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INTERACTION OF GOLD NANOPARTICLES WITH SYNTHETIC AND

BIOLOGICAL POLYMERS

By

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ABSTRACT OF THE DISSERTATION

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The focus of this research was to combine nanoparticles with polymers of synthetic and biological origin to create novel hybrid materials that could solve relevant unmet needs in the medical field. The first project explored the use of gold nanorods for the fabrication of healing patches while the second examined the role of nanoparticles in the improvement of the mechanical properties of polyisoprene (PI) and polystyrene (PS) for film applications.

The skin is an important biological barrier, which is why it is vital to protect its integrity, or to recover it as quickly as possible after injury. Fast wound healing enables the skin to regain the mechanical properties of normal skin; delayed or prevented wound healing due to age or medical conditions, such as hemophilia, can lead to deteriorated

mechanical properties of the tissues, severe scars, and ultimately death. In the most severe cases, in which the body is unable to produce the proteins necessary to the blood coagulation cascade, or when it cannot alone provide the necessary amount of proteins to heal extended wounds, it is necessary to externally intervene and regulate the wound healing processes. Our approach was to provide such external regulation via a wound healing patch synthesized with a biocompatible gel matrix that incorporated selected relevant proteins that drive wound healing. We bound the proteins to gold nanoparticles, and leveraged the optical properties of gold nanoparticles to selectively control the release of the proteins to the wound site.

Gold nanoparticles (AuNPs) possess unique mechanical and optical properties that make them useful in many applications. In this work their optical properties were harnessed to manufacture wound healing patches, while their mechanical properties were utilized to prepare polymer nanocomposites, such as puncture resistant surgical gloves. Some advantages of using AuNPs are their long term stability, their reproducibility in morphology, biocompatibility, and their ease of functionalization. Consequently, it is possible to synthesize, functionalize, and deliver them with pre-programmable accuracy to a targeted location in the body, or to incorporate them into a polymer matrix yielding a nanocomposite and, for instance, determine their effect on the mechanical properties.

In order to create the wound healing patches, gold nanorods (AuNRs) of varying aspect ratios were synthesized using a seed mediated growth method, and fibrinogen, a protein used in the blood coagulation cascade, was electrostatically bound to AuNRs. The advantage of using AuNRs is that their optical properties are strongly correlated to their geometry, which can in turn be leveraged to render them highly absorbing in the

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near infrared (NIR), which is the electromagnetic radiation most widely used in biomedical applications, due to its low interaction with tissues. The overall goal was to have the AuNRs thermally released into the wound upon contact with the skin. The NRs were synthesized with different aspect ratios to tune their absorption bands to different IR lasers. Fibrinogen and thrombin are important proteins in the blood coagulation cascade: Upon interacting, they form fibrin, which then leads to a blood clot. We have chosen to optimize the release of fibrinogen from AuNRs only. Ultimately two aspect ratios of AuNRs were synthesized with fibringen and thrombin electrostatically bound to them so that the proteins can be released selectively from each of the NRs when the irradiating laser is resonant with the longitudinal absorption band of the NRs. Finite element modeling of the electric field, carried out with COMSOL Multiphysics, was used to map the intensity and localization of the electric field around the NRs in response to the impinging incident light and the heat losses generated within the particles to ensure that the heat transferred from the nanoparticles to the surrounding environment does not cause a dramatic temperature increase, which would cause damage to the tissues.

Thus far, it has been shown in literature that using high energy pulsed lasers can initiate a release of proteins bound to AuNRs. However, the use of these types of lasers can cause damage to the body, can denature proteins adsorbed on the nanoparticles, and destroy the integrity of the nanoparticles by melting them. We demonstrate how using a low power 100 mW laser with a spot size of 0.0024 mm² initiates a plasmonic release of fibrinogen electrostatically bound to AuNRs without changing the morphology of the NPs. Theoretical and experimental calculations indicate that using a low power laser in

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the milliwatt regime does not significantly increase the local temperature around the nanoparticles, allowing for a safer targeted release of proteins than current approaches.

The second project was initially designed around the application of puncture resistant surgical gloves. Currently no puncture-resistant surgical gloves are available on the market, leading to critical issues for healthcare workers (HCWs), such as the contraction of diseases as a consequence of needlestick injuries. To reduce the likelihood of injury, in some instances HCWs wear two pairs of gloves, however there has been controversy about whether this procedure actually increases safety, or ultimately, it merely reduces dexterity, which is vital for procedures such as surgeries. Among various efforts focused on the development of puncture resistant gloves, one approach has been to incorporate nanostructured materials of various nature to increase the mechanical properties of polyisoprene (PI), the synthetic polymer most commonly used to manufacture surgical gloves. However, to optimize the mechanical properties of nanocomposites, there needs to be a deep understanding of the fundamental interactions between nanoparticles and the surrounding matrix. Our approach was to incorporate AuNR and AuNS in a PI matrix. The particles were capped with a common thermoplastic (polystyrene, PS) of two molecular weights, to retain colloidal stability. To facilitate the dispersion and generate favorable interactions between nanoparticles and matrix, a styrene-isoprene-styrene (SIS) block copolymer was also added. By varying the molecular weight of the capping agent, the shape of the nanoparticles, and the nature and amount of the block copolymer, we established a deeper understanding of how each of these parameters affects the mechanical properties of this nanocomposite.

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Once there is more knowledge on how to improve the interface between the nanoparticles and the polymer matrix, there will be more insight as to how to improve the mechanical properties of thin films. Typically, studies have utilized particles known for their hardness, such as graphene, graphene oxide, and carbon nanotubes. Although the addition of hard or tough reinforcement materials would seem to provide stronger materials, there is often the opposite result – degraded mechanical properties of the nanocomposite compared to the neat polymer due to poor matrix filler interactions: Producing a material with improved puncture resistance requires studying the interaction and interface between nanoparticles and the matrix.

We investigate nanoparticle-matrix interactions by studying two parameters that have been shown in literature to have a major effect on the mechanical properties of polymer nanocomposites. One of the key parameters in improving the mechanical properties of a nanocomposite is strong *bonding* between the nanoparticles and the surrounding matrix. Furthermore, it has been demonstrated that incorporation of anisotropic particles, such as nanoclay, in PI can increase tensile properties of the polymer thus suggesting that the *shape* of the nanofiller might be another key parameter. The increase in modulus has been attributed to the alignment of the particles in the matrix, which promotes strain-induced crystallization (SIC) in the material. In this work we demonstrate, for the first time, how gold nanoparticles are ideal nanofillers due to our ability to separately control their morphology and surface functionalization, allowing us to study independently the effect of filler shape and surface chemistry. Ceramic materials of various shapes, traditionally employed as nanofillers for polymer strengthening, do not

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allow for straightforward functionalization, thereby making it impossible to decouple the role of shape and interfacial interactions.

Studying the effect of bonding of nanoparticles with the matrix can be accomplished by grafting AuNS with thiolated PS of variable molecular weights. Our results indicate that incorporating high molecular weight (e.g. 11000 Da) PS-grafted AuNS in PS increases the modulus of PS due to increased chain entanglement and van der waals interactions compared to low molecular weight (e.g. 5000 Da) PS-grafted AuNS. We demonstrate how the shape of the nanoparticles has an effect on the mechanical properties of the nanocomposite materials by incorporating anisotropic particles, AuNR, and isotropic particles, AuNS into the matrix. We characterize the composites using two approaches: 1) straining the material uniaxially in tension, promoting alignment of anisotropic particles within the matrix and 2) locally deforming the composite at rest via nanoindentation. Using these different approaches allows us to decouple the shape from the surface chemistry effects. Our results show that incorporating AuNR in a PI/SIS block copolymer matrix results in an increased modulus under uniaxial tension, while the nanoindentation results do not show a significant improvement compared to AuNS, thus confirming that the alignment of the nanofiller achieved under tensile stress improves the mechanical properties of the bulk polymer. Our approach for incorporating grafted AuNPs of various shapes provides the foundation for a complete study necessary to begin understanding nanoparticle-matrix interactions.

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I am very grateful to have had the opportunity to work under the guidance of my advisor, Dr. Laura Fabris. Prior to graduate school, I was told that the most important relationship a graduate student can have is the relationship with his or her advisor. I wholeheartedly agree with this statement, and I am so glad to have had the opportunity to work under her. Laura, you are a wonderful mentor and you encouraged me to think outside the box. You always made yourself available to me in my times of need, listened to my ideas for my project, and were open-minded about the directions for my project. You went out of your way to support me and made sure I had the resources I needed. At times when I had trouble seeing the bigger picture, you made me feel like I was always within reach of our final goal. Your novel ideas and way of thinking are what our scientific community needs to grow and thrive.

