Mini Review

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Nanotubes reinforcement of degradable polymers for orthopedic applications

Abstract

Biodegradable polymers have been studied as scaffolds for bone tissue engineering applications. However, they possess insufficient mechanical properties to be considered as alternatives at sites of increased mechanical loading. To improve their mechanical properties, reinforcing with nanotubes has been investigated, as they reproduce the structure and length scale of bone native topography. The research advances in the use of nanotubes as reinforcing agents in developing nanocomposites with synthetic and natural biodegradable polymers are reviewed. The extent of mechanical reinforcement of the polymers and the corresponding biological response of the nanocomposites are presented.

Keywords: nanotubes, polymeric composite materials, bone tissue engineering, orthopedic applications

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Abbreviations: BNTs, boron nitride nanotubes; CNTs, carbon nanotubes; CS, chitosan; HA, hydroxyapatite; HNTs, halloysite nanotubes; MWCNT, multi walled carbon nanotube; PCL, polycaprolactone; PEO, polyethylene glycol; PET, polyethylene terephthalate; PGA, poly glycolic acid; PLA, poly lactic acid; PLLA, poly-l-lactic acid; PMMA, poly methyl methacrylate; PPF, poly propylene fumarate; PP, polypropylene; SWCNT, single walled carbon nanotube; TiNTs, titanate nanotubes

Introduction

Scaffolds for regenerative medicine provide a suitable substrate for the initial adhesion of cells, their proliferation, migration, and the differentiation process for each specific tissue. Thus, it is necessary to have certain properties in order to perform their role. In particular, scaffolds intended for bone tissue engineering should exhibit appropriate mechanical behavior, suitable shape and geometry.

Biodegradable polymers have been studied as scaffolds for bone tissue engineering applications.[1–4] Moreover, they are potentially a good alternative to the traditional metallic fracture fixation devices. Mismatch between the elastic modulus of bone and the metal implant leads to stress shielding in the surrounding bone tissue, resulting in absence of mechanical stimulus to the regenerating bone. However, they possess insufficient mechanical properties to be considered as alternatives at sites of increased mechanical loading.

Several strategies for improving the mechanical properties of polymers have been reported with a main direction to develop nanoparticle reinforced-biodegradable polymeric composites. A focus is to use nanotubes, which reproduce the structure and length scale of bone native topography and may act as an exceptional substrate for cell growth and differentiation.[5,6]

The research advances in the use of nanotubes as reinforcing agents in developing nanocomposites with synthetic and natural biodegradable polymers are reviewed. The extent of mechanical reinforcement of the polymers and the corresponding biological response of the nanocomposites are presented.

Degradable polymers for hard tissue engineering applications

In many cases human body needs a device, such that biocompatible and biodegradable polymeric materials are better alternatives than biostable ones. Moreover, the treatment of certain diseases aims not only to repair but also regenerate the damaged tissue. It has only recently become possible for scientists to construct complex two and three-dimensional tissue grafts in order to deal with diseases such as malformation, osteoporosis or damaged tissues. Actually, scaffolds, made of natural and synthetic polymers that they exhibit the structural properties of natural extracellular matrix, have been developed.[7] Some of the artificial polymers, used in hard tissue regeneration applications, are PLA, PGA and PCL.[2]

PLA is a widely used polymer in packaging and biomedical engineering applications.[8,9] It can be found in many forms,[10] with elastic modulus ranging from 0.35 to 4 GPa and tensile strength from 15 to 150MPa.[11] Its slow biodegradation rate can be attributed to the high transition temperature.[12] Osteoblasts' growth, differentiation and adherence were enhanced when cultured on PCL surfaces.[13,14] PCL is a biodegradable, biocompatible and non-toxic synthetic aliphatic polyester, produced by petroleum, with small manufacturing cost and easy manipulation.[15] Due to its excellent properties and its viscoelastic and rheological behavior, it has sparked the interest of researchers for use in biomedical engineering.[16]

Direct or indirect polymerization of glycolic acid can form PGA polymer. With approximately 6 GPa Young's modulus and 60-100MPa tensile strength,[11] PGA degrades faster than PCL or PLA in the human body,[10] Because of its appropriate mechanical and physical properties for use in bone tissue engineering, it has been approved by US Food and Drug Administration for certain applications.

