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In Vitro Cytotoxicity Testing of Carbon Dot Nanoparticles

Sharath R*

Department of Biotechnology, M.S. Ramaiah Institute of Technology (Affiliated to Visvesvaraya Technological University, Belgaum) Bangalore, India

Abstract

Cancer remains as one of the deadliest disease, causing large number of deaths worldwide. The expanding use of cancer immunotherapy has revealed that the survival patients suffer from various serious side effects due to the use of available anti-neoplastic medicines. The development of nanoparticle-based drugs seems to be effective providing low side effects and targeted action on cancer cells. The metal nanoparticles pose some side effects due to their toxicity whereas the Non-metallic Carbon dots (C-dots) are non –toxic and their applications in the field of medicine are less known. In the present study C-dots were synthesized by a simple single step wet chemical method from three different compounds namely Glucose (GCD), Sucrose (SCD) and Fructose (FCD) and characterized with UV-Visible and fluorescence spectroscopic techniques. The C-Dots were screened for the cytotoxicity against two cancer cell lines human Liver carcinoma cells (HepG2) and human breast carcinoma cells (MCF -7) by the MTT assay. The IC_{50} of the C-dot nanoparticles (SCD, GCD, FCD) on HepG2 cells were 67.24, <50 and and<50 µg/ml respectively and on MCF-7 cells were 105, <50 and <50 µg/ml respectively. The study reveals that the C-dots possess cytotoxic activity against the cancer cell lines and can be further used for the anticancer drug discovery.

Keywords: C-dot nanoparticles (SCD, GCD, FCD); IC_{50}; Cytotoxicity

Introduction

Cancer is one of the first causes of mortality worldwide. As a result, the principle challenges faced in anticancer therapy using nanotechnology have been the design and development of nanomaterials with multiple functions [1]. The anticancer activity of metal nanoparticles like zinc oxide and are much explored. The silver nanoparticles are well known for their antimicrobial properties and studies have shown that they possess anticancer properties; hence researches are going on to extract these nanoparticles via Green Synthesis. The Pharmaceutical activities of non-metals like carbon dots are not much known. In one of the recent studies carbon dot supported atomically dispersed gold can be used as a mitochondrial oxidative stress amplifier for cancer treatment. Anticancer therapy using multifunctional nanomaterials can target a cancer or tumour, deliver therapeutic drugs, and monitor the tumour tissues. In recent years, the use of various types of biological labelling probes such as gold nanoshells semiconductor quantum dots, iron oxide nanoparticles carbon nanotubes and polymer nanocarriers has resulted in the development of sensitive and specific targeted cell imaging, sensing, and therapy for invitro and in vivo applications. However, many of those probes possess properties that considerably decrease their therapeutic efficacy, including poor photostability and solubility in aqueous media, poor bio distribution, and a lack of target selectivity. The Pharmaceutical activities of non-metals like carbon dots are not much known. C-Dots are small carbon nanoparticles (less than 10 nm in size) with various surface passivation schemes in which chemical functionalization with organic molecules have been most effective.

Alternatively, carbon-based multifunctional water-soluble nanomaterials can be fabricated from bulk biological sources into fluorescent probes, thus reducing ecological concerns and economic problems. Recently, our group reported green carbon dots (G-dots) from food waste-derived sources as novel fluorescent probes for cell imaging. Although G-dots do not possess any targeting ligands on their surfaces, they are biocompatible and stable in aqueous systems, permitting their use in biomedical applications [2]. Compared to previous metallic or inorganic nanoparticles, carbon dot delivery systems can be used to minimise cytotoxicity and improve clinical outcomes and comprise the next generation of multifunctional nano medicine. Nevertheless, there are several critical issues involved in the design of carbon nano materials, such as surface functionalization.

Past attempts have involved attachment of gold nanoparticles to carbon nanotubes, resulting in the production of large clusters, which were unsuitable for biomedical applications. While these various imaging and therapeutic nanoparticles represent an exciting improvement in the field of bio sensing and cell imaging, it would be ideal to engineer 'advanced' versatile carbon dots that are capable of performing biological functions (e.g. sensing, imaging, and drug delivery) by a very simple manufacturing method. Nano biotechnology has introduced a new perspective for using nano sized elements against cancer diseases. Nanoparticles, because of their size (<100 nm), have unique physiochemical features including a large surface-to-mass ratio, easy surface functionalization, quantum characteristics and consequently, novel biological properties. The cellular transport of nanoparticles is also significantly different from that of chemical compounds or drugs because they are able to move across cells, reach nuclear membranes, and target specific structures such as proteins or gene sequences.

