Hybrid bionanomaterials based on nanocrystalline TiO_2 and catechol-grafted polymers: the effect of composition and morphology on photo and bioactivity

C. Cadman*, L. Ragupathy*, N.M. Zaki***, N. Tirelli**, F. Cellesi*

* School of Pharmacy and Pharmaceutical Sciences and ** School of Biomedicine and School of Materials, University of Manchester. Laboratory for Polymers and Biomaterials, Stopford Building, Manchester M13 9PT, United Kingdom. ***Department of Pharmaceutics, Faculty of Pharmacy, Ain Shams University, Cairo, Egypt.

ABSTRACT

We here present a synthetic strategy for generating hybrid nanoparticles composed of a size-controlled, crystalline TiO_2 core, coated with a synthetic biocompatible polymeric layer, which was designed to tailor their final photo and bioactivity. A bio-inspired approach was used to graft hydrophilic polymers (*i.e.* poly(ethylene glycol) or poly(glycerol methacrylate) based polymers), on the TiO_2 nanoparticles, utilising terminal catechol groups as chelating enediol ligands. After purification, these hybrid nanoparticles showed satisfactory stability at physiological conditions, a photoactivity which depends on the grafting density of the polymeric layer and a safe cytotoxic profile. The characteristics of these bionanomaterials provide interesting advantages for biomedical applications, particularly for imaging, cell targeting and immunostimulation.

Keywords: TiO_2 nanoparticles, dopamine, pegylation, photoactivity, cytotoxicity

1 INTRODUCTION

Hybrid inorganic oxides/polymeric bio nanomaterials have drawn intensive research over the past few years, due to their great potential of combining the typical characteristics of inorganic materials, such as optical, magnetic and photocatalytic properties, with the bioactivity and responsiveness of biopolymers.

Among different biocompatible metal oxides, nanocrystalline TiO_2 provides interesting advantages for imaging and cell targeting [1]. Its radio-opacity can potentially be utilized in X-ray bioimaging applications[2], while its photoactivity can trigger local formation of reactive oxygen species, thus inducing toxicity and death of targeted cells *in vivo*[3].

We proposed a synthetic strategy for producing nanoparticles composed of a TiO_2 core of highly controlled crystallinity, size and surface composition [3] which were further coated with a synthetic biocompatible polymeric layer (scheme 1). The robust and versatile chemical procedure designed for the synthesis of these nanomaterials offers a wide degree of freedom to control physical and chemical properties independently.

Core nanoparticles.

Crystalline (anatase) size-controlled titania cores were synthesized by employing a sol-gel process in organic solvent, which involved titanium (IV) alkoxy precursors dissolved in benzyl alcohol with traces of HCl/H_2O under mild temperature conditions. Interestingly, these organic-soluble alkoxylated TiO_2 nanoparticles were easily re-dispersible in water. The rapid hydrolysis of the alkoxy groups on the nanoparticle surface generated a stable dispersion of fully hydroxylated nanoparticles, ready for further polymer adsorption.

