# INTERNATIONAL SCIENCE REVIEWS

AIU

No. 2 (1) 2020

Natural Sciences and Technologies series

www.aiu.kz

ISSN: 2707-4862

ASTANA INTERNATIONAL UNIVERSITY

# INTERNATIONAL SCIENCE REVIEWS
# Natural Sciences and Technologies series

*Has been published since 2020*

## №2 (1) 2020

Editorial address: 8, Kabanbay Batyr avenue, of.316, Nur-Sultan,
Kazakhstan, 010000
Tel.: (7172) 24-18-52 (ext. 316)
E-mail: natural-sciences@aiu.kz



**International Sciense Reviews NST - 76153**
**International Science Reviews**
Natural Sciences and Technologies series
Owner: Astana International University
Periodicity: quarterly
Circulation: 500 copies

## CONTENT

# EFFICIENCY OF USING ALAMARBLUE CELL VIABILITY REAGENT TO STUDY EFFECTS OF CARBON NANOPARTICLES ON CANCER CELLS

**Qing Yang**

**Abstract.** *Background:* Nanoparticles have been widely used to deliver drugs, image tumors inhibit cancer cells, and to apply in other diseases. In cancer treatment, we usually need to test viability on cell lines *in vitro*. Cell counting or viability testing reagents have been applied. However, special characteristics of nanoparticles such as color, fluorescence, and organelle binding may interfere the dye binding. Here we studied the effects of alamarBlue Cell Viability reagent staining conditions on cell viability measurement efficiency.

*Objective:* To test more effective staining time to investigate the cytotoxicity of human cancer cell using alamarBlue Cell Viability reagent.

*Methods:* The carbon nanodots were used as nanoparticles and from alamarBlue Cell Viability Reagent staining method was used to measure cell viability. The A549 cells were used for measurement their cell growth inhibition and survival.

*Results:* In cancer cells, alamarBlue Cell Viability Reagent showed some efficiency of measurement of cell viability upon nanoparticles treatment with the best optimal time of 3hrs. The 1hr and 24hr staining did not show the better results of measurement of cell viability according to the concentration dependent trend.

*Conclusion:* Our results confirm alamar Blue Cell Viability Reagent method can be used by optimized condition to detect cell survival in cells *in vitro*.

## INTRODUCTION

Cell viability assay is widely used in anti-cancer drug screening and pre-clinical testing *in vitro*. Differential reagents are used based on cell characteristics upon drug treatment. For example, cell counting, MTT assay, crystal violet assay. Many methods are based on the characteristics of live cells with high energy release of mitochondria, or cell DNA intact, or metabolic intact.

Nanoparticles have been used for drug delivery, bioimaging and anti-cancer drugs. Carbon-based materials have been applied in nanomedicine. To test the nanotoxicity and anti-cancer effect, nanoparticles cannot be removed from cells and the measurement of cell survival may not be accurate. This is because of potential fluorescence from nanoparticles. When use fluorescence dyes to bind organelle or metabolite, there might be organelle or metabolic competitive binding if nanoparticle can cause cell death through these mechanisms. Therefore, it is needed to test the efficiency of the viability measurement when studying nanoparticles effects.

The mechanism of cell viability interruption by nanoparticles can vary. For

example, Carbon nanodots (CDs) can cause DNA damage, cell cycle arrest, DNA conformation switch even more details remain unclear. Here we applied tomato derived CDs to test their effect on inhibition of cell growth.

## MATERIALS AND METHODS

Alamar Blue Cell Viability Reagent was obtained commercially from ThermoFisher (https://www.thermofisher.com/order/catalog/product/DAL1025#/DAL1025). It is used for regular cell viability assay of drugs. The alamarBlue Cell Viability Reagents are ready-to-use that detect the reducing of living cells to test cell survival.

Here we applied alamarBlue™ Cell Reagent to measure the cell viability to optimize the best staining condition and test the potential efficiency of nanoparticles from tomato on anti-cancer effect.

1. Cell viability testing reagent: alamar Blue™ Cell Viability Reagent

Alamar Blue Cell Viability Reagent (ThermoFisher https://www.thermofisher.com/order/catalog/product/DAL1025#/DAL1025). It is used for cell viability assay using carbon nanoparticles drugs.

