Eurasian
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FULL PAPER

Polymeric nano-biomaterials in regenerative endodontics

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Applying nano-scaffolds for pulp regeneration is another use of nanotechnology in endodontics that creates impressive development in reconstruction of pulp structure. This study aimed to review the application of polymeric NPs in different stages of the conventional endodontic process and regenerative endodontic therapy. Accordingly, the studies over last ten years were searched by an electronic and manual search via PubMed and Google Scholar search motors. The search was conducted by using these keyword: "polymers","nanoparticles","endodontics", "polymeric nanoparticles","root canal disinfection", and "regeneration". The results showed that different polymeric nanoparticles (NPs) have different advantages and disadvantages but the point is the superiority of nanomaterials in comparison with conventional ones. Using polymeric NPs is a new concept in endodontics' procedures, which could take part as a promising method rather than conventional root canal therapy. Therefore, the future perspective of endodontic seems very promising by using these novel nanomaterials.

KEYWORDS

Polymers; nanoparticles; endodontics; polymeric nanoparticles; root canal disinfection; regeneration.

Introduction

Regenerative dentistry signifies a new method including biomaterials, several molecules and mesenchymal stem cells (MSCs), partly derived from oral tissues. It can be categorized into two main groups including endodontics (hard tissues) and periodontics (soft tissues) [1-4].

Nanotechnology is a broad scientific field that consists of several fields, such as physics, optics, material, and medicine. It is directly concerned with the management of structures in atomic-scale or nanometers in at least one dimension [1]. Though nanotechnology's presence returns to beginnings of the time, as in the format of synthesizing various molecular structures in the body; its discovery is ascribed to the American physicist Dr. Richard Phillips Feynman, due to his outlook on the several subjects as accommodation of data on a very tiny scale, miniaturization of the computer, or producing small devices. However, the term nanotechnology was employed for the first time by Taniguchi in 1974 [2,3]. Afterward, Dr. K. Eric Drexler, who used Feynman's theory and appended the concept of making extra copies of themselves, by machine processor rather than a human processor, and popularized the idea of nanotechnology in 1986. Nanotechnology provides improved properties of materials that contain between 1 and 100 nm [4].

Chemical aspects affected by larger surface area cause better functionalities and catalysis,

mechanical properties improved by better elements such as strength hardness, [5] crack and fatigue strength [6] and finally better mechanical interlocking [7], absorption, and fluorescence of nanocrystals causing better optical properties [8], fluidic properties enhanced by a higher flow using nanoparticles. Thermal properties are affected by raised thermoelectric performance [4]. Also, biodegradability is controlled better compared with conventional composite materials [8,9].

Nanotechnology has been applied in dentistry to enhance dental practice and restriction of oral diseases [4]. Also, it has helped dental material with modeling ultrafine objects at the nanoscale. Nano-dentistry will be the leading dental treatment to an efficient and highly effective treatment, creating a broad range of opportunities of more beneficence for both the dentist and the patient [10].

Root canal treatment is a common procedure with a significantly great success degree of 96%. However, the failure still happens as a result of inappropriate cleansing and forming the shape of canals. Amongst the current improvements in material sciences in endodontics, nano-sizing is an influential step in improving the bioavailability and bioactivity of the materials in order to partake in every clinical procedure from filing to filling materials [10,11]. Applying nano-scaffolds for pulp regeneration is another use of nanotechnology in endodontics to preserve the pulp tissue and stimulate the process of pulp repair instead of removing the pulp and obturating the canal [10,11].

There are several types of nanoparticles: Polymeric NPs, ceramic NPs, silica NPs, metallic NPs, magnetic NPs, carbon NPs, liposome NPs. Two main divisions in nanoparticles are organic and inorganic NPs. Polymeric NPs were covered in both groups. Numerous studies have discussed the polymeric nanoparticles and their benefits in dentistry and endodontics. In this review, we focused on the application of polymeric NPs in different stages of the conventional endodontic process and regenerative endodontic therapy.

Materials and methods

A primary search was performed within articles of the last ten years using PubMed and Google Scholar search motors and a total of 194 articles were recognized. The search was conducted by using these keywords: "polymers","nanoparticles","endodontics", "polymeric nanoparticles", "root canal disinfection", and "regeneration". After rejecting duplicated papers using EndNote software (version 8) and reviewing the articles, 103 articles remained. Through the next step, the abstract of the remaining papers was screened and 56 articles were accepted due to excluding non-related articles. A manual search resulted in 8 additional articles and the number of total articles reached 64. Then, the studies were classified in the following order: root canal irrigation and disinfection, obturating materials, root-repair materials and pulp capping, regenerative endodontics therapy.

