Histol Histopathol (2019) 34: 1195-1203

http://www.hh.um.es

Histology and Histopathology

From Cell Biology to Tissue Engineering

Review

Small molecule GSK-3 antagonists play a pivotal role in reducing the local inflammatory response, in promoting resident stem cell activation and in improving tissue repairing in regenerative dentistry

Marco Tatullo[1,2], Irina Makeeva[2], Sandro Rengo[3], Carlo Rengo[3], Gianrico Spagnuolo[2,3] and Bruna Codispoti[1]

[1]Tecnologica Research Institute, Stem Cells Unit, Crotone, Italy, [2]Department of Therapeutic Dentistry, "Sechenov" University of Moscow, Moscow, Russia and [3]Department of Neurosciences, Reproductive and Odontostomatological Sciences, University of Naples "Federico II", Naples, Italy

Summary. Regenerative dentistry is attracting growing interest in the scientific community, mainly because of its translational and promising therapeutic approach.

The latest research carried out by the scientific community are aimed at triggering the local cellular response, in order to induce a physiological self-repairing of damaged oral tissues. Such physiological processes mainly involve the activation of local stem cell populations: mesenchymal stem cells, in fact, retain the ability to proliferate and to differentiate towards functional mature elements, thus leading towards healing of damaged tissues.

Glycogen Synthase Kinase-3 (GSK-3) is a key-regulator of the Wnt/β-catenin pathway; it phosphorylates β-catenin, that then is degraded in the cytosol. The activation of such signalling, mediated by Wnt ligand/receptor association, inhibits GSK-3, leading to translocation of β-catenin to the nucleus and to gene transcription. Selective inhibitors of GSK-3 have been linked to the activity of Wnt signalling and to the regeneration of injured tissues, including complex dental and oral structures.

Small Molecule GSK-3 Antagonists are the most interesting class of molecules acting with a "Bystander effect": reducing local inflammation and local bone resorption and triggering the activity and differentiation of resident "sleeping" MSCs. The aim of this narrative topical review is to describe the current knowledge on the role of small molecule GSK-3 antagonists in regenerative dentistry, with strategic insights towards the translational applications in nanomaterials in dentistry and in dental repairing.

Key words: GSK-3 inhibitors, Tissue engineering, Dental regeneration, Nano-carriers, Small molecules

Offprint requests to: Prof. Marco Tatullo, Scientific Director - Tecnologica Research Institute, St. E. Fermi - Crotone, Italy, Professor - IM Sechenov First Moscow State Medical University, Moscow, Russia. e-mail: marco.tatullo@tecnologicasrl.com

DOI: 10.14670/HH-18-133

Introduction

Glycogen synthase kinase-3 is a serine, threonine kinase, originally discovered as regulator of glycogen synthase. Subsequently, it has been implicated in various cellular processes, such as apoptosis, cell survival and proliferation.

Furthermore, GSK-3 has been involved in phosphorylation of several substrates in a number of pathways, including Wnt, Hedgehog and insulin signalling (Cormier and Woodgett, 2017). GSK-3 exists in both the GSK-3α and GSK-3β isoforms, that encode for a 51kDa and a 47kDa protein respectively: these 2 proteins share the 98% of their sequence of the kinase domains (Meijer et al., 2004). Any alteration of the activity of this enzyme leads to a wide number of pathologic conditions, including cancer, neuro-

degenerative disorders, diabetes and cardiovascular diseases. For this reason, GSK-3 is attracting a growing interest as important therapeutic target (Pandey and DeGrado, 2016). Different natural molecules have been shown to inhibit GSK-3 activity with different degrees of sensibility and specificity; recently, a surprising number of novel GSK-3 antagonists have been designed and synthesized.

Recent findings showed the effects of blocking GSK-3 in dental tissues with interesting results. GSK-3 antagonists are able to stimulate resident oral stem and progenitors cells to activate their programs of tissue regeneration and repair damaged structures. Moreover, GSK-3 inhibition limits bone resorption, due to inflammatory response after severe oral infections.