2. Cell lines A549 cells were originally developed 50 years ago through the explant tumor of a 58-year-old white male, metastasized and cultured lung tumor tissue in medium. The cells are squamous, which can spread substances such as water and electrolytes through alveolar diffusion. The cell line was purchased from ATCC (ATCC® CCL-185™) and can be used to screen for anti-cancer drug or test.

3. Cell culture method Cells were seeded in Dulbecco's Modified Eagle Medium (DMEM). DMEM is the most widely applicable medium for culturing adherent cells. Here we added 10% serum of FBS, and antibiotics of penicillin/streptomycin based on protocol provided by the reagent manufacture (Life Technologies).

4. Cell density for cell growth inhibition assay

Usually in 96-well plates, cells were seeded as 5 000 and 2 500 per well in 24hrs in advance followed by treatment of nanoparticles drug. Due to many concerns of cell attachment due to nanoparticles targeting cell surface, we applied 5 000 cells per well in this test.

5. Cell staining method by alamarBlue™ Cell Viability Reagent

Cells were stained for 1hour, 3hours and 24 hours respectively and followed the standard protocol from manufacturer by spectrophotometer.

6. Carbon nanoparticles

Carbon nanodots were used as nanoparticles which were synthesized by Chemistry Department. Cells were undergone treatment in a 100 ul total volume in 96-well plates by differential concentration of nanoparticles calculated and medium used for making up total same volume finally.

## RESULTS AND DISCUSSION

We used carbon nanodots to test the differential times effect on cell staining for cell viability assay. As shown on Figure 1, cells stained for 1hour showed the significant toxic effect on cells at low concentration but not high concentration. It is likely due to nanoparticles uptake efficiency in cells. High concentration may cause cluster of nanoparticles and big enough to be blocked by cell membrane.

A549 cells are big sized compared to other cells. There may be other factors for drug effect in different cells, such as cell signaling and surface protein receptors or transporters. Moreover, the dye itself may interfere the stain and update of dyes due to cell size, nanoparticles size, cell membrane signaling.

As shown on Figure 2, at 3hrs staining, we found the same problem. However, 3hrs staining is much better for concentration trend.

As shown on Figure 3, 24 hrs staining may cause over lone stain and 1-3 hrs significant different toxicity showed no significant difference in 24 hrs. Thus, based on Figure 1 and 2, 24 hrs data is not consistent and over stain may cause false effect.

In conclusion, our data suggest 3hrs staining is the most effective time among tested and more time points maybe tested in future to get the best optimized results.

