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ANTIBODY-LABELED GOLD NANOCONJUGATES IN EXPERIMENTAL PHYSIOLOGY AND CANCER RESEARCH

I. Pantic[1] and L. Markovic[2]

[1]University of Belgrade, Faculty of Medicine, Institute of Medical Physiology, Visegradska 26/II, 11000, Belgrade, Serbia
[2]University of Belgrade, Faculty of Medicine, Institute of Pathophysiology, Dr Subotica 1, 11000, Belgrade, Serbia

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Abstract. During the past decade, gold nanoparticles (Au NPs) have been the focus of numerous experimental and clinical studies. Surface functionalization of Au NPs with various antibodies, enables them to have an adequate tissue biodistribution, and to effectively target cancer cells. In this concise review, we present the recent research data regarding the synthesis of antibody-labeled gold nanoconjugates, as well as their potential application in medical imaging and photothermal cancer therapy.

1. INTRODUCTION

During the past decade, gold nanoparticles (Au NPs) have been the focus of numerous experimental and clinical studies [1-5]. Gold-based nanomaterials have been used to design and improve novel drug delivery systems which could represent a significant future improvement of the existing cancer chemotherapy. Also, many researchers consider Au NPs to be a valuable addition to the present diagnostic, imaging, and bioassay techniques.

The surface of Au NPs, due to its unique properties can easily be modified with ligands containing various functional groups [6,7]. Resulting gold nanoconjugates based on the type of surface functionalization include citrates, amines, nucleic acids, peptides, antibody-labeled conjugates, and lipid ligand associated conjugates [6]. Surface functionalization of Au NPs enables them to have an adequate biodistribution in various living tissues and organs as well as to specifically target and accumulate in cells with certain membrane receptors.

In this short review we present the recent research data regarding the synthesis of antibody-labeled gold nanoconjugates, as well as their potential application in medical imaging and photothermal therapy.

2. SYNTHESIS OF ANTIBODY-LABELED GOLD NANOCONJUGATES

There are several effective ways to attach an antibody to the surface of a gold nanoparticle. Antibodies can be adsorbed to the NP surface by the thiol groups present in their primary structure [6]. Also, various moleculs such as hydroxysuccinimide derivates can form ester bonds and act as linkers that connect the gold surface and the amine group of the antibody [6]. In recent years, many new “linkers” have been suggested to be effective in increasing the stability of antibody-gold nanoconjugates.

Gold-gold sulfide nanoparticles conjugated with anti-HER2 and anti-IgG antibodies were successfully created using orthopyridyl-disulfide-poly (ethylene glycol)-N-hydroxysuccinimide (OPSS-PEG-NHS) as a linker [8]. These PEG-antibodes were formed as a result of a stable amide bond between primary

Corresponding author: Igor Pantic, e-mail: igorpantic@gmail.com

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amines on the antibody and carboxyl groups on the PEG molecule [8]. On the other hand, a connection between sulphur and gold enabled an efficient assembly and binding of a PEG-antibody and gold NP.

Another promising method based on PEGylation chemistry is the attachment of hydrazide-polyethylene glycol-dithiol to the Fc fragment of the antibody, thus creating a stable link to the Au NP surface [9]. Some researchers state that using this technique, various glycosylated antibodies can be mounted on a single surface, which makes the nanoparticle multifunctional [9]. Multifunctional gold NPs can then be used not only as drug carriers, but can also boost the efficiency of cancer cell targeting in tumor tissue. 3-mercaptopropionic acid (3-MPA), can also be used as a linkage between the Au NP and the primary amine group of an antibody [10]. 3-MPA is usually firstly self-assembled on the Au NPs via sulfur–gold bond, after which secondary antibody is connected to 3-MPA via amino-carboxyl reaction [10]. This way, scientists were able to attach Anti-Carcinoembryonic antigen antibody to Au NPs in order to design new and modernize existing immunolabeling techniques for sensitive and lowcost analysis of biological samples.

Because of the structural properties of some antibodies, an efficient gold-thiolate bond is not always achievable. Therefore, in some cases, Fv antibody fragment can be selectively mutated to present an exposed cysteine residue [11]. In the article in question, the authors described the conjugation of glutathione monolayer-protected gold clusters (more stable and functional than ordinary gold NPs) with a mutated antibody [11]. The bond was achieved as a result of the gold cluster activation by chemical oxidation.

Another way to increase the stability of antibody-labeled gold nanoconjugates is by hybridization of specific oligonucleotides attached to the antibodies with complementary oligonucleotides attached to the gold NP [6,12]. This way a variety of biomolecules (both small and large) can be conjugated to the gold surface. It has been demonstrated that this way, an anybody against epidermal growth factor receptor (EGFR), which has a relatively high molecular mass and bulk (150 kDa), can successfully be binded to gold surface [12].