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Dedication

I dedicate this dissertation to my family.

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1 Gold Nanoparticles in Wound Healing

1.1 Introduction

The second leading cause of death in the civilian community, and the first leading cause of death on the battlefield is severe hemorrhage from trauma. In a study conducted by Eastridge et al. on approximately 4,600 battlefield fatalities between the years 2001 and 2011, the injuries were categorized into explosive (73.7%), gunshot wounds (22.1%), and other (4.2%). 87.3% of deaths occurred before the people reached a medical treatment facility (MTF). 35.2% of the fatalities were instantaneous, 52.1% were acute (meaning the subjects died within minutes to hours after sustaining the injury), and 12.7% of people died after reaching an MTF. Nearly a quarter of the injuries that occurred before the soldiers reached an MTF, were classified as potentially survivable, 90.9% of which were from hemorrhage. In addition, it was reported that 40% of the soldiers in Vietnam that died from exsanguinations, or blood loss, could have survived if their bleeding was controlled using a hemostatic agent. Amongst civilians, the most common cause of death that Emergency Medical Services, EMS, personnel encounter upon arrival is exsanguination, signifying how crucial hemostasis is. Hemorrhage control will always be an issue for civilians and soldiers, and if people receive the medical treatment necessary to achieve hemostasis, or blood clotting, as soon as possible, they will have had a better chance of survival. Even after achieving hemostasis, other problems can lead to mortality, however controlling the bleeding is the first step. ¹⁻³

1.2 Approach Using Laser Release

Commercially available hemostatic agents have many drawbacks, including the extensive time necessary to achieve hemostasis and the need of significant externally applied pressure needed to achieve wound healing. Our patch will contain the major proteins involved in the blood coagulation cascade attached to gold nanorods. Our work focuses on using the optical properties of gold nanoparticles, as a method to thermally release the proteins *ad hoc* by using low laser power (such as that of a laser pointer) in the NIR region of the electromagnetic spectrum. Ultimately, the release of proteins in a sequence mimicking that of the natural healing processes will trigger the blood coagulation cascade and facilitate wound repair.

1.3 Background

1.3.1 Types of Wounds

There are two ways in which the body responds to tissue damage: tissue regeneration and tissue repair. Tissue regeneration, or epidermal wound healing, occurs when there is minor damage to the top layer of the skin. This results in epithelial cells replicating and the epidermis being restored quickly with good cosmetic appearance and without much loss in skin functionality. Tissue repair occurs when there is injury to the dermis, a deeper skin layer, and is a more complex process. These repair mechanisms can be seen in **Figure 1.1**. There are two types of wounds: acute wounds, which can repair relatively quickly even if large in dimensions, and chronic wounds, which heal very slowly or not at all even with the help of treatment. These are termed healing and non-healing wounds, respectively.³





Delayed wound healing can occur because of local and systemic factors; if these factors are not addressed they can turn an acute wound into a chronic wound. Examples of local factors are wound infection, which leads to an extended inflammatory response that delays the wound healing process, and excessive protease release, which degrades the extracellular matrix (ECM). Systematic factors depend more on the individual's conditions, and include factors such as obesity, age, and smoking. Our patches will be designed for acute wound healing in healthy subjects, applied by first responders. For example, a person who has a laceration would benefit from our patch. Although burns do fit under the acute wound category, they would not be our target users, since the goal of our patch is to stimulate blood coagulation via hemostasis, by forming a blood clot.³

Chronic wounds have thirty times the amount of protease and have a deficit of growth factors that disrupts the wound healing process. These abnormalities cause the ECM to break down and for the process to be held back in the inflammatory stage of the wound healing process. The ECM, a gel-like matrix, has an important role because it gives the skin its mechanical properties such as strength, elasticity, and compressibility. It consists mostly of the protein collagen. The wound healing process cannot move into the next stage until the normal levels of growth factors and proteases are established, which enables cells to communicate.³ The body of a person with hemophilia does not produce the factors necessary to form a clot and stop bleeding. Their wound healing process would fall into chronic wounds, and will be unresolved with the application of this or other similar patches.

For acute wounds, the coagulation cascade is initiated by the exposure of tissue factors, which trigger the extrinsic pathway. This is the pathway that the body follows in repairing the wound due to traumatic injuries.⁴ There are four stages of wound healing: hemostasis, inflammation, proliferation, and remodeling. The goal of the proposed patch is to achieve hemostasis as quickly as possible so that the patient has the best chances of survival and reduced scarring. Biopolymers have been used in this application because they are biodegradable and do not cause inflammatory response. Some examples of natural polymers that are used are fibrinogen, thrombin, and collagen.⁵ They come in forms such as powders, liquids, and sheets. Examples of synthetic polymers are polyethylene glycol (PEG), polyglycolic acid (PGA), and polylactic acid (PLA), and these usually come in a liquid form.⁵

1.3.2 Hemostatic Dressings

In a review by Naimer in 2014, many hemostatic dressings were compared, such as Quick Clot, Celox, and Hemcon.⁶ The disadvantage of many hemostatic dressings is that many of the hemostatic agents come in the form of granules and need to be poured into the wound. Not only is it difficult to direct the granules into the wound, but there is little

adherence to the wound surface, thus requiring the person who is applying the dressing to apply pressure on the wound until hemostasis occurs.⁶ Among the various treatments, fibrinogen-based dressings are the most effective and safe way to achieve hemostasis. Fibrin Sealant Dressing contains fibrinogen and thrombin, two of the main components (found in blood) needed to form fibrin, the network that creates the blood clot.⁷ The main disadvantage of using an injectable fibrin sealants is the time required to thaw, mix, and dissolve the components.⁸ Dry dressings are therefore ideal, compared to powder or foam forms. Once the dressing is applied, the proteins dissolve, and an adhesive fibrin layer forms to stop the bleeding.⁶ The dressing however needs pressure to be applied to the wound. This can be a problem because bleeding can occur again after forming a clot due to movement or friction that can dislodge it.⁶

Our modification to the commercially available fibrinogen-based dressing, will improve the delivery of the wound healing components by selectively controlling the release of fibrinogen and thrombin into the wound following a release sequence that mimics that of natural wound healing. ^{6,9} Unlike fibrin sealant dressings which require time to prepare our patches will be available for immediate use. The wound healing agents will be electrostatically bound to AuNRs that will be embedded in a biocompatible gel. Matrigel, a commercially available gel-like protein mixture. Matrigel is an ideal candidate because it contains the components of the extracellular matrix (ECM), such as fibronectin, collagen, and elastin, which could potentially facilitate the wound healing process since the role of ECM is to support and maintain cell growth. ¹⁰⁻¹²

1.3.3 Protein Coronas and Fibrinogen

An important aspect of utilizing gold nanoparticles for biomedical applications is the presence of various proteins in the body and their interaction with nanoparticles. When nanoparticles are introduced into the body, proteins adsorb to the nanoparticle surface, forming a protein corona. Previous literature has shown that thermal energy can be used to change the composition of the protein coronas, either by applying heat or by resonance heating with a 48 mW laser (785 nm).¹³ In the referenced work, gold nanorods were incubated in Fetal Bovine Serum (FBS), and the corona composition was monitored over a period of time with various conditions. These conditions were thermal release at 37 °C and 45 °C, and laser release for 27.5 minutes and 55 minutes.¹³ Since the formation of protein coronas is unavoidable upon nanoparticles being introduced in the body, we must address this phenomenon and use it to our advantage.

The proteins included in the patch are fibrinogen and thrombin. Fibrinogen converts to fibrin, in the presence of thrombin, a necessary step in the wound healing process (clot formation). We have chosen to focus our research on fibrinogen for proof of concept. Fibrinogen has a molecular weight of 340 kDa, and is composed of three peptide chains.^{14, 15} It is a long molecule with dimensions are 46 x 4 nm, and has been shown in literature to bind to nanoparticles with side-on and end-on configurations, with the side-on configuration having a weaker interaction with the nanoparticles. As the dimensions of the nanoparticles increase, the surface area increases, more binding sites become available, and number of fibrinogen molecules bound to the particles increases.

1.4 Plasmonic Release Application

In commercially available fibrinogen-based dressing, there is no control over the quantity of fibrinogen and thrombin in the dressing as well as when these proteins can be released. Our method provides control over both of these conditions by selectively releasing the proteins thermally using the optical properties of AuNRs. By irradiating AuNRs with a laser of a certain wavelength, their response is to heat up. Thus, the optical properties are connected to thermal properties, and we will discuss how they are related in the following chapter.

