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Design, synthesis and characterization of doped-titanium oxide nanomaterials with environmental and angiogenic applications

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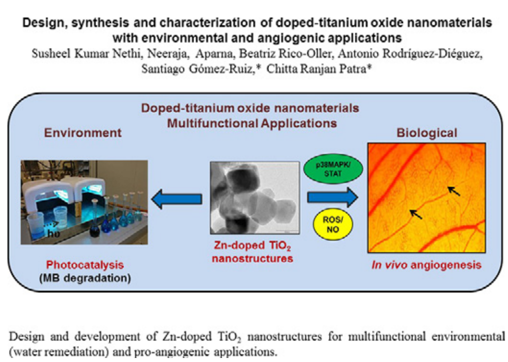
HIGHLIGHTS

- Zinc-doped titania-based nanoparticles have been synthesized and characterized
- The synthesized nanosystems have a potential application in water decontamination
- Some Zn-doped titania-based nanomaterials have shown improved pro-angiogenic properties *in vitro*.
- Nanomaterials have shown an interesting potential for the *in vivo* formation of new blood vessels in chick embryo model

GRAPHICAL ABSTRACT



Design and development of Zn-doped TiO_2 nanostructures for multifunctional environmental (water remediation) and pro-angiogenic applications.

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ABSTRACT

Since the last decade, the metal composite nanostructures have evolved as promising candidates in regard to their wide applications in the fields of science and engineering. Recently, several investigators identified the titanium based nanomaterials as excellent agents for multifunctional environmental and biomedical applications. In this perspective, we have developed a series of zinc-doped (2 and 5%) titanium oxide-based nanomaterials using various reaction conditions and calcination temperatures (**TZ1**–**TZ3:** calcined at 500 °C, **TZ4**–**TZ6:** calcined at 600 °C and **TZ7**–**TZ9:** calcined at 700 °C). The calcined materials (**TZ1** to **TZ9**) were thoroughly analyzed by several physico-chemical characterization methods. The increase of the calcination temperature results in significant changes of the textural properties of the nanostructured materials. In addition, the increase of the calcination temperature leads to the formation of anatase/rutile mixtures with higher quantity of rutile. Furthermore, incorporation of zinc changes the morphology of the obtained nanoparticles. The materials were studied in the photodegradation of methylene blue observing that materials calcined at lower temperatures (**TZ1**–**TZ3**) have higher photocatalytic activity than those of the materials calcined at 600 °C (**TZ4**–**TZ6**), rutile-based systems **TZ7**–**TZ9** are not active. Based on the background literature of titanium and zinc based nanostructures in therapeutic angiogenesis, we have explored the pro-angiogenic properties of these materials using various *in vitro* and

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in vivo assays. The zinc-doped titanium dioxide nanostructures (**TZ5** and **TZ6**) exhibited increased cell viability, proliferation, enhanced S-phase cell population, increased pro-angiogenic messengers (ROS: reactive oxygen species and NO: nitric oxide) production and promoted *in vivo* blood vessel formation in a plausible mechanistic p38/STAT3 dependent signaling cascade. Altogether, the results of the present study showcase these zinc doped-titanium oxide nanoparticles as promising candidates for environmental (water-remediation) and therapeutic angiogenic applications.

1. Introduction

Titanium dioxide (TiO_2) has gained immense importance owing to its wide applications in various fields such as cosmetics, paints, food and healthcare sectors (Chen and Mao, 2007). The initial discovery of the photocatalytic splitting of water exhibited by TiO_2 (Fujishima and Honda, 1972) has promoted its extensive applications in photocatalysis, photovoltaic and solar cells and hydrogen production (Ding and Nagpal, 2016; Feng et al., 2012; Rico-Oller et al., 2016). Recently, several research groups started working on the design, development and multi-functional applications of TiO_2-based nanomaterials (Chen and Mao, 2007; Giovannetti et al., 2015; Lázaro-Navas et al., 2015; Marelli et al., 2016; Nair and Elizabeth, 2015). The TiO_2 based nanostructures have been studied in several areas of environmental and biomedical applications attributed to their unique physico-chemical characteristics, catalytic efficiency, photochemical stability and biocompatibility properties (Bai and Zhou, 2014; Brammer et al., 2012; Venkatasubbu et al., 2013). For example, titanium oxide is one of the most important compounds used for advanced oxidation processes such as heterogeneous photocatalytic degradation of pollutants, which can be applied in water remediation. In this context, organic dyes like rhodamine-B or methylene blue (MB) are clear objectives for this kind of processes and the scientific community is always in search of novel nanostructured titanium oxide-based systems due to their low price and stability (Chong et al., 2010; Mahlambi et al., 2015; Tsydenova et al., 2015). Furthermore, in the last decade TiO_2 nanoparticles have emerged as potent candidates for various biomedical applications such as bone growth and regeneration (Brammer et al., 2012; Raines et al., 2010), drug delivery (Venkatasubbu et al., 2013), bio-medical devices (Kulkarni et al., 2015), biosensors (Bai and Zhou, 2014; Bao et al., 2008), vascular implants (Park et al., 2009) etc. Over the recent years many research groups have carried out the doping of various metallic ions into the lattice of TiO_2 and widely investigated their environmental and biomedical applications.

