes are discussed. Therefore, conducting in vivo studies, which are currently lacking, could help inform NT design and facilitate their application in clinical settings.

Another challenge of using NTs in biomedical materials is their potential cytotoxic effects and poor environmental compatibility. This issue can potentially be addressed through surface modification (functionalization) of the NTs, which can serve as a powerful tool to enhance material performance for specific applications. In some cases, further research is needed to improve the synthesis and purification processes of NTs, as residual impurities from these procedures—such as solvents used during synthesis or reagents from functionalization—may remain in the final product. These impurities can affect the properties of NTs, including cell viability and interactions with the surrounding environment. Nonetheless, early studies on NT applications in tissue engineering and dentistry demonstrate promising features for advancing bioengineering technology, paving the way for future research and potential treatment strategies.

Author's Contributions

DMS: Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Resources, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing. NVM, TCO, VMS, TLAM: Data Curation, Formal Analysis, Investigation, Methodology, Resources, Validation, Writing – Original Draft Preparation, Writing – Review & Editing. GPT: Investigation, Writing – Review & Editing, Visualization, Supervision. CYKI: Conceptualization, Investigation, Resources, Writing – Review & Editing, Visualization, Supervision, Project Administration and Funding Acquisition.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Regulatory Statement

This review was conducted utilizing electronic databases to identify previously published studies. As it solely depends on existing literature, no further ethical approval was necessary.

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