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2 Plasmonic Properties of Gold Nanoparticles

2.1 Optical Properties of Au (Bulk and Nanoparticles)

Our patch will selectively release wound healing agents by using gold nanoparticles, a relatively well characterized plasmonic material. Noble metals such as gold and silver have intriguing optical properties; for instance, silver appears shiny and colorless because it reflects almost 100% of incident light over the visible region of the electromagnetic spectrum, while gold reflects around 50% for wavelengths shorter than or equal to 600 nm. This is why the color of bulk gold is reddish-yellow. When longer wavelengths of incident light, around the near infrared (NIR), interact with a flat gold surface, almost 100% of the light is reflected, which makes it suitable for mirrors in the NIR. Gold nanoparticles have different optical properties from bulk gold. For example, gold nanospheres with a diameter of 20 nm absorb light around 520 nm (the green part of the electromagnetic spectrum), and therefore appear red. The color is attributed to a phenomenon called surface plasmon resonance. Gold nanoparticles have been used as early as the Roman times, where they were employed to stain glass red. Gold nanoparticles are currently being used in biomedical and solar applications.^{16, 17}

2.2 Dielectric Constant of Au

As mentioned earlier, bulk gold can be used as a mirror in the NIR, and gold nanospheres absorb light in the visible region of the electromagnetic spectrum, with the size being the determining factor of the color, implying that these materials have wavelength-dependent optical properties. The three optical properties are permittivity, conductivity, and permeability. Since noble metals are nonmagnetic, the permeability can be assumed to be equivalent to that of vacuum, and only the permittivity and conductivity need to be considered. Permittivity and conductivity can be grouped into a single term called the complex permittivity, which is also known as the dielectric constant.

The real part of the dielectric constant is related to the permittivity, and the imaginary part is related to the conductivity. Johnson and Christy carried out reflectivity experiments on thin films of noble metals and determined their optical constants, namely the refractive index *n*, and the extinction coefficient κ .¹⁸ With these parameters, the real and imaginary parts of the permittivity can be calculated, and they are plotted in **Figure 2.1**. Theoretically it can be stated that the unique optical properties of gold and silver originate from the fact that, in the visible region of the electromagnetic spectrum, the real part of dielectric constant is large and negative, and the imaginary part of the dielectric constant is small. If these conditions are met, metals can support surface plasmons, which will be leveraged in the wound healing application. It should be noted that the data collected and results reported by Johnson and Christy are representative of optical properties from bulk noble metals, and that they are a good approximation for noble nanoparticles as small as 20 nm in diameter.^{18, 19}



Figure 2.1 Dielectric function of bulk gold using optical constants from Johnson and Christy.¹⁸

2.3 Localized Surface Plasmon Resonances – Polarizability and Resonant (Fröhlich) Condition

The optical properties of gold nanoparticles are governed by how electrons in the nanoparticles respond to incident light. This project will focus on the phenomenon of localized surface plasmons (LSPs) (**Figure 2.2**), which are non-propagating plasmons that result from the interaction of the incident light with metallic nanoparticles. A description employing the electrostatic approximation can be used if the wavelength of incident light is much larger than the size of the nanoparticle, which causes a uniform polarization of the charges.



Figure 2.2 Illustration of the (dipole) localized surface plasmon resonance, in the quasistatic limit, showing a plane wave interacting with a metal sphere, uniformly polarizing charges in the particle, and displacing the electrons.

When the incident light interacts with the conduction band electrons in the nanoparticle, the electrons, being the lighter charged entities, are displaced from the positively charged nuclei. Another way of interpreting this phenomenon is that the electrons are polarized in the opposite direction of the applied field, thus forming a separation of positive and negative charges, called a dipole. The separation of charges, alone, imposes a restoring force on the electrons, like a mass on a spring. The localized surface plasmon resonance (LSPR), which is the resonant frequency at which the electrons collectively oscillate, is determined by the maximum polarizability of the particle, α , which, for spheres in the electrostatic approximation, is determined by the Fröhlich condition, shown in in equation 2.1. At the Fröhlich frequency, two conditions must be met for maximum polarizability, shown in equations 2.2 and 2.3. The complex permittivity is given by given equation 2.4.

$$\alpha = 4\pi a^3 \frac{\varepsilon - \varepsilon_m}{\varepsilon + 2\varepsilon_m}$$
 2.1

$$\varepsilon' = -2\varepsilon_m$$
 2.2

$$\varepsilon'' \approx 0$$
 2.3

$$\varepsilon = \varepsilon' + i\varepsilon'' \tag{2.4}$$

The surface of the particle, and therefore its size and shape, which determine the boundary conditions for the polarizability of the particle, as well as the permittivity of the surrounding medium, all contribute to the surface plasmon resonance.²⁰ In **Figure 2.3**a), it can be seen that the electromagnetic field lines are greatly deflected in the presence of an aluminum nanosphere at the resonance condition, while in Figure 4b), the field lines are not disturbed much by the nanoparticle and slightly beyond the particle the field lines are parallel again. By examining the range of convergence of the field lines in a), it can be observed how the absorption cross section of a nanosphere can be much larger than the geometric cross section. At resonance, for vanishingly small particles, there are both absorption and scattering of electromagnetic waves by the nanoparticle, with absorption being the dominant mechanism.^{20, 21}



Figure 2.3 Field lines of the Poynting vector (excluding that scattered) around an aluminum nanosphere a) where resonance occurs and b) not in resonance.²¹ Reproduced with permission.

2.4 Drude and Lorentz Models

The Drude model describes the response of the free conduction electrons in the presence of an applied field. The real and imaginary parts of the dielectric function are given and a term called the plasma frequency is introduced, this being the natural frequency at which the free electrons oscillate against the fixed positive core, the nuclei of the atoms. The Drude model accounts only for the electrons that undergo intraband

transition, meaning the electrons that stay within the conduction band. It is possible for the electrons to move to higher energy bands, and the Drude model can be modified to include an additional term that treats the electrons now as bound charges, called Lorentz oscillators, which accounts for the interband transitions that occur within the metal upon wave impingement. Often, interband transitions occur at higher frequencies, in which case the dielectric constant reduces to the Drude Model. When the interband transitions occur below or around the plasma frequency, in materials such as gold, the interband transitions interfere with the natural frequency of oscillation of the free electons, and need to be added to the optical response accordingly, as seen in equation 2.5 for the dielectric function where $\varepsilon_b(\omega)$ is the term for the interband transions and the second term accounts for the intraband transitions, where ε_{∞} accounts for the positive cores of the atoms, ω_p is the plasma frequency, and γ_0 is the damping term.²²

$$\varepsilon(\omega) = \varepsilon_b(\omega) - \varepsilon_{\infty} \frac{\omega_p^2}{\omega^2 + i\gamma_0 \omega}$$
 2.5

2.5 Mie Theory

In 1908, Gustav Mie, a German physics professor intrigued by the color of colloidal gold solutions, solved Maxwell's equations to determine the electromagnetic radiation scattered and absorbed by a small sphere. He assumed a linearly polarized plane wave incident on a small homogeneous sphere with a complex refractive index, with a size less than 200 nm in diameter.²³ In Mie's model, the particle is embedded in a nonabsorbing medium, in which there is no change in intensity of the wave (no attenuation) nor change in polarization upon interaction with the particle. Mie derived an equation for the extinction coefficient that included three terms, or multipoles: the electric dipole, the electric quadrupole, and the magnetic dipole. His calculations treated

light scattered by the particle as a series of partial waves corresponding to the magnetic and electric vibrations of the particles and estimated the amount of light absorbed by the spherical particle by subtracting the energy lost from the incident beam due to joule heating and scattering by the nanoparticle.^{24, 25}

It should be noted that Mie used the bulk optical constants of materials and applied them to nanoparticles, and his results were not too different from what is observed experimentally. Mie's calculations were valid for any material, however his work explained that the color of the colloidal gold solutions was due to the optical properties of materials. The main limitation of Mie theory is that it cannot be used for particle diameters much smaller than the wavelength of incident light (<20 nm) for which there is a size dependent dielectric constant and the bulk dielectric constant is no longer valid. Another limitation is that Mie theory assumes that the particles are isolated and noninteracting. Finally, Mie theory is not valid for ellipsoidal or irregularly shaped particles. ^{19, 26}

2.6 The Quasistatic Approximation

While Mie theory is valid for a large range of particle sizes, the solution for light scattering off a nanosphere can become simplified if an additional condition is imposed: The size of the nanoparticle must be much smaller than the wavelength of incident light. The condition implies that the applied field will uniformly polarize the particle, and calculations for the scattered electric field are performed according to the quasi-static approximation. The calculations for extinction, scattering, and absorption can be reduced to the dipole approximation since this dipole oscillation is the dominant contributor to the respective cross sections, as seen in Figure 5a.¹⁹ The dipole oscillation is considered the

first mode, as indicated by the summation index of the partial waves as L=1. L=0 is called the monopole term, and other modes are considered higher order modes such as the quadruple oscillation, L=2.²⁷ These higher order modes only need to be accounted for when the particle sizes become too large and the light cannot homogeneously polarize the particles. The near field effects of homogeneous and inhomogeneous polarizability can be seen in **Figure 2.4**.²⁸



Figure 2.4 a) Extinction spectra which shows the size dependence on modes and b-h) near field enhancement maps depending on the modes that are applicable.²⁸ Reproduced with permission.

