Chemoradiation strategy using PEGylated gold nanoparticles anchored with Doxorubicin against oral cancer-A nanomedicine *in vitro* approach

SYNOPSIS

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By

SHAMEER AHMED B

Under the Supervision of

VIJAYASHREE NAYAK, Ph.D.

BITS Pilani
Pilani | Dubai | Goa | Hyderabad

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<u>Synopsis Title</u>: "Chemoradiation strategy using PEGylated gold nanoparticles anchored with Doxorubicin against oral cancer-A nanomedicine *in vitro* approach"

Abstract: Squamous Cell Carcinoma (SCC) originating at oral cavity (OC) and oropharyngeal site both of these are separate entities which are included within the larger group of Head and Neck Squamous Carcinoma (HNSCC), which is the sixth most common form of cancer diagnosed worldwide. The aim of the present thesis is to develop and investigate more effective noble metallic nanoparticles which can serve as nanomedicine which is multifunctional, can also serve as nanotheranostics for intracellular drug delivery, radiosensitization and medical imaging. PEGylated AuNPs, Ag NPs and bimetallic Au-Ag NPs (BNPs) were prepared via one pot chemical reduction method where, PEG 600 was used as both reducing and capping agent. These PEG capped nanoconstruct were stable, with average hydrodynamic size being ~50±5nm. PEGylated AuNPs, Ag NPs and bimetallic Au-Ag NPs were found to be cytotoxic against KB oral cancer cells and has reduced the viability in MTT assay. When these 3 different metallic NPs exposed to oral cancer cells, they efficiently enter cells into the cytoplasm, and subsequently enhance the radiation effect as demonstrated by in vitro radiosensitization with enhancement ratio of ~1.5 and 1.2 and 1.4 respectively at 4Gy X-rays irradiation by LINAC. This current study confirms the radiosensitization effect of Au PEG NPs Ag PEG NPs and Au-Ag PEG NPs at 4Gy X-ray irradiation in (Oral cancer) KB cells. In phantom model, Au PEG NPs showed attenuated contrasting capacity higher compared to Ag PEG NPs, BNPs and clinically used CT imaging agent Omnipaque. This part of thesis showed that the Au PEG NPs is a better theranostics compared to Ag PEG NPs and BNPs.

Doxorubicin is an effective chemotherapeutic used to treat a variety of cancers including solid masses and leukemia. Clinically, its mechanism of action involves the intercalation of double-stranded DNA and the inhibition of important cellular replication enzymes. The Au Dox NPs and Au Dox PEG NPs nanoformulation was prepared using minimal chemicals and robust technique which is modified from literature survey. Doxorubicin reduces precursor of gold (Chloroauric acid) to form Au Dox NPs. PEG polymer is an FDA approved material with unprecedented advantage and biocompatible. PEGylation of NPs enhance circulation and evades immunogenic attack. Hence, Au NPs loaded with Dox and coated with PEG were synthesized. These two (Au Dox NPs and Au Dox PEG NPs) nanomedicines were compared

for their synergistic output in the chemoradiation strategy against oral cancers. The synergistic effect of the chemotherapy with radiation treatment was found to be higher in Au Dox NPs as the Dox loading was higher compared to Au Dox PEG. This was the major disadvantage found that Dox was attached by electrostatic adherence in Au Dox PEG NPs. The investigations described above help to advance the fields of nanotechnology, drug delivery and radiosensitization by first providing a robust doxorubicin loading on Au NPs and second by providing insights into the synergy of doxorubicin-gold conjugates in chemoradiation strategy. Together, these discoveries may influence future drug delivery research studies that utilize both doxorubicin and gold nanomaterials.

1. Introduction

Cancers of the oral cavity are a significant public health problem in India. The GLOBOCAN 2020, India fact sheet reports, cancer of the lip and oral cavity ranks second and showed a huge increase of 114.2%. Use of tobacco, betel nut/areca nut and its products have a direct impact on general health leading to oral cancers. Also, Alcohol consumption and life style changes are etiological factors of various cancers. At clinical and research level several cost effective and sophisticated treatment strategies like surgery, radiotherapy, chemotherapy, immunotherapy, hormone therapy, and targeted therapy have been developed to treat different oral cancer types, despite this the 5-year mortality rate is high with poor prognosis and recurrence. These morbidity and mortality are mostly due to genetic complexity and late diagnosis of the disease. The existing anticancer therapeutics have several side effects and leading to therapeutic resistance like chemoresistance and radioresistance. In cases of oral cancer surgical excision poses the challenges of function, aesthetics and prosthetic rehabilitations. Nanomedicine-nanoparticles based anticancer avenue has shown potential in both channels diagnostics and therapeutics making them a robust nano theranostics. The following doctoral thesis explores the radiosensitization effect of gold, silver and bimetallic gold-silver nanoparticles which are coated by PEG polymer which is FDA approved, to treat KB oral cancer cell line invitro model. Also, exploring the CT contrast capacity of 3 using phantom model. Finally, gold-based nanomedicine which coated with PEG and loaded with Doxorubicin is used as agent in chemoradiation strategy in an invitro model. The following finding and results could serve as preliminary step and this can be translated to invivo and ex vivo model to understand therapeutic in tumour microenvironment.