Results

Root canal irrigation and disinfection

One of the most significant stages of root canal therapy is removing infectious microorganisms and microbial components from root canals to preventing re-infection of canals. Various chemical and mechanical methods have been used to reach this purpose. Ethylene-diamine-tetra-acetic acid (EDTA), sodium hypochlorite (NaOCl) and chlorhexidine (CHX) are some of the best known chemical components for root canal disinfection [12]. Mechanical instrumentation is also used to decrease and remove a part of infected dentin from canals.

In recent years, the advent of nanomaterials and their ability in targeted

drug delivery have led to significant progress in the disinfection of root canal and accessory canals while many of the techniques and chemicals mentioned in the previous paragraph cannot penetrate in accessory canals [13]. Therefore, most of the recent reports about root canal disinfection have focused on the advantages and applications of using nanoparticles.

Perochena *et al.* examined the ability of bioactive chitosan nanoparticles (CS-NPs) to eliminate the smear layer and restrict bacterial recolonization on tooth dentin. The researchers found that these nanoparticles could enhance the resistance of the dentin surface against destruction by collagenase. And its resistance to biofilm formation is considerably better than other treatment groups (NaOCl and NaOCl-EDTA). Also, they found that chitosan has polycationic nature that interferes with the negatively charged surface of bacteria, distorting cell permeability and resulting in the leakage of intracellular ingredient [14,15]. CS-NPs can stop enzymatic degradation caused by bacteria. This biopolymer improves the mechanical features of root dentin. As a result, using CS-NPs as final irrigants in root canal therapy has the double benefit of inhibiting bacterial proliferation and removing the smear layer [14]. In an *in vitro* study, Shrestha *et al.* evaluated the efficacy of CS-NPs and ZnO-NPs to destroy the structure of a 7-day *E. faecalis* biofilm and long-term antibacterial activity of CS- NPs and ZnO- NPs following ageing process. Nanoparticles showed higher antibacterial potency because they have higher polycationic/polyanionic nature and higher charge density so their interaction with the bacterial cell is higher. It is identified that small particles have higher antibacterial activity compared with macro-sized particles so the size of nanoparticles plays an important role in their activity. In conclusion, this research showed that CS-NPs and ZnO-NPs had markable antibiofilm features and they had ability to disrupt biofilm architecture.

These nanoparticles were able to preserve their antibacterial property even after staying 90 days in saliva and PBS. CS-NPs and ZnO-NPs eliminated planktonic bacterial cells more rapidly and with lower concentrations in comparison to biofilm bacteria. In another study by Perochena et al., the efficacy of CS-NPs and ethanolic propolis extract (EPE) incorporated into a calcium hydroxide paste (Ca [OH]$_2$) to kill bacterial biofilms was evaluated. The study showed the bactericidal effect of Ca (OH)$_2$ despite the addition of CS-NPs or EPE when applied to *E. faecalis* biofilms for 7 or 14 days. Ca (OH)$_2$ was the only bacteriostatic when it was tested on polymicrobial biofilms but its ability to kill bacteria was markedly ameliorated in both 7 and 14 days. Its antibacterial strength diminished with time, indicating the ineffectiveness of Ca (OH)2/EPE paste [15]. In a relevant study, Elshinawy *et al.,* separately or combined, assessed the antimicrobial-biofilm performance of CS-NPs, silver nanoparticles (Ag-NPs), ozonated olive oil (O$_3$-oil) against endodontic pathogens. The findings concerning the bactericidal and fungicidal activity of CS-NPs indicated that while having the lowest values of MIC and MBC, CS-NPs' antibacterial activity against mutants of *Enterococcus faecalis* and *Streptococcus* were eight times higher than ozonated olive oil. Also, CS-NP's antifungal activity against *Candida albicans* was found to be four times higher, compared with O$_3$-oil. They observed that the mature, viable biofilm on premolars *ex vivo* model by 6-log declined relative to the double blend of CS-NPs and O$_3$-oil, indicating a probable application for root canal treatment. Therefore, the advantages of this combination are safety, novelty, and its potency to eradicate mature mixed-species biofilms [16]. Shrestha *et al.* evaluated the antibacterial/antibiofilm efficacy and dentin-collagen stabilization effect of CS-NPs. The findings suggested that bacteria's propagation considerably dropped as well as the relative disintegration of multilayered biofilm architecture [17]. The

findings of the study by Shrestha *et al.* indicated that photosensitizer's reactivity improved in response to the increased surface area due to the presence of nanoparticles [18].