Materials and methods

Literature search strategy

A careful literature search was performed by using Medline, via PubMed and Google Scholar. The main keywords used for the literature search were: "GSK-3 inhibitors", "GSK-3 AND Regeneration", "GSK-3 AND Stem Cells", "GSK-3 AND Dentistry". A total of 63 articles have been included in this narrative review. More in detail, 7 papers concerned the use of GSK-3 antagonists in neuropsychiatric and neurodegenerative diseases, 4 articles reported GSK-3 inhibitors in cardiovascular injuries, 2 papers in diabetes, 2 in cancer, 2 articles examined the effects of GSK-3 antagonists in the osteogenic differentiation of MSCs and 4 papers described the application of GSK-3 inhibitors in regenerative dentistry.

Clinical trials that involve the application of traditional and/or innovative inhibitors were also described.

Results

Glycogen synthase kinase-3 inhibitors

GSK-3 protein is composed of a conserved catalytic domain within the N-terminal end that contains the ATP binding site and the C-terminal that retains the domain (Hanks and Hunter, 1995). Tyrosine-residue located at the C-terminal tract mediates the kinase activity of GSK-3, through a process of chaperone-dependent auto-phosphorylation (Lochhead et al., 2006). GSK-3 activity is regulated by conserved sites at N-terminal and at C-terminal tract, external to the catalytic domain (Ilouz et al., 2008). Interactions with regulatory proteins also modulate GSK-3 activity; in fact, a binding of Axin protein with GSK-3 regulates the stability of the β-catenin in the Wnt signaling pathway (Wu and Pan, 2010). Therefore, GSK-3 could be regulated by exploiting different functional and/or interaction sites, which could be used for the creation of selective GSK-3 inhibitors.

Conventionally, GSK-3 antagonists are classified as ATP-competitive, non-ATP-competitive and substrate-competitive inhibitors. The first GSK-3 antagonist reported in literature was lithium, although it was initially discovered as an effective drug for the treatment of bipolar disorders (Mendlewicz et al., 1973). Lithium acts by a mechanism of competition with magnesium ions; in fact, eukaryotic cells exposed to lithium ions (Li+) show the inhibition of GSK-3-dependent phosphorylation, mimicking Wnt signalling activation (Stambolic et al., 1996). Furthermore, lithium showed neuroprotective effects, thus finding applications in neurodegenerative disorders and in the treatment of Alzheimer's disease (Munoz-Montano et al., 1997).

Afterwards, zinc ions were shown to reduce anxiety and to have antidepressant-like effects in mice (Kroczka et al., 2001). At the same time, zinc was found to be a valuable GSK-3 inhibitor, maybe superior to lithium (Ilouz et al., 2002).

In 2003, indirubin isomers derived from mollusks or synthetically produced, were demonstrated to selectively inhibit glycogen synthase kinase-3 by the interaction with their ATP binding pocket (Meijer et al., 2003).

The inhibition of GSK-3 by 6-bromoindirubin-3'-oxime maintains embryonic stem cells (ESCs) in an undifferentiated state by stimulating the expression of stemness genes, such as Oct and Nanog, and by up-regulating the Wnt pathway in a reversible way (Sato et al., 2004).

A series of other molecules derived from marine organisms were identified as ATP-competitive GSK-3 inhibitors. The brominated 3-(2-aminopyrimidine)-indoles purified from marine organisms, called meridianins, effectively inhibit GSK-3; however, at the same time, meridianins block cyclin-dependent kinases, cyclic nucleotide-dependent kinases and casein kinase 1, with the final effect of limiting cell proliferation and inducing apoptosis (Gompel et al., 2004). The fact that indole-based marine alkaloids interfere with cell growth and cell death in the cell cycle, triggered the production of synthetic GSK-3 inhibitors even more specific and selective. In 2000, two structurally different maleimides were synthesized as ATP-competitive inhibitors: these compounds selectively targeted GSK-3 but not a panel of kinases involved in the cell cycle. As a result, these molecules stimulated glycogen synthesis in human liver cells and activated Wnt/βcatenin signalling in HEK293 cells (Coghlan et al., 2000).

The studies focusing on the modelling of new bioactive compounds targeting GSK-3 are currently growing, and the main aims are related to the finding of highly selective inhibitors, able to overcome side effects, for a successful application on different pathological conditions (Sciu et al., 2019).