In theory, the quasistatic approximation is applicable to particles less than 20 nm in diameter, but in practice the equations are still a good approximation for spherical and ellipsoidal particles as large as 100 nm in diameter with visible and near IR light irradiation ²⁰. The effect of increasing the size of the NP is a red shift in the LSPR and broadening of the absorption peak. The peak broadening, quantified by the bandwidth, is due to the incoherent electron oscillation. ¹⁹
On the other hand, the quasistatic approximation no longer holds for particles smaller than 5 nm in diameter where the charge density is greatly reduced, resulting in a weak and broad absorption due to finite size effects. Some theoretical work has been done to incorporate a size dependent damping constant in the dielectric function so that the dielectric function can be adapted to Mie theory in the 5-20 nm size regime. Myroshnychenko et al. derived an equation to incorporate finite size effects into the dielectric constant for gold nanoparticles less than 10 nm in diameter. ²⁸ For particles larger than 5 nm but smaller than 20 nm, the electron-surface scattering increases due to the particles' small dimensions and limited electron mobility. The mean free path is between 40 to 50 nm for gold, thus the electrons cannot collectively oscillate in phase, and are damped, which results in a larger bandwidth with decreasing size. These results indicate that Mie's use of the bulk dielectric constant is size dependent. ^{19, 28}

2.7 Gans Theory

Mie recognized that his solution of light scattering off a small sphere was not applicable to ellipsoidal particles. The geometry of a nanoparticle is one of the most critical factors in determining the frequency at which the localized surface plasmon resonance occurs. Gans theory is an extension of Mie theory that calculates the extinction, absorption, and scattering of light of an ellipsoidal nanoparticle, which still uses the dipole approximation. When one of the axes is elongated, the plasmon resonance absorption band splits into two peaks. The blue shifted peak, at higher energies (around 520 nm), corresponds to the electrons oscillating along the minor or short axis, and the red shifted peak, at lower energies, corresponds to the electrons oscillating along the major or long axis. These localized surface plasmon resonances are referred to as the transverse localized surface plasmon resonance (T-LSPR) and longitudinal localized surface plasmon resonance (L-LSPR), respectively.²⁹

Gold nanorods are a good example of nanoparticles for which Gans theory need to be applied. There are two trends that are of significance when examining absorption spectra of gold nanorods. As the aspect ratio (AR=length/width) of the nanorod is increased, the L-LSPR redshifts to lower energies. This is the effect of the geometry, since the depolarization factors along each axis contribute to the resonance condition. The other trend is that as the dielectric constant of the surrounding medium is increased, the L-LSPR redshifts to lower energies. It can be seen by looking at equation 2.6 that the resonance condition is met when the denominator of the summation portion of the absorption cross section approaches zero.³⁰ This equation will be revisited in Chapter 3.2.

$$C_{abs} = \frac{2\pi V \varepsilon_m^{3/2}}{3\lambda} \cdot \sum_i \frac{\left(\frac{\varepsilon''}{(n^i)^2}\right)}{\left(\varepsilon' + \frac{1-n^i}{n^i} \varepsilon_m\right)^2 + {\varepsilon''}^2}$$
2.6

Ultimately the red shifting of the peaks is due to the maximum material polarizability at the resonance condition. The frequency at which the L-LSPR occurs is important because it shows that gold nanorods are very sensitive to the change in dielectric medium, therefore they can be used in sensing applications. The other value of using gold nanorods is that the L-LSPR can be tuned just by changing the dimensions of the nanoparticle. This is the reason why they can be used in biological systems, where it is critical to work in the NIR region of the EM spectrum. The T-LSPR is weakly/not dependent on the AR of the nanorod and the dielectric constant of the surrounding medium, and has a weaker intensity than the L-LSPR.

Recall that the L-LSPR is due to electrons oscillating along the major axis of the rod. Since the absorption band for the L-LSPR has a higher intensity than the T-LSPR, there is a high electric field enhancement at the tips of nanorods, due to a phenomenon known as the lightning rod effect. This field enhancement leads to a localized temperature increase and needs to be investigated theoretically, to ensure that the wound does not exceed the temperature range of 33 to 42 °C, the ideal temperature for tissue repair. ³ Theoretical calculations will determine what size of particles should be used, the concentration, and the laser pulse to optimize conditions of the release of wound healing proteins into the body.

2.8 References

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3 Theoretical Modeling of Nanoparticles

3.1 Overview

To address the development of wound healing patches, our approach was to form fibrinogen-based protein coronas around AuNRs and have their release triggered by the temperature increase brought about by the local heating of the AuNRs after exposure to resonant laser light. AuNRs are well suited for this application because, due to their dimension (20 nm to 100 nm), they can evade phagocytosis and remain localized in the injured tissue for a period of time sufficient to produce a therapeutic effect. It has been shown that having at least one extended axis reduces the rate of phagocytosis, which is why AuNRs have a longer circulation times than gold nanospheres.¹ The surface charge of the NPs also influences the circulation time of the NPs. Neutral particles circulate longer in the body than particles that are charged, and this can be accomplished by coating the AuNRs with a hydrophilic polymer such as polyethylene glycol (PEG).²

The steps that the body takes to achieve hemostatis are the cleavage of fibrinogen by thrombin, followed by the formation of fibrin, a fibrous mesh that leads to a blood clot by impeding blood flow.

The following steps need to be accomplished in order to make a wound healing patch:

Two different aspect ratios of nanorods need to be synthesized, one for each wound healing agent to be added (fibrinogen and thrombin);

Protein coronas need to be formed by incubating the particles with each aspect ratio AuNR, respectively; The nanorods need to be incorporated in Matrigel, a synthetic protein gelatin mixture that is rich of extracellular matrix (ECM) components.

Once in Matrigel, the electrostatically bound fibrinogen can be selectively released following a localized temperature increase. To irradiate the nanorods, a laser source that has a wavelength in the NIR matching their plasmon resonance should be used. Commercially available continuous low power laser pointers include 808 nm, 850 nm, and 980 nm lasers with 200 mW, 150 mW, and 5 mW lasers, respectively. We purchased two low power (5 mW and 100 mW) 808 nm lasers for our work. The nanorods will absorb the energy from the laser and will heat up, causing the proteins to desorb from the nanoparticles and releasing the proteins used for blood coagulation. In a study by Bendix et al. gold nanoparticles trapped in a lipid bilayer were irradiated with a laser and temperature profiles were created.³ Depending on the size of the nanoparticles and the laser power, the surface temperature changed by a few to hundreds of degrees Celsius. A schematic of our patch can be seen in **Figure 3.1**.



Figure 3.1 Schematic showing the multilayered AuNR system. Each protein will be attached to a NR of a specific AR, and the mixture of AuNRs with their respective protein groups will be embedded in a biocompatible polymer, Matrigel.

3.2 COMSOL Multiphysics Introduction

It is evident that both the size and shape of the nanoparticle as well as the dielectric constant of the surrounding medium determine the wavelength at which the L-LSPR occurs. Since many layers are being added to the gold nanorod, producing a multidielectric problem, it would be difficult to calculate the L-LSPR wavelength. In order to optimize source pulse duration, nanorod aspect ratio, and ligand selection, and to simulate cellular environment parameters, to implement in the experiment and in the fabrication of the patch, it is important to rely on theoretical calculations. Due to the absorption phenomenon, it will be crucial to determine the temperature right around the nanorod as tissue repair needs to occur when the body surface temperature is between 33 and 42 °C.⁴ This system will be modeled using the RF module of the COMSOL Multiphysics software through an implementation of the electromagnetic theory, with Maxwell's equations and the derived wave equation in equation 3.1, where \vec{E} is the electric field, μ_r and ε_r are the relative permeability and permittivity, respectively, σ is the conductivity, and k_0 and ε_0 are the wave number and permittivity of free space, respectively.

$$\nabla \times \mu_r^{-1} \left(\nabla \times \vec{E} \right) - k_0^2 \left(\varepsilon_r - \frac{j\sigma}{\omega \varepsilon_0} \right) \vec{E} = 0$$
 3.1

In these simulations, nanorods with different aspect ratios are modeled in water. The dielectric constant of CTAB is not reported in literature. Some groups who have modeled nanorods use a value of permittivity for the surrounding medium higher than that of water to account for the CTAB in the system. This approach of fixing the permittivity of the surrounding medium to a single value is inaccurate since CTAB forms a bilayer on the nanoparticle and also dissolves into the surrounding medium, forming micelles at the Critical Micelle Concentration (CMC). Due to the complexity of the surfactant system alone, we model the bare nanorod in water, and compare the experimental results to theoretical results. COMSOL solves for the total field (i.e. absorption and scattering) resulting from the interaction of electromagnetic waves with the various media. Once the geometry is built, the optical properties are defined for each domain (i.e. material). The incident field is implemented, and the electric field components are defined, including selected polarizations for the impinging radiation. The power of the laser can be included in the parameters of the impinging EM field, and meshing, which determines the size of the individual sub-domains in which each domain is divided, is defined last, before computing.

