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Mini Review

Nanoparticles in Regenerative Endodontics: A Current Status Review

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Nanotechnology has significantly evolved in the past few decades, compelling several possible applications in various biomedical fields. There have been noteworthy developments seen in the nanoparticles' utilization in endodontics due to their unique nanoscale dimensions, generating remarkable interest in dentistry. They hold many divergent properties that may augment the endodontic infection's treatment, such as enhanced reactivity and antibacterial activity, and their ability to be functionalized with other reactive compounds. In the search for the "ideal" material, there have been innumerable experimental research on materials incorporated with nanoparticles and devices coated with the same. Nanoparticles' use in the field of regenerative endodontics shows a very promising prospect as they are known for bioactive molecule's release support system and amplifying the scaffold's biophysical properties. Applying nano-scaffolds for pulp regeneration is another use of nanotechnology in endodontics that created a stirring development in the reconstruction of pulp structure. The persistently expanding research in this area has led to an enormous prospective of nanoparticle's translational applications in regenerative endodontics. This review article aims to study the application of nanoparticles in different stages of regenerative endodontic therapy and provide an overview of its current advancement.

Introduction

The technological leap in the field of nanotechnology brought about the development of nanoscaled controlled materials in the past decade making it the next new rage. It has led to its applications in numerous fields from chemistry to biology, optics, electronics, energy, and communications [1]. Regenerative dentistry denotes a new method including bio-materials, mesenchymal stem cells(MSCs), and biomimetic molecules, partly acquired from tissues of the oral cavity [2]. Regenerative endodontic procedures are defined as "biologically based procedures that are designed to replace injured structures that include dentin and root structures, and also cells of the pulp-dentin complex". Dr. B.W Hermann described in one of his case reports regarding regenerative endodontic procedures following the use of calcium hydroxide for vital pulp amputation. This led to what many believe to be, the origin of Regenerative Dental Surgical procedures in 1952. Immature teeth with pulpal necrosis can be treated with regenerative endodontic procedures, which form a suitable alternative to conventional endodontic treatments [3]. Iwaya and the team were the first groups to use the term "revascularization". Thereafter, the term revitalization was adopted over revascularization since the tissues that regenerated in the canals included hard and soft tissues as well, along with the vascular tissues. In 2007, the American Association of Endodontists introduced the term "regenerative endodontics" built on the idea of tissue engineering. Biomimetic scaffold, stem cells (SC), and bio-active growth factors are considered the triad of tissue engineering and are applied in the root canals as part of Regenerative endodontics, to regenerate the damaged dental pulp due to infection, injuries, or abnormalities in the root canal development [4]. European Society of Endodontology (ESE 2016) issued a position statement where the term 'revitalization' was used. Revascularization, revitalization, and regenerative endodontics are terms utilized interchangeably and synonymously in the endodontic literature. The term nanotechnology was employed for the first time by Taniguchi in 1974 [2]. Nano-technology is a broad scientific field that consists of several fields and is directly concerned with the management of structures at atomic-scale or nanometers in at least one dimension [5]. Though nanotechnology's presence returns to the beginnings of the time, as for its format of synthesizing various molecular

