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Review Article

Application of Gold in Biomedicine: Past, Present and Future☆

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SUMMARY

Gold has been used in medicine for thousands of years. Nowadays, gold compounds contribute to the treatment of rheumatoid arthritis; gold alloys are used in implants in various fields of medicine; and colloid gold is used in immunogold electron microscopy. Advances in nanotechnology have resulted in novel gold nanoparticles, which possess distinct physical properties such as fluorescence. We have developed fluorescent gold nanoclusters (FANCs) that contain gold nanoparticles with a core dimension < 2 nm. Initial studies have shown that FANCs are a highly biocompatible marker with a fluorescence half-life of 9 days, and are suitable for *in vitro* and *in vivo* tracking of endothelial and endothelial progenitor cells. FANCs may also have novel chemical properties involved in regulation of endothelial cell function. This minireview discusses the future directions of biomedical research for FANCs.

1. Introduction

Gold has been known to humans for more than 5000 years. Owing to its rarity and vivid color that is maintained without oxidization in air or water, gold is considered a symbol of fortune to its owners and has been used in coinage, jewelry and ornaments, and other art works. Later, the malleable, ductile and nontoxic properties brought gold into dentistry for use in tooth restoration. In modern medicine, implants made of gold alloys have been used in a variety of medical fields other than dentistry, including eyelid closure, middle ear reconstruction, and voice prostheses[1]. In these cases, the high biocompatibility of gold with minimal tissue reaction is an important advantage. In addition, gold is used in biomedical research. In immunogold electron microscopy colloidal gold particles are conjugated to antibodies to detect specific antigens for visualizing their location. Apart from implants and research tools, gold has pharmacological effects.

2. Pharmacological effects of gold

Gold was considered to be a precious drug and had multiple beneficial health effects in ancient times. In an important book of Tao entitled Bauputz, the author, Hung Kir (283–363 AD) stated: "ingest gold to live a gold-like longevity". Traditional Chinese medicine has used gold to treat palpitations, seizures, and skin infection. Nowadays gold leaf, flake or dust is used on or in some foods and drinks, however, the purpose is only for decoration. Although there is no evidence that the gold leaf, flake or dust is absorbed by the body, a recent study of zebra fish exposed to gold nanoparticles of two sizes (12 nm and 50 nm) at very low doses (36–106 ng gold/fish/day) showed alteration of genome composition, as well as expression of genes involved in DNA repair, detoxification processes, apoptosis, mitochondrial metabolism, and oxidative stress. These data suggest the toxicity of long-term low-dose gold exposure[2]. In addition, gold ions do have pharmacological effects. In patients with rheumatoid arthritis, gold ions have been used to reduce joint pain for nearly 80 years[3]. Several gold compounds have been developed for parenteral or oral administration. Among these, gold sodium thiomalate has been the most extensively studied[4]. For this purpose, gold compounds can be used alone or in combination with other drugs. In a recent randomized, double-blind, double-observer, placebo-controlled multicenter trial (the METGO study) in rheumatoid arthritis patients with a suboptimal response to methotrexate, adding weekly intramuscular gold caused significant clinical improvement[5]. The mechanisms are not fully understood but attenuation of inflammation in the diseased joints is though to play a major role. *In vitro* studies have shown that gold ions inhibit T lymphocyte activation and antigen presentation[6], as well as inhibit overproduction of proinflammatory cytokines, cyclooxygenase expression, and prostaglandin E2 production in