For the first part of the simulation, the goal is to verify that the experimental data agree with the theoretical data. This can be confirmed by using the RF module and simulating a gold nanorod in water, since our synthesis method yields CTAB-stabilized gold nanorods in water. We can compare this with theoretical results for the absorption cross section, C_{abs} , in literature as well as simulated absorption spectra using COMSOL. COMSOL will calculate the absorption spectrum by computing the absorption by the gold nanorod at 1 nm intervals, by taking the integral of the heat losses by the gold over the entire volume and using an equation for the absorption efficiency, Q_{abs} . ⁵ This absorption can be compared to the experimental results that are obtained to confirm that the LSPRs occur at the same wavelength for a given aspect ratio by scanning electron microscopy (SEM) and theoretically by using the equation below for the absorption cross section, where n^i is the depolarization factor, and a, b, and c are the three axes of the nanoparticle, with a being the major axis and b and c as the minor axes, R is the aspect

ratio, V is the unit volume of the nanoparticle, and ε_m is the permittivity of the surrounding medium.⁶ The model can then be changed to incorporate the additional layers that were mentioned above. Based on the wavelengths of our lasers, the aspect ratios of the nanorods will be synthesized according to match the L-LSPRs to wavelength of the lasers, which will be extracted from the absorption spectrum.

Theoretical equations for absorption cross section of AuNR from literature are shown in equations $3.2-3.4^{6}$:

$$C_{abs} = \frac{2\pi V \varepsilon_m^{3/2}}{3\lambda} \cdot \sum_i \frac{\left(\frac{\varepsilon''}{(n^i)^2}\right)}{\left(\varepsilon' + \frac{1-n^i}{n^i} \varepsilon_m\right)^2 + {\varepsilon''}^2}$$
3.2

$$n^{a} = \frac{2}{R^{2} - 1} \left(\frac{R}{2\sqrt{R^{2} - 1}} ln \frac{R + \sqrt{R^{2} - 1}}{R + \sqrt{R^{2} - 1}} - 1 \right)$$
3.3

$$n^b = n^c = \frac{1 - n^a}{2} \tag{3.4}$$

Theoretical equation for absorption efficiency of AuNS from literature are shown in equations 3.5 and 3.6⁷:

$$Q_{abs} = 12x \frac{\varepsilon_m \varepsilon''}{(\varepsilon' + 2\varepsilon_m)^2 + {\varepsilon''}^2}$$
 3.5

$$x = \frac{2\pi nr}{\lambda}$$
 3.6

The absorption cross section can be related to the absorption efficiency by equation 3.7, where G is the geometrical cross section.⁷

$$C_{abs} = G \cdot Q_{abs} \tag{3.7}$$

To simplify the geometry, first we modeled a gold nanosphere (AuNS) in water. The results of the heat losses are plotted in Figure 7, and the absorption efficiency is calculated from equation 3.8, where r_2 is the radius of the AuNS, E_{inc} is the amplitude of the incident electric field, and the integral term is the heat losses over the entire volume of the particle.⁵

$$Q_{abs} = \frac{1}{\pi r_2^2} \frac{2}{\sqrt{\varepsilon_0/\mu_0} E_{inc}^2} \int (U_{av}) \, dV \qquad 3.8$$

In this project, we investigate how protein coronas can be formed around AuNRs with the final application of constructing a wound healing patch. The proteins are selectively released upon thermally heating the nanorods using lasers tuned to their L-LSPRs. As a consequence of the photothermal effect, the nanorods will heat up upon absorbing the incident light. This temperature increase will thermally release the proteins into the wound to promote the blood coagulation cascade. Our main aims are to theoretically model that nanoparticle system and determine the heat generated around the particles. The experimental results include synthesizing the particles, forming the protein corona, and releasing the proteins. The theoretical work will help to determine the optical and thermal properties of the system, so it will be discussed first.

3.3 Nanoparticles Used in Synthesis

Theoretical modeling is important to determine the wavelength of the lasers and study the temperature profiles generated by the incoming radiation. We need to model AuNRs (of different aspect ratios) with the various layers in a biological environment to study how they will respond to the laser light. We will be using commercially available lasers to study the release of the proteins, and for this reason we need to optimize the aspect ratios of the AuNRs depending on the available wavelengths. Once we alter the geometry accordingly to excite the L-LSPRs, we need to study the temperature around the AuNR, so that we do not exceed 42 °C, the maximum temperature for ideal wound

healing. In order to correctly model the nanoparticles properly in COMSOL, several particles were synthesized, which will be discussed further in the experimental section in Chapter 4. The dimensions are reported in **Table 3.1**. The bigger spheres and both types of rods are capped with the surfactant, CTAB.

Table 3.1 Size results for various particles that were synthesized. The particle size was characterized by TEM, where 100 particles were counted for the analysis. The experimental LSPR was obtained from UV-Vis with particles that were purified once for spheres and twice for rods and subsequently resuspended in water.

Particles	Ler	ıgth (nm)	Width or Diameter (nm)		
	Avg	Std dev	Avg	Std dev	
Citrate AuNS	NA	NA	15.6	1.3	
CTAB AuNS	NA	NA	24.3	5.8	
Short AuNR	40.5	6.4	20.5	2.6	
Long AuNR	53.2	5.1	15.3	1.6	

3.4 Modeling of Nanospheres

In the software COMSOL Multiphysics (CMP, RF Module), the particles were modeled according to the size results in **Table 3.1**. As lasers will be used for the final application, the electric field was implemented by introducing a laser source used as a Gaussian beam with laser powers of 5 mW and 100 mW using the equation 3.9 and 3.10, where S_{in} is the Poynting Vector, P is the power of the laser, r_{spot} is the laser spot size, E_0 is the electromagnetic field, and Z_0 is the impedance of free space:

$$S_{in} = \frac{P}{r_{spot}}$$
 3.9

$$E_0 = \sqrt{2S_{in}Z_0} \tag{3.10}$$

The wave is propagating in the y direction with the electric field along the x direction and magnetic field in the z direction. The laser spot size of 1.5 mm^2 was chosen such that the results would be comparable to a previously cited reference, where there

was a change in the composition of the BSA protein corona around nanorods.⁸ For the cited work, the temperature of the irradiated system was 35 °C and 39.3 °C after 27.5 and 55 min, respectively, showing that the temperature is well within safe temperatures of wound healing.

In our work, the particles were modeled as bare gold nanospheres without any capping agents, surrounded by water (n=1.33). The results in **Figure 3.2** show the theoretical cross sections of gold nanospheres with the dimensions of the citrate and CTAB spheres (given in **Table 3.1**) solved by CMP and the comparison of absorption efficiency of a gold nanosphere with 20 nm with that in literature.⁷ The study is computed for 1 nm intervals of the incident light wavelength and b) Theoretical results for absorption efficiency using the equation by Bohren and Huffman (blue) and CMP (red). Figure 7a shows that the cross sections are much greater for a larger gold nanosphere simply because it has a larger geometric cross section. In **Figure 3.2** it is apparent that the absorption cross section is greater than the scattering cross section as the absorption contribution dominates for small particles.