In an *in vitro* investigation, Pagonis *et al.* analyzed the antibacterial impact of light-synergized poly lactic-co-glycolic acid nanoparticles (PLGA NPs) on *E. faecalis* using TEM and photosensitizer methylene blue (MB). The outcomes demonstrated that nanoparticles decreased the amount of *E. faecalis* colonies in cultures significantly by generally acting on its cell wall. They concluded that PLGA NPs enclosed with protecting medications could be an advantageous point in root canal disinfection [19]. Likewise, another study revealed that the positive-charged photosensitizer could disable the number of microbial biofilms such as *E. faecalis* and cut off the biofilm architecture [20]. Makkar *et al.* ran a study to assess the effectiveness of fabricating Poly (Lactic-co-Glycolic Acid) (PLGA)-moxifloxacin nanoparticles and evaluate the supported antimicrobial efficacy with calcium hydroxide of it and chitosan-moxifloxacin hydrogel facing *Enterococcus faecalis* was tested. These authors found that PLGA reprised moxifloxacin nanoparticles were more effective than other formulations because of their effectiveness and sustained antibiotic release with meaningful results. Its grayish release makes them specific contenders for more evaluation *in vitro* and *in vivo* models as possible intracanal medicaments. The study showed future avenues for the application of polymer briefed antibiotics with importance to Moxifloxacin and its possible role in preventing diverse and pathogenic endodontic microflora with no provoking resistance [21]. A group of researchers accomplished an experiment to evaluate the antibacterial activity of ciprofloxacin (CIP) loaded PLGA nanoparticles (S_2) and CIP-PLGA nanoparticles covered with chitosan (S_3) against ciprofloxacin solution (S_l) as a control on Enterococcus faecalis. S_1, S_2, S_3, and chitosan (CS) were evaluated in vitro using the agar diffusion technique and biofilm inhibition analysis. All the qualified nanoparticles conferred a drug release pattern that was controlled. Also, the covered nanoparticles with cationic chitosan showed higher encapsulation reaction, the zone of inhibition, and antibiofilm effect than the antibiotic alone and the nanoparticles with zero chitosan [22].

A study in 2016 on drug delivery aimed to design nanoparticles containing chlorhexidine that could unvaryingly release the chemical as a continued bacterial repression in the root canal system [23]. In an *in vitro* study, the antimicrobial effect of 3D tubular-shaped triple antibiotic-eluting polymer (TAP) nanofibers against multispecies biofilm on dentin were assessed using infected dentin slices. In this study, they divided infected dentin slices into 4 groups (2 control groups, 3D antibiotic eluting nanofibers, and TAP). The results showed a significant bacterial reduction on the surface of dentin and the inner part of the dentinal tubules. It was concluded that 3D tubular-shaped antibiotic eluting is effective in multispecies biofilm and has clinical potential as a disinfectant [24]. Later, Albuquerque *et al.* conducted a study about nanoparticles-associated drug delivery resembling the previous study. In this investigation, the antimicrobial effect of triple antibiotic-containing polymer nanofibers (metronidazole, minocycline, and ciprofloxacin) on dual species biofilm and the capability of dental pulp cells to bond and accelerate on dentin upon nanofiber exposure was assessed, using three different solutions including saline (control), antibiotic-free nanofibers (control), and TAP. Bacterial development was assessed with the use of the LIVE/DEAD test and confocal laser scanning microscopy. The results monitored a meaningful bacterial death in the antibiotic-included group and a similar proliferation rate on days 1 and 3 in antibiotic-containing groups and antibiotic-free nanofibers. The study concluded that TAP causes remarkable

bacterial expiration, but it does not affect DPSC appliance and proliferation on dentin [25]. In an *in vitro* study done by Quiram *et al.,* the value of a trilayered nanoparticle (TNP) drug release system that encapsulated chlorhexidine digluconate was examined, proposed at increasing the root canal system disinfection [26].

In an investigation in 2014, the antimicrobial effect of SCHNC (Silver-crosslinked Hydrogel Nanocomposite) against Sodium hypochlorite and chlorhexidine on *E. faecalis* was assessed to determine the efficiency of SCHNC as root canal disinfectant. After producing SCHNC under vacuum, TEM images were used to determine the size and shape of these nanoparticles. TEM images showed that they were round in contour, and their common size was 20-30nm. The results showed that sodium hypochlorite and chlorhexidine act better as disinfectants than SCHNC [27]. In research by Hafez *et al.,* the antimicrobial effect of Salvadora persica root nanoparticles versus sodium hypochlorite was examined counting *E. fecalis* before and after irrigation by these solutions in two groups of extracted anterior teeth to determine the efficiency of Salvadora persica as a disinfectant. The results showed that the highest bacterial reduction percentage (94%) was done by sodium hypochlorite and *Salvadora persica* had the least bacterial reduction percentage (85%). There was no substantial distinction between both groups, so the study concluded that *Salvadora persica* performed as an efficient disinfectant. [28].