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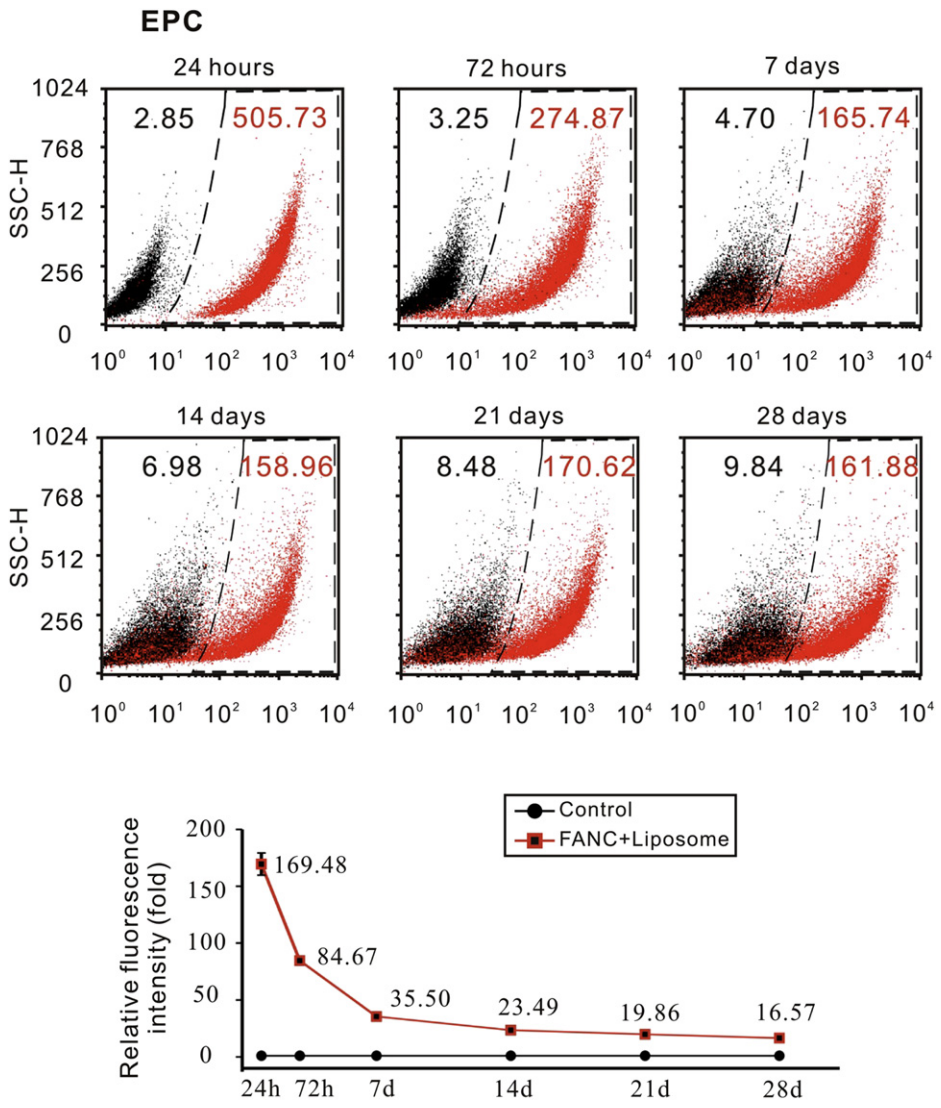

Fig. 1. Duration of FANCs in EPCs. After 4 hours of FANC delivery, medium and FANC−liposome complexes were replaced with fresh medium. Cells were trypsinized and subject to flow cytometry at indicated time points. The fluorescence distribution profile and mean fluorescence intensity of control cells (liposome only) and cells loaded with 500 nmol/L FANCs are shown in black and red, respectively. The relative fluorescence intensity of FANCs compared to liposomes only was calculated. During the period of maintenance, medium was changed every 2 days. EPC = endothelial progenitor cell; FANC = fluorescent gold nanocluster.

macrophages[7]. Interestingly, whether the anti-inflammatory effect of gold ions occurs at the cell membrane or requires entrance of gold into the cytoplasm remains unclear. Different compounds may vary in their effects. Concern has been raised about long-term use, for example, immune suppression may occur after administration of auranofin, an oral gold compound[4].

In patient with coronary artery disease undergoing percutaneous balloon angioplasty with stenting, inhibition of inflammation after stent placement has been demonstrated to be an effective way to attenuate neointima formation and hence prevent in-stent restenosis. The anti-inflammatory effect of gold has been tested. Clinical studies comparing gold-coated stainless steel stents with uncoated bare stents showed worse outcome in the gold-coated stent group[8]. This implies that other effects of gold promoting in-stent restenosis outweigh the anti-inflammatory properties.