Figure 3.2 a) Theoretical results, solved by COMSOL Multiphysics (CMP) RF Module, for cross sections for 15.6 nm and 24.3 nm bare gold nanospheres in water irradiated with a 5 mW laser with laser spot size of 1.5 mm², and absorption efficiency for a light interacting with a 20 nm diameter bare gold nanoparticle in water.

a)

3.5 Modeling of Nanorods

To compare how the shape and size affects the heat losses of nanoparticles, rods were also modeled in CMP, with the geometry of the rods built as cylinders with hemispherical endcaps with the dimensions listed in Table 1. The modeling domain can be seen in **Figure 3.3**, which shows the domains and the meshing from the outside in: Perfectly Matched Layer (PML), surrounding medium (water), and nanorods. The PML and surrounding medium were modeled as spheres, and the cross sectional view is shown in Figure 3.3. Figure 3.4 shows the theoretical heat losses of the nanoparticles with a 5 mW and 100 mW laser, and demonstrates that the heat losses scale linearly with the laser power. The heat losses for the bigger sphere is greater than the small sphere, and the heat losses are greater for rods, with the longer rod having the highest heat losses. The explanation for the increase in the heat losses from small spheres to larger sphere and short rods to long rods, is due to the polarization of the electrons within the nanoparticle. In the presence of an electric field, the electrons get displaced from the neutral axis, in the opposite direction of the electric field. For rods, there are two maxima in the heat losses spectra, with the lower peak relatively constant at 520 nm, and the higher peak at lower energies, ranging from the visible region towards the IR. The electrons along the long axes of the rods experience a greater degree of polarization than the electrons along the short axes, hence why the heat losses and absorption are greater.



Figure 3.3 Model of short AuNRs (length: 40.46 nm, width: 20.51 nm), a) showing the geometry and meshing of all domains (Outer to inner domains: PML, surrounding medium, and AuNRs) and b) meshed area of AuNRs and the surrounding medium.



Figure 3.4 Theoretical heat losses for various nanoparticles irradiated by a) 5 mW laser and b) 100 mW laser calculated by CMP RF module based on experimental size for citrate spheres (15.63 nm diameter), CTAB spheres (24.28 nm diamter), short rods (length: 40.46 nm, width: 20.51 nm), and long rods (length: 53.15 nm, width: 15.3 nm) in water. The L-LSPR corresponds to 522 nm, 604 nm, and 759 nm for both types of spheres, short rods, and long rods, respectively. The laser spot size is 1.5 mm². The heat losses linearly scale with the laser power.

3.6 Gans Theory - Effect of Aspect Ratio and Surrounding Medium

As a comparison to literature, **Figure 3.5** plots simulated absorption spectra for gold nanorods based on Gans theory, which approximates particles as ellipsoids. Gans theory provides valuable information on trends that can be observed, such as changing aspect ratios of particles and changing the surrounding medium, but does not predict the

L-LSPR of particles accurately since it does not represent the actual shape/geometry of the synthesized particles. The result of changing the permittivity of the surrounding medium to a higher value is a redshift in the L-LSPR. As the aspect ratio of the AuNR increases, the L-LSPR redshifts and the absorption increases.



Figure 3.5 Simulated absorption spectra using Gans theory which approximates gold nanorods as ellipsoidal particles. The permittivity of the surrounding medium is fixed at a) 1.77, b) 2, and c) 2.71. Changing the permittivity of the surrounding medium to a higher value redshifts the L-LSPR. As the aspect ratio of the AuNR increases, the L-LSPR redshifts and the absorption increases.

Aspect	L-LSPR (nm)				
Ratio	n=1.33	n=1.415	n=1.645		
2	586	602	648		
2.3	616	635	693		
2.7	649	671	742		
3.1	683	710	790		
3.5	719	751	838		

Table 3.2 The L-LSPR for AuNR showing a redshift in the L-LSPR with increasing AR and increasing refractive index of the surrounding medium.

3.7 Comparison of Theoretical and Experimental Results

A summary and comparison of the theoretical and experimental results can be seen in **Table 3.3**. To determine only the effect of the shape on the L-LSPR, Gans Theory can be compared to CMP, as the surrounding medium was fixed at 1.33. Gans Theory correctly predicts the L-LSPR for isotropic particles, such as spheres, but underestimates the value of L-LSPR for nanorods. It is difficult to exactly compare the experimental results to CMP. For the long rods, CMP estimates the L-LSPR well (with a slight overestimation), while for short rods, it understimates it greatly. The discrepancy between the results is due to the presence of capping agent, surfactant and impurities in the system, as well as a size distribution of particles. The biggest discrepancy can be seen for the short AuNRs that show the experimental results are significantly red-shifted compared to the results predicted by CMP. Combined with the reasons for the descrepancy above, the particles that were modeled oversimplify the shape of the experimental AuNRs. The surfactant will be discussed further in the experimental section. Our group is currently modeling the exact morphology of AuNPs in COMSOL by using high resolution microscopy.

Table 3.3 Summary of the size for various particles that were synthesized. The particle size was characterized by TEM, where 100 particles were counted for the analysis. The experimental LSPR was obtained from UV-Vis with particles that were purified once for spheres and twice for rods and subsequently resuspended in water. The theoretical results were for bare gold nanoparticles in water (n=1.33). Gans theory LSPR was predicted using an equation outlined by Link, Mohamed, and El-Sayed^{9, 10}, and the CMP LSPR was from the maximum in the heat losses curve.

Particles	Length (nm)		Width or Diameter (nm)		Aspect	LSPR (nm)		
	Avg	Std dev	Avg	Std dev	Ratio	Experimental	Gans Theory	СМР
Citrate AuNS			15.6	1.31	1.00	521	522	522
CTAB AuNS			24.3	5.83	1.00	525	522	522
Short AuNR	40.46	6.41	20.5	2.62	1.97	648	586	603
Long AuNR	53.15	5.05	15.3	1.59	3.47	751	719	759

3.8 Temperature Calculation

3.8.1 Electric Field Confinement

The final step in the theoretical calculation is to determine the local temperature around the nanoparticle. The electric field distribution could give an idea of what the temperature profile might look like. Figure 3.6 shows the normalized electric field when the long and short axes of a nanorod are in resonance at 759 nm and 522 nm with values of 9.98 x 10^8 V/m and 8.29 x 10^6 V/m, respectively. This figure shows the electric field confinement to the tips of the nanorods with the electric field on the long axis being orders of magnitude higher than the short axis. Figure 3.7 shows the normalized electric field for all particles irradiated by 5 mW and 100 mW lasers. The normalized electric field is defined in equation 3.11, and the incident electric fields are 1584 V/m and 7087 V/m for 5 mW and 100 mW lasers, respectively. The higher laser power induces a higher normalized electric field, which is expected because there is a higher count of incident photons. The longer rods show the highest electric field, followed by the short rods, citrate spheres, and CTAB spheres. It is interesting that even though the geometric and absorption cross sections are greater for the bigger nanospheres (CTAB), the curvature of the smaller (citrate) particles are greater, which leads to the higher normalized electric field.

$$E_{normalized} = \sqrt{E_x^2 + E_y^2 + E_z^2}$$
 3.11



Figure 3.6 Normalized electric field for a long AuNR (length: 53.15 nm,width: 15.3 nm) in water irradiated with a 5 mW laser a) in resonance with the long axis at 759 nm showing a maximum E field norm at 9.98×10^8 V/m, and b) in resonance with the short axis at 520 nm showing a maximum E field norm of 8.29 x 10^6 V/m. The incident field has a magnitude of 1584 V/m. The wave is propagating in the y direction with the electric field along the x direction and magnetic field in the z direction. This gives a possible visual of the temperature distribution around the nanoparticle.



Figure 3.7 Normalized electric field (V/m) maxima for various nanoparticles irradiated by a) 5 mW and b) 100 mW lasers calculated by CMP based on experimental size for citrate spheres (15.63 nm) CTAB spheres (24.28 nm), short rods (length: 40.46 nm,width:20.51 nm), and long rods (length: 53.15 nm,width: 15.3 nm). The L-LSPR corresponds to 522 nm, 522 nm, 604 nm, and 759 nm for spheres, short rods, and long rods, respectively.

3.8.2 Absorption Cross Section and Heat Losses

To quantify the temperature around the nanoparticle, we can approximate the temperature around the nanoparticle using equations 3.12 and 3.13, where temperature can be calculated as a function of distance from the nanoparticle center, r, where k is the thermal conductivity of water, and T_{room} is the initial temperature, which was set to 298.15 K, and Q_{abs} is the heat losses within the particle ¹¹:

$$T(r) = \frac{Q_{abs}}{4\pi kr} + T_{room}$$
3.12

$$Q_{abs} = C_{abs} \cdot S_{in} \tag{3.13}$$

As the above temperature is only valid for spheres, the temperature was only calculated for spheres, at the surface of the nanoparticle, which is shown in the table below. As previously mentioned, the heat losses within the particle scale linearly with the laser power. As it is straightforward to calculate the heat losses generated within the particle using the maximum absorption cross section, it was calculated for a 100 mW laser with the 1.5 mm² spot size (which was used for the other theoretical calculations), a 1 W laser with the equivalent spot size, and finally a 100 mW laser with a smaller spot size that was used for the experimental work. In **Table 3.4**, it can be seen that the laser power and spot size determine the heat on the nanoparticle surface. The 100 mW does not prompt a major temperature increase around the NPs. Increasing the laser power by an order of magnitude only increases the temperature slightly by a few thousandths of degrees. The smaller spot size with an equivalent laser power increases the laser fluence, so the photons are concentrated in a small area. The smaller spot size laser is equivalent to the laser we purchased, and using these conditions, it does not show a drastic increase around the NPs, although changing the spot size does influence the temperature around

the NPs. Using the equation to calculate temperature does not calculate the temperature around the small (citrate) AuNS to be higher than the large (CTAB) AuNS. The equation seems to be an approximation, and does not take into account the electric field distribution around the nanoparticles and the size of the particles as r in the equation is the distance from the nanoparticle center. The temperatures were not calculated for the short and long AuNRs because the equation only applies to AuNS. Our group is currently working on correlating the heat losses in the RF module of CMP to local temperature around NPs.