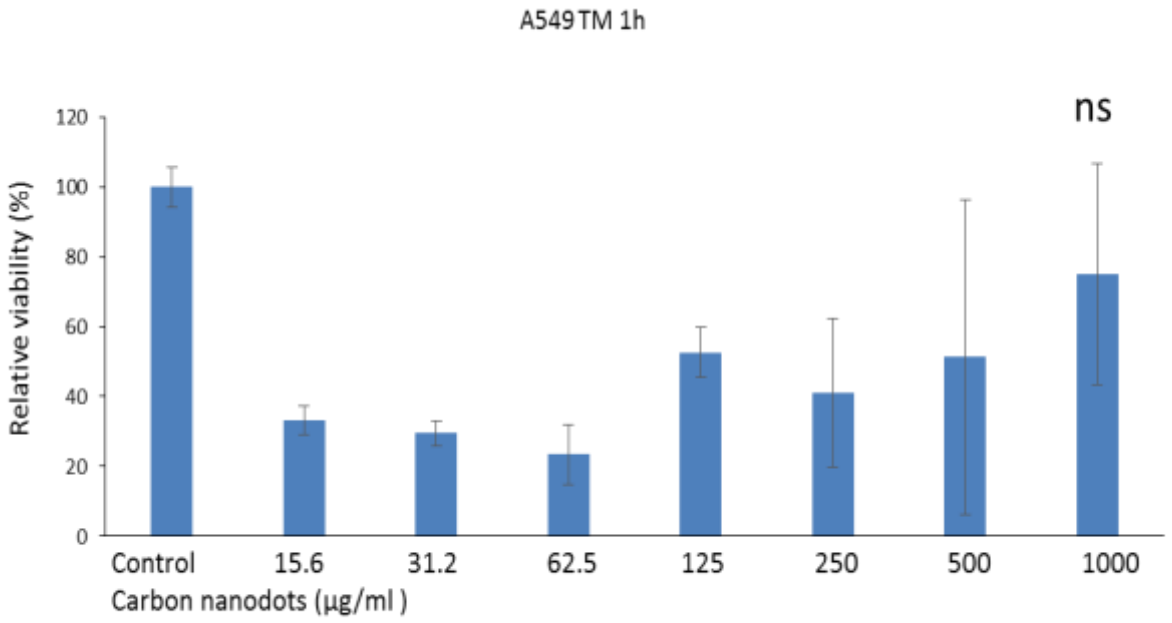


Figure 1

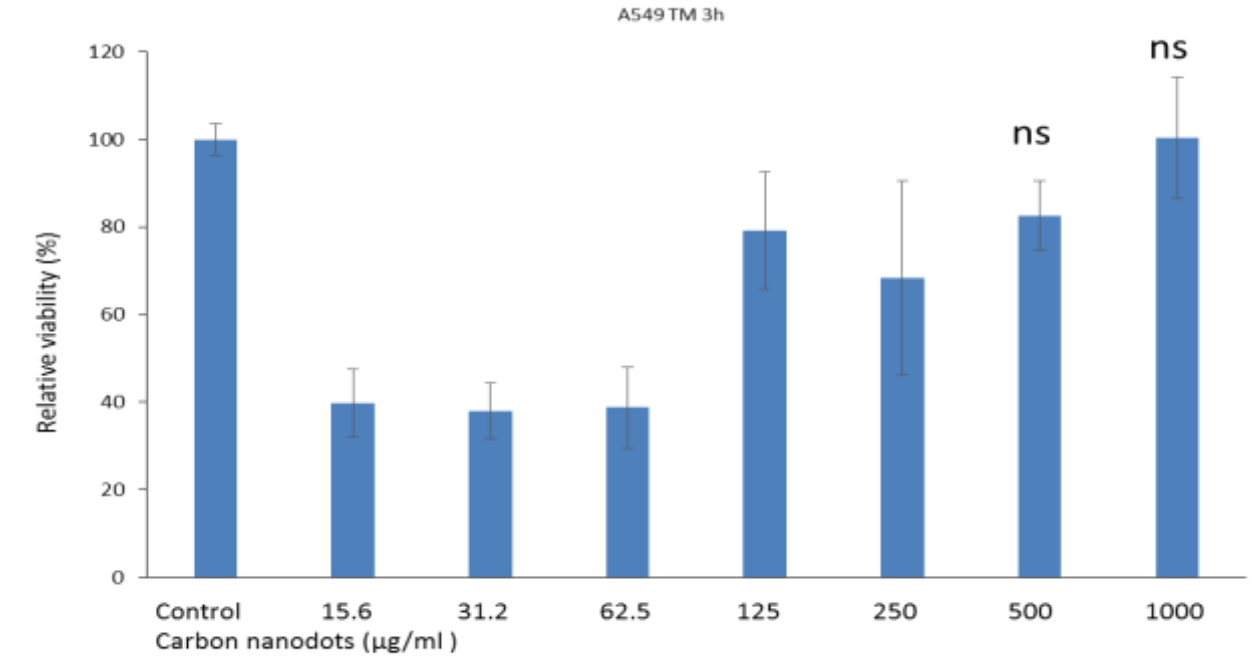


Figure 2

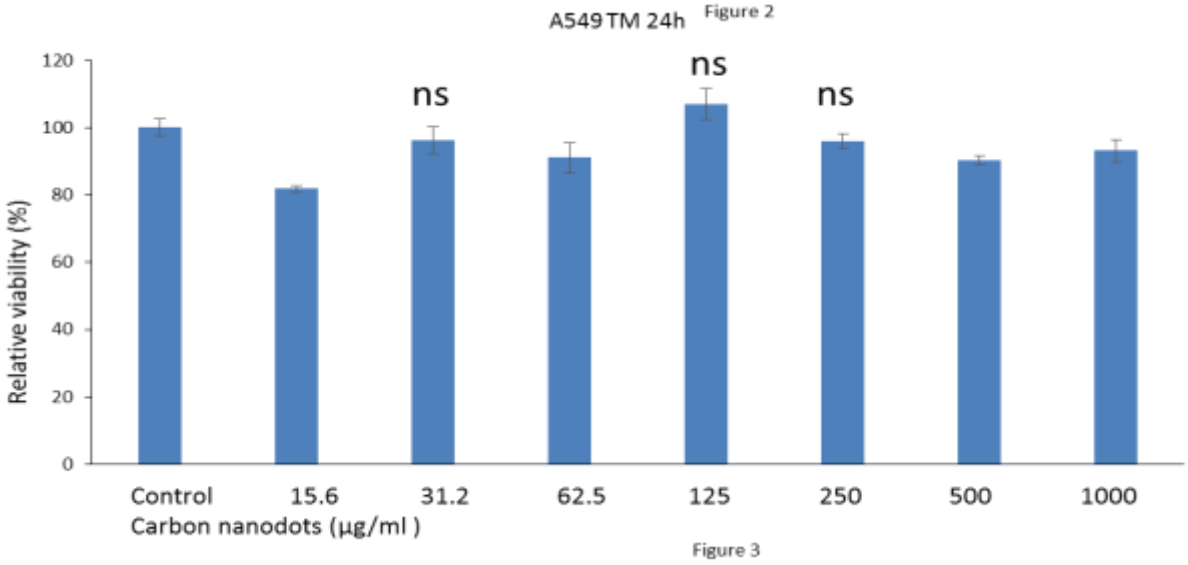


Figure 3

Figure 1-3 Carbon dots of tomato (TM) effect on A549 cancer cell by dye staining of 1hr, 3hr and 24hrs. Not significant results are shown as ns, while all others are significant without marker shown.

## REFERENCES

1. Panwar N, Soehartono AM, Chan KK, Zeng S, Xu G, Qu J, Coquet P, Yong KT, Chen X. Nanocarbons for Biology and Medicine: Sensing, Imaging, and Drug Delivery. Chem Rev. 2019 Aug 28;119(16):9559-9656.
2. Chung S, Revia RA, Zhang M. Graphene Quantum Dots and Their Applications in Bioimaging, Biosensing, and Therapy. Adv Mater. 2019 Dec 12:e1904362.
3. Shi X, Wei W, Fu Z, Gao W, Zhang C, Zhao Q, Deng F, Lu X. Review on carbon dots in food safety applications. Talanta. 2019 Mar 1;194:809-821.
4. Chen BB, Liu ML, Li CM, Huang CZ. Fluorescent carbon dots functionalization. Adv Colloid Interface Sci. 2019 Aug;270:165-190.