3. Distinct physical properties of gold nanoparticles

Although colloidal gold particles used in immunogold electron microscopy are nanoparticles, the size is rarely < 5 nm. Recent advances in nanotechnology have paid attention to gold nanoparticles < 2 nm. Such a small size renders the behavior of gold intermediates between that of atoms and nanoparticles (> 2 nm), with alteration of various fundamental properties[9]. For example, gold nanoparticles show a size-dependent plasmon absorption band when their conduction electrons are confined to dimensions smaller than the electron mean free path length ($\sim$20 nm)[10]. However, gold nanoparticles < 2 nm no longer possess plasmon resonance and Mie's theory no longer can be applied[11–13]. Instead, when the size goes down to the Fermi wavelength of electrons ($\sim$0.7 nm), the gold nanoparticles possess molecule-like properties including size-dependent fluorescence and discrete size-dependent electronic states. The fluorescent properties of gold nanoparticles make them potential labels in biomedical research. Initial studies showed that most thiol-related gold nanoclusters protected by glutathione[14], tiopronin[15], meso-2,3-dimercaptosuccunic acid[16], and phenylethylthiolate[17], emit fluorescence ranging from red to infrared red. However, the quantum yield was reported at $< 1\%$ and was not bright enough for the nanoclusters to be of interest as fluorochromes. Recently, we used bovine serum albumin as the

template and reductant to develop a novel water-soluble fluorescent gold nanocluster (FANC) with negatively charged surface modification, unique optical properties, and a higher quantum yield (~2%)[18]. The quantum yield is even improved after thermal treatment (up to 7%)[19]. The FANC has high colloidal stability, and can be readily conjugated with biological molecules. The core diameter of the FANC is < 2 nm. In addition, FANCs with different modifications[20] are able to emit blue, green and red fluorescence.

4. FANCs are biocompatible markers for *In Vitro* and *In Vivo* tracking

To date, FANCs have been successfully used to label primary cultured cells, including human aortic endothelial cells and endothelial progenitor cells (EPCs)[19]. For both types of cells, simple addition of FANCs in the culture medium followed by incubation for several hours delivers the FANCs into the cells. However, the fluorescence is not bright enough for clear visualization. With the help of liposome complexes, up to 1000 nmol/L of FANC can be effectively delivered. The fluorescence at 500 nmol/L is strong and can be easily detected by both fluorescence microscopy and flow cytometry. In addition, it lasts for at least 28 days. Measurement of the fluorescence by flow cytometry has shown that the half-life is nearly 9 days *in vitro* (Fig. 1). Whether such a strong fluorescence is associated with cytotoxicity has been tested by a series of comprehensive examinations (Table 1). Only vascular cell adhesion molecule 1 and vascular endothelial cadherin were downregulated at a high concentration (500 nmol/L). Otherwise, there was neither activation of apoptosis nor proliferation, nor change of cell viability, unless > 500 nmol/L, at which level, there was a minor reduction in viability due to liposomes. For human EPCs, no impairment of angiogenesis was seen *in vitro*. In an *in vivo* study using hindlimb ischemia, mice with intramuscular administration of FANC-labeled human EPCs showed that the cells preserved angiogenic potential and exhibited traceable signals after 21 days. These findings demonstrated that FANCs are promising biocompatible fluorescent probes.

With such a high biocompatibility and not being toxic to the environment, the future of FANCs for labeling is promising, and if the quantum yields can be further improved, FANCs may totally replace quantum dots, which possess intrinsic toxicity for living cells and the environment, although the latter have stronger fluorescence intensity (quantum yields 30–50%). Table 2 compares the properties of FANCs and quantum dots for biomedical applications.

Table 1
Effects of FANC on expression profiles and function of human aortic endothelial cells and endothelial progenitor cells.

	Effect	Unchanged
mRNA level	Downregulation of VCAM and VE-Cad	Ang-1, Cx43, eNOS, PAI-1, VEGF, KDR, ZO-1, JAM-1, IL-8, MMP-9
Protein level		Cx43, PAI-1, eNOS, VE-Cad, KDR, caspase-3, PCNA
Cytotoxicity	Occurs above 500 nmol/L	
Labeling using liposome	> 98% efficiency and 130-fold induction of fluorescence intensity at 500 nmol/L	
Angiogenic activity (*in vitro*)		Tube formation in Matrigel
Angiogenic activity (*in vivo*)		Limb salvage (hindlimb ischemic mice)

Ang-1 = angiopoietin 1; Cx43 = connexin 43; eNOS = endothelial nitric oxide synthase; FANC = fluorescent gold nanocluster; IL-8 = interleukin 8; JAM-1 = junctional adhesion molecule 1; KDR = kinase insert domain receptor; MMP-9 = matrix metallopeptidase 9; PAI-1 = plasminogen activator inhibitor 1; PCNA = proliferating cell nuclear antigen; VCAM = vascular cell adhesion molecule; VE-Cad = vascular endothelial cadherin; VEGF = vascular endothelial growth factor; ZO-1 = zona occludens 1.

