Radiosensitizatioin of cancer cells using gold nanoparticles

Bachelor Thesis

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Abstract

abstract

Results And Discussion

1 Introduction

Each year 8.2 million people die from cancer, that is an estimated 13% of all deaths worldwide. Moreover the number of new cases is expected to increase by 70% over the next two decades. [**?**] These numbers are a clear indication of the importance of cancer research, which includes the development of new treatments and the enhancement of existing treatments.
Cancer is a general term for a large group of diseases that are all characterised by a rapid creation of abnormal cells that grow beyond their usual boundaries. The cells grow out of control and thereby form tumors, because the part of the DNA responsible for cell death is disturbed.
The three major cancer treatments that exist today are chemotherapy, surgery and radiation therapy. In chemotherapy anti-cancer drugs are used to damage the cancer cells. These drugs only affect rapid dividing cells, which is a typical characteristic of cancer cells. Cancer can also be treated by removing the tumor by surgery. A third important cancer treatment is radiation therapy, where ionizing radiation is used to damage the DNA of the cancer cells. Because in most cases combinations of these treatments are used, it is estimated that 50% of all cancer patients undergo radiation therapy as part of their treatment. [**?**]
The problem with ionizing radiation is, that it does not discriminate between malignant and normal tissues. This means that normal tissue is also irradiated. Therefore radiation therapy has an unintentional toxic effect on the healthy tissue surrounding the tumor. These effects range from those that cause mild discomfort to others that are life-threatening. The radiation dose has to be carefully chosen: it has to be high enough to bring damage to the cancer cells, but it cannot be too high because of the negative effect on the healthy tissue.
The toxic effect of radiation therapy can be reduced in different ways. For example instead of delivering the total dose of radiation at once, the dose can be fractionated in smaller doses that are delivered over a longer period of time. Dose fractionations offers the opportunity for healthy cells to recover from the previous dose before the next dose is delivered. [**?**] Another technique to reduce the effect on healthy tissue is radiosensitization. With this technique it is possible to make the cancer cells more sensitive to ionizing radiation, so that lower radiation doses can be used, which reduces the effect on the surrounding tissue. Here radiosensitization using gold nanoparticles (GNP)is discussed. The absorption of photons is higher for elements with high mass numbers. Therefore because of the high mass number of gold (Z=79), when the GNP can be brought inside the tumor, the ionizing radiation will mainly interact with the nanoparticles. Thereby causing

local secondary radiation, which delivers its energy locally, so inside the tumor. [**?**] The reason gold is used instead of other element with high atomic mass, is that gold biocompatible, which makes it suitable for medical treatment. [**?**]
The first experimental evidence of the use of GNP to enhance radiation therapy was provided by Hainfeld et al. [**?**]. Mice with cancer tumors were injected with 1.9 nm diameter GNP and then radiated with 250 kVp X-rays. The combination of GNP and radiation resulted in a one-year survival of 86% compared to 20% with radiation therapy alone. Other experiments showed similar evidence of the radiosensitizing effect of GNP. !REFERENTIES! These results provide a motivation for further research within the field of nanoparticle enhanced radiation therapy.
The main goal of this project is to synthesize GNP of different sizes and to characterize them using different methods. Next the GNP are functionalized with a polyethylene glycol (PEG) coating, which provides stability to the GNP solution, i.e., prevents them from aggregating. The PEG coating also increases the probability of delivering the nanoparticles to the cancer cells. Finally a mixture of DNA and GNP is irradiated and the effect on the DNA is analysed.

2 Theoretical background

2.1 Radiation physics

Opzoeken gebruikte straling bij radiation therapy A. Zuppinger. Particle Beam Therapy. Proceedings of the Royal Society of Medicine, 58(March):151160, 1965.
There are three possible ways photons can interact with matter: photoelectric absorption, Compton scattering and pair production. In the photoelectric effect, the energy of an incoming photon is transferred to an electron, which is then ejected. The vacancy left by this electron is then filled with another electron from a higher shell. This electron then gives of its excess energy as an characteristic X-ray photon. In some cases the excess energy may be transferred to an outer-shell electron. As a consequence this electron is ejected and is called an Auger electron. The cross section for photoelectric absorption τ increases for increasing mass number Z and in decreases sharply with the photon energy E_γ: Formule

$$\tau C^{te} \cdot \frac{Z^n}{{E_\gamma}^3.5} \tag{1}$$

with n varying between 4 and 5.
The Compton effect is an inelastic scattering between a photon and an electron, where part of the energy of the incoming photon is transferred to the recoiling electron. The cross section for Compton scattering grows linearly with Z and falls of gradually with increasing energy. With pair production, a photon creates an electron-positron pair. The cross section for this process varies approximately with Z^2 and increases for increasing energy.
Since the cross sections for each of these processes increases with increasing Z, it is clear that gold, with its high atomic mass, is suitable for radiosensitization.

