Available online on 15.10.2022 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Full Text Article

Mini Review

Nanoparticles of Cerium Dioxide and Pristine (Unmodified) Fullerene C_{60} Protect Living Cells against Adverse Environmental Exposure: Does it Work?

Oksana V. Falko[1]*, Viktor V. Chizhevskiy[1], Vladimir K. Klochkov[2], Viktoria V. Yevlash[3]

1 – Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

2 – Institute for Scintillation Materials of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

3 – Kharkiv State University of Food Technology and Trade, Ukraine

Article Info:

Article History:

Received 21 August 2022
Reviewed 18 Sep 2022
Accepted 22 Sep 2022
Published 15 Oct 2022

Cite this article as:

Falko OV, Chizhevskiy VV, Klochkov VK, Yevlash VV, Nanoparticles of Cerium Dioxide and Pristine (Unmodified) Fullerene C_{60} Protect Living Cells against Adverse Environmental Exposure: Does it Work?, Journal of Drug Delivery and Therapeutics. 2022; 12(5-S):1-4

DOI: http://dx.doi.org/10.22270/jddt.v12i5-s.5697

*Address for Correspondence:

Oksana V. Falko, Institute for Problems of Cryobiology and Cryomedicine of the National Academy of Sciences of Ukraine, Kharkiv, Ukraine

Abstract

We believe that unmodified hydrated C_{60} fullerene (C60FWS) and nanocrystalline cerium dioxide (NCD) are promising for biological research. It is knowns that these nanoparticles protect living cells from damaging environmental factors such as radioactive and ultraviolet radiation, temperature, hypoxia, toxic substances, etc. However, little attention has been paid in scientific reports to the use and study of these nanoparticles as possible adaptogens. In our studies, we tried to protect the culture of cyanobacteria *Spirulina platensis* with C_{60} and NCD from adverse conditions. The choice of *Spirulina platensis* is due to its sensitivity to adverse environmental factors. The results obtained show that the absence of toxic effects of C_{60} and NCD of selected concentrations on the *Spirulina platensis* culture has been found. Nanoparticles C_{60} and NCD maintained the culture of *Spirulina platensis* for at least 4 weeks in the absence of nutrients, light, low pH storage conditions and low temperature. Cyanobacteria stored for 1–4 weeks in distilled water with nanoparticles showed increased proliferative activity compared to samples stored at the same time in the standard Zarrouk's nutrient medium.

Keywords: fullerene C_{60}, nanocrystalline cerium dioxide, *Spirulina platensis*, adaptogens.

1. INTRODUCTION

Recently, the rapid development of nanotechnology has made it possible to create innovative nanomaterials with fundamentally new properties and great potential to be used in biology, medicine and pharmacy. Unique in structure and chemical qualities the nanoparticles demonstrate a wide range of biological action and capabilities as a tool for influencing the functional systems of cells and the body as a whole [1,2,3,4,5].

However, the toxicity of these compounds remains a serious problem [6,7,8,9]. Undoubtedly, the disputes and misunderstandings in this regard are due to the fact that the studying of biological properties of nanoparticles does not always comprehensively assume the conditions of synthesis, methods of stabilizing the particles in colloidal systems, their size, transport to biological targets etc. [5,10,11] *i.e.* the parameters depending on which the biological effects of nanoparticles can vary from cytoprotective to cytotoxic ones.

For example, there are quite a number of water-soluble derivatives of fullerene C_{60}, used in various medical and biological experiments. However, it should be borne in mind that there is a significant difference between the physicochemical and, consequently, biological properties of the solutions of unmodified fullerene and those of its water-soluble derivatives. When converting a hydrophobic fullerene molecule into an aqueous solution, the final products may strongly differ by properties; demonstrate new aspects of activity due to changes in the acquisition of biological activity by them. In our opinion, the use of pure unmodified fullerene is promising for the detection of a direct biological action of fullerene C_{60}.