Table 2
Comparison of FANC and quantum dot for biomedical application.

	FANC	Quantum dots
Core	Gold	Cadmium selenide, cadmium sulfide, or indium arsenide
Size	2–20 nm	5–50 nm
Quantum yield	7%	30–50%
Fluorescence stability[a]	Less photobleaching	Much less photobleaching
Cytotoxicity	Suitable for long-term labeling	Highly toxic
Environmental impact at synthesis and usage	Environmental friendly	Toxic byproducts
Biomedical application	Feasible	Restricted by the nature of toxic heavy metal

FANC = fluorescent gold nanocluster.
[a] Compare to traditional fluorescent dyes.

5. Unresolved issues of FANCs

Although FANCs are effectively delivered into cells with the help of liposome complexes, their location in the cells is not clear. Immunoconfocal microscopy shows that FANCs inside cells are away from the cell membrane and only a few are co-localized with endosomes or lysosomes. In addition, FANCs are not found in the cell nucleus. Questions that remain are: (1) how are FANCs metabolized? (2) what are the mechanisms underlying the decay of fluorescence? (3) Does the decay of fluorescence parallel the removal of FANC from the cells? Further investigation is required to clarify these issues.

6. Other potential application of FANCs in biomedicine

Existing knowledge is helpful to guide the direction of research for future applications of gold compounds. Examples have been demonstrated in traditional medicine for providing clues[21]. The anti-inflammatory properties of gold compounds used for treatment of rheumatoid arthritis may also exist in FANCs. The finding that FANCs downregulate vascular cell adhesion molecule 1 and vascular endothelial cadherin is consistent with this view, because expression of both molecules is known to attract inflammatory cells[22]. In contrast, negatively charged poly(acrylic acid)-conjugated gold nanoparticles have been reported to bind to and induce unfolding of fibrinogen, which promotes activation of macrophage receptor 1 (Mac-1) and the nuclear factor-κB signaling pathway, resulting in the release of inflammatory cytokines[23]. These data indicate the presence of cell-type-specific effects of gold nanoparticles, and that the molecules conjugated to gold nanoparticles play an important role in determining the properties. Gold nanoparticle treatment has been reported to induce oxidative-stress-mediated genomic instability in lung fibroblasts[24]. Furthermore, calcined gold has been reported to attenuate stress-elicited neuroendocrine hyperactivity[25]. Whether FANCs possess all the properties requires clarification in the future. However, from a practical point of view, the metabolism of FANCs and their interaction with cell organelles, such as in human aortic endothelial cells and EPCs, have a higher priority, to understand the biomedical effects of FANCs.