2. Gaps in existing research

i. The radiosensitization capacity of PEGylated noble metallic nanoparticles – gold and silver were not tested in oral cancer model.

ii. Novel PEGylated bimetallic Au-Ag NPs synthesis by facile one pot method and radiosensitization potential of nanoalloy intermix was not tested in oral cancer model.

iii. Chemoradiation strategy and efficiency of Dox loaded gold nanoparticles with PEGylation was not evaluated in oral cancer model.

3. Objectives of the thesis work

i. To design a novel bimetallic PEGylated gold-silver nanoalloy as a potential radiosensitizer against KB cell line.

ii. To design one step synthesis technique for Au-Dox NPs and Au-Dox conjugated PEG polymer to achieve drug delivery in KB cell line.

iii. To characterize synthesized NPs and evaluate the drug release pattern by dialysis method.

iv. To test the chemoradiation potential of Au Dox NPs and Au Dox PEG NPs in KB cell line.

4. Results and discussion

4.1. Chapter 1: This chapter describes the cancer and various treatment strategy. Further, epidemiology of cancer worldwide and in India was described on the basis of recent reports. The characteristic hallmarks and emerging hallmarks & enabling characteristics of the cancerous cells were discussed. In addition, the chapter outlines a brief introduction to the oral oncology and aetiology. It also describes the new avenue of therapeutics nanomedicine against oral cancers. The present chapter also discussed the gaps in the proposed doctoral thesis and objective and finally outlined the thesis structure.

4.2 Chapter 2: This chapter describes the brief background of oral oncology and comparison of Au PEG NPs and Ag PEG NPs as radiosensitizers in KB cell line. This chapter describes the PEGylated nanoparticles synthesis by one pot technique using PEG 600 as reducing and capping agent, thorough physico-chemical characterization to establish nanostructure. The

cytotoxicity of both NPs was evaluated. 4Gy X-ray irradiation from LINAC source was the dosage used. The radiosensitization of both Au PEG NPs and Ag PEG NPs was calculated. The nuclear damage resulting from NPs exposure and radiosensitization from Au PEG NPs and Ag PEG NPs was imaged by confocal microscopy Hoechst stain was used. In phantom model, Au PEG NPs and Ag PEG NPs was evaluated for contrasting capacity and compared with clinically used CT imaging agent Omnipaque.

4.3. Chapter 3: This chapter describes the brief background of nanoradiosensitizer and use of bimetallic PEGylated Au-Ag NPs as radiosensitizers in KB cell line. This chapter describes the novel method for synthesis of bimetallic PEGylated Au-Ag NPs by one pot technique using PEG 600 as reducing and capping agent, thorough physico-chemical characterization to establish nanostructure. The cytotoxicity of NPs was evaluated. 4Gy X-ray irradiation from LINAC source was the dosage used. The radiosensitization of bimetallic PEGylated Au-Ag NPs was calculated. The nuclear damage resulting from NPs exposure and radiosensitization from bimetallic PEGylated Au-Ag NPs was imaged by confocal microscopy Hoechst stain was used. In phantom model, bimetallic PEGylated Au-Ag NPs was evaluated for contrasting capacity and compared with clinically used CT imaging agent Omnipaque.

4.4 Chapter 4: This chapter describes the brief background of Oral squamous cell carcinoma and comparison of Au Dox NPs and Au Dox PEG NPs as nanomedicine employed for chemoradiation strategy in KB cell line. This chapter describes the facile and robust one pot synthesis of nanoformulations, thorough physico-chemical characterization to establish nanostructure. The drug release pattern was measured by invitro dialysis method. The cytotoxicity of both NPs was evaluated. 4Gy X-ray irradiation from LINAC source was the dosage used. The chemoradiation potential of both Au Dox NPs and Au Dox PEG NPs was calculated. The nuclear damage resulting from NPs exposure and chemoradiation from Au PEG NPs and Ag PEG NPs was imaged by confocal microscopy Hoechst stain was used.

5. Conclusion

In conclusion, a summary on the basis of review of all three chapters and the future scope of the work has been discussed followed by references and appendices.

6. Future scope of the work

1. Advancing the synthesize of AuNPs by making it a targeted drug delivery system as Au-Dox-PEG-Cetuximab NPs.

2. Thorough characterization of Au-Dox-PEG-Cetuximab NPs along with Drug release pattern.

3. Extending the whole chemoradiation strategy in *ex vivo* model to understand therapeutics in TME (Tumour Micro Environment) or 3D Cultures.

4. Therapeutic efficiency and efficacy can be evaluated in animal model along with accumulation and imaging.

7. Publication(s)

1.**Shameer Ahmed B,** Gunjan Baijal, Rudrappa Somashekar, Subramania Iyer and Vijayashree Nayak- One pot synthesis of PEGylated bimetallic gold - silver nanoparticles for imaging and radiosensitization of oral cancers (Accepted on 5th Oct 2021 International Journal of Nanomedicine- IF=6.4 Dove Press) https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8545617/ (Output from chapter 3).