Due to the physicochemical structure of fullerenes C_{60} and nanocrystalline cerium dioxide (NCD) have pronounced antioxidant properties, as they are able to participate in biological processes as the ROS regulators and free radical acceptors [12,13,14].

In particular, the protective effect of cerium dioxide nanoparticles has been shown in a model of oxidative stress induced by tobacco smoke toxicants [15].

NCD and water-soluble unmodified C_{60} fullerene exhibit radioprotective properties and can strongly reduce the harmful effects of ionizing radiation [16,17]. Cerium dioxide nanoparticles also have a photoprotective effect to the cells against ultraviolet light [18,19]. There are some reported data that nanoparticles of unmodified C_{60} fullerene protect skeletal muscles from ischemic lesions [20,21], have a stabilizing effect on

enzymes, protecting them from thermal and oxidative inactivation [22].

That is, the protective properties of the presented nanoparticles can be promising when searching the effective means of artificial protection of living cells from the negative effects of environmental factors.

The aim of this research was to study the biological effect of aqueous solutions of nanocrystalline cerium dioxide and unmodified C_{60} fullerene on the culture of *Spirulina platensis* when stored in distilled water at moderately low temperatures (4-5°C).

2. MATERIALS AND METHODS

(a) Subjects

The research was performed in the culture of *Spirulina platensis* cyanobacteria and aqueous solutions of nanocrystalline cerium dioxide (NCD) as well as unmodified hydrated fullerene C_{60} (C_{60}FWS).

In our studies we used a water-soluble hydrated pristine (unmodified) fullerene C_{60} (C_{60}FWS - C_{60}HyFn), (manufactured by LLC «Institute of Physiologically Active Compounds (IPAC)», Ukraine, Kharkiv).

Also in these investigations we applied the nanocrystalline cerium dioxide (NCD) with a particle size of 2 nm obtained by wet synthesis (produced by the Institute for Scintillation Materials of the National Academy of Sciences of Ukraine, Kharkiv).

(b) Protocol

Before the experiments, the culture of *Spirulina platensis* was washed from the growth medium with distilled water using a nylon filter and placed in aqueous solutions of fullerene C_{60} with a final concentration of 14.4 mg / l ($2x10^{-5}$ M) and NDC – 20 mg / l. Each experimental sample contained 300 µl of *Spirulina platensis* biomass in 1.0 ml of the studied solution of nanoparticles (number of trichomes made ~ 3.75 $x10^9$ / l). The selected concentration of fullerene solutions C_{60} and NCD was the maximum allowable, taking into account the possible toxic effects of nanoparticles [6, 23].

Experimental samples of cyanobacteria were stored in distilled water at a temperature of 4–5°C in a household refrigerator. To assess the safety of cells we used the method of quantitative counting of *Spirulina platensis* intact trichomes.

Biomass growth of *Spirulina platensis* was evaluated by the suspension optical density using a Solar PV1251C spectrophotometer (Belarus) at a wavelength of 750 nm. Measurements were performed in cuvettes with a working side length of 1 cm.

Washed from the Zarrouk's nutrient medium the culture of *Spirulina platensis* was divided into the following experimental groups, which were stored: 1 - in distilled water; 2 - with adding the C_{60}FWS; 3 - with the NCD adding; 4 - in the Zarrouk's cultural medium. Observations were performed for 28 days. These results were statistically processed according to Student's criterion with 0.95 reliability.

3. RESULTS

(a) Results of morphology analysis

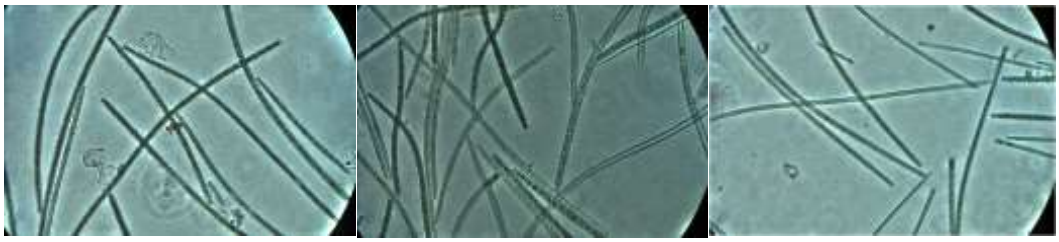
An important point in favor of choosing the *Spirulina platensis* cyanobacteria as a biological model in our studies was its increased sensitivity to any environmental changes. The response of *Spirulina platensis* to any changes is an instant death of the trichomes. That is why this object could be used as a biological indicator of the first link in the food chain when studying an environmental pollution [24].