GSK-3 inhibitors in regenerative medicine

Currently, clinical trials are testing the effects of GSK-3 inhibitors for the treatment of various diseases in human patients. More in detail, the effects of lithium

carbonate involved in the work of several scientists, in order to understand its usefulness in the treatment of acute kidney injury, or in such patients undergoing cardiac surgery after a cardiopulmonary bypass (NCT03056248). A selective GSK-3 inhibitor, defined LY2090314, is under clinical experimentation on patients with metastatic pancreatic cancer, in association with the well-known classic chemotherapeutic agents (NCT01632306). Another trial is exploring the effects of 9-ING-41, a GSK-3β inhibitor, as a single agent, or also in combination with traditional chemotherapy, in patients affected by refractory hematologic malignancies or by different kinds of solid tumours (NCT03678883). Two different trials are focused on Alzheimer's disease (AD): a robust trial is based on lithium ability to prevent of the onset of AD (NCT02601859); on the other hand, another trial is investigating the effects of a novel GSK3 inhibitor, NP031112, on mild to moderate AD patients, under stable anti-cholinesterasic treatment (NCT00948259). A recent study reports the effects of two different posologies of Tideglusib (NP031112) a novel GSK-3 inhibitor, compared to placebo in the treatment of patients with mild-to-moderate progressive supranuclear palsy (NCT01049399) (listed on *ClinicalTrials.gov*).

Recent studies *in vitro* and on animal models are investigating the potential application of GSK-3 inhibitors in regeneration of different tissues.

GSK-3, mainly its β isoform, is ubiquitously expressed in neurological cells and consequently is implicated in different dysfunctions of the nervous system (Leroy and Brion, 1999).

The role of GSK-3β in neurological disease is particularly relevant in AD, because of the involvement of this kinase in Tau-protein phosphorylation. Moreover, GSK-3β also promotes the amyloidogenic process by phosphorylation of the amyloid precursor protein (Zhang et al., 2016). For these reasons GSK-3 inhibitor drugs have been proven as effective in counteracting AD and currently lithium and a novel molecule NP031112 (Tideglusib) are under investigation in clinical trials (Table 1).

Table 1. Clinical and experimental application of GSK-3 inhibitors.

GSK-3 Antagonist Definition	Human Disease / Animal Model / *In Vitro* System	Main Effects	References/ Clinical Trial Number Identifier
Lithium Carbonate	Patients with acute kidney injury		NCT03056248
LY2090314	Metastatic pancreatic cancer patients		NCT01632306
9-ING-41	Patients with refractory hematologic malignancies or solid tumors		NCT03678883
Lithium	Human Alzheimer disease		NCT02601859
Tideglusib, NP031112	Human Alzheimer disease		NCT00948259
Tideglusib, NP031112	Patients with mild-to-moderate progressive supranuclear palsy		NCT01049399
Lithium and kenpaullone	Murine TSM1 neuronal cells, C57BL/6 mice	Protection against mitochondrial stress-induced cell death	Nagao and Hayashi, 2009
6-bromoindirubin-3'-oxime (BIO)	Cardiomyocytes from rats	Stimulation of proliferation in mammalian cardiomyocyte	Eschenhagen et al., 2017
BIO associated with IGF-1	Myocardial infarction rat model	Restoration of lesion in infarcted myocard	Tseng et al., 2006
Gsk-3β knockout mice, Lithium cloride	Mice with beta cell deficiency of GSK-3β (β-Gsk-3β -/-), MIN6 cells	Expansion of beta islet mass and resistance to fat feeding-induced diabetes in mice	Liu et al., 2008b
Metavert	MIA PaCa-2, Bx-PC3, HPAF-II, and HPDE6 cell lines, B6.129J mice with tumors grown from UN-KPC961-Luc cells	Induction of apoptosis of pancreatic ductal adenocarcinomas cells, slow growth of tumors and metastases in mice	Liu et al., 2010
CHIR99021 in association with MEK inhibitor PD184352	human mammary epithelial cells	Induction of cancer stem cells transformation	Edderkaoui et al., 2018
603281-31-8	Murine pre-osteoblasts C3H10T1/2, osteopenic rats	Increased osteoblast differentiation *in vitro*, and augmented bone mass and strength *in vivo*	De Boer et al., 2004a
AR28	BALB/c mice	Decreasing in bone marrow adiposity and relative increasing of bone mass	De Boer et al., 2004b
Lithium Chloride	Embryos mouse first molar	Retarded differentiation of ameloblast and odontoblast and in a limited mineralization in first molars	Jarvinen et al., 2006
SB-415286	Rats subjected to premaxillary suture expansion	Augmented new bone formation in the expanding suture	Aurrekoetxea et al., 2011
SB216763	Periodontitis mouse model	Restoration of the damaged soft and hard tissues surrounding the teeth	Jiang et al., 2013
BIO, CHIR99021 and Tideglusib	Mouse models of tooth damage and pulp exposure	Augmented formation of reparative dentine, increasing in mineralization	Adamowicz et al., 2012

In a study by Selenica et al. five GSK-3β inhibitors were tested in depressing the phosphorylation of tau-protein, that is believed to be central in Alzheimer's disease pathogenesis, with promising results in *in vivo* experiments (Chase and Vemuri).