2. **Shameer Ahmed B,** Gunjan Baijal, Rudrappa Somashekar, Subramania Iyer and Vijayashree Nayak- Comparative study of one pot synthesis of PEGylated gold and silver nanoparticles for imaging and radiosensitization of oral cancers (Accepted on 1st Feb 2022 Journal of Radiation Physics and Chemistry - IF=2.8 Elsevier Ltd) https://www.sciencedirect.com/science/article/pii/S0969806X2200032 (Output from chapter 2).

Brief Biography of the candidate

Name: Shameer Ahmed B

Date of Birth: 23rd February, 1988

Education: M Tech (Materials Science- R&D based Programme), 2014-2016 from University of Mysore, Mysore, Karnataka, India

BDS (Bachelor of Dental Surgery), 2006-2011 from RGUHS University, Bangalore, Karnataka, India

Email: p20170006@goa.bits-pilani.ac.in shameerahmed88@gmail.com

Research Experience (6 years)

1. Presently working on doctoral thesis with a position of Ph.D. Scholar, Department of Biological Science, BITS, Pilani-K.K. Birla Goa Campus, Goa, India. Supervisor: Prof. Vijayashree Nayak. Scheme CSIR-SRF (April 2021-present)

2. Institute Research Scholar in the Department of Biological Sciences under the supervision of Prof. Vijayashree Nayak, Biological Sciences, BITS Pilani, K.K Birla Goa Campus (August 2017- April 2021).

3. Hairline Research Institute| Skin Clinic, Bangalore (2012-2014) Research coordinator and HR: To organize Ethical committee for clinical trials

4. Vydehi Institute of Dental Sciences | Rajiv Gandhi University of Health Science (2011 – 2012) [1 year]: Rotatory Internship

Received: Grants

The Goa Cancer Society |NGO| Goa funded research grant to conduct Research project as Principal Investigator- Ref No.2/Research/ GCS/2019-2020 for a period of 3 years 6lacs as initial funding. Total 6lacs.

05 research publications in the international journals

Peer-Reviewed Publications

1. Shameer Ahmed.B, Rao, A. G., Sankarshan, B. M., Vicas, C. S., Namratha, K., Umesh, T. K.,... & Byrappa, K. (2016). Evaluation of Gold, Silver and Silver–Gold (bimetallic) nanoparticles as radiosensitizers for radiation therapy in cancer treatment. Cancer Oncol. Res, 4, 42-51.

2. Shameer Ahmed, B., Nandaprakash, M. B., Namratha, K., Byrappa, K., & Somashekar, R. (2018). Structure and electrical conductivity of irradiated BaTiO3 nanoparticles. physica status solidi (b), 255(6), 1700581.

3. Shameer Ahmed, B., Namratha, K., Nandaprakash, M. B., Somashekar, R., & Byrappa, K. (2017). Effect of gamma irradiation on hydrothermally synthesized barium titanate nanoparticles. Radiation Effects and Defects in Solids, 172(3-4), 257-270.

4. Shameer Ahmed B, Gunjan Baijal, Rudrappa Somashekar, Subramania Iyer and Vijayashree Nayak- One pot synthesis of PEGylated bimetallic gold - silver nanoparticles for imaging and radiosensitization of oral cancers (Accepted on 5th Oct 2021 International Journal of Nanomedicine- IF=6.4 Dove Press)

5. Shameer Ahmed B, Gunjan Baijal, Rudrappa Somashekar, Subramania Iyer and Vijayashree Nayak- Comparative study of one pot synthesis of PEGylated gold and silver nanoparticles for imaging and radiosensitization of oral cancers (Accepted on 1st Feb 2022 Journal of Radiation Physics and Chemistry - IF=2.8 Elsevier Ltd).

Brief Biography of the Supervisor

Name: Prof. Vijayashree Nayak

Designation: Professor, Department of Biological Sciences

Present affiliation: Dr. Vijayashree Nayak, Professor, Department of Biological sciences, BITS-Pilani K. K. Birla Goa Campus, NH 17- B, Zuarinagar, Goa 403726.

Educational Qualification: Ph.D. from MAHE Manipal, Year of award: 1998, Thesis title: "Anticancer activity of Tenosporacordifolia on HeLa cells in response to ^{60}Co gamma radiation and without radiation".

Dr. Vijayashree Nayak has worked in different capacities at BITS Pilani-K.K. BIRLA Goa Campus from 2009 onward in different positions. She has 21 years of research and teaching experience. Her lab focuses on a couple of aspects like herbal medicine, such as cancer therapeutics and their molecular mechanisms in Cancer. Additionally, gold nanoparticles mediated drug delivery and nanomedicine is one of the areas she interested in. Her lab also worked on therapeutic applications of Tissue engineering. Her interest also lies in developing novel scaffolds for cell culture in tissue engineering applications and drug screening for anticancer activity. She has completed a couple of project sanction by DST, India and BRNS. She has more than 20 publications in National and International journals of repute. She has guided 5 Ph.D. and another two are pursuing under her guidance. As a teacher, she has taught courses/subjects for both undergraduate (first degree) and M.E. Biotechnology (higher degree) students.