In our previous studies, we demonstrated that the addition of fullerene C_{60} nanoparticles at a final concentration of 14.4 mg / l or NCD of 20 mg / l did not show toxic effects on *Spirulina platensis*, stored in Zarrouk's medium at a temperature of 18 ÷ 20ºC (Table 1).

Table 1: Preservation of *Spirulina platensis* culture in the studied media at 18 ÷ 20ºC

Media studied	Number of non-damaged trichomes, %	
	day 1	day 7
Zarrouk's medium	99.0 ± 1.0	98.0 ± 2.0
Zarrouk's medium + fullerene C_{60} (14.4 mg / l)	99.0 ± 1.0	95.0 ± 1.0
Zarrouk's medium + NCD (2nm, 20 mg /l)	99.0 ± 1.0	92.0 ± 1.0

The morphology of *Spirulina platensis* trichomes after storage for 7 days at 18 ÷ 20ºC in the studied media is shown in Figure 1.



а) б) в)

Figure 1: *Spirulina platensis* culture, stored: a) in the Zarrouk's cultural medium; b) with the C_{60} adding; c) with NCD adding, magnification x40.

Thus, the morphology of *Spirulina platensis* trichomes and their number after storage at a temperature of 18 ÷ 20°C for 7 days in a nutrient medium with the addition of nanoparticles of cerium dioxide and hydrated fullerene C_{60} did not differ from the samples stored just in Zarrouk's medium. Therefore, the obtained experimental results indicated the absence of toxic effects of NCD and hydrated fullerene C_{60} on *Spirulina platensis* culture.

Based on the main idea of our study, to determine the possible biological protective effect of nanoparticles against the negative influence of the environment, we intended to create unfavorable living conditions for the *Spirulina platensis* culture, namely: lack of nutrients, reduced pH of the storage medium, lack of light and lower temperatures. Therefore, later the culture of *Spirulina platensis* of the four studied groups was stored in a household refrigerator; the condition of the samples was examined at intervals of 7 days. Morphology of *Spirulina platensis* trichomes and their number after storage at 4-5ºC for 21 days in groups 2 and 3 did not differ from the samples stored in Zarrouk's medium ($p > 0.05$), while in group 1 all the trichomes were damaged. After 3-4 weeks of storage there was a decrease in the number of *Spirulina platensis* trichomes in the samples of group 2, compared with those of groups 3 and 4 ($p < 0.05$) (Table 2).

Table 2: Preservation of *Spirulina platensis* culture trichomes in NCD and hydrated fullerene C_{60} presence

Experimental groups	Number of non-damaged trichomes, %				
	day 1	day 7	day 14	day 21	day 28
№1	97.0±1.0	12.0±2.0	1.0±1.0	–	–
№2	99.0±1.0	95±1.0	90.0±3.0	70.0±4.0	52.0±2.0
№3	98.0±1.0	97±1.0	86.0±3.0	80.0±3.0	65.0±6.0
№4	98.0±1.0	95.0±2.0	90.0±6.0	80.0±3.0	69.0±2.0

(b) Results preservation the *Spirulina platensis*

To determine the preservation rate of the experimental samples, the *Spirulina platensis* culture proliferative activity was examined. Cells of each experimental group after each storage step (first day; 1 – 4 weeks) were subsequently cultured for 5 weeks in Zarrouk's medium. Optical density Indices of *Spirulina platensis* culture suspension, significantly changed depending on the period of previous hypothermic storage of cyanobacteria and the composition of the storage is shown in Figure 2.