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structures in the body; its discovery is ascribed to Dr. Richard Phillips Feynman, an American physicist, due to his outlook on several subjects as accommodation of data on a very tiny scale, miniaturization of the computer, or producing small devices [2]. Nanomaterials can be synthetic, natural, or incidental, comprising of particles aggregates, agglomerates, or in unbound states, with exterior dimensions of 1 nm to 100 nm for 50% or more of the particles [6]. Particles with a size of 5-100 nanometers are more commonly utilized in nanomedicine and nanodiagnosis. The high surface-to-volume ratio of nanoparticles is related to their distinct properties, which include biochemical, optical, magnetic, and electronic alterations at the molecular, cellular, and atomic levels, proving their significant biomedical utility. Biomaterials-based nanoparticles are gaining traction because they have the ability to transform the current medical care setup by enhancing their performance [7]. Because of their amplified and distinct physicochemical features, including ultra-small size, significant surface-area to mass ratio, and elevated chemical reactivity, nanomaterials have promising outcomes in antibacterial and regenerative therapies. The terminology “nano dentistry” was first used around the beginning of the twenty-first century. Since then, nanotechnology has been applied in a wide range of clinical dentistry procedures, including direct restorative materials, dental prosthesis materials, guided tissue regeneration, periodontal treatment, implant surface modifications, and also in endodontic treatment. Throughout the last ten years, researchers have been paying special attention to the use and application of nanoparticles in regenerative endodontics. Since its inception, the shared comprehension of this area has heightened significantly, with current research studies persistently annexing the existing body of knowledge [6]. Nanoparticles could provide new treatment and prevention techniques for dental infections and provide regeneration of pulpal tissues and pulp dentin complex [8]. 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 [2]. As a result, the purpose of this review article is to consolidate, integrate and summarise existing studies on nanoparticles’ potential translational applications in regenerative endodontics [6].

Nanoparticles

Nanomaterial is defined as a “material with any external dimensions in the nanoscale or having an internal structure or surface structure in the nanoscale (1-100nm)” as given by the International Organisation of Standardization [9]. Biomaterials-based nanoparticles and nanostructured materials have assumed greater importance in technological advancements and received notable attention as they hold promise to revolutionalize medical treatment due to their tunable physicochemical characteristics making them less toxic, more potent, and with their smart therapeutics resulting in enhanced performance [7,10]. Polymeric nanoparticles are conventionally divided into 3 components, that is the outermost (surface) layer which can be functionalized with other active agents; the second layer which is the shell layer (intermediate layer), and the core which is the innermost and the most important material (figure 1) [11].

Categories of Nanoparticles

Nanomaterials can be generically classified as natural and man-made based on their composition [12]. Nanoparticles can be sub-grouped predominantly into numerous categories based on their size, chemical properties, and morphology. Based on the morphology they can be categorized into cubical, spherical, or needle-shaped particles within the nanoscale range [13]. Nanoparticles presently in function are nanopores, carbon nanotubes, nano-capsules, nano rings, nanospheres, and dendrimers [14]. Some well-known classes of nanoparticles are based on physical and chemical characteristics are given in figure 2.

Mechanism of Action

It was discovered that nanoparticles coated onto biomaterial surfaces or coupled with polymers have an improved antibacterial capability in the oral cavity [8]. This included improved reactivity, solubility, biomimetic properties, and the capacity to be able to be functionalized with other materials such as medicines, bioactive compounds, and photosynthesizers [1,11,15]. Moreover, antimicrobial nanoparticles have better penetrating capability into biofilms, are more powerful at lower doses, and may assist to reduce the overuse of antibiotics [16]. Nanoparticles interact with the cell wall of bacteria electrostatically and enter the biofilm, causing damage to the cell membrane, increasing cell permeability and formation of reactive oxygen species, encroachment with cellular activities, protein degradation, DNA damage, and, eventually, cell death [13,16,17]. Given their numerous intrinsic benefits, the prospective use of nanoparticles in general health and dentistry has