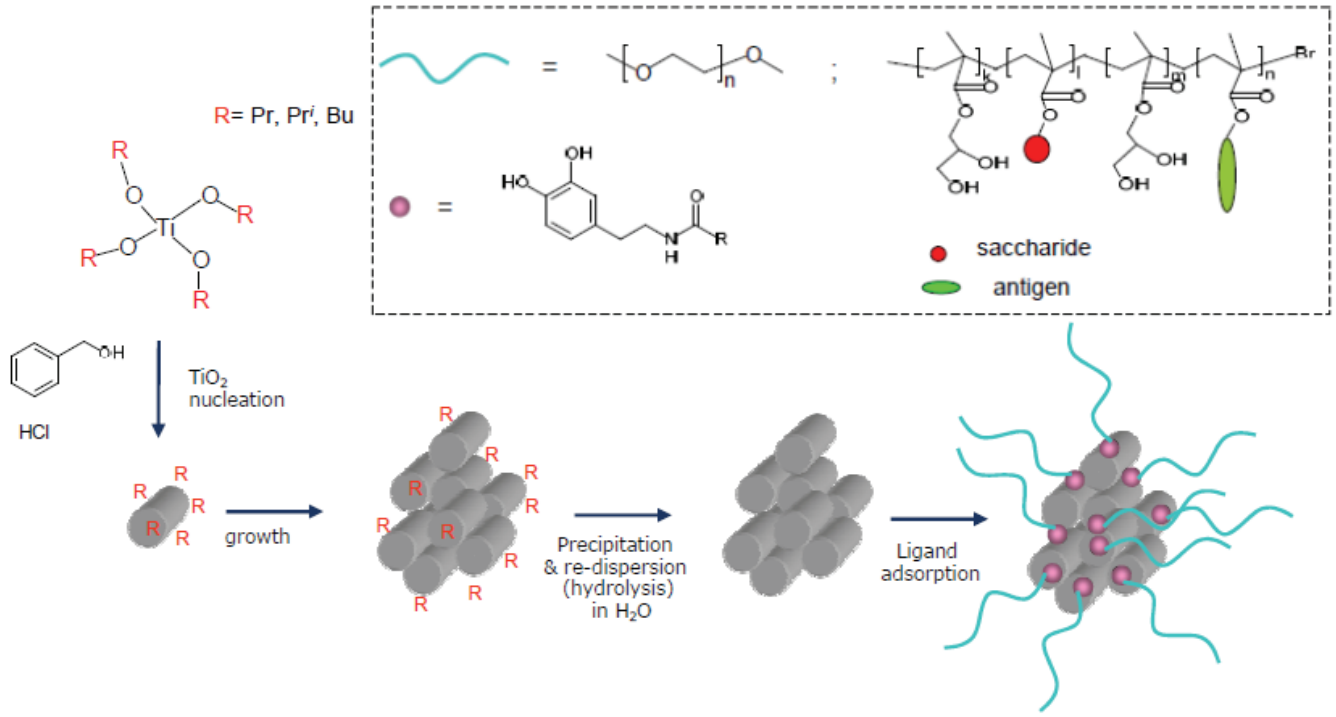
Polymeric shell.

A bio-inspired approach was used to graft hydrophilic polymers on the TiO_2 nanoparticles, utilising terminal catechol groups as chelating enediol ligands, which provided an irreversible surface functionalisation.

The bioactivity of these colloids may be regulated by the chemistry of the polymeric surface. For instance, “stealth” nanomaterials can be produced from poly(ethylene glycol) conjugated with terminal dopamine groups, while immunoactive nanoparticles can be achieved by using poly(glycerol methacrylate) containing dangling antigenic peptides and/or immunogenic sugar moieties (scheme 1). Controlled living radical polymerisations, combined with thiol-ene click chemistry, was used to vary polymer composition and architecture.

2 METHODS

$Ti(OR)_4$ (R = propyl, butyl or isopropyl) was added to benzyl alcohol which contained traces of water and hydrochloric acid at fixed molar ratios. The suspension was then heated (80 °C) under nitrogen for up to 90 minutes depending on reaction conditions. The growth of the nanoparticles was monitored via dynamic light scattering (DLS). The reactions were stopped when the average diameter of the nanoparticles reached 8-9 nm. The nanoparticles were precipitated in diethyl ether, centrifuged, and washed again with diethyl ether. The precipitate was then re-dispersed and dialysed in water at pH 1.5 . The final concentration of the solution was calculated by freeze drying.



Scheme 1. Synthetic route for the preparation of hybrid TiO_2/polymeric nanoparticles

A fixed amount of polymeric ligand (methoxy poly(ethylene glycol) dopamine, poly(ethylene glycol) di-dopamine, pluronic dopamine, or multifunctional poly(glycerol methacrylate)) was dissolved and added to the TiO_2 suspension to allow fast adsorption. The suspension was then neutralised at physiological pH, purified in presence of boric acid gel, and isolated for further characterisation.