Obturating materials

Obturation is an important step in root canal treatment. Most of the failures in endodontic treatment are because of performing this step improperly and incomplete obturation. Using a proper sealer can lead to a reliable seal, prevent microleakage, fill the spaces between the obturating substances and canal, and get into accessory or lateral canals; therefore, it

can show magnificent performance in microbial control of the root canal system. Antimicrobial property of a sealer is one of the most important factors that has always been the focus of researchers [29,30].

In an *in vitro* study, the antimicrobial effectiveness of routine endodontic sealers including chitosan nanoparticles or Ag nanoparticles related to chlorhexidine, Calcium hydroxide with propylene glycol and CSNPs+chlorhexidine were evaluated against *Enterococcus faecalis*. The authors discovered that all sealers showed an enhanced inhibition action when they were combined with other materials particularly the bactericidal activity developed when the sealer composed CSNP-chlorhexidine. The highest values of antibacterial action increase were for the sealers with CSNPs+chlorhexidine. It was also mentioned that CSNPs showed an inconsiderable bactericidal activity but it can be applied as a vehicle for other bactericidal matters because of its biocompatibility [31]. Also, the benefits of chitosan nanoparticles joined to a zinc oxide eugenol (ZOE) based sealer was evaluated in the prohibition of biofilm organization at the sealer-dentin junction in another study. The study showed that CSNPs+ZOE based sealer hindered biofilm organization within the sealer-dentin junction. When the surface of canals was operated with chitosan combined with Rose Bengal followed by PDT, biofilm formation was reduced but it was maintained when phosphorylated chitosan applied [32].

An *in vitro* study was designed to assess the antimicrobial action using propolis and propolis-loaded polymeric NPs (ProE-loaded NPs) as root canal sealers owing to its antimicrobial and antioxidant characteristics. The result of the study showed that ProE-loaded NPs sealers revealed antimicrobial action against strains of *Enterococcus faecalis* and *Streptococcus mutans* and anti-fungal activity against *Candida albicans.* Prolonged-release and improved cytocompatibility were also collected from this research [33].

In a study, the nano diamond gutta-percha (NDGP) implementation was tested for future endodontic therapy improvements. One of the reasons that researches carried out this study was the limitations such as leakage and canal reinfection owing to using conventional gutta-percha (GPs). As a result, it was clear that the amoxicillin function was saved using NDGP and NDGP inhibits bacterial infection. X-ray images showed no obvious void creation after the conventional technique. In micro-computed tomography (micro-CT) images, a minimal number of small voids were recognized in the central third of the canal which is admissible as a successful root canal operation [34].

In an *in vitro* study, a well-diffused endodontic sealer including quaternary ammonium polyethyleneimine (QPEI) nanoparticles is expressed by a system for manufacturing optimized nanoparticles tailored to epoxy-based substances. Based on the results, a strong and continued antibacterial effect was shown by this endodontic sealer that made it a therapeutic choice. Also, they found that the novel sealer has a meaningful antibacterial influence on *E. faecalis* that come in close connection with the material's surface, as can be observed in SEM micrographs and the discrete cosine transform (DCT) outcomes and viable cell counts. Furthermore, the sealer's physical features, such as flow and solubility, were not influenced by the incorporation of the QPEI nanoparticles in comparison to those of the regular sealer. They concluded that the utilization of polycationic nanoparticles with antimicrobial attributes in an endodontic sealer reveals continuing antimicrobial effects, presenting an efficient antimicrobial option [35]. An *in vitro* study aimed to modify and improve the antibacterial attributes of endodontic sealers by combining low concentrations of QPEI NPs as an insoluble antibacterial nanoparticle (IABN). They found that incorporating IABN to accessible endodontic sealers displayed an antibacterial influence on E. faecalis, that was sustained for 4 weeks and reduced *E. faecalis* bacterial counts significantly. The antibacterial experiments and DCT displayed cumulative bacterial growth repression when they used IABN+sealers [36].

It is noteworthy that several non-polymeric nanoparticles were employed as nano-based root canal sealers, for instance, nanohydroxyapatite (NHA) crystals [37], nanocrystalline tetracalcium phosphate [38], nanoparticles of amorphous calcium phosphate [39,40], nanoparticles of Ag/ZnO [41], EndoSequence BC [42], which we did not mention because of their non-polymeric structure. These materials exhibited excellent properties, so researchers can use them alone or in composition with polymeric nanoparticles to achieve better results. consequently, incorporating polymeric nanoparticles into the conventional sealers can lead to better antimicrobial properties, better remineralization properties, reducing the biofilm CFU and as a result, reducing treatment failure of root canal treatment. These proper properties may be useful in designing endodontic sealing materials.