Elevated levels of GSK-3 have been found in the brain of patients suffering from Parkinson's disease (PD). PD patients display dysfunctions in mitochondrial protein complexes that finally result in neuronal damage, GSK-3β signalling is involved in the intrinsic apoptotic cascade, thus it may contribute to mitochondrial dysfunction and indirectly may lead to dopaminergic cell death (Nagao and Hayashi, 2009).

Different *in vitro* evidence and investigations in animal models demonstrated that targeting GSK-3 signalling supports the prevention of neuronal loss in PD. MPP+, a selective inhibitor of mitochondrial complex I, is able to induce depolarization of mitochondrial membrane potential, leading to cell death. Inhibition of GSK-3β activity by inhibitors such as lithium and kenpaullone, prevented mitochondrial membrane potential changes caused by MPP+ in two neuronal cell models. As a result inhibition of GSK-3β might offer protection against mitochondrial stress-induced cell death (Petit-Paitel et al., 2009). Moreover, GSK-3 is involved in other injuries of the nervous system such as in schizophrenia (Li et al., 2007) and in amyotrophic lateral sclerosis (Yang et al., 2008). Since the discovery of the efficacy of lithium treatment for bipolar disorders and depression (Adli et al., 2007) lots of studies investigated the effects GSK-3 inhibition in neurodegenerative and neuropsychiatric diseases underlying the central role of GSK-3 in the pathogenesis of these clinical conditions.

GSK-3 is crucial in the regulation of cardiac homeostasis (Zhou et al., 2016). Hearth failure, determined by cardiomyocyte dysfunctions, is extremely dangerous and diffused; these cells have, notably, a very short proliferation degree (Eschenhagen et al., 2017). Various efforts are trying to induce cardiac regeneration *in situ*, by stimulating resident cardiomyocytes to proliferate to a more robust rate. Tseng et al., demonstrate that the small molecule 6-bromoindirubin-3'-oxime (BIO) up-regulates cell cycle modulators and proliferation in mammalian cardiomyocyte, by pharmacological inhibition of GSK-3 (Tseng et al., 2006). Co-administration of BIO associated with IGF-1 has been shown to be able to restore myocardial infarcted heart in rats (Fang et al., 2015).

Although pharmacological inhibition of GSK-3 seems to be efficient in inducing cell cycle progression of cardiomyocytes, some evidence described that this pharmacological non-isoform specific inhibition of GSK-3α/β could be harmful (Zhou et al., 2016). Thus, the application of GSK-3 inhibitors for repairing of the injured heart needs more investigation to better understand their role in a cardiac setting.

GSK-3 is implied in glucose homeostasis and in insulin action. Indeed, overexpression of GSK-3β in mice depresses beta cell proliferation (Liu et al., 2008a,b); conversely beta cell specific deficiency of GSK-3β in mice promotes cell proliferation, increasing islet mass and conferring resistance to fat feeding-induced diabetes (Liu et al., 2010).

GSK-3 is also involved in cancer. Increased levels and activity of GSK-3β are associated with development of pancreatic ductal adenocarcinomas (PDAC) and with drug resistance onset. Experiments in PDAC cell lines and in mouse model, elucidate that the dual inhibition of GSK-3β and HDACs with the synthetic molecule Metavert reduces the growth of tumours, decrease cell migration and metastases and promote cancer cell apoptosis. These effects are synergic in association with gemcitabine (Edderkaoui et al., 2018). An innovative drug is under clinical experimentation for the treatment of metastatic pancreatic cancer patients.

Some studies reported the participation of GSK-3 inhibition in promoting neoplastic transformation. The concomitant inhibition of GSK-3 and of the MAPK mitogen-activated protein kinase (MEK) by the small molecules CHIR99021 and PD184352, induces the transformation of immortalized human mammary epithelial cells in cancer stem cells (CSCs). Thus, the role of GSK-3 in cancer is unclear (Fortunato et al., 2018) due to the potential dichotomous effects in oncology (Liao et al., 2018).

