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Sona Gold Nanorods:

A Potential Breakthrough in Biomedical Science

Exploring the Context for Sona Nanotech's Toxin-free, Bio-compatible Gold Nanorods

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GLOSSARY

Gold Nanorod(s)	GNR(s)	Nanometer	nm
Cetyltrimethylammonium Bromide	CTAB	Near Infrared	NIF
Gold Nanoparticle(s)	GNP(s)	Mercaptopolyetheleneoxide	PEG-Thiol
Pegylation	PEG	Real Time *in-vivo*	RTiV

INTRODUCTION

Nanotechnology involves the fabrication and manipulation of materials on the near-atomic scale, and so it is not surprising that this emerging area of science is little understood outside of the realm of chemists, physicists and other scientists. While a highly technical field of science, the application of gold nanorods (GNRs) in nanotechnology is poised to play an important role in a broad spectrum of areas in including within medical practice.

Among the many different types of nanoparticles created, gold nanoparticle fabrication, in particular, has been extensively studied since the 1850's by pioneers including Faraday, Turkevitch, Lens, and Brust. The production of gold nanorods emerged at the start of the 21st century facilitating an even larger array of applications for nanotechnology. There are now hundreds of researchers around the world pursuing the advancement of GNRs as part of solutions to various technical and medical problems.

This White Paper provides readers possessing little to no scientific background with a basic overview of the technology surrounding gold nanoparticles and gold nanorods, and how these particles can be leveraged to enable important scientific and medical innovations. Throughout the paper, we also aim to briefly put into context Sona Nanotech's proprietary gold nanorod technology and highlight the role it could play in enabling the adoption of GNRs in multiple biomedical applications.

Specifically, Sona's biocompatible gold nanorods address a primary concern, that of toxicity, in the development and adoption of medical therapies involving the use of GNRs within the body, or 'in vivo', as the manufacture of Sona's GNRs uniquely does not involve the use of CTAB (cetyltrimethylammonium bromide), a substance well-known to be toxic.

BACKGROUND ON GOLD NANOPARTICLES

Nanoparticles are interesting molecular entities because the properties of materials change as their size approaches the atomic scale. This is a result of the increasing surface area to volume ratio, which results in the material's surface atoms having a greater impact on the material's characteristics. As a result, nanoparticles can have different optical, physical, and chemical properties from the larger scale form of their base material, as they are small enough to confine their electrons and produce quantum effects. As an example, gold nanoparticles have a much lower melting point than bulk gold.

Before exploring further, it is helpful to first put in context the relative size of the nanoscale. One nanometer (nm) is equal to one millionth of a millimeter and the atomic scale begins below one nanometer, so most nanoparticles are made up of only a few hundred atoms. Here are several relative size benchmarks to illustrate scale:

- a human hair is between 60,000 – 100,000 nm wide;
- a fingernail grows ~1 nm per second;
- a DNA molecule is ~2.5 nm in width; and
- 50 million, 10 nm gold nanoparticles can fit within a single red blood cell.

Gold nanoparticles (GNPs) have also attracted tremendous scientific interest due to other attributes including ease of synthesis, their unique optical properties, and their chemical stability. Nanoparticles, despite inhabiting the near-atomic scale, can actually be created and modified through widely available chemical processes, making them relatively accessible. As well, because of their attractive optical properties, expansive colour spectrum, and surface resonance abilities, gold nanoparticles, have demonstrated utility in applications such as lateral flow diagnostic tests and are now the component of choice for these types of tests, including the majority of COVID-19 rapid tests currently on the market. Furthermore, as bulk gold is well known to be chemically inert, and therefore inherently biocompatible, GNPs have received much interest with regard to use in biomedical applications. The interactions of GNPs with biological systems are primarily associated with their physiochemical properties which can see them internalized within cells or allow them to cross biological thresholds such as the blood-brain barrier, attributes that larger particles are not likely to have. This has led to many studies exploring their use in important bioengineering *in vivo* applications including cancer treatment, drug delivery, chemical sensing, and biological imaging.