Imaging: Transmission Electron Microscopy (TEM), Atomic Force Microscopy (AFM). Crystallinity: X-Ray Diffraction Analysis (XRD). Colloidal stability: Dynamic light scattering (DLS) and ζ-potential analysis. Adsorption, photoactivity and cytotoxicity: UV-Vis spectrophotometric analysis.

3 RESULTS AND DISCUSSION

Growth kinetics, crystallinity and colloidal stability of the titania cores were investigated, with respect to varying titanium alkoxy precursors and reaction conditions.

After redispersion in water, TEM images of the colloidal samples showed isolated TiO_2 nanoparticles with a size of 5-7 nm (Figure 1a and 1b), and a typical anatase structure, with a lattice spacing of 3.2Å (Figure 1c), which corresponds to the (101) lattice plane of anatase. Crystallinity and size distribution were also confirmed by XRD and DLS analysis respectively (Figure 2).

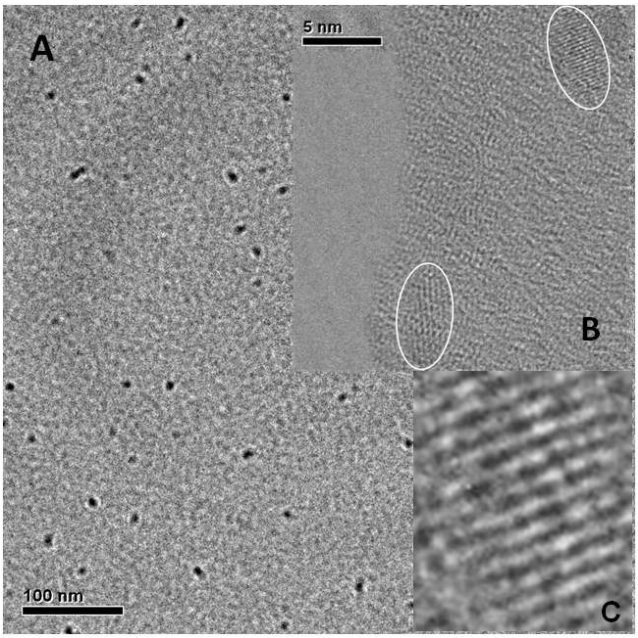


Figure 1. A) TEM of anatase nanoparticles after synthesis in benzyl alcohol and redispersion in water. B) At higher magnification, isolated nanoparticles showed a size range of 5-7 nm. C) A lattice spacing of 3.2Å was measured, which corresponds to the anatase (101) lattice plane.

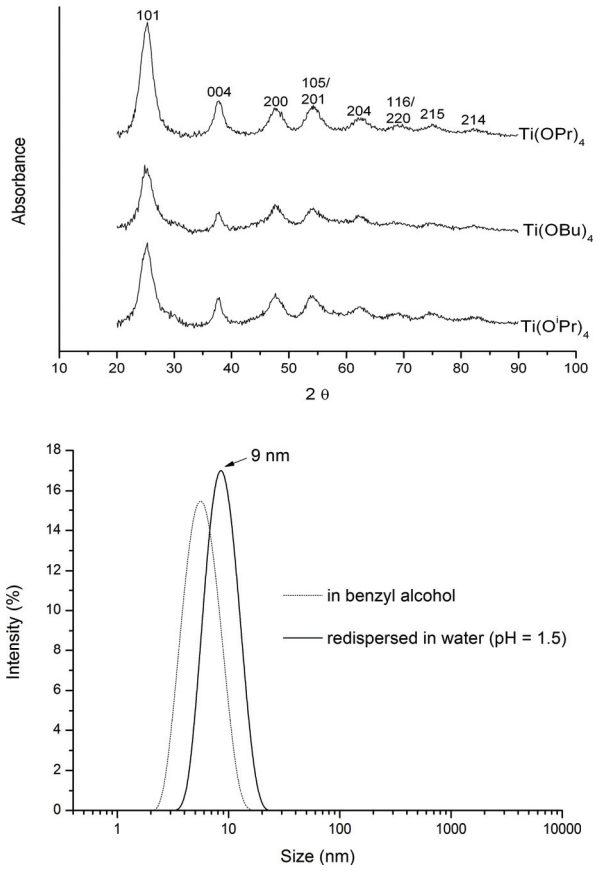


Figure 2. *Above,* XRD of TiO_2 nanoparticles prepared using different titanium alkoxy precursors. *Below,* DLS of TiO_2 nanoparticles prepared using $Ti(O^iPr)_4$ as the precursor.