PPF is also a biodegradable polymer with mechanical properties comparable to cortical bone and biocompatibility used especially in bone tissue engineering.[1]

Natural biopolymers such as collagen, fibrin, alginate, silk, hyaluronic acid, and chitosan are also highly biocompatible, ideal for tissue engineering scaffolds.[2]

CS is a linear polysaccharide and derivative of chitin, the most abundant polysaccharide in nature along with cellulose and starch. It is a multilayer polymer found in shellfish exoskeleton, such as crabs and shrimps. It is highly biocompatible and biodegradable, cationic stimulates haemostasis and accelerates tissue regeneration. This could be explained by CS's monomeric unit N-acetyloglycosamine, which occurs in hyaluronic acid being an important substance for wound reconstruction.[17]

Collagen has been tested in various regenerative studies because it exhibits remarkable similarities to the dominant protein which builds the extracellular matrix of bone. Collagen is biocompatible and bioactive, as it naturally allows cell adhesion and enhances migration and proliferation. It is a material that can be degraded, in particular by the enzyme, collagenase, and at the same time exhibits high tensile strength.

Alginate is a natural, non-toxic, biocompatible polysaccharide. It can be degraded at a fairly high speed in *in vivo* applications and exhibits a low mechanical rigidity. However, it can be strengthened with calcium, increasing its mechanical strength. In fact, the enhanced arginine-glycine-aspartic acid (RGD) alginate hydrogen allows cell adhesion, proliferation and differentiation. Alginate hydrogels provide a highly suitable matrix for dental applications.

Hyaluronic acid belongs to the class of glycosaminoglycans that make up the extracellular matrix of connective tissue. It is biocompatible but displays poor mechanical strength and rapid degradation rate *in vivo* which can be controlled in a specific way. At the same time, it is enzymatically degraded by hyaluronidase, which is easily processed by the human body. A composite of alginate and hyaluronic acid gel has shown improved properties. RGD peptides in hyaluronic acid hydrogel enhance cell adhesion, differentiation and proliferation just like alginate. Consequently, the construction of hyaluronic acid-based scaffolds leaves great prospects for regenerative medicine.

Additionally, silk scaffolds exhibit certain properties that favor the manufacture of scaffolds for application in clinical applications. More specifically, they are biocompatible, non-toxic, and enhance the proliferation of cells. It has been found that silk gel material is capable of generating a sustained three-dimensional increase in soft molecules, useful in periodontal and maxillary therapies.

Fibrin is a natural biological material that has many advantages over synthetic polymers when it comes to cost, biocompatibility and cell attachment properties. Unfortunately, fibrin is weak in strength, but this disadvantage can be overcome if combined with other materials such as hyaluronic acid. Its ability to form three-dimensional scaffolds makes it suitable for use in regenerative medicine applications.

Types of nanotubes-mechanical reinforcement and biological response

Nanotubular structures can form from a variety of different materials such as inorganic, carbon, synthetic polymers, proteins, carbohydrates, DNA, lipids,[18] for the production of bulk quantities of well-defined nanostructures. Here we review the types of nanotubes that have been used to reinforce degradable polymers and discuss the mechanical reinforcement and the biological response of cells, cultured on these nanocomposites.

Carbon nanotubes CNTs: CNTs are helical structures, approximately 1-30nm in diameter with lengths greater than100 nm. A range of CNTs have been refined including single wall nanotubes (SWNT), multi walled nano-tubes (MWNT), and functionalized nanotubes.[19] MWCNT has modulus and strength in the range 200-1000GPa and 200-900MPa, respectively, and even small amounts can improve the mechanical properties of polymers.[20]

In tissue engineering scaffolds, carbon nanostructures are implemented in order to improve the mechanical properties by increasing the Young's modulus and tensile strength of the structure. Furthermore, the combination of carbon nanomaterials with substrates supports cell attachment, growth and differentiation.[21] Cells, in their biological environment, interact with ECM components at the nanometer scale and nanoscaled biomaterials should have an effect on the cell functions.[22]

MWCNTs have been used to enhance the properties of polymers for bone tissue engineering, such as PLLA, using a phase separation/particle leaching method;[23] PET, using twin-screw extrusion;[24] PMMA using ultrasonic disintegration[25] and PCL using solvent using a solvent evaporation technique.[3]

CS's compressive modulus has been increased from 1 to 15MPa due to the presence of MWCNTs[26] Increase of Young's modulus at 77% and of ultimate stress at 60% has been achieved by Kroustalli et al.[27] Tensile strength of PCL/CNTs electrospun composite fibers can be enhanced from 3 to 8 MPa.[28] Higher modulus of elasticity at 7.1 % of the PLA/PCL/CNT nanocomposite has been achieved in comparison to the pure PCL.[29]