Root-repair materials and pulp capping

Retrograde root filling while periapical surgery and root repair materials are discussed in several studies. A strong and long-lasting seal of retrograde root fillings is so important in clinical works and the lack of a fair root canal filling would lead to surgical therapy. Although polymer nanocomposites (PNCs) are not polymeric nanoparticles, they are polymeric materials that are combined with nanoparticles in a minimum amount. Accordingly, significantly improved mechanical and thermal features are shown by PNCs. In an *in vitro* study, two new polymer nanocomposites (NERP$_1$ and NERP$_2$) were tested for the initial apical seal besides a commonly used polymer. Significantly, the results illustrated the apical micro-leakage

decreased in the presence of NERP1 [43]. The cytotoxicity of two kinds of the novel root-end filling materials, polymer nanocomposite resins was assessed in another study in comparison with ProRoot® MTA and Geristore®. No meaningful diversity in cytotoxicity was observed between Geristores, ProRoots MTA, and PNC resin on 24 h, 1, 2, and 3 weeks specimens [44].

During tooth preparation or removal of caries, the pulp can be exposed. In such cases, the treatment of choice is direct pulp capping. This procedure includes preserving the vitality and function of pulp via a biocompatible material. To succeed in direct pulp capping, capping substances should have some properties such as sealing strength, ability to cause dentin bridge development to support the pulp against bacterial leakage or other external provocations. In an in-vitro study, authors provided poly (d,l-lactide-co-glycolide acid) (PLGA) nanoparticles that carry lovastatin for application in direct pulp capping. The study aimed to obtain the release of lovastatin in long-term over 72 days by incorporating it with PLGA nanoparticles. The result was that PLGA–lovastatin nanoparticles showed an effective controlled release to the 44th day. High-grade biocompatibility and excellent osteogenic and odontogenic ability to dental pulp cells were observed in the presence of these nanoparticles in rat teeth. Also, improvement in the organization of tubular reparative dentin was recognized at the section of pulp exposure, while a complete dentinal bridge was formed. Consequently, direct pulp capping could be enhanced by the application of this local delivery agent [45].

Regenerative endodontic therapy

When the severe injuries happen in pulp tissues, a severe inflammatory reaction has begun or has displayed necrosis; the preservation of the pulp becomes impossible. A pulpectomy is the treatment of choice in such cases, and the whole root canal system must be disinfected and filled to inhibit bacterial infiltration. Although conventional root canal methods are usually prosperous, they have several restrictions such as lack of vitality, feeble mechanical features, and complicated disinfection [10,46]. Moreover, the conventional endodontic therapy of permanent teeth with pulpal necrosis and immature root formation is associated with major challenges observed principally in children and adult cases. Fragility occurs in forming roots due to open apices and fragile dentinal surfaces. Root canal disinfection, by mechanical instruments, becomes more difficult due to this characteristic. The apexification process does not improve root formation; the root canal walls will remain thin and fragile that make these teeth sensitive to cervical fracture. The integrity of the afflicted tooth is improved if an alternative treatment procedure can strengthen the root against fracture, thus prepares an acceptable function for patients. Regenerative endodontic therapy (RET) is considered a much better treatment modality. There are kinds of definitions possible for tissue engineering. In a simple word, tissue engineering uses the principles of biology and engineering to improve practical replacements for hurt tissue [46].

Three principal elements in regeneration consist of stem cells, scaffolds, and growth factors. However, stem cell dimensions cannot be decreased to nanosize; many nano-based scaffolds and drugs can be applied in regenerative systems. Numerous scaffolds are also packed with drugs that are discharged gradually [10].

RET engage dental pulp stem cells (DPSCs) in producing revascularization of the root canal and maintained root growth. Two main methods in RET differ in scaffolds types. After conservative preparing of the root canal and disinfection, in one method, clinicians insert injectable scaffold, which is merged with seeded DPSCs into the canal space [47]. In the second procedure that is the conventional

clinical standard for regenerative systems, they instrument the canal with an endodontic file to provoke blood flow from the apical zone into the canal area and coagulate the blood. Stem cells emigrate into the root canal due to this operation [30]. The root lengthening and dentinal wall thickening result from both procedures, while pulpal revascularization occurs underneath an MTA seal and restoration material [47].