The polymer grafting onto TiO_2 nanoparticles was characterised by UV-Vis spectroscopy, i.e. investigating the optical properties of the suspension which develops a deep red-orange colour instantaneously upon exposure to dopamine-based ligands (Figure 3). A model of the adsorption mechanism, based on Langmuir isotherms, was proposed for different polymeric ligands.

After purification (boric acid gel extraction was used to retain any unadsorbed catechol-grafted polymers), the covered nanoparticles showed satisfactory stability at physiological conditions. A distinct increase in particle size was observed by DLS and AFM (Figure 4), which was moderate for monofunctional polymers but much more pronounced for the bi-functional polymers (such as poly(ethylene glycol) di-dopamine). The bifunctional polymer chains may have the characteristic of "anchoring" their two available DA end groups on two different nanoparticles, thus forming larger aggregates.

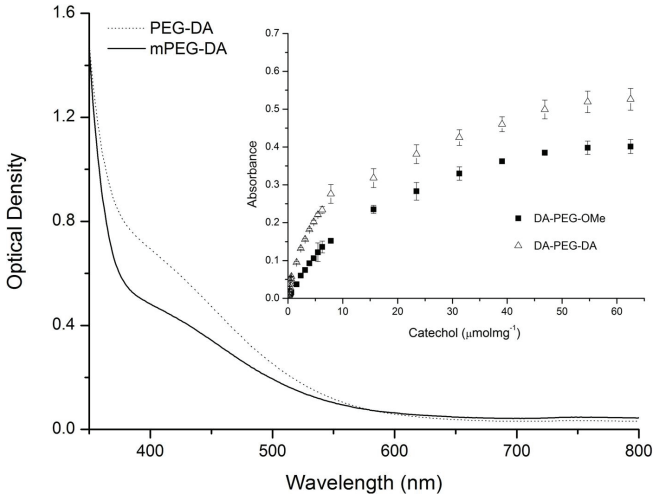


Figure 3. UV-Vis curves obtained following addition of dopamine-grafted ligands to titania nanoparticles. The scattering contribution can be removed through baseline subtraction, leading to "pure" absorption spectra peaked at 435nm. *Inset*. The absorption at 435nm as an indication of the concentration of catechols in the colloidal system.

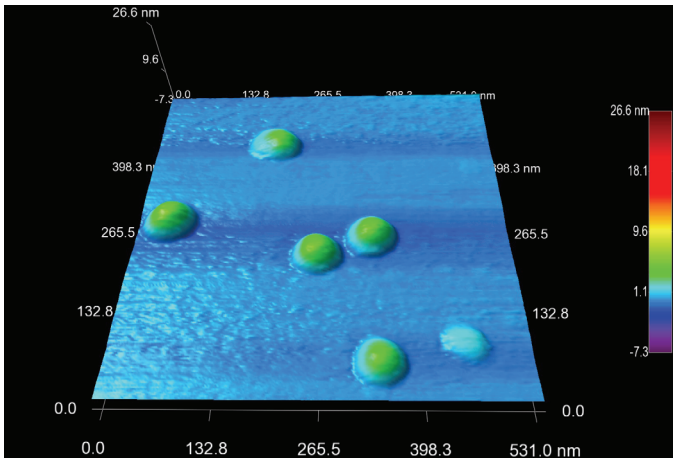


Figure 4: AFM of purified TiO_2 nanoparticles coated with PEG di-dopamine (average particle size 90 nm).

The photoactivity of the nanoparticles was investigated before and after functionalisation. While uncoated TiO_2 nanoparticles showed photooxidative properties typical of nanocrystalline anatase, the fully covered nanoparticles (i.e at the maximum polymer grafting density) showed a negligible photocatalytic degradation of methylene blue (Figure 5).