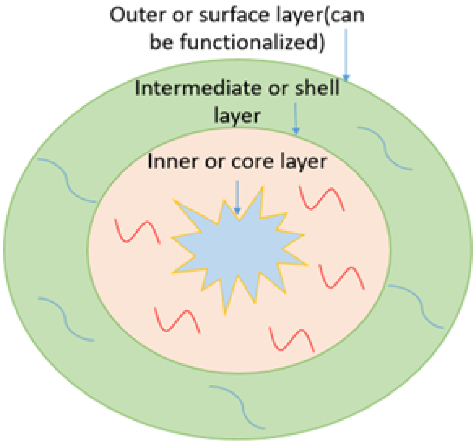

Figure 1: Structure of polymeric nanoparticles

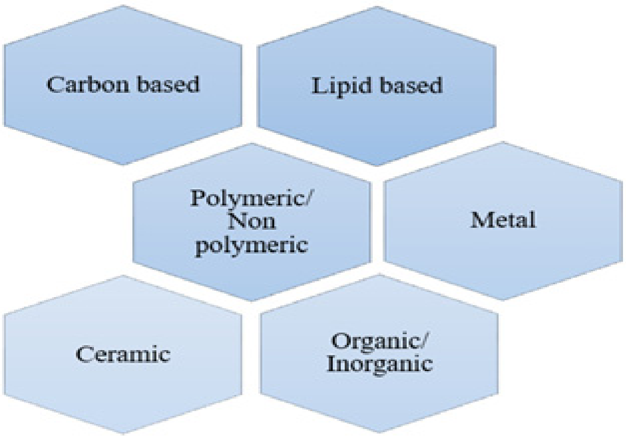

Figure 2: Categories of nanoparticles

sparked considerable interest [6]. For delivery of the drugs, nanoparticles encapsulate or covalently bind to the hydrophobic drugs, significantly enhancing their solubility in an aqueous solution. They have the capability to encapsulate bio-macromolecules (like proteins, peptides, and nucleic acids) or hydrophilic compounds to improve their in vivo stability, increase their time of blood circulation, and allow their transmembrane administration. The diameter of nanoparticles being on the nanoscale (10^{-9}) enables specific tumor targeting through the increased retention (EPR) effect and permeability, significantly minimizing the toxicity of non-specific chemo drugs. Furthermore, delivering the contrast agent or imaging probe to the target tissues or cells, showed high stability and provided a stage for highly sensitive and selective molecular imaging. Various ligands or antibodies can be grafted onto the surfaces of the nanoparticles to improve targeting efficacy and specificity. On the other hand, some nanoparticles have intrinsic imaging or therapeutic qualities, particularly inorganic nanoparticles with desirable optical properties. By themselves producing imaging contrast and mediating photothermal treatment through photon-to-heat conversion, certain nanoparticles, such as superparamagnetic iron-oxide nanoparticles or gold nanoparticles might permit multimodal imaging [7].

Applications in regenerative endodontics

Due to various restrictions and concerns regarding the transfer from laboratories and animals, new methods need to be researched for it to be delivered in an environment that directly regenerates tissues. The process of stem and progenitor cell propagation, types of scaffolds, signaling mechanisms, differentiation, and growth all without causing any changes in the genetic make and tissue toxicity can be very overwhelming. The human cells are over and above the nanoparticle's size normally manufactured making it easily penetrable. Incorporating them into restorative materials will eliminate the chances of microleakage and reinfection thus halting the need for pulp protection [18]. Numerous applications of nanoparticles were seen in regenerative endodontics such as the growth of new tissue using various nanomaterials and nanoscaffolds.