To summarize, various methods exist for an effective conjugation between antibodies and Au NPs. The future design strategies will be most definitely focused on how to improve the stability of the conjugate, so it may reach its target in the concentrations sufficient for an effective action. These novel techniques will also enable further functionalization of Au NPs which will make them even more valuable for medical research and clinical practice.

3. ANTIBODY-LABELED GOLD NANOCONJUGATES IN IMAGING STUDIES

One of the main applications of nanoparticles, regardless of the materials used for their synthesis or the conjugation status, is cancer research [13]. Gold nanomaterials have so far been used in a number of studies related to chemotherapy drug delivery, malignant cell targeting and cancer bioassay design. As for antibody-labeled gold nanoconjugates, much of the research effort has so far been focused on developing a diagnostic imaging assay by which a physician would be able to visualize cancer cells and distinguish them from the normal, unchanged tissue. Most of the imaging studies have been focused on designing a conjugate that would be capable of selectively binding to a certain growth factor receptor or other membrane molecule specific for the cancer cells, making it visible and detectable.

It was found that the anti-EGFR antibody conjugated nanoparticles were able to specifically attach to oral cancer cells (with 6 times greater affinity than to non-malignant cells), changing their spectroscopic and surface plasmon resonance characteristics [14]. The authors describe this method as simple, accurate and affordable. Moreover this and similar approaches could lead to the design of an imaging procedure capable of early detection of oral cancer cells before they become visible using traditional imaging techniques [15].

Antibody-labeled gold nanoconjugates have also been applied in photoacoustic imaging. Photoacoustic imaging represents a novel, non-invasive method that is based on so-called photoacoustic effect, a physical phenomenon in which the absorbed energy from the light is converted to kinetic energy resulting in sound (wave) formation [16]. It is thought that using this method, gold nanorods conjugated with HER2 and CXCR4 monoclonal antibodies, could be useful for estimation and measurement of oncogene expression in cancer cells [17].

These and other imaging studies, although promising for future cancer diagnosis research, have certain limitations. It is often hard to design a conjugate that is 100% specific for the cancer cell, since many normal calls may also possess the molecule

that is being targeted (although in smaller concentrations). Also, as previously mentioned, much of the research is presently conducted *in vitro*, and there is still insufficient data regarding factors that may affect biodistribution of the conjugates in a living organism and availability at the tumor site. Nevertheless, we assume that in the future, antibody-labeled gold nanoconjugates will become an important addition to the existing cancer diagnosis methods in clinical practice.

4. APPLICATIONS IN PHOTOTHERMAL THERAPY

Photothermal therapy is a new cancer treatment approach in which electromagnetic infrared radiation is used to selectively target and destroy abnormal tissue. Photothermal effect takes place when a specific compound called photosensitizer, after being excited with light, emits thermal energy. Nanoparticles as photosensitizers can absorb and scatter light strongly at a characteristic wavelength which is called plasmon resonance [18]. It is known that nanoparticles with certain geometrical shapes, such as rods and shells, can have their plasmon resonance tuned to the near infrared (NIR) region of the spectrum, which is of great clinical importance, because light at these wavelength is able to penetrate deep into the tissue [18].

Anti-EGFR antibody-conjugated gold nanoparticles were successfully used for photothermal destruction of cancer cells [19]. Due to the selective antibody binding to EGFR overexpressing cells, laser power thresholds for cancer cells were significantly lower than the thresholds for normal tissue [19]. This, and other studies concerning Anti-EGFR Au nanoconjugates [14,20] set the basis for further research in *in vivo* conditions. One of the first studies investigating the photothermal ablation properties of anti-EGFR Au NPs *in vivo* was carried out in mice, by injecting viable tumor cells that eventually developed detectable tumor tissue [21]. Among many parameters, the authors also analyzed the biodistribution and cellular uptake of labeled Au NPs, which might have great clinical significance in the future [21].

Anti-HER2 Au NPs are another promising candidate for efficient photothermal therapy design. Since HER2 is a cell membrane surface-bound receptor tyrosine kinase and is a clinically important breast cancer biomarker, some authors assume that the nanoconjugates can exhibit sufficient absorption to enable effective photothermal ablation of breast cancer tissue [22]. Recently, the study of Chen J *et al.* demonstrated that anti-HER2 gold nanocages strongly absorb light in the NIR region of the spectrum [23]. These and other findings give us hope that existing breast cancer treatment may soon be significantly improved, providing that an adequate clinical trial is conducted.

5. FUTURE OF ANTIBODY-LABELED GOLD NANOCONJUGATE RESEARCH

Immunotargeted gold nanomaterials represent a novel, efficient and relatively affordable addition to the present knowledge regarding cancer imaging and treatment. However, many issues remain unresolved. Firstly, many of the studies previously mentioned fail to address the potential toxicity of gold nanomaterials in a living organism. There is insufficient data regarding both nephrotoxicity and hepatotoxicity, particularly having in mind that standard rules concerning biodistibution and membrane transport do not always apply to nanoscale objects. Immunotoxicity and immune response modulation of certain nanomaterials must also be taken into account when considering their clinical value [24,25].