GSK-3 inhibitors and their influence on resident stem cells

Intrinsic self-renewal ability of pluripotent mouse embryonic stem cells (ES) has been studied by using a selective GSK-3 inhibitor, CHIR99021 (Bain et al., 2007). CHIR99021 alone improves cell growth but also induces non-neural differentiation. It has been investigated if Wnt ligand was able to imitate the effect of CHIR99021; the obtained results show that treatment with recombinant Wnt3a alone promotes non-neural differentiation, as observed for CHIR99021 administration (Ying et al., 2008). Moreover ES cells deleted for both GSK-3α and GSK-3β present an important deficiency in neural differentiation (Doble et al., 2007).

Taken together, the described findings demonstrate that blocking of GSK-3 exerts pleiotropic effects on embryonic stem cells, such as enhancing of cell proliferation, encouraging non-neural differentiation and restraining neural differentiation by mimicking canonical Wnt signalling.

GSK-3 has a central role also in adult stem cell behaviour, adult stem cells from different sources, and particularly from oral sources, representing a very attractive tool for regenerative dentistry purposes (Spagnuolo et al., 2018). Inhibition of this GSK-3 kinase activates Wnt signalling and the canonical Wnt pathway has been widely demonstrated to be implicated in the self-renewal of adult stem cells. Expansion of stem cells is often required for transplantation purposes (Codispoti

et al., 2017); the administration of GSK-3 inhibitors in NOD/SCID mice has been shown to increase the self-renewal capacity of hematopoietic stem cells (HSCs) (Trowbridge et al., 2006); these data suggest that administration of GSK-3 inhibitors could be proposed as a promoting agent for stem cell expansion.

In mesenchymal stem cells (MSCs) the effect of blocking GSK-3 is unclear. Different attempts are trying to explore and to exploit the great potential of these mesenchymal immature cells derived from various tissue sources (Tatullo et al., 2017; Codispoti et al., 2018). Activation of Wnt signalling has been shown to inhibit MSCs proliferation (Qiu et al., 2007); while a paper by de Boer and colleagues showed that activation of the Wnt signal transduction pathway, by lithium or Wnt3A, induces proliferation of human MSC while retaining pluripotency (De Boer et al., 2004a,b).

Administration of lithium chloride to MSCs promotes activation of Wnt signalling and suppression of dexamethasone-induced osteogenesis (de Boer et al., 2004). However, the effects of an α/β dual inhibitor of GSK-3 tested on murine mesenchymal cells and in osteopenic rats, consisted of an increase in levels of β-catenin, and consequently of Wnt signalling, and encouraged osteogenic differentiation *in vitro*, and improved bone mass and strength *in vivo* (Kulkarni et al., 2006).

The new molecule AR28, which selectively inhibits GSK-3α/β, also promotes activation of Wnt pathway, supports the amplification of mesenchymal progenitor cells, and stimulates *in vivo* bone formation. The explanation of these results correlates with the expansion of a population of multipotent mesenchymal progenitors that shift their differentiation fate through osteogenesis at the expense of adipogenesis. The final interesting result is the establishment of an increased bone mass in BALB/c mice (Gambardella et al., 2011).

Discussion

Dental regeneration is a central topic of modern dentistry. The isolation of stem cells from aboundant sources such as adipose tissue have been extensively investigated for oral regeneration (Paduano et al., 2017a,b), and novel sources such as stem cells from inflamed periapical cysts represent promising clinical tools (Tatullo et al., 2017). The understanding of the molecular mechanisms of oral cells is a very useful tool for repair of bone defects (Paduano et al., 2016).

Furthermore, innovative scaffolds are fundamental instruments for regenerative dentistry, and the research for biocompatible resorbable biomaterials is actively ongoing (Aulino et al., 2015; Paduano et al., 2017a,b).