In this context, earlier reports suggest that the incorporation of metals ions especially Zn^{2+} have significantly enhanced the photocatalytic activity in water remediation (Rico-Oller et al., 2016; Sánchez-Muñoz et al., 2013) and the photoelectric and photovoltaic properties of TiO_2 for solar cell applications (Zhu et al., 2010). On the other hand, Zn-doped titania nanostructures have been well studied for various biomedical applications as zinc is an essential element required for physiological growth, metabolism, neurological development, immunity etc. (Frassinetti et al., 2006). Recent reports demonstrated the anti-bacterial properties of the Zn-doped titania nanostructures against various Gram positive and Gram negative bacterial strains (Amna et al., 2012; Arunachalam et al., 2015). Another study demonstrated the osteogenic, bone growth and regeneration properties of Zn-incorporated TiO_2 coatings in animal model (Qiao et al., 2014). Very recently, the role of TiO_2 based nanostructures in modulating the angiogenesis process is being well-investigated (Beltran-Partida et al., 2017; Gong et al., 2017; Jo et al., 2014; Raines et al., 2010). Angiogenesis, the development of newer blood vessels from existing vasculature, plays major role in the embryonic growth, development and several other important patho-physiological processes (Carmeliet and Jain, 2011). Our group including others have thoroughly demonstrated the pro-angiogenic properties of zinc oxide nanoparticles in various cellular and animal model systems (Augustine et al., 2014; Barui et al., 2012).

In this context, the present study was aimed at incorporation of zinc into the TiO_2 nanostructural network and evaluating their photocatalytic and pro-angiogenic responses. The influence of these nanomaterials on both the environmental (photocatalysis: MB degradation) as well as the pro-angiogenic responses in endothelial cells (HUVECs and EA.hy926 cells) was performed employing several *in vitro* [cell viability, cell cycle, ROS (DCFDA), NO (Griess) estimation and immunoblot] and *in vivo* angiogenesis assays [chick chorioallantoic membrane (CAM)]. Henceforth, from the observations of the present study, we strongly believe that the Zn-doped titania nanostructures could be developed as potential candidates for environmental and pro-angiogenic applications.

2. Materials and methods

2.1. Materials

Titanium(IV) isopropoxide - 97%, succinic acid - 99.7%, 2-propanol - 99%, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), vascular endothelial growth factor (VEGF), RIPA, PIC (protease inhibitor cocktail), DCFDA (2′,7′-dichlorofluorescein diacetate) and propidium iodide (PI) were purchased from Sigma-Aldrich, USA and used without any further purification. Nitric acid and zinc nitrate hexahydrate - 98% were procured from Fluka Analytical and Acros-Organics, respectively. Methylene blue (MB) purchased from Scharlau, was chosen as a model pollutant for testing photocatalytic activities of the synthesized materials. Milli-Q water (18.2 MV cm, Millipore Waters, USA) was used in all the syntheses. The primary phospho-STAT3 and STAT3 antibodies were procured from Cell Signaling Technologies and the secondary anti-rabbit IgG horse radish peroxidase (HRP) antibody was purchased from Thermo Pierce. The Nitrocellulose/PVDF membrane and the super signal west picochemiluminescent substrate were procured from Thermo Scientific, USA.

2.2. Synthesis of TiO_2 and TiO_2-Zn-based materials

TiO_2 and TiO_2-Zn-based materials were prepared using a slight modification of the synthetic method previously described by our group (Rico-Oller et al., 2016; Sánchez-Muñoz et al., 2013). The hydrolysis of titanium(IV) isopropoxide is carried out in Milli-Q water at a controlled pH of 2.2 which is achieved by adding succinic acid. The Zn-doping quantity is controlled by adding different amounts of zinc nitrate. In addition, the crystallization of the titanium oxide materials in form of either anatase-based or rutile-based nanostructured systems is controlled by using different calcination temperatures.

In brief, for a regular synthesis, a 100 mL solution containing the desired molar amount of zinc nitrate hexahydrate was prepared in Milli-Q water. Subsequently, a 100 mL aliquot of a titanium(IV) isopropoxide solution in dry 2-propanol (prepared immediately before addition, in order to avoid uncontrolled hydrolysis) is prepared. Both solutions were added dropwise (1 mL/min) to 500 mL of the acidic solution using a peristaltic pump *Perimax 12/4*. The pH of the acidic solution was previously adjusted to pH 2.2 using succinic acid.