2.2 Biological effects

2.3 Targeting

In order to have a beneficial effect of GNP in radiation therapy, it is important to bring the nanoparticles as close as possible to the DNA of the cancer cells. The uptake of GNP into the nucleus of the cells is only possible below a certain upper size limit.
http://onlinelibrary.wiley.com/doi/10.1002/smll.201000134/full Therefore the size of the nanoparticle is a very important parameter. GNP are known to passively accumulate in cancer cells because of the enhanced permeability and retention (EPR) effect. Because cancer cells are rapid growing cells, tumors have leaky, immature vasculature, so that their blood vessels are more permeable.
http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3473940/ This effect can be enhanced by functionalizing the naked gold nanoparticles with PEG. This PEG coating sterically hinders nonspecific binding of proteins to the surface of the particle and delays the recognition of the particles by the reticuloendothelial system. This increase the circulation time of the GNP in the blood and as a

result increases the probability of delivering the nanoparticles to the tumor.
http://onlinelibrary.wiley.com/doi/10.1002/smll.201000134/full The hydrodynamic radius of the particles increases because of this PEG coating, therefore the optimal size (of the naked GNP) for uptake is smaller when the particle is functionalized. Finally the functionalization also has a positive effect on the stability of the GNP solution.
Besides PEGylation, the GNP can also be coated with antibodies, which actively bind to receptors that are specific for cancer cells. An example of a receptor that is overexpressed in tumors is the epidermal growth factor receptor. Nanoparticles coated with an antibody that corresponds to this receptor are guided to the tumor and bind on its surface. http://www.mdpi.com/2079-4991/1/1/31/htm

3 Theoretical backround

The project consists of three major parts. First the gold nanoparticles are synthesized using the Turkevich [2] method followed by thorough characterization of the particles using several techniques such as TEM, UV-VIS, DLS and ζ-potential. Finally the radiosensitization effect of the GNP is tested by irradiation of samples containing circular DNA and GNP.
This section gives an overview of all techniques and their mechanism used in this project.

3.1 Synthesis GNP

3.1.1 Method of Turkevich

The synthesis of gold nanoparticles is a two step process. First there is the nucleation step. Small seeds of atomic gold are formed after the addition of an reducing agent to a solution of gold ions in an aqueous environment. Secondly there's the growth process where small particles aggregate together to form bigger particles.

As mentioned above the Turkevich method is used. Sodiumcitrate ($NA_3C_6H_5O_7$) is added to an tetrachlorauric acid (H_AUCl_4) aqueous solution to reduce the AU^{3+} ions (see figure 1). The fist seeds are formed and the nucleation process has started. After the reduction the negative citrate ions remain on the surface of the newly synthesized gold seed causing a negative surface potential. At first the electrostatic repulsion is low and the Van der Waals forces cause the seeds to aggregate and form bigger particles. During this growth process more and more citrate ions cover the surface of the particles and eventually establish an sufficiently high electrostatic potential to prevent further growth of the nanoparticles. In that way the sodiumcitrate both starts and end the synthesis of the nanoparticles, controlling the size of the nanoparticles. Adding more citrate to the tetrachlorauric acid solution will stop the growth process sooner creating smaller nanoparticles.

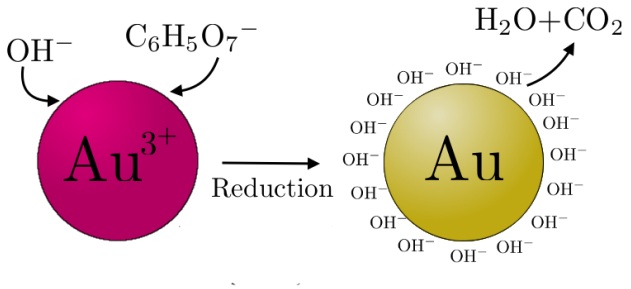

Figure 1: The synthesis of GNP using the sodiumcitrate ($NA_3C_6H_5O_7$) as an reducing agent.