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References

1. Demann ET, Stein PS, Haubenreich JE. Gold as an implant in medicine and dentistry. *J Long Term Eff Med Implants*. 2005;15:687–698.
2. Geffroy B, Ladhar C, Cambier S, et al. Impact of dietary gold nanoparticles in zebrafish at very low contamination pressure: the role of size, concentration and exposure time. *Nanotoxicology*. 2011:1–17.
3. Forestier J. Copper and gold salts in rheumatoid arthritis. *Ann Rheum Dis*. 1949;8:132–134.
4. Kean WF, Kean IR. Clinical pharmacology of gold. *Inflammopharmacology*. 2008;16:112–125.
5. Lehman AJ, Esdaile JM, Klinkhoff AV, et al. A 48-week, randomized, double-blind, double-observer, placebo-controlled multicenter trial of combination methotrexate and intramuscular gold therapy in rheumatoid arthritis: results of the METGO study. *Arthritis Rheum*. 2005;52:1360–1370.
6. Griem P, Panthel K, Kalbacher H, et al. Alteration of a model antigen by Au(iii) leads to t cell sensitization to cryptic peptides. *Eur J Immunol*. 1996;26: 279–287.
7. Han S, Kim K, Kim H, et al. Auranofin inhibits overproduction of pro-inflammatory cytokines, cyclooxygenase expression and pge2 production in macrophages. *Arch Pharm Res*. 2008;31:67–74.
8. Fort S, Kornowski R, Silber S, et al. 'Fused-gold' vs. 'Bare' stainless steel NIRflex stents of the same geometric design in diseased native coronary arteries. Long-term results from the NIR TOP study. *EuroIntervention*. 2007;3: 256–261.
9. Zheng J, Zhang C, Dickson RM. Highly fluorescent, water-soluble, size-tunable gold quantum dots. *Phys Rev Lett*. 2004;93:077402.
10. Link S, El-Sayed MA. Optical properties and ultrafast dynamics of metallic nanocrystals. *Annu Rev Phys Chem*. 2003;54:331–366.
11. Alvarez MM, Khoury JT, Schaaff TG, et al. Optical absorption spectra of nanocrystal gold molecules. *J Phys Chem B*. 1997;101:3706–3712.
12. Schaaff TG, Shafigullin MN, Khoury JT, et al. Isolation of smaller nanocrystal Au molecules: robust quantum effects in optical spectra. *J Phys Chem B*. 1997;101: 7885–7891.
13. Hostetler MJ, Wingate JE, Zhong C-J, et al. Alkanethiolate gold cluster molecules with core diameters from 1.5 to 5.2 nm: core and monolayer properties as a function of core size. *Langmuir*. 1998;14:17–30.
14. Negishi Y, Takasugi Y, Sato S, et al. Magic-numbered Au(n) clusters protected by glutathione monolayers (n = 18, 21, 25, 28, 32, 39): isolation and spectroscopic characterization. *J Am Chem Soc*. 2004;126:6518–6519.
15. Wang G, Huang T, Murray RW, et al. Near-IR luminescence of monolayer-protected metal clusters. *J Am Chem Soc*. 2005;127:812–813.
16. Negishi Y, Tsukuda T. Visible photoluminescence from nearly monodispersed Au12 clusters protected by meso-2,3-dimercaptosuccinic acid. *Chem Phys Lett*. 2004;383:161–165.
17. Lee D, Donkers RL, Wang G, et al. Electrochemistry and optical absorbance and luminescence of molecule-like au38 nanoparticles. *J Am Chem Soc*. 2004;126: 6193–6199.
18. Lin CA, Yang TY, Lee CH, et al. Synthesis, characterization, and bioconjugation of fluorescent gold nanoclusters toward biological labeling applications. *ACS Nano*. 2009;3:395–401.
19. Wang HH, Lin CA, Lee CH, et al. Fluorescent gold nanoclusters as a biocompatible marker for *in vitro* and *in vivo* tracking of endothelial cells. *ACS Nano*. 2011;5:4337–4344.
20. Lin CA, Lee CH, Hsieh CT, et al. Synthesis of fluorescent metallic nanoclusters toward biomedical application: recent progress and present challenges. *J Med Biol Eng*. 2009;29:276–283.
21. Chen RS, Lu MC, Wang SD, et al. Two Chinese herbal regimens safe for the elderly on inhibiting liver and bladder tumor cell growth and regulating gene expression. *Int J Gerontol*. 2011;5:30–40.
22. Schofield L, Novakovic S, Gerold P, et al. Glycosylphosphatidylinositol toxin of plasmodium up-regulates intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and e-selectin expression in vascular endothelial cells and increases leukocyte and parasite cytoadherence via tyrosine kinase-dependent signal transduction. *J Immunol*. 1996;156:1886–1896.
23. Deng ZJ, Liang M, Monteiro M, et al. Nanoparticle-induced unfolding of fibrinogen promotes mac-1 receptor activation and inflammation. *Nat Nano*. 2011;6:39–44.
24. Li JJ, Lo SL, Ng CT, et al. Genomic instability of gold nanoparticle treated human lung fibroblast cells. *Biomaterials*. 2011;32:5515–5523.
25. Shah ZA, Gilani RA, Sharma P, et al. Attenuation of stress-elicited brain catecholamines, serotonin and plasma corticosterone levels by calcined gold preparations used in indian system of medicine. *Basic Clin Pharmacol Toxicol*. 2005;96:469–474.