Wnt signalling is crucial in tooth organogenesis and represents a key pathway for regulation of dental development, and for patterning the precise shape of individual teeth (Liu et al., 2008a,b). Initiation of Wnt pathway activates the development of de novo ectodermal appendages related to teeth formation (Thesleff et al., 1995). Blocking of Wnt signalling by the ectopic expression of Dkk1 prevented tooth development at the lamina-early-bud-stage in the oral epithelia of mouse embryos (Andl et al., 2002) while constitutive activation of β-catenin in the oral epithelium determined multiple teeth generation (Jarvinen et al., 2006). Wnt genes are largely expressed in dental epithelium, and some of those are up-regulated during tooth development. Inhibition of the Wnt activator LEF1 led to loss of the expression of *Fgf4a* gene, a direct LEF1/β-catenin target, causing limited tooth development at the late bud stage, and loss of dental epithelial cells (Kratochwil et al., 2002).

Taken together, the described data support the importance of the Wnt pathway in odontogenesis. As previously described, GSK-3 inhibitors have been widely used as canonical Wnt pathway activators. Thus, GSK-3 inhibitors have been exploited as an interesting tool for regenerative dentistry investigations.

In a recent work, aimed at characterizing the implication of the Wnt signalling pathway on tooth development, organotypic cultures of embryo mouse first molar have been prepared at different stages of dental development. The culture has been treated with lithium chloride. An increase of Lef1 expression has been revealed by *in situ* hybridization analysis, on the dental mesenchyme of molars treated with lithium chloride. These experimental results confirmed the overactivation of Wnt signalling. The final effects of continuous activation of Wnt signalling by lithium administration lead to a retarded differentiation of ameloblast and odontoblast and in a limited mineralization in first molars (Aurrekoetxea et al., 2011).

Physical and mechanical forces influence mesenchymal stem cell behaviour (Marrelli et al., 2018); mechanical loading influences osteogenesis, trough GSK-3α/β-catenin signalling.

Rats subjected to premaxillary suture expansion have been injected with a small-molecule GSK-3β inhibitor: SB-415286. Immunohistochemical staining revealed an increase in β-catenin expression, along with an improvement of the number of the overall proliferating osteoblasts in the active sutures. Such early effects definitely resulted in an augmented new bone formation in the suture area; in this light, local delivery of a GSK-3β inhibitor could be proposed as a pharmaceutical support for improving the effect of orthodontic treatments (Jiang et al., 2013).

GSK-3β is involved in the promotion of proinflammatory response during bacterial infections (Wang et al., 2014). Periodontitis is a chronic inflammatory disease characterized by the irreversible deterioration of the soft and hard tissues surrounding the teeth. The main cause of tissue loss following a periodontitis is related to the immunologic response to bacteria, hence, GSK-3 inhibition has been proposed as protective action against alveolar bone loss in patients affected by periodontitis. A cell permeable GSK-3

specific inhibitor, SB216763, has been tested in mouse infected with *P. gingivalis*. As a result, pathogen-induced alveolar bone loss has been reduced in treated mouse with consequent significant decreasing of neutrophil infiltration and limited production of proinflammatory cytokines (Adamowicz et al., 2012).

A recent paper reported an innovative method for the stimulation of reparative dentin production in teeth affected by deep carious lesions, by gradual releasing of GSK-3 inhibitors. Wnt pathway is up-regulated following tooth damage, with the consequence of stimulating the cellular-based repairing of damaged tissues (Minear et al., 2010). The effects of three small molecule GSK-3 inhibitors, named BIO, CHIR99021 and Tideglusib, agonists of Wnt-signalling, have been tested for dentine restoration. In mouse models of tooth damage and pulp exposure, a collagen sponge has been used to deliver low doses of GSK-3 inhibitors. The effects of the treatment consist of an increased mineralization, due to the augmented formation of reparative dentine, promoted by the stimulation of resident stem cells mainly located in dental pulp (Neves et al., 2017).

Nanomedicine exploits the plasticity and the physical properties of materials to interact at the molecular level with biological structures. Nanomaterials offer the possibility of being designed and functionalized as carriers, detectors, and deliverers applied to cells and tissues as systems characterized by a controlled size and shape in the nanoscale range (1 to 100 nm) (Feynman, *There's plenty of room at the bottom*. Science. 1991)

Nanomaterials are at the leading edge of the expanding landscape of nanotechnology, one of the most relevant application fields of nanotechnology is drug delivery.

Nanotechnology-based drug carriers could permit the delivery of molecules with biological activity at a controlled rate into the target tissue or even in a cell-specific manner. This system allows the maximization of therapeutic effects and, at the same time, minimizing the consequences of systemic delivery such as limited solubility, poor distribution within the body, and unfavourable pharmacokinetics behaviour. Furthermore, the design of nanocarriers requires the evaluation of all the parameters involved in its interaction with the target structure such as size, shape, and the surface charge (Kumar et al., 2013).