Table 3.4 Theoretical results for absorption cross section and heat losses calculated by CMP, and temperature calculations using equation from literature, with a initial temperature of 298 K.

	Geometric Cross Section (m^2)	Absorption Cross Section (m^2)	Heat Losses 100 mW laser (W)	Temperature (K)			
Particles				100 mW (1.5 mm^2)	1 W (1.5 mm^2)	100 mW (0.002394 mm^2)	
Citrate AuNS	1.92×10 ⁻¹⁶	3.32×10 ⁻¹⁶	2.21×10 ⁻¹¹	298.1504	298.1537	298.3853	
CTAB AuNS	4.63×10 ⁻¹⁶	1.28×10 ⁻¹⁵	8.50×10 ⁻¹¹	298.1509	298.1593	298.7320	
Short AuNR	7.40×10 ⁻¹⁶	4.38×10 ⁻¹⁵	2.92×10 ⁻¹⁰	-	-	-	
Long AuNR	7.63×10 ⁻¹⁶	1.06×10 ⁻¹⁴	7.07×10 ⁻¹⁰	-	-	-	

3.9 References

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4 Plasmonic Nanoparticle Synthesis, Characterization, and Applications in Wound Healing

4.1 Synthesis of Gold Nanorods

We have synthesized AuNRs with various aspect ratios, for the 2 different proteins we will use for the patch. Gold nanorods (AuNRs) were synthesized using a seed mediated growth method developed by Nikoobakht and El Sayed.¹ In this method, gold seeds, acting as nucleation points for AuNRs, are added to a growth solution where additional gold atoms can bind. Rods are stabilized using the surfactant hexadecyltrimethylammonium bromide (CTAB), which preferentially binds to specific facets of the AuNPs and directs their growth.² Chlorauric acid, CTAB, and deionized (DI) water, are mixed, and form gold seeds upon addition of sodium borohydride. After aging the seeds, they are added to a growth solution that contains more chlorauric acid, CTAB, silver nitrate, DI water and ascorbic acid. The silver ions provided by the silver nitrate are believed to control the aspect ratio of the AuNR, and the ascorbic acid serves as a weak reducing agent of the gold salt. The NPs do grow in all directions, however due to the preferential binding of CTAB to {110} facets, the growth along the longitudinal direction is accelerated. This yields rods that have a diameter of about 20 nm (depending on seed aging time), along with other impurities such as gold nanospheres, cubes, and irregularly shaped particles. The rods are then purified by centrifugation, which will reduce some of the impurities. UV-Visible spectroscopy indicates that some impurities and a size distribution of rods still exist by looking at the shoulder of the T-LSPR and the width of the L-LSPR peak, which is dependent on aspect ratio of the nanorods. CTAB is toxic to cells and ligand exchange must be performed to remove the CTAB and replace it with another ligand, for example polyethylene glycol, PEG. The thickness and the relative permittivity of the attached ligand will shift the L-LSPR, so this will need to be taken into account when selecting and synthesizing the final rod aspect ratios.

Figure 4.1 shows an SEM micrograph of a batch of AuNRs and UV-Vis spectra showing our ability to tune the L-LSPR towards the NIR. Ultimately, the removal of the surfactant, CTAB is necessary due to its toxicity, but our work shows the proof of concept of using a capping agent with a charge and forming a protein corona with a protein of the opposite charge. CTAB capped particles measure a positive zeta potential, and fibrinogen, with a negative charge electrostatically binds to the particles. As thrombin has a positive surface charge, it would be ideal to cap the AuNR with a negatively charged surfactant, such as citrate, using a method by Mehtala et al. ³ Another option is to perform layer-by-layer polyelectrolyte deposition to adsorb negative charges on the CTAB layer, described by DeVetter et al. ⁴



Figure 4.1 a) Scanning electron micrograph of gold nanorods synthesized using the seed mediated method and b) experimental absorption spectra (normalized) for various 25 mL batches of AuNRs, purified 1x, size tuned by changing the volume of silver nitrate added indicated in the legend. c) Colloidal AuNRs arranged in order of increasing aspect ratio.

4.2 Influence of CTAB on LSPR

To demonstrate the role that CTAB has on the L-LSPR, **Figure 4.2** shows how the L-LSPR blueshifts upon each centrifugation step of 8000 *g* for 30 minutes. This is a consequence of the dielectric constant of the surrounding medium changing along with the removal of some larger particles. The latter is more unlikely in the first 2 centrifugation cycles as the larger particles have a higher likelihood of forming a pellet and there is still enough CTAB in the system to stabilize the particles. The last three rows in the table (higher aspect ratio rods), show much lower intensities for the L-LSPR (if any) as the purification cycles increase, indicating that too much CTAB was removed, thus destabilizing the particles. This is expected since the high aspect ratio rods generally

have a smaller width and longer length, which need more CTAB to cover the surface area. Centrifuging many times removes the CTAB bilayer, and the size results confirm the instability of the higher aspect ratio rods.



Figure 4.2 Experimental absorption spectra (normalized) for various 25 mL batches of AuNRs, purified 1x, 2x, and 3x, size tuned by changing the volume of silver nitrate added indicated in the legend. As the purification cycles increase, the particles destabilize and crash out of solution to due to the removal of the CTAB bilayer.

The numerical values for the LSPR are given in **Table 4.1**. These values show the blue shift in LSPR as the particles are purified. Although many references in literature provide equations to calculate or predict the aspect ratio based on the LSPR wavelength, they predict different values, and the equations themselves do not correlate well with experimental values due to the presence of the capping agent and dielectric constant of

the surrounding medium. The validity of this is shown by the discrepancy between the LSPR for CMP results for rods in water and the experimental results for rods with a capping agent in water with surfactant and other reagents present in the surrounding medium. These results also show the difficulty in purification and characterization of CTAB rods, as CTAB is toxic, but removal of too much of the surfactant destabilizes the particles. As the dielectric properties of CTAB are unknown, it was not possible to accurately model the nanoparticle capped with CTAB with and without CTAB in the surrounding medium.

Table 4.1 L-LSPR for various 25 mL batches of AuNRs with purification cycles. The size results are given for the particles purified 3x, which were obtained using SEM and measuring 100 particles using ImageJ.

Volume		L-LSPR (nm)	Size after Centrifuging 3x			
AgNO ₃ (mL)	Centrifuged	Centrifuged	Centrifuged	Length (nm)	Width (nm)	Aspect Ratio
0.15	682	674	653	34.01	16.14	2.11
0.3	742	729	706	40.49	13.91	2.91
0.45	784	783	763	48.84	15.09	3.24
0.6	796	760	764	39.77	14.49	2.75
0.75	840	825	540	33.19	11.66	2.85
0.9	831	831	797	42.77	14.04	3.05

4.3 Characterization of Particles

As it was mentioned in Chapter 3, spheres and rods were synthesized for the experiments, and below are the TEM micrographs for the particles that the size was given in Table from Chapter 3. All of the particles were stabilized using CTAB as the surfactant.



Figure 4.3 TEM micrographs for a) spheres, b) short rods, and c) long rods.