Regenerative endodontic stratergies

Regeneration and repair form a strong link with embryonic SC growth for restoring and sustaining its original structures and functions in endodontics [12]. More researchers are turning their attention to regenerative medicines as a result of Banchs and Trope's groundbreaking report. Removal of the infection, stimulating the closure and development of immature root apices, and re-establishing pulpal vitality, are the techniques attempted to reinstate the functionality of the tooth [6]. The three most pivotal steps for endodontic regeneration include disinfection and elimination of the microorganisms of the damaged tissues, control of inflammation at various levels, and regeneration of lost pulpal tissue through the development of a collagen matrix (type I) with innervation, vascularization, fibroblasts and also with the odontoblastic layer. Endodontic regenerative active biomaterials must encourage proliferation and colonization of various capable cells at the tissue injury for this final step. The status of the tooth pulp, the stage of inflammation, and the amount of injured and infected tissues all influence regeneration strategies [19]. Regenerative endodontics is based on biological techniques and tissue engineering utilizing bioactive compounds, SC, and also scaffolds. One of the most important entrusted features of regenerative endodontics is formed by nanoparticle-based carrier systems which form a method for prolonged release of bioactive molecules which balances cellular activities like proliferation, migration, and differentiation. Improvement in the dissolution, bioactive molecule, and medications absorption is seen owing to their increased solubility, high surface-area-to-volume ratio, and smaller dimensions. Various research were tried and tested. Dental pulp stem cells (DPSC) were implanted on nano-hydroxyapatite and nanofibrous poly (L-lactic acid) scaffolds which showed increased odontogenic differentiation both in-vivo and in-vitro of the same [20,21]. Smith et al. and various other teams of researchers led to the examination and development of nanostructured polymer scaffolds [22]. These teams lead to the establishment of the fact that the structural features of the tissue-engineered scaffolds should lead to cell response and must support cell adhesion, proliferation and differentiation. Tests were formulated with various components identical to synthetic extracellular matrix in combination with nanofibrous scaffolds which in turn interacted with cells forming new tissues. Ultimately their group led to the development of a biodegradable polymer of nanofibers of the same diameter and size as collagen fibers found in the extracellular matrix i.e 50-500 nm. A change in quantity and schedule of the apatite crystals was brought about while growing them onto biodegradable polymer scaffolds. In an attempt to improve cell adherence, proliferation, and differentiation ability of the cell numerous researchers are testing formulations of these crystals throughout 3-dimensional nanofibrous and nanocomposite scaffolds. It was shown to be possible with differentiation and growth factors [12]. Scaffolds, which are provisional structures that resemble the extracellular matrix have also been modified and created by various nanoparticles which help for the differentiation and growth of stem cells allowing the medications and bioactive substances to be released in a regulated manner [23]. They can also be paired with nanocarriers to allow for the release of numerous bioactive molecules [24]. In a study done by Gupte et al, highly permeable nanofibrous poly-L-lactide scaffolds with and without the utilization of dexamethasone and bone morphogenic protein 7 were tested. Their study concluded that the manipulation of both dexamethasone and bone morphogenic protein 7 together induced differentiation of DPSCs more effectively than dexamethasone alone, making it a high-quality environment for regeneration of dental pulp, enamel, and dentin by the DPSCs [12,25]. Development of new tissue for regeneration and repair could be brought about by the fabrication of an engineered replica of a naturally occurring extracellular matrix [12]. In a study done by Yang et al, DPSCs in-vivo and in-vitro behavior on various scaffolds like poly(epsilon-caprolactone) (PCL)/gelatin scaffolds with or without the addition of nano-hydroxyapatite were tested. The in vitro evaluation revealed alkaline phosphatase activity, osteocalcin levels, and DNA content revealed that these scaffolds aided the adhesion, odontoblastic differentiation, and proliferation of the DPSCs [12,20]. Both in vivo and in vitro studies concluded that the nanohydroxyapatite crystals along with nanofibres were found to increase the DPSCs differentiation into odontoblast-like cells [12]. Bovine serum albumin-loaded chitosan nanoparticles boosted the viability and alkaline phosphatase activity of SC from the apical papilla [26]. SC from apical papilla's odontogenic differentiation was enhanced by dexamethasone-loaded chitosan nanoparticles (CN). Conditioning of dentine with CN or dexamethasone-modified CN has the potential to reduce the negative effects of sodium hypochlorite and lipopolysaccharides while also increasing SC from apical papilla's survival, differentiation, and adherence [27,28]. It has been observed that dexamethasone condensed in poly (å-caprolactone)-forsterite nanocomposite fibrous membranes promotes osteogenic differentiation and proliferation of stem cells from exfoliated human deciduous teeth [29]. It was reported by Bellamy and his team that transforming growth factor-β1-loaded CN nanoparticles along with carboxymethyl CN-based scaffold were