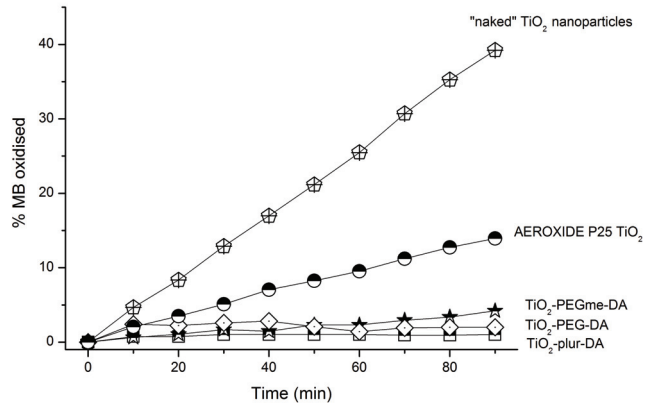


Figure 5: Methylene blue decomposition by TiO_2 nanoparticles under UV illumination.

This result may be ascribed to a change of the optical properties of nanoparticles [4], as well as the depletion of photocatalytic active sites (surface titanols), due to the binding of the bidentate ligands.

These purified hybrid nanoparticles also showed a safe cytotoxic profile, demonstrated by *in vitro* studies on murine fibroblasts and macrophages. In general, hybrid TiO_2/polymeric nanoparticles were found to have a relatively low toxicity. For instance, in *in vitro* cultures of macrophages the half maximal inhibitory concentration (IC50) was above 20mg/mL of nanoparticles (Figure 6).

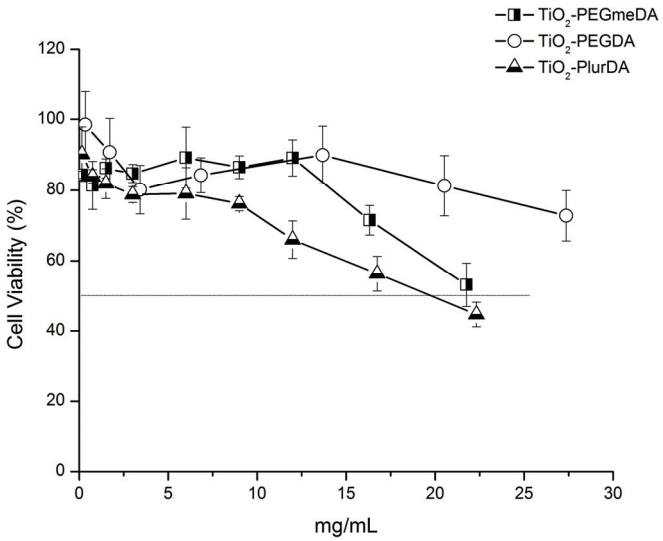


Figure 6: J774 macrophage viability (MTT assay) tested under exposure of hybrid TiO_2/polymeric nanoparticles at different concentrations.

4 CONCLUSION

We succesfully synthesized nanoparticles composed of a TiO_2 core of highly controlled crystallinity and size, coated with a hydrophilic and biocompatible polymeric layer, obtained by adsorption of catechol-terminated polymeric ligands. These bionanomaterials showed interesting advantages for biomedical applications, particularly for imaging, cell targeting and immunostimulation.

After purification, these colloids present satisfactory stability at physiological conditions, a tuneable photoactivity, and a safe cytotoxic profile, demonstrated by *in vitro* studies on different murine cell lines.

REFERENCES

[1] Matsui K et Al., MedChemComm 2010, 1 (3), 209 -211
[2] Ashcroft, J. M. et Al.,Chemical Communications 2008, (21), 2471-2473.
[3] Kotsokechagia, T. et Al., Langmuir 2008; Vol. 24, pp 6988-6997.
[4] Dimitrijevic, N. M. et Al., Journal of the American Chemical Society 2009, 131, (8), 2893-2899.