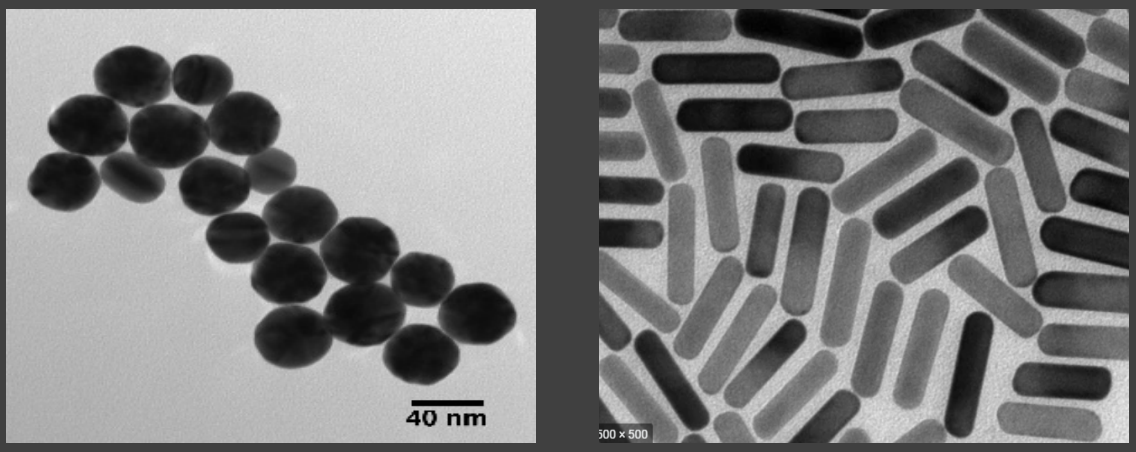
GOLD NANORODS – UNIQUE PROPERTIES, ASPECTS, AND CHALLENGES

At the start of the 21st century, scientists began growing spherical gold nanoparticles in an axial direction to create a new sub-class of GNPs called gold nanorods. This represented a paradigm shift for gold nanoparticles, as changing the shape had profound effects upon this nanoparticle's spectral characteristics. Unlike bulk gold, gold at the nanoscale level can appear to have different colours because of the interaction of their electrons' incident light.

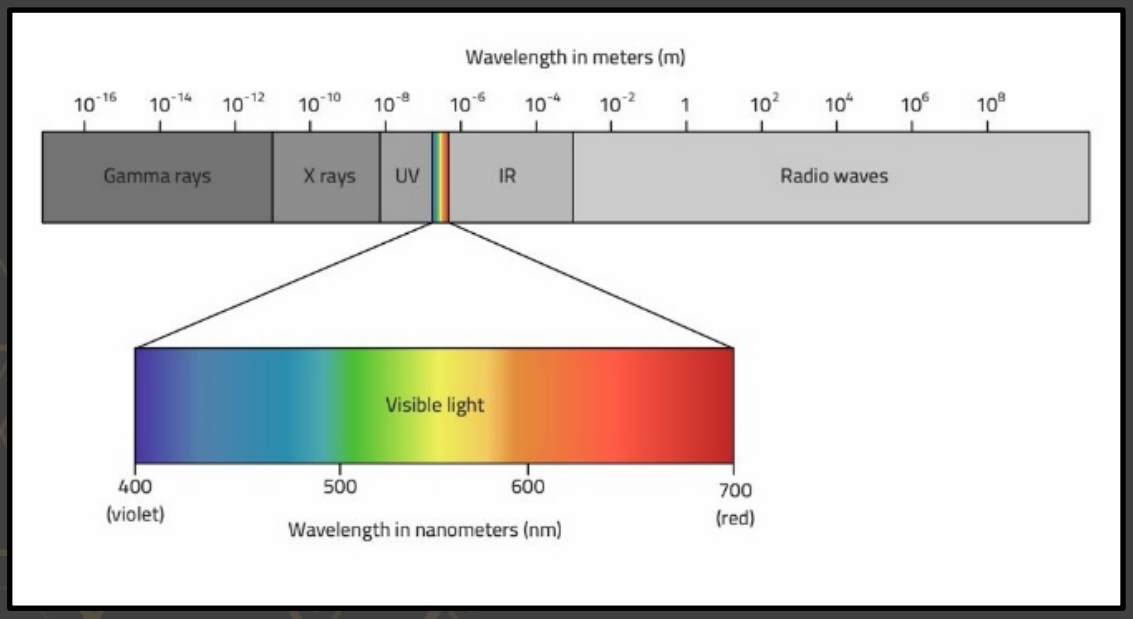
Tuning the length and width of the GNR's allows scientists to manipulate the wavelength at which they absorb and scatter light, creating different coloured nanoparticles and allowing the conversion of light to heat better than other types of GNPs. Surface chemistry modifications also provide further enhancement capabilities over standard GNPs allowing positive, negative, and neutral charges to be created on their surface, as well as the ability to produce both soft and rigid surface coatings, enabling control over the position and distance of various biologics conjugated to them. Given their ability to present different longitudinal and transverse properties, and effectively be "programmed" through their manufacture, GNRs represent a great potential for use in important bioengineering applications.

Other important attributes that GNRs offer include:

- Large surface area relative to size giving a strong ability to conjugate with drugs/molecules;
- Stability – the avoidance of aggregation over longer time; and
- High cellular membrane permeability – cells internalize GNRs through a process called endocytosis with the effectiveness of the process being a function of the GNR's shape and size, and surface charge and decoration.