Table 1: Commonly used nanoparticles in regenerative endodontics

Nanoparticle/Nanomaterial	Site of Action*	Mechanism of Action
Nano-hydroxyapatite and nanofibrous poly (L-lactic acid) scaffolds with DPSCs	Dental pulp	Showed increased odontogenic differentiation
Nanofibrous poly-L-lactide scaffolds with dexamethasone and bone morphogenic protein 7; along with DPSCs	Dental pulp	Regeneration of dental pulp, enamel, and dentin
Bovine serum albumin-loaded chitosan nanoparticles	Dental pulp	Apical papilla's odontogenic differentiation was enhanced
Dexamethasone condensed in poly (ε-caprolactone)-forsterite nanocomposite fibrous membranes	Dental pulp	Promotes osteogenic differentiation and proliferation of stem cells
Transforming growth factor-β1-loaded CN nanoparticles along with carboxymethyl CN-based scaffold	Dental pulp	Increased the viability, differentiation, and migration of SC from apical papilla
Bioactive glass nanoparticles to scaffolds		Enhancement of mechanical characteristics, mineralization, and bioactivity through the deposition and release of calcium in hard tissues

*based on in vivo and in vitro studies

seen to boost the viability, differentiation, and migration of SC from apical papilla by getting the benefit of chitosan's "swell to fit" configuration adaptability property at any given site and its preparedness to be conjugated with other molecules [30,31]. The inclusion of bioactive glass nanoparticles and dexamethasone into a nanofiber scaffold system has also been shown to inflate the DPSC's odontogenic differentiation [32]. The introduction of bioactive glass nanoparticles to scaffolds may improve mechanical characteristics while also encouraging mineralization and bioactivity through the deposition and release of calcium [33]. A study was done on reinforced hydrogel scaffolds using cellulose nanocrystals, resulting in enhanced stiffness and stability. Platelet lysate was also added to reinforced hydrogel as they are high in proangiogenic and chemotactic factors that improved the ability of pulpal tissue revascularization and regeneration [34]. Nanoparticles have also been used in unique regenerative outcome assessment methodologies. In a study done by Biz and his team, gold nanoparticles were combined with biodegradable organic L-lysine. They were used to make compounds that SC could easily absorb, which in turn increased the radiopacity of the cells which allowed microtomography to detect the existence of feasible cells after regeneration procedures with no obvious cytotoxic effects [35]. It was established through in-vitro and animal study models that for disinfection and the stimulation of tissue regenerative process bioactive molecule releasing nanoparticles were effective.The commonly used nanoparticles in terms of research regarding regenerative endodontics are summarized in table 1 [27].

Conclusions

This review article aims to acquaint the readers with a detailed overview of the potential translational applications of nanoparticles, their types, physiochemical properties, applications, and their advances in regenerative endodontics. Nanoparticles range from a few nanometers to about 500 nanometers. Their morphology is controllable, making them useful in a broad-spectrum range. The reduced size of nanoparticles gives them a greater surface area, making them a good contender for a variety of applications [11]. They may be produced for a variety of applications in endodontics, including disinfection techniques, photodynamic therapy, obturation materials, and regenerative procedures, according to the present literature. The data obtained from the studies reviewed earlier have illustrated that these new concepts could play an impressive role to improve the upcoming endodontic treatment. Different nanoparticles have different pros and cons but they provide superiority in comparison with conventional ones. Despite several studies about them, clinical and in vivo studies are recommended for their further uses on day to day basis. The future perspective of regenerative endodontics seems very promising by using these novel nanomaterials [18]. Stimulation of SCs' differentiation, proliferation, and migration is served by a nanoparticles-based carrier system. It also serves as a temporary structure known as a scaffold which promotes the SC's growth and differentiation [12]. Due to the complicated and diverse anatomy of the tooth along with the poor approachability of the object of interest, Endodontics continues to remain one of the most taxing fields of specialization in dentistry [36]. With groundbreaking developments of nanotechnology in dentistry, it also comes with certain concerns, which include ethical issues, issues with biocompatibility, human safety, economic mass production of robots, and its expertise [12]. Because different materials, formulas, and combinations will produce varied qualities, both useful and detrimental, the versatility of nanoparticles should be used with caution. As a result, the capability of the nanoparticle's use in regenerative endodontics is immense, but there are still miles to go before they are translated into clinical investigations [6].

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