**Photosensitizer potential of doped and undoped nanostructured TiO2**

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Treating cancer remains a major challenge, despite the development of many therapies and advances in general knowledge about the disease. The treatments commonly used are invasive and non-selective, leading to severe side effects and unsatisfactory long-term outcomes. Nevertheless, external stimuli activating therapeutic agents in the affected area can be more beneficial than these aggressive therapies. Photodynamic therapy (PDT) is a minimally invasive, selective treatment that uses photosensitizer (PS) to damage cancer cells. The PS is activated by light, triggering a series of processes that produce reactive oxygen species (ROS), ultimately leading to cancer cell death. Numerous types of nanomaterial possess the capability to act as PS, one of which is TiO2 [1]. Although nanostructured TiO2 is biocompatible in the absence of light, its valence band electrons can be stimulated only by ultraviolet (UV) light irradiation. Since the penetration of UV light into tissue is limited, for application in PDT, nanostructured TiO2 can be doped with heteroatoms like N or C to allow visible light responsiveness [2,3].

This work evaluated the PS properties of unmodified nanostructured TiO2 (spherical nanoparticles TiO2 NPs and prolate nanospheroids, TiO2 PNSs) and doped TiO2 (N- and C-TiO2 NPs). After the synthesis, the size of TiO2 was confirmed to be in the nanoscale range (5-104 nm) by transmission electron microscopy [3,4]. The doped TiO2 was found to absorb visible light, as demonstrated by UV-Vis spectroscopy and bandgap calculations. Additionally, hydroxyl radicals were detected in water suspensions of TiO2 PNSs by electron paramagnetic resonance (EPR) spectroscopy, both with and without UV light illumination [4]. However, this radical was observed only with blue light stimulation of the water suspensions of N- and C-TiO2 NPs [3].

Cell experiments further revealed the internalization process of nanostructured TiO2 within cells, their cytotoxicity profiles, and the different death modalities triggered by their uptake. After confocal microscopy indicated the successful internalization of the investigated TiO2, viability tests on different cell lines confirmed their good biocompatibility without light [3,4]. The PDT's efficacy using nanostructured TiO2 and appropriate light stimuli was evaluated on various cancer cell lines. The most significant viability reduction (60%) was observed in the HeLa cell line with the combined treatment of C-TiO2 NPs-blue light. In addition to EPR results, blue light-induced C-TiO2 NPs-catalyzed generation of ROS was confirmed intracellularly, implying that oxidative stress was the leading cause of HeLa cell death. Fluorescent labeling allowed distinguishing morphological changes inside the cells after the C-TiO2 NPs, blue light, and the combined C-TiO2 NPs-blue light treatment. Blue light exposure led to the appearance of large necrotic cells with deformed nuclei, cytoplasm swelling, and membrane blebbing. In contrast, the combined therapy with C-TiO2 NPs-blue light resulted in controlled cell death, such as autophagy. Since programmed cell death is the desired cancer cell death mechanism, the combined treatment presented here can provide a better outcome of local anticancer therapy.

REFERENCES

[1] Z. Haijun et al., J. Biomed. Nanotechnol. 10, 1450 (2014).

[2] Z. Li et al., Nano. Res. Lett. 6, 356 (2011).

[3] M. Matijević et al. Photochem. & Photobio. Sci. 20, 1087 (2021).

[4] M. Matijević et al., J. Nanopart. Res. 22, 175 (2020).