3.1.2 Functionalisation with PEG

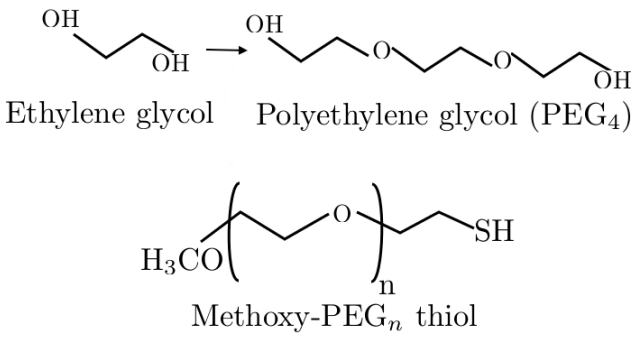

Figure 2: The synthesis of methoxy-PEG$_n$ thiol

In order to have an optimal targeting of the GNP to the tumor the particles are coated with an layer of PEG derivates (Polyethyleen glycol). One of the end hydroxyl groups is substituted by an sulfhydryl group (SH). This substitution ensures an favorable PEG-GNP bound. In an aqueous environment this group deprotonates forming an radical (RS·). The PEG$_n$ thiol is now negatively charged. It will substitute the citrate ions on the surface of the GNP since an thiolategold bound is comparable in strength to that of the gold-gold bound[1]. The second hydroxyl substitution will determine the surface

properties of the particles in the colloid. In this project we substitute with an methyl-group creating an neutral surface potential. Hence, the GNP are coated with an layer of Methoxy-PEG$_n$ thiol, see figure 2.

4 Hydrodynamic Radius (DLS)

Table 1: DLS measurement results for three different particles, of expected size 15nm, 30nm and 45nm, with and without functionalisation using PEG. The length of the PEG chain varies from 20k to 1k .

Size (nm)	No PEG	1k PEG	5k PEG	10k PEG	20k PEG
15	28.37 ± 1.53	135.72 ± 38.01	40.26 ± 1.25	54.03 ± 0.56	66.00 ± 4.71
	9.70 ± 1.44			14.05 ± 4.13	
30	30.03 ± 1.25	99.61 ± 17.33	45.41 ± 1.26	53.28 ± 1.66	61.29 ± 1.50
	4.99 ± 1.55	25.37 ± 1.84	7.85 ± 1.05	5.90 ± 0.97	7.36 ± 1.73
45	50.75 ± 2.05	109.54 ± 0.69	70.04 ± 3.06	71.56 ± 1.87	76.14 ± 0.66
	3.28 ± 0.79		10.67 ± 1.44	10.74 ± 1.13	5.15 ± 0.74

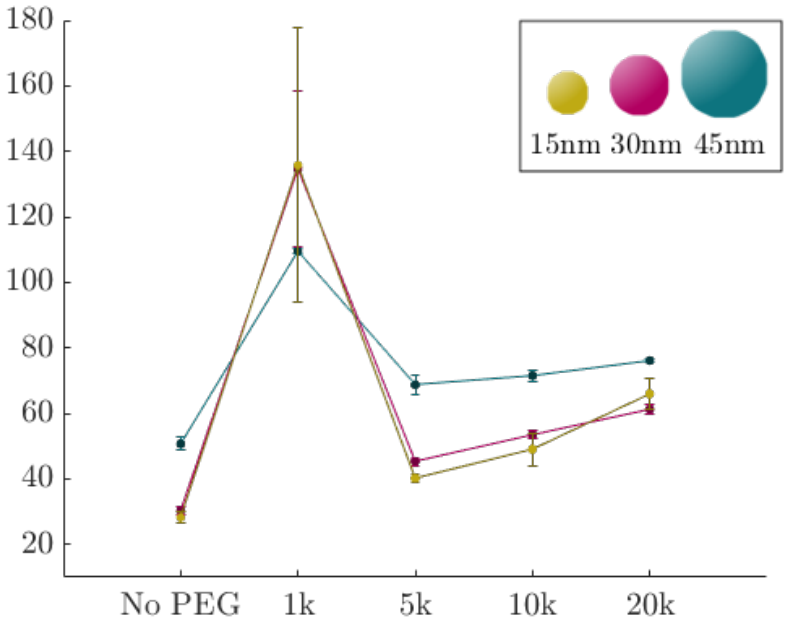

Figure 3: DLS measurement results for three different particles, of expected size 15nm, 30nm and 45nm, before (transparent) and after PEG functionalisation.