Moreover, it has been demonstrated that MSCs expand and spread on layers of MWCNTs.[30] MWCNTs can also induce osteogenic differentiation of MCSs.[31] Increasing the surface area can promote cell growth and migration. MWCNTs in CS/β- Glycerophosphate nanocomposites have increased the alkaline phosphatase activity in comparison to neat CS.[32]

Another important aspect of the use of MWCNTs for biomedical applications is the cytotoxicity they can cause to surrounding tissue. It has been proven that concentrations from 0.8-10μg/ml exposed to epithelial cells for 24 and 48h were non-toxic. It is suggested that such concentrations are safe for biomedical applications and also that cell viability can be altered by higher MWCNTs concentrations and in a dose dependent matter.[33]

Inorganic nanotubes

Titanium nanotubes-TiNTs: Titanium and titanium-based alloys are known for their high mechanical strength and corrosion resistance[34] and are widely used materials for orthopedic applications. Highly ordered nanoporous arrays of titanium dioxide that form on titanium surface by anodic oxidation are receiving increasing research interest due to their effectiveness in promoting osseointegration. On the surface of Ti substrates, a highly ordered TiO_2 nanotube array with dimensions in the sub-100 nm range can be formed, when it is anodized in fluoride-containing electrolytes.[35,36] TiO_2 NTs have proven effective in promoting bone formation. They can accelerate the growth of osteoblasts by as much as 300%-400% compared to non-anodized Ti.[37]

Titanate Nanotubes (TiNTs) are a relatively new class of nanotubes that have similar morphology to CNTs[38] but lower production cost, and their synthesis is carried out at far lower temperatures. Moreover, TiNTs compositions and structures may be practically unlimited

and, by functionalization, their adhesion to polymer matrices can be improved.[38]

A few studies have been performed for the use of TiNTs in reinforcement of polymer matrices. Reinforcement of a polymer composite film PEO/CS with 25 wt% content of TiNTs was found to be 2.6 times harder than the neat polymer blend and 3.4 times stiffer. Further addition of nanotubes had a negative effect on the mechanical properties due to a poor distribution and nanotube aggregation.[39]

Silanized titanate nanotubes have been found to significantly increase the glass transition temperature and the modulus in the rubbery state of epoxy-based nanocomposites. A smaller addition (0.19-1.52wt%) of the nanofiller provided a larger increase, as larger added amounts form smaller and larger aggregates. It was also found that TiNTs coating biodegradable photopolymer scaffolds affect cell viability and proliferation, demonstrating that TNT coatings enhance cell growth on the scaffolds by further improving their surface topography. TiNTs, used to reinforce PCL 3D scaffolds, resulted in enhanced mechanical properties suitable for load baring applications.[40,41]

Enhanced interaction and strong adhesion between the functionalized n-TiO_2 tubes and polymer matrix (resin cements) allows external mechanical stress to be more effectively transferred through the filler-matrix interface. This novel filler in conjunction with the existing ones can be used to reinforce orthopedic and dental cements as well as flowable dental composites.[42]

Titania nanotube arrays, coated by biocompatible polymers, CS and PGA, have been also used for improved drug elution and osteoblast adhesion.[43] Excellent osteoblast adhesion and cell proliferation on polymer-coated TNTs compared with uncoated TNTs were also observed.

Silica nanotubes: Another type of NTs used in biomedical applications is silica nanotubes. Silica nanoparticles are considered to be stable and their size, shape and porosity can be controlled.[44] This type of nanotubes is also known for being biocompatible, hydrophilic and to have a large specific surface area.[45] All these properties and its high mechanical strength make this material a promising choice for bone tissue engineering fabrication. Silica nanotubes have been use to reinforce PMMA polymer matrix and the results showed that the composite material had greater mechanical properties.[46] It has been shown[47] that scaffolds fabricated using silica nanotubes supported osteoblast cell spreading and proliferation.

Zirconia nanotubes: Zirconia oxide ZrO_2, so-called zirconia, has remarkable mechanical and chemical properties, as well as a high degree of biocompatibility.[48] For these reasons, zirconia is widely used in dental applications and in orthopedics, such as in femoral head prostheses and generally in aesthetic outcome of implant-supported rehabilitations.[49] Recent studies focus on the synthesis of zirconia nanotubes (ZrO_2 NTs,[48,50,51] with a diameter from 50 to 130 nm and a length from 17 to 190μm.

According to Yu et al.,[52] ZrO_2 nanotubes display a better reinforcement effect in PMMA matrix than ZrO_2 nanoparticles. More precisely, the composites reinforced with ZrO_2 nanotubes had higher elastic modulus and flexural strength, compared with the ZrO_2-nanofillers reinforced composites.