Different studies in the literature have proposed the application of nanomedicine approaches for the delivery of GSK-3 inhibitors in different pathological conditions.

Nanotechnology could allow the delivery of molecules with specific biological activity at a sustained and controlled rate. The targeting of heart tissue with nanocarriers, for delivery of the compound CHIR99021, has been proposed for inducing cardiac regeneration by direct cardiac reprogramming in a cell-specific manner. The mechanism of repair starts with GSK-3β inhibition (Lo Muzio et al., 2005) and with consequent activation of the canonical Wnt signalling, contributing to the direct reprograming of fibroblasts into cardiomyocytes by modulation of combined pathways (Mohamed et al., 2017).

The delivery of drugs in bone tissues is still challenging, mainly because of the high-dose required for the systemic administration of bone-acting drugs (Giudice et al., 2018). Poor bone uptake reduces the therapeutic efficacy of drugs and increases off-target tissue effects.

Nanotechnology will provide an efficient tool for target-specific delivery of drugs at a defined and sustained rate in bone tissues. In an experimental study, an innovative system for bone-delivery of a GSK-3β inhibitor was developed to enhance fracture healing. The bone-targeted nanoparticle consisted of a highly stable poly(styrene-alt-maleic anhydride)-b-poly(styrene) NPs combined with a peptide with high affinity for tartrate-resistant acid phosphatase (TRAP). The created nanocomplex showed enhanced pharmacokinetic profiles with an important accumulation of GSK-3β inhibitor at fractured bone level. Moreover, a significant uptake of the drug has been recorded in osteoblasts and mesenchymal stem cells. These regenerative cell types exhibited a 2-fold increase of β-catenin signalling compared to control, consisting of free drug administration. Furthermore, an augmented bone deposition was observed after 28 days of treatment, demonstrating accelerated fracture healing (Wang et al., 2017).

Nanotechnology approaches could be used not only for tissue and/or cell interaction but also at molecular level. Shah et al., described the delivery of proteins linked to hydrophobically modified 15-nm silica nanoparticles, in order to interact with specifically targeted cell signalling proteins. The authors created a chimeric protein, GFP-FRATtide, characterised by the FRATtide segment able to bind and block the active site of GSK-3β, wherein GFP was used as a biomarker for fluorescence detection (Shah et al., 2011).

Interestingly, the incorporation of graphene nanoplates in poly(lactic-co-glycolic acid) scaffolds increases the osteoinductive properties of scaffold by its ability to activate the PI3K/Akt/GSK-3β/β-catenin signalling pathway (Wu et al., 2018).

Concluding, inhibition of GSK-3 could be induced by specific delivery systems based on nanotechnologies. These approaches include the specific tissue or cell delivery of drugs that inhibit GSK-3, otherwise the enzyme could be blocked at the active site by molecular interaction with designed nanoparticles.

Conclusions

GSK-3 inhibition induces the activation of central pathways able to trigger the regenerative and reparative cellular processes (Neves et al., 2017). The blocking of GSK-3 activity influences mesenchymal stem cell (MSCs) proliferation and their differentiation fate. Our

objective was to provide an overview of the current literature, to better delineate the role of GSK-3 in stem cells and in tissue engineering. Furthermore, the effects of natural and synthetic molecules used as GSK-3 antagonists are described for different pathological conditions, with specific relevance to regenerative dentistry. The efficacy of GSK-3 inhibition in cancer, diabetes, cardiovascular disease and neurodegenerative disorders has been established *in vitro* and in animal models; moreover, different molecules are currently under investigation on human patients in promising clinical trials. Blocking of GSK-3 activity seems to show favourable results in the dental field: the main effect is related to the promotion of regeneration and repairing of several dental tissues. The mechanisms of dental repairing involve the massive activation of resident oral stem cells, which are stimulated to initiate the most effective cellular programs leading to tissue regeneration. Some of these processes still remain unclear, and more investigations are of course needed to justify the strategic step from the experimental research to the more useful clinical landscape. Scientific efforts must be improved to transfer what has been discovered in an *in vitro* scenario, to the patient, in terms of better efficacy and safety.

Acknowledgements. None.
All the authors equally participated to the drafting of this manuscript.

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Accepted June 6, 2019