There are different varieties of scaffolds used in regenerative endodontic therapy like host-derived [48], naturally-derived [49], and manufactured scaffolds [48]. Nanomaterials have a beneficial role in fabricating tissue engineering scaffolds due to great surface area and surface energy [46]. This section will identify and discuss numerous polymeric nanoparticles as a scaffold or a part of scaffolds in regenerative endodontic therapy. These scaffolds could carry DPSCs to the root canal environment or act as a component of the disinfection procedure before applying DPSCs.

Shrestha *et al.* (2014) in an *in vitro* study evaluated mineralization of stem cells from apical papilla (SCAP) via alkaline phosphatase (ALP) activity rate in the attendance of chitosan nanoparticles merged with bovine serum albumin (BSA). The research displayed the application of chitosan nanoparticle (CSNPs) as a bioactive controlled-release scaffold and a design for drug delivery. Two types of BSA-loaded CSNPs were synthesized in this study: (1) the encapsulation technique (BSA-CSnpI) and (2) the adsorption technique (BSA-CSnpII). Significantly higher activity of ALP was observed in the BSA-CSnpI after 3 weeks than in BSA-CSnpII [50]. Another *in vitro* study by the same team analyzed two modifications of CSNPs loaded by dexamethasone (Dex): Encapsulation (Dex-CSNPsI) and adsorption (Dex- CSNPsII) procedures. The investigation aimed to manufacture and compare these two modifications to the odontogenic differentiation of SCAP. Dex-CS CSNPsI led to a

more delayed release of Dex in comparison with Dex- CSNPsII, although both confirmed continued discharge of Dex for 4 weeks. Unlike the results from the previous study, Dex-CSNPsII presented meaningfully greater ALP gene expression than Dex-CsnpI. Also Dex-CSNPsII meaningfully presented more favorable Biomineralization of SCAP and odontogenic differentiation compared with Dex- CSNPsI. Collectively, these pieces of evidence propose that maintained discharge of Dex results in improved odontogenic differentiation of SCAP [51]. Regardless of some differences between the two studies, these studies illustrated chitosan nanoparticles as a potential bioactive controlled-release scaffold in regenerative endodontic.

Then Shrestha *et al.* designed a study to assess the influence of dentin conditioning materials on adherence, viability, and differentiation of SCAP on root dentin in contact with endodontic irrigants. Slab-shaped dentin samples were provided parallel to the root canal and administered with 5.25% sodium hypochlorite (NaOCl) for 10 minutes and/or 17% EDTA for 2 minutes. The researchers accomplished dentin conditioning in this order: (1) no nanoparticle therapy, (2) CSNPs, (3) Dex- CSNPsI, and (4) Dex- CSNPsII. This investigation concluded that CSNPs, DEX-CSNPsI, and DEX- CSNPsII may hold the capability to reduce the lack of SCAP viability and adherence in root canal systems that have been disinfected with NaOCl and could prepare a better local environment in regenerative endodontics [52]. Also, chitosan NPs role in releasing factors was studied in other research by Bellamy. The aim of this research was to produce and identify a novel modified chitosan-based scaffold including transforming growth factor (TGF)-β1–releasing chitosan nanoparticles (TGF-β1-CSNPs) to improve migration and differentiation of SCAP. The scaffold showed properties that stimulate ECM. The combination of TGF-β1 with CSNPs provided a

continued discharge of TGF-β1, presenting a crucial concentration of TGF-β1 at the proper time. TGF-β1 bioactivity was continued for up to 4 weeks. Higher viability, immigration, and biomineralization of SCAP happened in the attendance of TGF-β1-CSnp than in free TGF-β1. These investigations indicated the ability of carboxymethyl chitosan-based scaffold with growth factor releasing nanoparticles to encourage immigration and differentiation of SCAP. [53] In 2017, a study was carried out to assessed and analyze cytotoxicity and apoptotic changes caused by propolis, chitosan, and nanoforms of them on DPSCs. Several studies have studied the antimicrobial effect of propolis and CS, not yet their biocompatibility on DPSCs. The authors provided aqueous and ethanolic extract of propolis, chitosan, propolis nanoparticles, and chitosan nanoparticles. Higher cell viability and lower DNA fragmentation were observed in the presence of both nanoparticles compared with their unique form. The results of chitosan nanoparticles were affected by the type of vehicle, while time was the determinant influencing propolis nanoparticles. Consequently, both propolis and chitosan nanoparticles exhibited favorable biocompatibility and could be useful in endodontic regeneration [54]. An investigation compared the cytotoxicity and the biocompatibility of stem cells collected from human primary dental pulp, followed by culturing them in three different nanofibers scaffolds. These nanofibers scaffolds including polyhydroxy butyrate (PHB), PHB/chitosan, and PHB/chitosan/nano-bioglass (nBG) were prepared by electrospinning technique. The researchers evaluated Cell viability in 5 scaffold groups due to incorporation of mineral trioxide aggregate (MTA): (1) PHB (G1), (2) PHB/chitosan (G2), (3) the optimal PHB/chitosan/NBG (G3), (4) MTA, and (5) the G3 + MTA. Proper mechanical features and bioactivity were observed in the produced scaffold owing to the attendance of chitosan and nBG NPs. On the other hand, the results of

the evaluation of cell viability demonstrated that the scaffolds containing MTA and nBG nanoparticles had higher cell viability percentage in comparison with scaffolds without nanoparticles [55]. Therefore, combining polymeric nanoparticles like chitosan and non-polymeric nanoparticles like nano-bioglass could help in better properties in scaffolds.