Tungsten disulfide nanotubes-WSNTs: Recently, inorganic nanomaterials such as tungsten disulfide nanotubes (WSNTs) have been used as reinforcing agents to improve the mechanical and tribological properties of epoxy composites, electrospun poly (methyl methacrylate) fibers, and biodegradable PPF nanocomposites.[53–55] WSNTs have excellent mechanical properties (Young's modulus≈150GPa, bending modulus≈217GPa), The advantage of WSNTs in comparison to CNTs is that they possess functional groups, such as sulfide and oxy-sulfide, and can be readily dispersed in organic solvents, polymers, epoxy and resins.[56] Due to these potential benefits, the efficacy of WSNTs as fillers to improve the mechanical properties of biodegradable polymers used for bone tissue engineering needs to be investigated. It has been found that very low amounts (0.01-0.2 weight %) of WSNT, used to reinforce a biocompatible and biodegradable polyester, poly (propylene fumarate) (PPF), significantly increased the mechanical properties and the dispersion of the nanomaterial in the matrix in comparison to carbon nanotubes and neat polymer.[1]

Boron nitride nanotubes: The Boron Nitride nanotubes are characterized by the same exceptional mechanical properties, but better chemical resistance than CNTs.[56] More precisely, the axial tensile Young's modulus of BNNTs was found to be 1.18TPa,[57] which is comparable with the 1.33TPa of CNTs. Polymeric, ceramic and metallic matrices[58–61] have been reinforced with BNNTs and their mechanical properties has been remarkably improved.

When BNNTs are used as fillers in a hydrohyapatite matrix, 120% increment in elastic modulus, 129% higher hardness and 86% more fracture toughness were achieved.[62] Further, the osteoblast proliferation and viability were not influenced by the presence of BNNTs in the HA matrix. 1370% increase of Young's modulus was noticed of PLC co-polymeric matrix.[63] The tensile strength was also increased at 109%. In addition, the levels of the main regulator of osteoblast differentiation, Runx2 gene, were increased. According to these studies, the BNNTs are undoubtedly a potential reinforcement for composites for tissue engineering and orthopedic applications.

Halloysite nanotubes-HNTs: Halloysite nanotubes (HNTs) are composed of double layer of aluminum, silicon, hydrogen and oxygen. These unique and versatile nanomaterials are naturally formed in the earth over millions of years. Their diameters are less than 100 nm and their length can vary from 500 nm to over 1.2μm.[64] Their modulus of elasticity ranges between 230 to 340GPa.[65]

Recent studies have demonstrated the importance of HNTs in the improvement of the mechanical properties of polymer matrices. HNTs increased both the Young's modulus and the tensile strength of the chitosan matrix at 134% and 65%, respectively.[66] High structural performance in low cost was also observed by the teams of Olugebefola et al.[67] & Jia et al..[68]

The cellular response on polymeric-matrix, reinforced with HNTs, composite materials has been investigated by Zhou et al..[69] Osteoblasts' attachment has been enhanced thanks to HNTs, according to the *in vitro* study. The biocompatibility of the manufactured composites has also been proved by fibroblasts behavior.

Peptide nanotube: Biological proteins and peptides have the intrinsic ability to self-assemble into nanotubes, defined as an elongated nano--object with a definite inner hole elongated solid nanofibrils, which have attracted research interest as key components for nanotechnology.[18]

Peptide nanotubes have been constructed, using β-amyloid peptide, by Hartgerink et al.[70] As a result, chemical stable nanotubes

with elastic modulus of 19GPa have been achieved. It should be mentioned that, although the elastic modulus is not as high as in the case of CNTs, peptide nanotubes can be categorized in the stiffest biologically-based materials.[70]

In the research of Rubin et al.,[71] D,L-cyclic peptide has been used as the base bundle for the synthesis of peptide nanotubes. The nanotubes reinforced a polymeric matrix and led to more than 5-fold stiffer composite materials.

Peptide nanotubes are also synthesized in the study of Alam et al..[72] *In vitro* tests of the materials on mammalian cell lines and normal blood cells showed non-toxic activity and potential use in malarian drug delivery field.