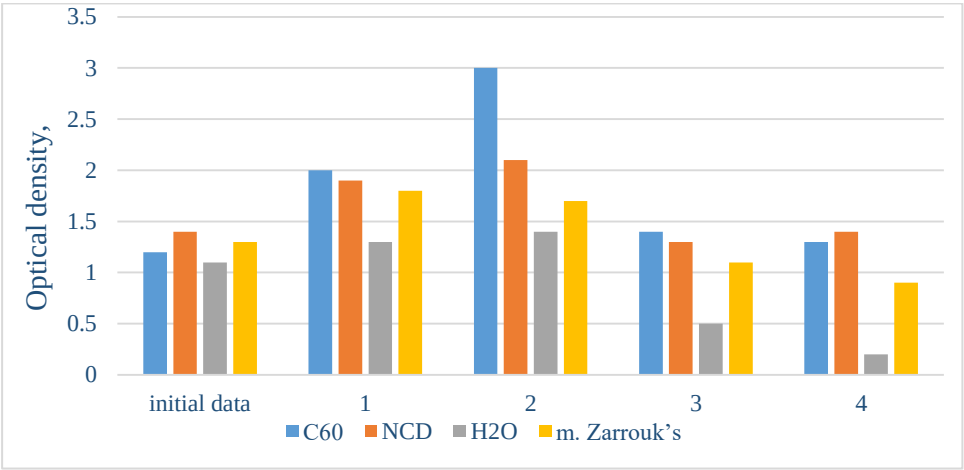


Figure 2: Optical density of *Spirulina platensis* suspension at different times of its cultivation in Zarrouk's medium after hypothermic storage.

In general, we can conclude that the culture of *Spirulina platensis* is able to survive in "deadly" conditions of existence in the presence of nanoparticles studied by us.

We noted above that the biological action of nanoparticles depended on many physicochemical factors, synthesis conditions, etc., the issue of the behavior of C_{60} fullerene and NCD in the presence of electrolytes in the dissolution medium deserves special attention. According to the published reports, the presence of, for example, Na + ions, K +, promotes the agglomeration of nanoparticles of unmodified fullerene C_{60} and NCD, which ultimately affects their bioavailability [10, 11, 25]. Therefore, our study of the effect of nanoparticles on the culture of *Spirulina platensis*, stored in distilled water, suggests that the nanoparticles of unmodified fullerene C_{60} and NCD"were able" to maximize their own biological action.

It is also important that the aqueous solution of unmodified fullerene C60FWS used in the research is registered and certified for application in Ukraine as a dietary supplements, namely as a "Fullerene C_{60} hydrated" concentrate (C60FWS). That is, it enables the application of research results for the needs of food industry, cosmetology and others.

We believe, that the biological effect of the solutions of nanosized compounds studied by us may be related to their antioxidant activity, the ability to prevent the negative effects of free radical compounds that occur during storage of cells. The same opinion is expressed by some authors, arguing that hydrated fullerene C_{60} is able to simulate free radical life processes at different levels of biological organization - to stabilize the state of enzymes under adverse conditions, blood parameters, etc. [26]. In turn, the protective effect of CeO_2 nanoparticles may also be due to their antioxidant activity, the ability to stimulate the expression of key genes involved in the cell response to oxidative stress [27]. However, our findings necessitate further study of the mechanisms of protective action

of nanoparticles and detailed development of techniques for the use of unmodified C_{60} fullerene and NCD in practical biology.

CONCLUSIONS

1. The presence of nanoparticles of cerium dioxide of 2 nm size in 0.02 g / l concentration and hydrated fullerene C_{60} with a concentration of 14.4 mg / l allows the storage of *Spirulina platensis* cells at f 4-5°C for 4 weeks without the use of Zarrouk's nutrient medium.

2. The use of nanosized particles of fullerene C_{60} and NCD stimulates the proliferation of *Spirulina platensis* after hypothermic storage.