5 UV-Vis

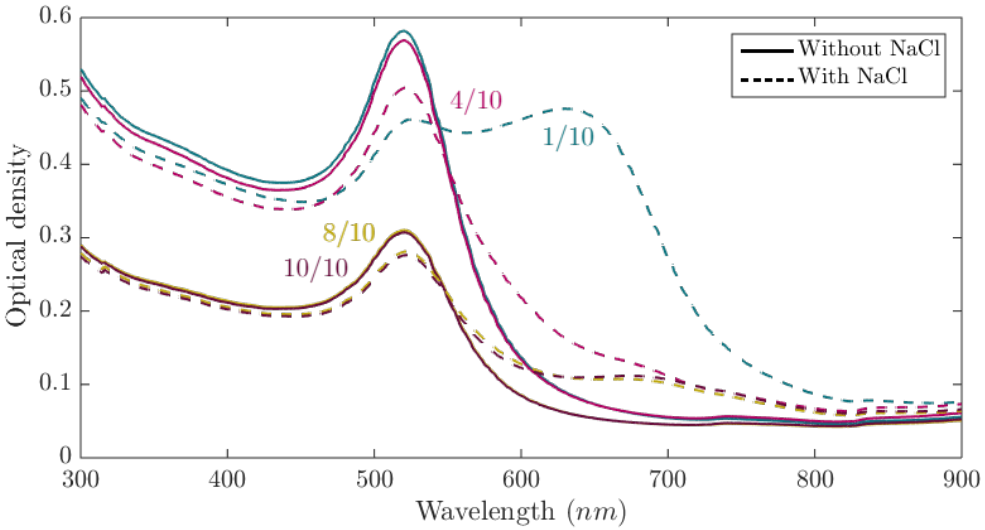

Figure 4: Optical density in function of wavelength for 15nm GNP with 20k PEG for different PEG/GNP proportions, with and without NaCl.

Appendix

References

[1] Hannu Hakkinen. The gold-sulfur interface at the nanoscale. *nature chemistry*, 4:443–455, 2012.

[2] Peter Copper Stevenson Jhon Turkevich and James Hillier. A study of the nucleation and growth processes in the synthesis of colloidal gold. *Discussion of the Faraday Society*, 11:55–75, 1951.

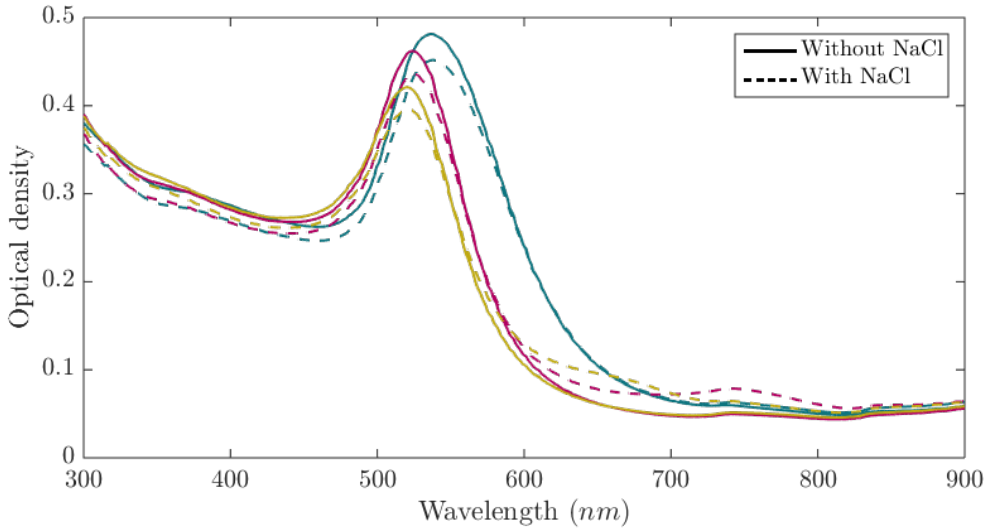

Figure 5: Optical density in function of wavelength for 15, 30 and 45nm GNP, with 20k PEG with PEG/GNP=8/10.