Discussion

In the process of developing new materials, researchers have come up with methods to combine different materials with distinct characteristics. In biomedical science, composite biomaterials have been used extensively, as they enable the blend of different properties. Nanoscale materials, in the context of composites, are being increasingly considered for tissue engineering given the unique properties acquired by matter when their dimensions are at the nanoscale.[73]

Bone ECM is a composite structure with two major constituents: organic collagen and inorganic hydroxyapatite. The two constituents of bone are hierarchically organized in multiple scale lengths. At the nano scale, human cancellous bone is composed of arrays of parallel fibrils of type I collagen along which crystals of hydroxyapatite are aligned.[74] The structural and dimensional characteristics of CNTs create a biomimetic analog for the proteins of the ECM, which have comparable length scales with CNTs (diameters of CNTs are in the range of 1-100 nm and lengths of several micrometers; diameter of the collagen molecule is 1.5nm and its length is 300nm.[74,75] For this reason, various types of nanotubes have been investigating to create cell supporting environments.

Reinforcing of polymers with nanotubes, possessing high strength and high modulus of elasticity, creates an important class of lightweight materials, which are characterized by excellent specific mechanical properties. In order to succeed effective reinforcement of polymers, high interfacial strength and homogeneous dispersion of the nanotubes in the matrix, to overcome the sliding between them and increase of bonding between NTs and polymer matrix, are necessary.

Low loading concentrations of CNTs and other NTs are implemented for polymer reinforcement. This has to do with the difficult dispersion of CNTs due to strong vander Walls interactions and π-π stacking. Pristine CNTs create aggregates in the form of bundles, ropes, and networks in the polymeric matrix resulting in absence of interaction between them and stress concentration. To fully exploit the properties of CNTs as fillers in polymer matrices, their chemical modification is necessary. A simple way to disperse the CNTs in the polymer solution is the use of surfactants, like sodium dodecyl sulphate[76] or the addition of carboxyl, hydroxyl, amino or alcohol groups. Another way to increase the reinforcement of polymers is the use of mechanically interlocked derivatives of single walled carbon nanotubes (SWNTs). Formation of mechanically interlocked derivatives of SWNTs can be achieved by templating the ring-closing metathesis of the U-shaped precursor around the nanotubes.[77] On the other hand, Tungsten Disulfide Nanotubes possess functional groups, and can be readily dispersed in polymers without further functionalization.[1]

Bulk crystalline TiO2 is a relatively durable compound, which tends to react only with strong acids or bases under heating. In contrast, nanostructured TiO2 and protonated titanates tend to be much more reactive due to their increased surface area. Functionalization of TiNTs is often necessary to improve adhesion with matrix, e.g. functionalization with poly dopamine (PDA) improves the interfacial compatibility between the polymer matrix and inorganic material.[38] Silicon(Si) doped into the titanium dioxide nanotubes on Ti surface, using plasma immersion ion implantation, significantly enhanced the expression of genes related to osteogenic differentiation and improved bone-Ti integration.[78] Ion exchange of one-dimensional titanate nanotubes (TiONTs) can alter their biological activities. It has been found that Ag ion exchanged TiONTs exerted potent antibacterial and antifungal effects. Incorporation of various ions into nanotube architectures lead to mild, moderate, or even to a massive loss of human cell viability.[79]

A drawback in the use of NTs in bone regenerative medicine is their potential toxicity that includes oxidative stress and inflammatory pathways to living organisms and their removal from the environment. For this reason, *in vitro* and *in vivo* investigations have studied possible biological transformations, clearance and fate of NTs. It has been found that there exist pathways to biodegradation of CNTs and that peroxidase, either plant, horseradish, animal, myeloperoxidaseor eosinophil peroxidase, catalyze the degradation/biodegradation of carbon nanomaterials.[80–88] Nanomaterial degradation is being explored in the direction of expanding the repertoire of microbes and enzymes and optimal conditions that work toward this aim. Degradation of nanomaterials is important for targeted delivery and regulatable life-span of drugs in circulation applications.

Conclusion

Various types of NTs are used as reinforcing nanofillers in synthetic and natural biodegradable polymers, resulting in nanocomposites with remarkably improved mechanical properties (i.e. compressive modulus, compressive yield strength, flexural modulus, and flexural yield strength) for bone tissue engineering applications. Moreover, NTs can create cell supporting environments, due to their structure and dimensions. Although low filler fractions of NTs are used, in some cases, aggregation of NTs and particularly CNTs, was observed. Some strategies have been proposed to succeed better dispersion of NTs and higher adhesion strength with the matrix. The current progress in nanotechnologies is the development of new nanomaterials, which include the "safe-by-biodegradation" component, providing for the optimized life-time and clearance of nanoparticles from the body.

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Conflict of interest

The author declares no conflict of interest.

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