

# Testing the photo-sensitive nanocomposite system for potential controlled metallo-drug delivery

Maja Nešić<sup>1</sup> · Iva Popović<sup>1</sup> · Andreja Leskovic<sup>1</sup> ·  
Zoran Šaponjić<sup>1</sup> · Marija Radoičić<sup>1</sup> · Milutin Stepčić<sup>1</sup> ·  
Marijana Petković<sup>1</sup>

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**Abstract** Photo-responsive drug release systems are promising for drug delivery applications due to many benefits compared to conventional chemotherapy such as targeted, controlled release of a drug and reduced toxicity to healthy tissues. In this work, we report synthesis of the nanocomposite system based on carrier TiO<sub>2</sub> nanoparticles and potential anticancer ruthenium complex, with light controllable release properties. Nanocomposite system showed biological activity and induced the generation of free radicals, which are implied in the efficient cell killing. The drug release tests demonstrated sustained release of the transition metal complex from the surface implying the potency for the controlled drug delivery system. Taking into account photoactivity of the Ru-complex, in the next step we have investigated the influence of green light on the rate of the complex release, and the results showed dependence of the Ru-complex release from the surface of TiO<sub>2</sub> nanoparticles on the applied laser energy. Therefore, these characteristics make this nanocomposite system promising for the photo-responsive chemotherapy.

**Keywords** Photo-responsive release · Nanocomposite system · Anticancer drug

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✉ Maja Nešić  
maki@vinca.rs; maki@vinca.com

<sup>1</sup> Vinča Institute of Nuclear Sciences, University of Belgrade, Mike Petrovića Alasa 12-14, Belgrade 11 000, Serbia

## 1 Introduction

Conventional drugs, primarily transition metal complexes, used in chemotherapy fail to target the cancerous cells selectively without interacting with the healthy tissue. Therefore they cause serious side effects including toxicity and resistance (Piulats et al. 2009). There is an increasing interest of scientists in the last decades for developing stimuli-responsive drug delivery systems to overcome these limitations (Mura et al. 2013). The main goal is to make targeted, stimuli-responsive drug delivery, which could optimize the drug concentration at the diseased site. Light is an attractive choice as an external stimulus since its intensity and penetration depth can be controlled (Alvarez-Lorenzo and Concheiro 2013). As a result, light-sensitive carriers and drugs become increasingly popular as drug delivery systems.

TiO<sub>2</sub> nanoparticles (NPs) are biocompatible, readily available, environmental friendly but also possess exclusive photoactivity and possibility for surface modification (Xu et al. 2015). These properties tend to allow TiO<sub>2</sub> NPs to be used as a drug carrier. TiO<sub>2</sub> NPs absorb UV light which is very harmful. A major challenge in photodynamic therapy is to use less invasive light and to try to control the rate of a drug release with a less harmful means. It is known that visible light is less damaging to cells than UV and also penetrates deeper in human tissue (Elisseff et al. 1999). Indeed, only a few millimeters of tissue are penetrable by visible light but it is sufficient for treatment of skin cancer. On the other side, drugs used in cancer therapy are predominantly transition metal complexes which are intensively colored and absorb in visible region. This opens up ability to use visible light to modulate drug release properties.

The aim of the study was to test nanocomposite system (NCS) made of colloidal TiO<sub>2</sub> NPs and transition metal complex, which might be applied in the anti-tumor therapy. NCS and its components were tested for biological application and also visible light photo-responsive characteristics for potential controlled drug delivery were also checked.

## 2 Materials and methods

### 2.1 Synthesis of NCS

Complex *cis*-dichlorobis(2,2'-bipyridyl-4,4'-dicarboxylic acid)ruthenium(II), was synthesized using the procedure described earlier (Nazeeruddin et al. 1999) and stored in closed dark colored bottle in the freezer. Colloidal TiO<sub>2</sub> NPs (with diameter  $d \sim 5$  nm) were synthesized by the modified method of Rajh et al. (1996). Ethanolic solution of complex was added to aqueous suspensions of TiO<sub>2</sub> NPs pH 2.5, estimating that the fraction of exposed Ti atoms on surface of TiO<sub>2</sub> NPs is 30 % (Chen et al. 1997). The reaction mixture was kept overnight in dark at room temperature to construct nanocomposites of complex-TiO<sub>2</sub> NPs.

### 2.2 Biological activity

Blood was obtained from 35 years old healthy donor. Lymphocyte cultures were treated with three different concentrations of complex, TiO<sub>2</sub> NPs and NCS (final concentrations were 25, 15 and 5  $\mu$ M) and then were incubated at 37 °C for 72 h in the dark. Detail

procedure is described earlier (Fenech 1993). All lymphocyte cultures were set up in triplicate. Non-treated cell cultures served as a control.

A cytokinesis-block proliferation index (CBPI) was calculated using the equation:

$$\text{CBPI} = [\text{MI} + 2\text{MII} + 3(\text{MIII} + \text{MIV})]/N \quad (1)$$

where *MI–MIV* represents the number of cells with one to four nuclei, respectively, and *N* is the number of cells scored (Surralles et al. 1995).