Electron microscope image of gold nanospheres (left) and gold nanorods (right)



Electromagnetic spectrum for white light. Light become safer for human body as we move from Gamma rays to Radio waves (left to right direction)

GNRs can be manufactured in a variety of ways, but, at present, seed-mediated synthesis is the most common method. During the seed-mediated approach, nanorods are grown from small spherical gold nanocrystals, utilizing a 'surfactant' to facilitate their growth and shaping. Also called a 'surface-active agent', a surfactant is a substance such as a detergent that, when added to a liquid, reduces surface tension, thereby increasing the liquid's spreading and wetting properties. The addition of a surfactant thereby stabilizes the growth and guides the nanorod formation in one direction based on a desired aspect ratio. Beyond making this process easier than other methods, the seed-mediated approach offers a robust

and tunable suite of techniques for the tailoring of aspect ratios without reducing throughput, increasing reaction time, or limiting flexibility of the process.

The challenge with seed mediated GNR manufacturing, however, is that it typically relies upon the surfactant cetyltrimethylammonium bromide (CTAB), which is toxic to cells. As a result, CTAB's toxic effects are typically sought to be reduced by repeated cleaning, filtration, and chemical substitution by nontoxic modifiers, lowering the endotoxin level to below allowable limits. Nonetheless, the CTAB molecules that remain have still been identified as a source of cytotoxicity, potentially damaging cell structures and functioning, thereby calling into question the long-term safety of their use *in vivo*. As a result, the question of how to manage the toxicity of the surfactant CTAB, at any concentration, is an urgent problem, and although many surface modifiers have been found, there is still a desire for a simple, inexpensive, and fully biocompatible alternative, more appropriate for clinical use. Sona Nanotech's proprietary biosafe GNRs represent one such GNR synthesis method for which no toxic material is used at any point in their manufacture. Before expounding on Sona's technology and potential applications, however, let's briefly discuss other current approaches to mitigating the toxic effects of using CTAB for the manufacture of GNRs.

Current Approaches to Dealing with Gold Nanorod Toxicity

While Sona's biocompatible GNRs do not require treatment to mitigate toxicity, to use other GNRs for biological applications, surface modification is crucial, and several steps are needed to prepare CTAB-mitigated GNRs. The most common method is bioadaptive molecular ***wrapping*** or Pegylation.

Bioadaptive molecular wrapping involves the GNR being encapsulated in a polymer that serves to both mitigate the toxicity impact of CTAB and provide certain stabilization properties for the GNR. The most common method uses a polymer called PEG-Thiol (Mercaptopolyetheleneoxide). Studies indicate that while CTAB-GNRs create issues such as pulmonary inflammation, PEG-GNRs have been shown to improve biocompatibility. Cytotoxicity, however, is still present when using high concentrations of PEG-GNRs, typically required for many medical applications.

Bioadaptive molecular ***substitution***, on the other hand, involves the introduction of small molecules, following GNR development through the CTAB method, that have the capability to bind strongly to gold surfaces, ultimately replacing CTAB with alternative bioadaptive molecules during the final step of the manufacturing process. It has been found that this bioadaptive molecular substitution is capable of eliminating acute toxicity, however, long term toxic effects have not been extensively studied and the evidence is limited on whether, in fact, all CTAB is removed using this process.

These approaches to dealing with the toxicity of CTAB are helpful but have limitations, as CTAB still likely remains on the GNR surface in each case, and Pegylation is also subject to degradation over time, leading to a possible future toxicity event. This biocompatibility concern should be fully addressed in order to facilitate the many potential *in vivo* medical applications that GNRs are thought to safely enable, some of which will now be discussed.

MEDICAL APPLICATIONS FOR GOLD NANORODS

The novel physio-chemical properties of GNRs discussed above have generated significant attention for developing both diagnostic and therapeutic approaches in various medical applications. Given GNR's unique ability to be 'tuned' to specific wavelength resonance, their larger surface area for conjugation of activating molecules, and their refractive properties, they lend themselves to several uses in medical nanotechnology applications extremely well. For each of these applications, size, shape, and surface modification of the implemented GNRs are key factors that should complement their overall success.

Photothermal Therapy

Drugs and radiation used in treatment for cancer, while effective at killing tumor cells, cause damage to organs and healthy cells. Evidence suggests that GNRs could be more effective at killing tumors with less or no adverse reactions on healthy cells.

Tumor cells typically can be killed through heat treatment at temperatures between 42-45 degrees Celsius over 15–60-minute intervals, however, traditional methods involve non-selective irradiation, simultaneously damaging the normal tissue surrounding the tumor.