In the present investigation, non-metallic C-dots were synthesized by wet chemical method from three different compounds namely glucose, sucrose and fructose. The synthesized nanomaterials were characterized with UV-Visible and fluorescence spectroscopic techniques. The cytotoxicity of the synthesized nanoparticles was tested

***Corresponding author:** Sharath R, Department of Biotechnology, M.S. Ramaiah Institute of Technology (Affiliated to Visvesvaraya Technological University, Belgaum) Bangalore, India, E-mail: sharatsarati@gmail.com

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on theHepG2and MCF-7 cell lines by the MTT method. MTT assay is a colorimetric assay used for the determination of cell proliferation and cytotoxicity, based on reduction of the yellow colour water-soluble tetrazolium dye MTT to formazan crystals. Mitochondrial lactate dehydrogenase produced by live cells reduces MTT to insoluble formazan crystals, which upon dissolution into an appropriate solvent exhibits purple color, the intensity of which is proportional to the number of viable cells and can be measured spectrophotometric ally at 570 nm [3].

Materials and Methods

Cell lines and medium

HepG2 and MCF-7 cell lines were from (NCCS, Pune). Foetal Bovine Serum [#RM10685], MTT Reagent [5 mg/ml, # 4060], D-PBS [#TL1006] were from Himedia. EMEM [#56416C], DMSO [#PHR1309] and were from Sigma. 96-well plates for culturing cells were from Corning, USA

Chemicals used

Sucrose, Glucose, Fructose, Camptothecin (# C9911, Sigma) for HepG2, Oxaliplatin (# O9512, Sigma) for MCF7.

Synthesis of C-Dots

C-dots were synthesized by acidic oxidation of sucrose, glucose and fructose in separate glass beaker with H_3PO_4at 60^OC till color changes to the formation of yellow C-dots. In this method 0.698g of sucrose, 0.1396g of glucose and fructose were dissolved in 150ml distilled water in three different glass beakers, then 0.106ml of phosphoric acid was added to each beaker and kept on the heating mantle at 60oC till the colour changed to pale yellow. The synthesized nanoparticle solutions were then transferred to dialysis bag and subjected for 24 hours of dialysis treatment. Detailed synthesis and characterization of C-dots published in our earlier article [4, 5].

Cell Culture

The cell lines MCF-7 and HepG2 were obtained from the NCCS Pune and maintained in Dulbecco's modified Eagle's culture medium supplemented with 10% Fetal bovine serum (Sigma-Aldrich), 1% penicillin, and streptomycin (Sigma-Aldrich) at 37 ^{0} C in a humidified atmosphere of 5% CO2/95% air in a CO_2 Air-Jacketed Incubator until confluent before used for cytotoxicity assay.

Cytotoxic activity by MTT assay

Cytotoxicity studies with normal cell culture systems of C-dots have not been studied extensively and this is vital for the safety evaluation of these nanoparticles. Therefore, the objective of this study was to evaluate the potential cytotoxic activity of C-dots against HepG2 and MCF-7 cell lines.

The cytotoxicity study was carried out for C-dots (Test Compound) from Sucrose (SCD), Fructose (FCD) and Glucose (GCD). They were screened for their cytotoxicity against HepG2 and MCF-7 cell lines at different concentrations to determine the IC50 (50% growth inhibition) by MTT assay. In-Vitro assay C-dots formulation was carried out for their confirmation of cytotoxic effect on HepG2 and MCF-7 cell lines. Percentage of viable cell can be obtained by performing try pan blue dye exclusion technique. The cytotoxicity activity is carried out by using MTT assay. Cell lines derived from NCCS, Pune were free from any kind of bacterial and fungal contamination.