Malondialdehyde (MDA) assay; The lipid peroxidation product in human lymphocytes was assayed by measurement of the malondialdehyde (MDA) production by the procedure of Aruoma et al. (1989).

*Statistical analysis* was carried out using Student's *t* test and linear regression analysis. Differences at  $p < 0.05$  were considered statistically significant.

### 2.3 Light stimuli complex release study

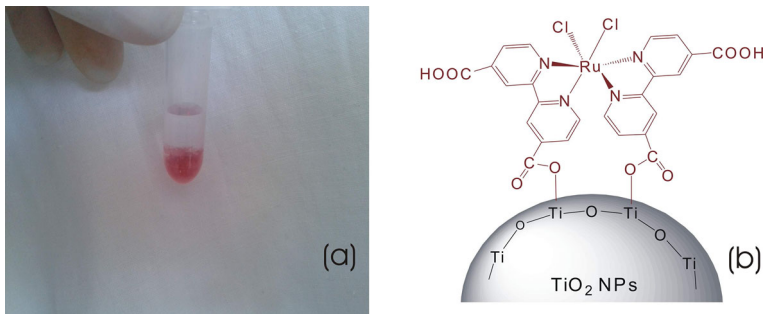
500  $\mu\text{L}$  of NCS were placed into the dialysis cassette (Thermo Scientific Dialysis cassette, 7.000 MWCO, 0.1–0.5 mL capacity). Then the cassettes were transferred into the beakers with 60 mL of phosphate buffer saline (PBS) solution, which were stirred with a magnetic stirrer and kept at room temperature in the dark. At indicated time intervals, 500  $\mu\text{L}$  of samples were removed from outer medium and replaced with equal volumes of PBS solution. Then, aliquots were analyzed by measuring absorbance at 310 nm. UV–Vis spectrometer (Perkin Elmer Lambda 35) was used for acquisition of the spectra and absorbance at room temperature. Percentage of complex release were calculated and plotted against the time. Non-irradiated NCS complex release profile was served as control.

Light stimuli complex release test was performed under the same experimental conditions with one difference. NCS solution in dialysis bag was irradiated with green light from diode-pumped solid state CW Verdi 5 laser (Coherent, 5 W, 532 nm). Laser's beams were focused to dialysis cassettes with sample. Light power that reached the samples were (1, 3, 50 and 100 mW) The light intensities were measured with laser power meter NOVA II (Ophir) with UV enhanced silicon photodiode sensor PD300-UV with installed filter (CW power up to 300 mW). Light intensities were measured in front of the beakers on the laser's beams path to the samples. Light stimuli NCS complex release profile was then compared with control.

## 3 Results and discussion

### 3.1 Binding of complex to the $\text{TiO}_2$ NPs carrier

It is already well known that  $\text{TiO}_2$  surfaces have a strong affinity for carboxyl groups (Savić et al. 2013). Therefore, in the first part of our work, we have synthesized ruthenium complex, which contains ligands with four carboxyl groups (Fig. 1b) which are able to bind to the surface of  $\text{TiO}_2$  NPs. On the other side, the central metal ion is free for the interaction with target biomolecules which is important for expression of its anticancer feature. After mixing these two components, NCS appeared in the form of red pellet (Fig. 1a) and potential binding structure is given in Fig. 1b. The binding through carboxylic groups to the surface of the  $\text{TiO}_2$  NPs, was also confirmed by Fourier transform infrared (FTIR) spectroscopy (data not shown).



**Fig. 1** Photo of the tube with synthesized NCS (a). Schematic presentation of binding of complex and  $\text{TiO}_2$  NPs in NCS (b)

### 3.2 Biological activity of Ru-complex, $\text{TiO}_2$ NPs and NCS

In the next step we have checked NCS and its components for their potential applications as systems for metallo-drug delivery. We used readily available cells, blood lymphocytes, for that purpose.

As the measure of their activities, we have determined the following:

- *The cell proliferation index (CBPI)*, which is a measure of the number of cells that are dividing (proliferating) in the treated cell culture compared with control cells. It is known that cancer cells grow and divide in an uncontrolled manner, invading normal tissues. Therefore, it is of great importance to check CBPI of NCS and its components. All tested concentration showed statistically significant decreases in the cell proliferation (Table 1) which indicated that NCS and its components could reduce cell dividing. Given results confirms the potency of the use of the NCS and its components as anti-proliferative agent. Also, numerous in vitro studies on different cell types have shown cytotoxicity and genotoxicity to cells treated with  $\text{TiO}_2$  NPs (Park et al. 2008; Acar et al. 2015).
- *Antioxidative activity* It has been previously demonstrated that the main mechanism of  $\text{TiO}_2$  NPs toxicity is via oxidative species, which damages proteins and DNA, as well as electrical conductivity of cells (Hirakawa et al. 2004). On the other hand, it has been demonstrated that ruthenium complexes influence the cellular oxidative status (Anchuri et al. 2012). Accordingly, our next goal was to determine the cellular MDA levels, which reflect the degree of membrane lipid damage induced by free radicals and reactive oxygen species. In summary, increased MDA levels were determined only in the cells treated with the highest concentration of complex, NPs and NCS (Table 1), but also at the lowest concentration of NCS. This might imply the potency of the system in the cell killing, probably by the mechanism which involves the generation of free radicals and the damage of the cell membrane. Most probably, higher concentrations of NCS might trigger other mechanisms of the cellular defense, but also, both NCS components can act as free radical scavengers.