Secondly, there is insufficient evidence to draw a definite conclusion on effectiveness and potential side effects of antibody-Au NPs-based thermotherapy. Although serious research efforts are being made towards this goal, still many questions remain unanswered. It is known that the certain cancer molecular markers targeted by antibodies, are also expressed on normal non-malignant cells, and therefore it is reasonable to assume that normal tissue could be also significantly affected by thermotherapy, if proper precautions are not taken. Future research will have to focus on designing new strategies in order to make thermotherapy more specific to cancer tissue, as well as to precisely define the optimal temperature and nanoparticle concentrations that would minimize the negative thermal effects on healthy tissue. In order to achieve this goal, multidisciplinary approach is needed, including the cooperation of biophysics experts, molecular biologists and medical practitioners.

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REFERENCES

[1] L.H. Bac, J.S. Kim and J.C. Kim // *Rev. Adv. Mater. Sci.* **28** (2011) 117.

[2] Y. Yamamoto and H. Hori // *Rev. Adv. Mater. Sci.* **12** (2006) 23.

[3] A. Zeleňáková, V. Zeleňák, J. Degmová, J. Kováč, K. Sedláčková, M. Kusý and J. Sitek // *Rev. Adv. Mater. Sci.* **18** (2008) 501.

[4] M.A. van Huis, A. van Veen, H. Schut, S.W.H. Eijt, B.J. Kooi, J.Th.M. De Hosson and T. Hibma // *Rev. Adv. Mater. Sci.* **4** (2003) 60.

[5] N. Kannan and S. Subbalaxmi // *Rev. Adv. Mater. Sci.* **27** (2011) 99.

[6] D.A. Giljohann, D.S. Seferos, W.L. Daniel, M.D. Massich, P.C. Patel and C.A. Mirkin // *Angew. Chem. Int. Ed. Engl*. **49** (2010) 3280.

[7] M.C. Daniel and D. Astruc // *Chem. Rev*. **104** (2004) 293.

[8] E.S. Day, L.R. Bickford, J.H. Slater, N.S. Riggall, R.A. Drezek and J.L. West // *Int. J. Nanomedicine* **5** (2010) 445.

[9] S. Kumar, J. Aaron and K. Sokolov // *Nat. Protoc*. **3** (2008) 314.

[10] X. Yang, Y. Guo and A. Wang // *Anal. Chim. Acta*. **666** (2010) 91.

[11] C.J. Ackerson, P.D. Jadzinsky, G.J. Jensen and R.D. Kornberg // *J. Am. Chem. Soc.* **128** (2006) 2635.

[12] N. Nitin, D.J. Javier and R. Richards-Kortum // *Bioconjug. Chem*. **18** (2007) 2090.

[13] I. Pantic // Rev. Adv. Mater. Sci. **26** (2010) 14.

[14] I.H. El-Sayed, X. Huang and M.A. El-Sayed // *Nano Lett*. **5** (2005) 829.

[15] J.C. Kah, K.W. Kho, C.G. Lee, C. James, R. Sheppard,Z.X. Shen, K.C. Soo and M.C. Olivo // *Int. J. Nanomedicine.* **2** (2007) 785.

[16] H. Wang, D. Xing and L. Xiang // *J. Phys. D: Appl. Phys.* **41** (2008) 095111.

[17] P.C. Li, C.W. Wei, C.K. Liao, C.D. Chen, K.C. Pao, C.R. Wang, Y.N. Wu and D.B. Shieh // *IEEE Trans. Ultrason. Ferroelectr. Freq. Control.* **54** (2007) 1642.

[18] S. Krishnan, P. Diagaradjane and S.H. Cho // *Int. J. Hyperthermia* **26** (2010) 775.

[19] X. Huang, P.K. Jain, I.H. El-Sayed and M.A. El-Sayed// *Photochem. Photobiol.* **82** (2006) 412.

[20] X. Huang , I.H. El-Sayed, W. Qian and M.A. El-Sayed // *J. Am. Chem. Soc.* **128** (2006) 2115.

[21] M.P. Melancon, W. Lu, Z. Yang, R. Zhang , Z. Cheng, A.M. Elliot, J. Stafford, T. Olson, J.Z. Zhang and C. Li // *Mol. Cancer Ther.* **7** (2008) 1730.

[22] C. Loo, A. Lowery, N. Halas, J. West and R. Drezek // *Nano Lett.* **5** (2005) 709.

[23] J. Chen, D. Wang, J. Xi, L. Au, A. Siekkinen, A. Warsen, Z.Y. Li, H. Zhang, Y. Xia and X. Li // *Nano Lett.* **7** (2007) 1318.

[24] M.A. Dobrovolskaia, D.R. Germolec and J.L. Weaver // *Nature Nanotechnol.* **4** (2009) 411.

[25] I. Pantic // *Sci. Prog.* **94** (2011) 97.