In the following of chitosan nanoparticle roles in tissue engineering, a recent study in 2018 was carried out to evaluate crosslinked biopolymeric chitosan nanoparticle's effect on the reduction of compressive strain distribution in the post-instrumented root dentine. Root canal instrumentation caused a definite rise in radicular compressive strain distribution and caused tensile root strain in the orientation similar to dentinal tubules. The authors found out microtissue engineering of the root canal dentine among crosslinked biopolymeric chitosan nanoparticles solution reduced compressive strain distribution in the post-instrumented root dentine [56].

Gelatin is another natural polymeric nanoparticle that could be utilized in endodontic regeneration. In 2009 a study was conducted to synthesize 3D nanofibrous gelatin (NF-gelatin) scaffolds. Since collagen (type I) is the principal natural element of a normal dentin matrix, gelatin was chosen as the scaffolding material to simulate the chemical organization of collagen fibers in dentin matrices. The researchers illustrated that the biomimetic NF-gelatin scaffolds may afford more desirable surroundings for kinds of tissue engineering purposes [57]. Another research was carried out by the same team. They incorporated silica bioactive glass to the previous 3D scaffold. This study concludes that the NF-gelatin/SBG hybrid scaffolds provide a more desirable surroundings for DPSCs and are assuring applicants for dentin/pulp tissue regeneration [58]. Another scaffold was investigated by a group of authors, suggesting combining gelatin is a composite nanofibrous matrix made of

biopolymer blend polycaprolactone-gelatin (BP) and mesoporous bioactive glass nanoparticles. They found out this nano-matrix could be a helpful scaffold to seed HDPCs and dental tissue engineering purposes [59].

One subsequent naturally derived scaffold is Gutta-percha nanocomposites. In a study, three types of Gutta-percha (GuttaCore™, ProTaper™, and Lexicon™) was used to fabricate flat scaffolds by spin coating Si wafers. All three substances included an elastomeric polymer matrix and PI were packed with inorganic nanoparticles to supply mechanical qualities and radiopacity. They cultured DPSC on these substrates and observed that despite some primary obstacles in adhesion, tissue formation began after 21 days. Consequently, Gutta-percha nanocomposites may be a useful substance for pulp regeneration system [60].

Poly (l-lactic acid) (PLLA) is a common synthetic polymer that could be applied in nano-form and have the ability to participate in tissue engineering. Wang *et al.* (2010) investigated the odontogenic differentiation of DPSCs on nanofibrous (NF) PLLA scaffolds *in vitro* and *in vivo*. The compound of a phase separation procedure and a porogen leaching technique was employed in fabricating NF-PLLA scaffolds. The authors assessed novel scaffold properties through several tests. Consequently, the NF-PLLA scaffold promoted odontogenic differentiation of human DPSCs and dentin like tissue organization, confirming its ability for dental tissue engineering purposes. Besides, the study showed that the incorporation of BMP-7 and DXM properly improves the odontogenic differentiation than DXM solitary.[61] PLLA nanofibrous microspheres (NF-MS) are another potential scaffold for regenerative endodontics. Previous research assessed the efficacy of PLLA NF-MS as a cell delivery vehicle, in blending with PLGA microspheres for controlled BMP-2 discharge, and the development of odontogenic differentiation of

human SCAP. A combination of phase separation method and the emulsification procedure has been used to build NF-SMS. In conclusion, using injectable NF-MS encouraged odontogenic differentiation of human SCAP and dentin-like tissue organization both *in vitro* and *in vivo*, expressing their ability as dental tissue construction use. Also, this scaffold revealed a promising use in developing the regeneration of dentin tissues [62].