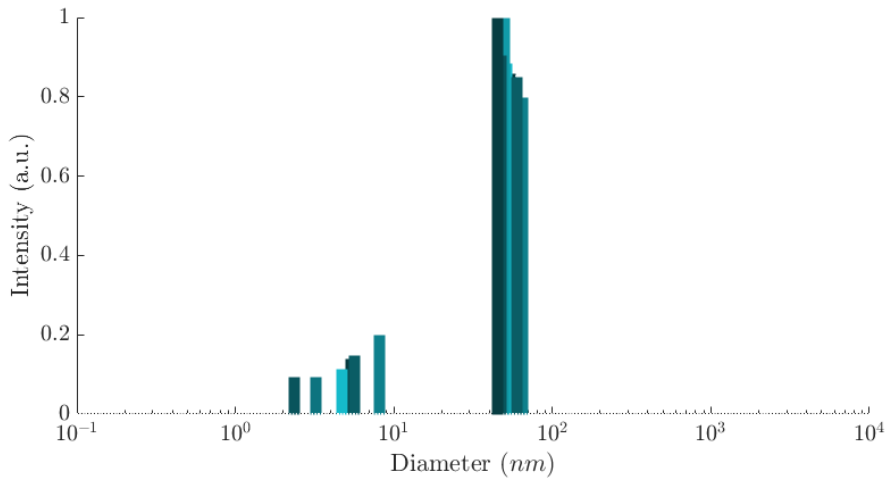

Figure 6: DLS measurement results for GNP with expected size 45nm, no PEG added

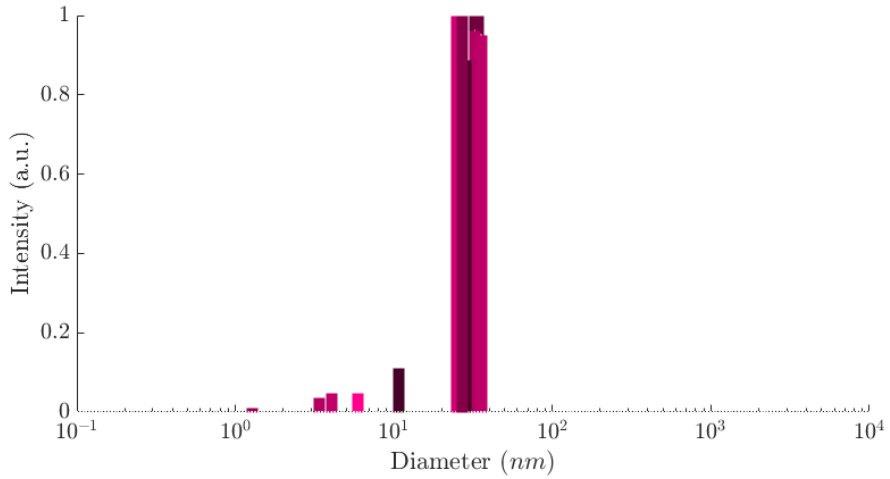

Figure 7: DLS measurement results for GNP with expected size 30nm, no PEG added

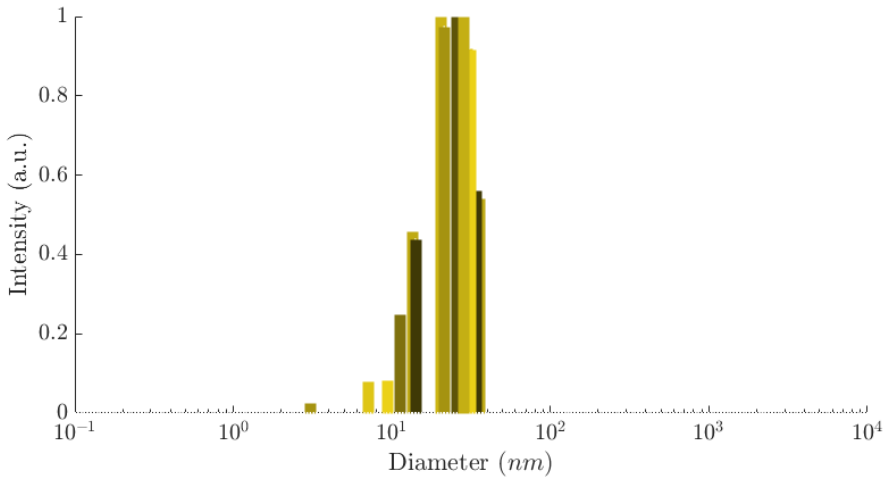

Figure 8: DLS measurement results for GNP with expected size 15nm, no PEG added