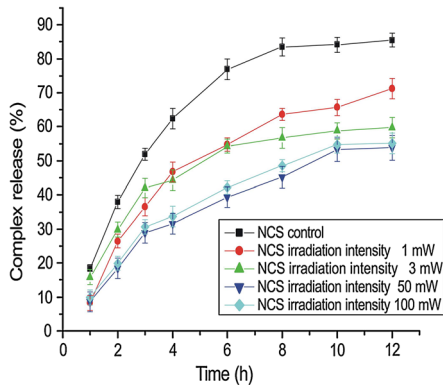
### 3.3 Photoinduced complex release study

Since photodynamic therapy represents attractive mode of therapy, after we have confirmed NCS potential biological applications, we have tested whether the light can be used

**Table 1** CBPI and MDA level in cultures treated with complex, TiO<sub>2</sub> NPs and NCS with respect to control

	Control	Complex 25 $\mu$ M	Complex 15 $\mu$ M	Complex 5 $\mu$ M	TiO <sub>2</sub> 25 $\mu$ M	TiO <sub>2</sub> 15 $\mu$ M	TiO <sub>2</sub> 5 $\mu$ M	NCS 25 $\mu$ M	NCS 15 $\mu$ M	NCS 5 $\mu$ M
CBPI $\pm$ SD	1.76 $\pm$ 0.01	1.61 $\pm$ 0.02	1.62 $\pm$ 0.02	1.70 $\pm$ 0.02	1.59 $\pm$ 0.01	1.62 $\pm$ 0.02	1.62 $\pm$ 0.01	1.64 $\pm$ 0.03	1.66 $\pm$ 0.02	1.62 $\pm$ 0.02
MDA $\pm$ SD	2.79 $\pm$ 0.77	14.45 $\pm$ 1.89	2.95 $\pm$ 0.94	5.33 $\pm$ 1.35	17.93 $\pm$ 8.49	5.84 $\pm$ 0.09	7.32 $\pm$ 3.65	12.64 $\pm$ 1.12	4.57 $\pm$ 1.32	16.54 $\pm$ 5.65

All data are presented as percentage (mean  $\pm$  SD)



**Fig. 2** Comparative complex release profiles of non-irradiated NCS (control) and NCS irradiated with *green light*. The experiment conditions for the release tests were the same and they were carried out at the room temperature. (Color figure online)

for modulation of the time release profiles of complex from the NCS. Both components of NCS,  $\text{TiO}_2$  NPs and complex, are photoactive substances.  $\text{TiO}_2$  NPs absorbs the UV light, while the complex absorbs UV and visible light. Ru-complex has a red color and absorbs green light, giving us the possibility to affect its binding properties to the surface of  $\text{TiO}_2$  NPs by the light with the wavelength of 532 nm. To check whether binding properties of the complex and its release kinetics from the  $\text{TiO}_2$  surface can be manipulated by the green light we have performed the complex release tests under various laser intensities. Results are shown in Fig. 2. When compared to the non-irradiated system, irradiation of the NCS results in the sustained complex release, which is dependent on the laser power. Irradiated NCS achieved maximum release of around 50 % merely after 12 h. For the same time interval, 85 % of free complex passed through the membrane in non-irradiated NCS sample. Increase in the light intensity, from 3 to 50 mW, induces increase in complex retention, while slight increase from 1 to 3 mW, and 50 to 100 mW did not lead to significantly different retention profile.

Described effects are mediated by the Ru-complex, since the  $\text{TiO}_2$  does not absorb visible light. It is possible that the complex, undergoes some structural changes after irradiation, which lead to increased binding to the surface. Similar effect has been previously observed with modified hollow mesoporous silica nanocomposites which can release drugs in a “release-stop-release” manner by converting light irradiation (Mei et al. 2012). To check this, we have recorded UV–Vis spectra, which indicate *cis*–*trans* isomerisation process, which might lead to the increased binding to the surface. Photoinduced *cis*- to *trans*- isomerization is common for many Ru(II) bis-bipyridyl complexes (Walsh and Durham 1982; Zakeeruddin et al. 1999).

## 4 Conclusion

We synthesized smart light-controlled potential anticancer drug delivery systems whose drug release properties can be manipulated by various light intensities. Due to the presence of the Ru-complex, the system has ability to respond to green light by sustaining complex release. Light-controlled sustained release provides promising way of decreasing the side

effects, i.e. keeps the drug bound to the surface until it reaches the target tissue. This increases effectiveness of the drug by its localization at the target tissue and provides uniform drug delivery, which makes this system of interest for further investigation.

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