Xiangwei Li *et al.* incorporated vascular endothelial growth factor (VEGF) with heparin and then encapsulate it in heparin intermixed gelatin nanospheres, later immobilized in the injectable PLLA NF-MS [63]. Another research was conducted to produce a novel injectable cell carrier, nanofibrous spongy microspheres (NF-SMS), for dentin regeneration. NF-SMS were formed from a star-shaped poly (L-lactic acid)-block-polylysine (SS-PLLA-b-PLYS) copolymer. The authors assumed that the NF-SMS could improve the proliferation and odontogenic differentiation of human DPSCs, in comparison with NF-MS without pore formation and conventional solid microspheres (S-MS) with neither nanofibers nor pore formation. NF-SMS group demonstrated significantly better results in examined odontogenic factors. Besides, higher dentin-like tissue development was found in NF-SMS in comparison with NF-MS and S-MS. In sum, the NF-SMS has showed ability as an injectable cell vehicle for dentin regeneration.[64] A subsequent study carried out by the same team investigated the same NF-SMS used to carry hDPSCs inside the pulp cavity to reconstruct dental pulp tissues. Cleansed pulp cavities of rabbit molars were fulfilled by Hypoxia-primed hDPSCs/NF-SMS composites. After 4 weeks, the hypoxia group displayed meaningfully improved angiogenesis inside the pulp chamber and superior production of odontoblast-like cells lining along with the dentin-pulp junction, in comparison with the control groups including

hDPSCs alone, NF-SMS alone, and hDPSCs/NF-SMS group pre-planted under normal circumstances. Also, it showed a histological structure like the natural pulp. Concluding from the results, NF-SMS could be used as a proper carrier in regenerative endodontic therapy [65].

Several research groups have been exploring for scaffolds that have further promising effects in cell arrangement and structure than the normal blood clot [66]. Generally, the destruction of residual infection was obtained by calcium hydroxide, triple antibiotic paste including ciprofloxacin (CIP), Metronidazole (MET), and minocycline or double antibiotic paste, including CIP and MET. This conventional disinfection materials have some disadvantageous such as root weakening and unfavorable effects on dental pulp cells [67]. An antimicrobial scaffold was fabricated and discussed in research by Palasuk *et al.* This investigation aimed to evaluate both the antimicrobial effectiveness and cytocompatibility of bi-mix MET and CIP antibiotic-carrying polydioxanone (PDS)-based polymer scaffolds. The scaffolds were characterized as a micro/nanofibrous arrangement with the attached pores. Antimicrobial efficacy was examined against *Enterococcus faecalis*, *Porphyromonas gingivalis*, and *Fusobacterium nucleatum*, and cytotoxicity was assessed on hDPSCs. The results indicated that the inclusion of multiple antibiotics in a nanofibrous scaffold could be a desirable drug delivery method for regenerative endodontics [68]. Further study in 2015 was conducted to assess drug release and the impacts on hDPSCs generation as well as viability of same bi-mix antibiotic-carrying scaffolds that fabricated through electrospinning. Control groups in that study were pure PDS scaffolds and a saturated CIP/MET solution. A sharp increase in CIP and MET releasing was observed in the initial 24 h. although drug(s) concentration was maintained in stable amounts for 14 days. In conclusion, antibiotic-carrying scaffolds

supported meaningfully less cytotoxic impacts on dental pulp cells in comparison with the clinically used saturated CIP/MET solution (DAP). Moreover, the results showed decreased cell proliferation and viability owing to raising the CIP concentration [67]. They also found that CIP not only supported the antimicrobial attributes but also diminished the negative effect on hDPSCs viability/proliferation [69].

Shiehzade *et al.* (2014) investigated poly (lactide-co-glycolide)-polyethylene glycol (PLGA-PEG) nanoparticles as an injectable scaffold for SCAP. This study was conducted in cases with large periapical lesions. These three case reports represent the treatment of necrotic or immature teeth with periradicular periodontitis, not treated with routine apexification procedures. Consequently, adverse effects were not observed on the tissues around the injured tooth using the PLGA-PEG scaffold, and periapical bone reconstruction was hastened within 6 months while the tooth continued to function. Minimum radiographic sign of continued root development in the length and canal wall thickness was noted in these cases that showed the PLGA-PEG scaffold could induce apexification at the same time with helping periapical healing. These results indicate PLGA-PEG nanoparticles as a potential scaffold applying in RET [70].

Conclusion

The data obtained from the studies reviewed earlier have illustrated that the new concepts like nanotechnology could play an impressive role to improve the upcoming of endodontic treatment. In this paper, we reviewed the applications of polymeric nanoparticles in various sections of endodontics, from canal irrigation to novel topics like tissue regeneration. Different polymeric NPs have different cons and pros but the point is the superiority of nanomaterials in comparison with conventional ones. Despite several

studies about these materials, clinical and *in vivo* studies are recommended in this field to reach more promising results. The future perspective of endodontic seems very promising by using these novel nanomaterials.

Acknowledgements

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