5. Zhao DL, Chung TS. Applications of carbon quantum dots (CQDs) in membrane technologies: A review. Water Res. 2018 Dec 15;147:43-49.
6. Ehtesabi H, Hallaji Z, Najafi Nobar S, Bagheri Z. Carbon dots with pH- responsive fluorescence: a review on synthesis and cell biological applications. Mikrochim Acta. 2020 Jan 27;187(2):150.
7. Iravani S, Varma RS. Green synthesis, biomedical and biotechnological applications of carbon and graphene quantum dots. A review. Environ Chem Lett. 2020 Mar 10:1-25.
8. He P, Shi Y, Meng T, Yuan T, Li Y, Li X, Zhang Y, Fan L, Yang S. Recent advances in white light-emitting diodes of carbon quantum dots. Nanoscale. 2020 Feb 27;12(8):4826-4832.
9. Ali H, Ghosh S, Jana NR. Fluorescent carbon dots as intracellular imaging probes. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2020 Feb 10:e1617.
10. Ding C, Zhu A, Tian Y. Functional surface engineering of C-dots for fluorescent biosensing and in vivo bioimaging. Acc Chem Res. 2014 Jan 21;47(1):20-30.
11. Liang W, Bunker CE, Sun YP. Carbon Dots: Zero-Dimensional Carbon Allotrope with Unique Photoinduced Redox Characteristics. ACS Omega. 2020 Jan 6;5(2):965-971.
12. Dong X, Liang W, Meziani MJ, Sun YP, Yang L. Carbon Dots as Potent Antimicrobial Agents. Theranostics. 2020 Jan 1;10(2):671-686.
13. Radnia F, Mohajeri N, Zarghami N. New insight into the engineering of green carbon dots: Possible applications in emerging cancer theranostics. Talanta. 2020 Mar 1;209:120547.
14. Ashrafizadeh M, Mohammadinejad R, Kailasa SK, Ahmadi Z, Afshar EG, Pardakhty A. Carbon dots as versatile nanoarchitectures for the treatment of neurological disorders and their theranostic applications: A review. Adv Colloid Interface Sci. 2020 Feb 19;278:102123.
15. https://www.sigmaaldrich.com/life-science/cell-culture/classical-media-salts/dmem.html?gclid=EAIaIQobChMI-YqwlJ3A6AIVyoeyCh1nyAQ2EAAYAiAAEgIH3_D_BwE
16. https://www.lgcstandards-atcc.org/products/all/CCL-2.aspx?geo_country=kz#characteristics
17. https://www.thermofisher.com/order/catalog/product/DAL1025#/DAL1025
18. https://www.lgcstandards-atcc.org/products/all/CCL- 185.aspx?geo_country=kz
19. Nurkesh AA, Sun Q, Fan H, Dukenbayev K, Tsoy A, Altaikyzy A, Wang K, Xie Y. Date Pit Carbon Dots Induce Acidic Inhibition of Peroxidase and Disrupt DNA Repair in Antibacteria Resistance. Glob Chall. 2019 Aug 27;3(11):1900042.
20. Anand A, Manavalan G, Mandal RP, Chang HT, Chiou YR, Huang CC. Carbon Dots for Bacterial Detection and Antibacterial Applications-A Minireview. Curr Pharm Des. 2019;25(46):4848-4860.
21. Dhenadhayalan N, Lin KC, Saleh TA. Recent Advances in Functionalized Carbon Dots toward the Design of Efficient Materials for Sensing and CatalysisApplications. Small. 2020 Jan;16(1):e1905767.

# ЭФФЕКТИВНОСТЬ ИСПОЛЬЗОВАНИЯ РЕАГЕНТА VIABILITY КЛЕТОК ALAMARBLUE ДЛЯ ИЗУЧЕНИЯ ВЛИЯНИЯ УГЛЕРОДНЫХ НАНОЧАСТИЦ НА РАКА

**Цин Ян**

**Аннотация.** *Справочная информация*: наночастицы широко используются для доставки лекарств, опухолевые изображения подавляют раковые клетки и применяются при других заболеваниях. При лечении рака нам обычно необходимо тестировать жизнеспособность на клеточных линиях in vitro. Были использованы реагенты для подсчета клеток или тестирования жизнеспособности. Однако особые характеристики наночастиц, такие как цвет, флуоресценция и связывание с органеллами, могут препятствовать связыванию красителя. Здесь мы изучили влияние условий окрашивания реагента alamarBlue Cell Viability на эффективность измерения жизнеспособности клеток.

Qing Yang

*Цель:* раковых клеток человека с использованием реагента alamarBlue Cell Viability.

*Методы:* углеродные наноточки были использованы в качестве наночастиц, а из метода окрашивания реагентов alamar Blue для определения жизнеспособности клеток был использован метод измерения жизнеспособности клеток. Клетки A549 использовали для измерения их ингибирования роста клеток и выживания.
Результаты: В раковых клетках реагент жизнеспособности клеток alamar Blue показал некоторую эффективность измерения жизнеспособности клеток после обработки наночастицами с наилучшим оптимальным временем 3 часа. 1-часовое и 24-часовое окрашивание не показывало лучших результатов измерения жизнеспособности клеток в соответствии с зависимой от концентрации тенденцией.

*Заключение:* наши результаты подтверждают, что метод реагента Alamar Blue Cell Viability Reagent может быть использован в оптимизированных условиях для определения выживаемости клеток в клетках in vitro.

**Information about the author:** Qing Yang, *Education:* BS in oral medicine, Hebei North University (former Zhangjiakou Medical College), Zhangjiakou, Hebei Province, China, 2003-2008, *Professional Appointments:* Department of Biology, SSH, Nazarbayev University; 2014-2015, Vanderbilt University, Nashville, TN; 2011-2014, Department of Biochemistry and Cancer Biology, Meharry Medical College, Nashville, TN