Percentage cell viability of cell line was carried out by using try pan blue dye exclusion technique. 200µl cell suspension was Seeded in a 96-well plate at required cell density (20,000 cells per well), without the test agent. The cells were allowed to grow for about 12 hours. Final concentrations of C-dots (Test Compound) from Sucrose (SCD) Fructose (FCD) and Glucose (GCD) 50, 150, 250, 350 and 450 µg/ml were added. The plate was incubated for 24hrs at 37°C in a 5% CO2 atmosphere. After the incubation period, the plate was removed from the incubator; spent media was removed followed by addition of MTT reagent to a final concentration of 0.5 mg/ml. The plate was wrapped with aluminum foil and placed in the incubator for 3 hours, after which MTT was removed and 100 µL of DMSO was added. Absorbance was measured in an ELISA reader at 570 nm.

The Absorbance of medium control was deducted from Absorbance in treatment wells before estimation of viability. The mean and SD % viability was estimated for each concentration .

Statistical Analysis

All the invitro experiments were performed in duplicates using two replicate samples for each formulation. The cytotoxicity of each formulation was expressed in terms of it IC_{50} (concentration causing 50% if death of cell population) calculated from concentration – response curves. The IC_{50} value was determined by using linear regression equation i.e. y= mx+c. Here, y = 50, m and c values were derived from the viability graph.

The percent viability of cells was estimated using the following equation:

% Viability = [Absorbance of treated cells/Absorbance of untreated cells] * 100

Results and Discussion

Cytotoxicity activity of C- Dots

The present study carried out for C-dots from Sucrose (SCD), Fructose (FCD) and Glucose (GCD) showed that the % viability of HepG2 and MCF-7 cell line is 90.85%, which is most suitable to perform cytotoxicity study. These C-dots were screened for its cytotoxicity against HepG2 and MCF-7 cell lines at different concentrations to determine the IC_{50} (50% growth inhibition) by MTT assay. IC_{50} of the C-Dot nanoparticles (SCD, GCD, FCD) on HepG2 cells were 67.24, <50 and and<50 µg/ml respectively. Results of % Viability of HepG2 cell line of GCD, FCD & SCD were presented in and graphically represented.

The IC_{50} of the C-Dot nanoparticles (SCD, GCD, FCD) onMCF-7 cells were 105, <50 and <50µg/ml respectively. Results of %Viability of MCF-7 cell line of untreated/control, GCD,FCD and SCDwere presented in and graphically represented. The percentage viability was found to be increasing with decreasing concentration of test compounds.

Conclusion

A Wet chemical method adopted for the synthesis of Carbon Dots has been reported. The method is very simple, cost effective and environmental benign and can be used for various applications. These nanoparticles are advantageous with their well-known intrinsically nontoxic *in vitro* and *in vivo*, eco-friendly and can be used in the field of medicine for their efficacy. The present work reveals the potential application of C-dots for *in vitro* cytotoxic activity against two cancer cell lines human liver carcinoma cells (HepG2) and human breast carcinoma cells (MCF -7) and further it recommends advanced studies

of anticancer drug discovery using the Carbon dot nanoparticles as its pharmaceutical applications are less explored.

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Conflicts of Interest

There are no conflicts of interest among the authors.

References

1. Ashley CE (2011) The targeted delivery of multicomponent cargos to cancer cells by nanoporous particle-supported lipid bilayers. Nat Mater: 10: 389-397.
2. Aladahalli Shivanna (2019) Synthesis and characterization of Zinc oxide nanoparticles utilizing seed source of Ricinus communis and study of its antioxidant, antifungal and anticancer activity. Mater Sci Eng C 97: 842-850.
3. Anithaa K (2018) Cytotoxicity, antibacterial and antifungal activities of ZnO nanoparticles prepared by the Arto carpus gomezianus fruit mediated facile green combustion method. J Sci Adv Mater Devices (4): 440-451.
4. Gurumallesh Prabua (2019) Green synthesis of anisotropic silver nanoparticles from the aqueous leaf extract of Dodonaea viscose with their antibacterial and anticancer activities. Process Biochemistry 80: 80-88.
5. Ningqiang Gong (2019) Carbon-dot-supported atomically dispersed gold as a mitochondrial oxidative stress amplifier for cancer treatment. Nat Nanotechnol l (14): 379-387.