GNRs can theoretically be placed at the tumor site via intravenous or local injections and accumulating the GNRs within the cancer cells by attaching specific proteins to their surface that recognize the tumor cells. Then, using a near infrared (NIR) light generating laser that is harmless to skin, the local temperature of the cancerous cells can be elevated by causing the GNRs to vibrate at a specific frequency, thereby killing the tumor cell safely. This method is less invasive and can be more precise than surgery.

Targeted Drug Delivery

The ability of GNRs to be activated via surface modifications, and conjugated to specific molecules or drugs, enables the possibility of using them as drug delivery vehicles *in vivo*. In fact, studies have now demonstrated how NIR light can be used to successfully trigger GNRs to release drugs that had been conjugated to them, offering an efficient, targeted release of drugs within human cells. In this example, the concentration of drugs released can be controlled by the NIR light irradiation time. It is the GNR's unique rod structure that permits them to be tuned, carry, and enable the release of, biological 'payloads'.

Imaging Applications

GNPs serve as great contrast agents for cell image enhancement and super-resolved imaging due to their effective surface plasmon resonance (SPR) properties, thus are not affected by photobleaching. Yet, while most GNPs appear to offer potential benefits in this case, their optical signals are still insufficiently strong for many important real-life applications. These limitations hamper the progress in cell research by direct optical microscopy and narrow the range of phototherapy applications. By modulating the light polarization of GNRs, however, super-resolution in live cell imaging could be achieved. Taking advantage of the polarization-dependence of gold nanorod optical properties, the 'lock-in amplification', widely used in electronic engineering, can be achieved in live cells and in cells that undergo apoptotic changes. This has distinct advantages in advancing real time in-vivo (RTiV) applications for monitoring inflammation in patients with cardiovascular and auto-immune diseases.

Further Consideration

In all three of these applications of GNRs, beyond toxicity, there remains one further common concern: the ability of the body to expel the GNRs after they have served their purpose. According to FDA guidelines, pharmaceutical drugs should be eliminated via metabolism or excretion processes after they enter the body. Drug elimination reduces toxicity effects and prevents drug accumulation. Like other pharmaceutical agents, nanoparticles should be designed to be eliminated from the body. Indeed, nanoparticle elimination should be considered seriously since they are more resistant to elimination routes such as metabolism and renal excretion. Irrespective of the biocompatibility of the materials used, their ability to 'clear' the body will be key to progressing these three important therapies towards clinical practice. Further study in this area is therefore warranted.

SONA'S BIOSAFE GOLD NANORODS

The value of GNRs in each of the above applications is clear but mitigating the toxicity in GNR manufacturing will be essential to the advancement of their adoption for clinical use. The best way to minimize any potential toxicity effects of CTAB is to eliminate it from the manufacturing process altogether and replace it with a non-toxic alternative. Taking this approach, Sona has generated patent pending fabrication methods to create biosafe GNRs with the same properties, tunability and activation potential as GNRs currently produced with CTAB. Sona's methods of producing GNRs are not based on blocking CTAB or replacing CTAB after manufacturing. Our production process relies on the use of in-house proprietary materials and an alternative surfactant to those used in traditional methods, combined with metal cations (which are positively charged ions) to form a mixture that undergoes a reduction reaction to form the GNRs.

Sona is currently undertaking research to validate and further understand its technology's biocompatibility and efficacy in key applications and, therefore, its potential to enable important nanomedical advancements. To this end, the company has entered into a collaboration with the University of Toronto's Institute of Biomedical Engineering, through Dr. Warren Chan, distinguished professor, and Canada Research Chair in Nanobioengineering & Director of the Institute of Biomedical Engineering at the University of Toronto. Through research collaborations such as this, the company aims to leverage the expertise and scientific leadership of third-party, respected scientists and entrepreneurs to substantiate the technology's biocompatibility and provide a foundation for further research programs, with a view to identifying the most promising potential medical applications.

SUMMARY

It is clear that nanoparticles, and GNRs in particular, have the potential to play a critical role in advancing cancer and other medical treatments. Determining the safest materials and route to get there, however, will take further study, especially as it pertains to the long-term safety of the use of GNRs in these *in vivo* applications.

Key Takeaways

- **The toxicity associated with the functionalization of GNRs and the long-term cytotoxicity of nanomaterials must be understood before they can be fully applied clinically.**
- **Sona Nanotech's biosafe GNRs uniquely do not rely upon toxic CTAB for their manufacture, like all other current products do, so they make an ideal candidate to be considered for *in vivo* studies and applications.**
- **Greater knowledge about Sona GNR biocompatibility and functionality is essential before these nanomaterials can be used in real clinical settings, and as such, strategic research collaborations are being developed with respected scientific and engineering teams in various fields.**
- **The list of target applications for Sona GNRs within the medical and scientific fields continues to grow, as more is understood about the functionality of these unique nanoparticles, broadening their potential impact.**

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