REFERENCES

1. Eswaran SV. Water soluble nanocarbon materials: a panacea for all? Curr Sci. 2018; 114:1846-50 https://doi.org/10.18520/cs/v114/i09/1846-1850

2. Matyshevska OP, Prylutska SV, Grynyuk II. Fullerenes C60 - biologically active molecules I. Physicochemical properties and bioavailability. Biotechnologia Acta. 2010; 3(1):18-26.

3. Goltsev A, Bondarovych M, Babenko N, GaevskaYu, Dubrava T, Ostankov M. Use of Nanomaterials in Cryobiology and Cryomedicine. Probl Cryobiol Cryomed 2020; 30(4):313-330 https://doi.org/10.15407/cryo30.04.313

4. Larner SF, Wang J, Goodman J et al. In vitro neurotoxicity resulting from exposure of cultured neural cells to several types of nanoparticles. J Cell Death. 2017 Mar 23; 10:1179670717694523. https://doi.org/10.1177/1179670717694523

5. Goodarz S, Da Ros T, Conde J, Sefat F, Mozafari M. Fullerene: biomedical engineers get to revisit an old friend Materials Today. 2017; 20(8):460-80 https://doi.org/10.1016/j.mattod.2017.03.017

6. Andrievsky GV, Klochkov V, Derevyanchenko L. Is C60 fullerene molecule toxic? // Fuller. Nanotub. Carbon Nanostruct. 2005; (13): 363-376. https://doi.org/10.1080/15363830500237267

7. Paresh Chandra Ray, Hongtao Yu, Peter P Fu Toxicity and Environmental Risks of Nanomaterials: Challenges and Future Needs J Environ Sci Health C Environ Carcinog Ecotoxicol Rev. 2009; 27(1):1-35. https://doi.org/10.1080/10590500802708267

8. Kumar V, Dasgupta N, Ranjan S. Environmental Toxicity of Nanomaterials, CRC Press; 2018. 537 p. https://doi.org/10.1201/9781351252966

9. Sforzini S, Oliveri C, Barranger A, Jha AN, Banni M, Moore MN, Viarengo A. Effects of fullerene C60 in blue mussels: Role of mTOR in autophagy related cellular/tissue alterations Chemosphere. 2020; (246):11-3. https://doi.org/10.1016/j.chemosphere.2019.125707

10. Grynyuk II, Prylutska SV, Slobodyanik NS, Chunikhin OYu, Matyshevska OP. The aggregate state Of c60-fullerene in various medi. Biotechnologia acta, 2013; 6(6):71-6.

11. Kavok N, Grygorova G, Klochkov V, Yefimova. The role of serum proteins in the stabilization of colloidal LnVO4:Eu3+ (Ln = La, Gd, Y) and CeO2 nanoparticles. Colloids and Surfaces. 2017; (529):594-599. https://doi.org/10.1016/j.colsurfa.2017.06.052

12. Ivanov VK, Shcherbakov AB, Usatenko AV. Structure-sensitive properties and biomedical applications of nanodispersed cerium dioxide. Russ chem rew. 2009; 79(8): 924-43 https://doi.org/10.1070/RC2009v078n09ABEH004058

13. Shcherbakov AB, Zholobak NM, Ivanov VK, Tretyakov YuD, Spivak NYa. Nanomaterials based on the nanocrystalline ceric dioxode: properties and use perspectives in biology and medicine. Biotechnologia Acta. 2011; 4(1):9-28.

14. Shirinkin SV, Volkova TO, Shaposhnikov AA, Andreivskiy GV, Davidovskiy AG. Hydrated fullerene as an instrument to understand the role of special structural characteristics of aqueous medium of a living organism for its normal functioning. Belgorod State University. Scientific Bulletin. Natural sciences. 2012; 128(9):34-50. Russian.

15. Niu J, Wang K, Kolattukudy PE.Cerium oxide nanoparticles inhibit oxidative stress and nuclear factor-κB activation in H9c2 cardiomyocytes exposed to cigarette smoke extract. J Pharmacol Exp Ther. 2011; 338(1):53-61. https://doi.org/10.1124/jpet.111.179978

16. Gudkov SV, Guryev EL, Gapeyev B, Sharapov MG, Bunkin NF, Alexey V Shkirin AV, et al. Unmodified hydrated C60 fullerene molecules exhibit antioxidant properties, prevent damage to DNA and proteins induced by reactive oxygen species and protect mice against injuries caused by radiation-induced oxidative stress. Nanomedicine: Nanotechnology, Biology, and Medicine. 2019; (15):37-46 https://doi.org/10.1016/j.nano.2018.09.001

17. Colon J, Herrera L, Smith J, Patil S, Komanski C, Kupelian P, Seal S, Jenkins DW, Baker CH Protection from radiation-induced pneumonitis using cerium oxide nanoparticles. Nanomedicine. 2009; 5(2):225-31 https://doi.org/10.1016/j.nano.2008.10.003

18. Fujita N., Kamada K. Protective effect of Ce02 nanoparticles on photo-induced oxidative damage of DNA. Journal of the Ceramic Society of Japan. 2014; (122):141-5 https://doi.org/10.2109/jcersj2.122.141

19. Kong L, Cai X, Zhou X, Wong LL, Karakoti AS, Seal S, McGinnis JF. Nanoceria extend photoreceptor cell lifespan in tubby mice by modulation of apoptosis/survival signaling pathways. Neurobiol Dis. 2011; 42(3):514-23. https://doi.org/10.1016/j.nbd.2011.03.004

20. Kartal H, Küçük A, Kiliçarslan A, Polat Y, Süngü N, Kip G, Arslan M. The effect of fullerenol C60 on skeletal muscle after lower limb ischemia reperfusion injury in streptozotocin-induced diabetic rats. J Surg Med. 2020; 4:451-5, https://doi.org/10.28982/josam.756665

21. Nozdrenko D, Matvienko T, Vygovska O, Bogutska K, Motuziuk O, Nurishchenko N,et al. Protective effect of water-soluble C60 fullerene nanoparticles on the ischemia-reperfusion injury of the muscle soleus in rats. Int. J. Mol. Sci. 2021; 22(13):6812. https://doi.org/10.3390/ijms22136812

22. Yablonskaya IO, Voeikov VL. Effect of hydrated C60 fullerenes on enzyme activity (in Russian) In: Proceedings of III Eurasian Congress on Medical Physics and Engineering "Medical Physics - 2010". 21-25 of June, 2010 At: Moscow V.3. p. 311-313.

23. Popov AL, Popova NR, Ivanov VK. Cerium oxide nanoparticles are not toxic for human mesenchymal stem cells and are able to stimulate their proliferation in vitro (in Russian) In: Proceedings of XI International Science-Technical Conference BPPC - 2016. 25-29 of April, 2016 At: Sevastopol V.2, p. 47-51.

24. Zholobak NM, Lysenko TG. Cyanobacterium Synechococcus cedrorum - the test-culture for toxic bioindication of new chemically synthesized medicinal substances. Reports of the National Academy of Sciences of Ukraine. 2009; (7):123-5. Russian.

25. Chen K. L., Elimelech M. Aggregation and deposition kinetics of fullerene (C60) nanoparticles. Langmuir. 2006; (22):10994-11001, https://doi.org/10.1021/la062072v

26. Novikov KN, Berdnikova NG, Novikov AK, Lyusina OY, Muhitova OG, Yablonskaya OI, Minh HD, Voeikov VL. Changes in chemiluminescence of whole blood of COPD patients treated with Hypoxen® and effects of C60 fullerenes on blood chemiluminescence. Med Sci Monit. 2012; 18(2):76-83. https://doi.org/10.12659/MSM.882460

27. Popov AL, Popova NR, Selezneva II, Ivanov VK. Modulation of the expression of antioxidant enzymes and proinflammatory cytokines by cerium oxide nanoparticles in vitro. European Journal of Human Genetics. 2015; 23(1):450.