4.4 Formation of Protein Corona Using Fibrinogen

Ideally, long rods that have an L-LSPR around 800 nm, would be ideal for the experiments as the overall goal is to use a NIR laser to plasmonically release the proteins bound to the rods. To determine if the release of proteins was possible on different aspect ratio particles, spheres, short rods, and long rods were synthesized with the use of the surfactant, CTAB and subsequently purified. UV-Vis was taken on particles after the purification step, directly after the addition of fibrinogen and sonication, and then the particles were incubated for 8 hours at 37 °C, shown in Figure 4.4a). Figure 4.4b) shows the UV-Vis for spheres, short rods, and long rods with LSPRs 525 nm, 648 nm, 751 nm before incubation in fibrinogen, respectively, and redshifts in LSPRs to 538 nm, 677 nm, 759 nm, respectively. There was no additional redshift observed from directly adding the fibrinogen and after the incubation step. The fibrinogen solution was prepared by adding 0.9% saline solution to fibrinogen (10 mg/mL) and agitating the solution at 37 °C, which dissolves the fibrinogen as it does not dissolve in water alone. The control samples prepared were the same nanoparticles used for the fibrinogen incubation, except these samples were incubated in a 0.9% saline solution only. Figure 4.4a) shows that addition of the fibrinogen to nanoparticles causes an immediate redshift in LSPR, and the incubation period does not shift the LSPR. This demonstrates how quickly proteins

adsorb to the nanoparticles in the body, and shows that a long incubation time is unnecessary for protein corona formation. The same results were observed for rods, proving that the addition of saline solution does not cause a redshift in LSPR, as opposed to fibrinogen. Incubation of the samples at 37 °C does not further redshift the LSPR but does broaden the peak. The DLS results in **Figure 4.5**a) show the intensity results for rods (estimated as spheres), and their relative size difference after incubating in fibrinogen. These results combined with the SEM micrograph in **Figure 4.5**b) shows that the fibrinogen caps individual results as well as forming clusters of rods with fibrinogen. The very large sizes could either be a result of clusters of rods or fibrinogen aggregation (which cannot be seen in SEM).



Figure 4.4 a) CTAB spheres before and after addition of fibrinogen solution. The addition of fibrinogen causes an immediate redshift in the plasmon resonance. The incubation period does not further shift the LSPR but does broaden the peak. b) UV-Vis for CTAB spheres, short rods, and long rods before and after fibrinogen incubation.





Figure 4.5 a) DLS for rods in 0.9% NaCl solution and 0.9% NaCl solution and fibrinogen and b) SEM for rods incubated in fibrinogen which shows the clustering of rods upon addition of fibrinogen. It can be observed that, for the two clusters represented in this micrograph, the diameter can be estimated to be between 200 and 300 nm, in agreement with the DLS data.

The adsorption of fibrinogen to the nanoparticles' surface was confirmed by zeta potential in **Table 4.2**. The CTAB stabilized particles initially had a positive charge. The controls used in this experiment were the same nanoparticles incubated in 0.9% NaCl (saline) solution, and all particles with fibrinogen have a lower charge. Fibrinogen has a negative charge, which is why some of the particles have a negative charge upon fibrinogen adsorption, while some have a positive charge, which indicates the incomplete coverage of fibrinogen on the nanoparticles' surface. This could be due to the side on configuration of fibrinogen, and wrapping of fibrinogen around the nanoparticle as a consequence of the smaller dimensions for width of the rods (15.3 nm) compared to the short rods (20.51 nm) and the diameter of the CTAB spheres (24.28 nm), a mechanism described in Chapter 1.

Sample	T (°C)	ZP (mV)	Mob (µm cm/Vs)	Cond (mS/cm)
CTAB AuNS with NaCl	25	41.4	3.247	0.526
CTAB AuNS with fibrinogen	25	0.517	0.04053	0.672
Short AuNR with NaCl	25.1	38.1	2.988	0.542
Short AuNR with fibrinogen	24.9	-6.74	-0.5281	0.742
Long AuNR with NaCl	24.9	50	3.92	0.609
Long AuNR with fibrinogen	25	18.7	1.466	0.668

Table 4.2 Zeta potential for AuNPs capped with CTAB incubated in normal saline solution (0.9% NaCl) or fibrinogen (10 mg/mL in normal saline solution).

4.5 Laser Release Study

To determine whether a laser release was possible, the first experiment was to synthesize AuNRs, incubate them in fibrinogen and saline solution (control), and irradiate them with a low power laser for different times. **Figure 4.6** shows the power of two handheld low power lasers (5 and 100 mW) tested with a power meter. Due to the fast decay of the lasers, a stable high power laser with an attenuator was used to lower the laser power to 5 mW, which was monitored using a power meter. The laser irradiated three samples in quartz cuvettes. Irradiation times of 1 and 5 minutes were chosen, and the particles were then characterized with UV-Vis. There was no major difference in the UV-Vis spectra for any of the samples for either time, indicating that no damage to the particles occurred as a consequence to irradiation. Furthermore, UV-Vis may not be able to provide a direct confirmation that proteins were released from the corona as this may not cause a distinctive change in the number of proteins bound and thus to the overall dielectric function of the nanostructured system.



Figure 4.6 Power of 808 nm a) 5 mW and b) 100 mW lasers. The power decays over a period of time.

We therefore decided to probe alterations in the protein corona employing NanoSight LM 10. This instrument, sold by Malvern, is equipped with various lasers, and allows to carry out nanoparticle tracking analysis, by visualizing and analyzing their Brownian motions. The equation used to calculate the size of the particle is the Stokes-Einstein equation, thus assuming that all particles are spherical in shape. The available lasers are 405 nm (violet), 488 nm (blue), 532nm (green) or 642 nm (red). Small volumes of dilute samples are injected into the NanoSight LM with a syringe, and the particles flow through the system, while the scattering signal is detected. To determine whether a laser could trigger the plasmonic release of the fibrinogen bound to the nanoparticles, the 532 nm laser was used with the CTAB nanospheres incubated in fibringen. Even though the particles still maintained their plasmon resonance, the particles had agglomerated and overwhelmed the detector with its scattering signal. As an alternative, the long aspect ratio nanorods were used for the remainder of this experiment. The nanorods without fibrinogen were tested followed by the particles incubated in fibrinogen. Although the software calculates the particles' hydrodynamic

diameters using the Stokes-Einstein equation, a relative size difference can be observed between the different samples.

In the **Figure 4.7**a), the size increase is apparent between the particles without fibrinogen and with fibrinogen at room temperature, which are 39 nm and 43 nm respectively. The particles with fibrinogen were then exposed to a 100 mW handheld 808 nm laser for 3 minutes to determine if this would trigger the release of the fibrinogen. The 100 mW laser was chosen for this experiment as there was no significant plasmonic difference after irradiating particles with a 5 mW laser in the previous study. The inability to characterize the data during the irradiation process is not ideal, as results from literature show that protein corona formation is instantaneous, and there is a constant adsorption and desorption of proteins to and from the nanoparticles. Nevertheless, the particles were irradiated with the 100 mW laser, and the system does indeed show a difference in size shown in **Figure 4.7**b). After laser irradiation, the highest intensity peak at 43 nm decreases to 39 nm, which is the original size of AuNR without fibrinogen. The decrease in size indicates that the laser irradiation frees individual AuNR from their protein coronas.

To determine what type of configurations of AuNR and fibrinogen were formed upon laser irradiation, the UV-Vis spectra were collected after different irradiation times of 3, 6, and 9 minutes, as shown in **Figure 4.7**c). The raw data did not show a decreased absorbance nor a shift in the plasmon resonance, which indicates that the nanorods maintained their morphology. As it was seen in the SEM micrograph in **Figure 4.5**b), fibrinogen has a tendency to group nanoparticles together in clusters. Two phenomena may be occurring that can explain the data observed: 1) The released fibrinogen molecules may be clustering, forming protein-only aggregates, while 2) the particles already clustered in protein-nanoparticle assemblies retained enough relative separation to avoid inducing shifts in the LSPR. The larger agglomerates can be explained by looking at literature. Kirichenko et al. have studied fibrinogen at room temperature via DLS and have reported 2 size peaks for fibrinogen in solution at 13 nm and 200-300 nm, the latter being attributed to aggregates, which accounts for the larger agglomerates observed in **Figure 4.7**.⁵

Finally, since the application of the patch exposes the particles to body temperature, the particles were heated within the NanoSight LM to 37 °C and 50 °C, the temperature just below the denaturation temperature of fibrinogen, and simultaneously measured shown in **Figure 4.7**d). The higher temperature, 50 °C, produces smaller agglomerates which is unexpected as higher temperatures typically produces unstable particles which aggregate together. A possible explanation for these smaller agglomerates at higher temperatures is that the faster kinetics, which free proteins from the AuNR, forms small agglomerates due to mobility of the fibrinogen while at a lower temperature, the kinetics are slower, and the fibrinogen released from the AuNR bind to already existing protein coronas.

Further tests will be needed to confirm and quantify the release of fibrinogen. This test reveals, however, that temperature has a major influence on the nanoparticle-fibrinogen system and that it has to be accounted for - even exposing the nanoparticles to body temperature, 37 °C causes a size difference in the system. Upon introducing nanoparticles to the body, there is a burst in the release of fibrinogen in the system. One method that could be used to avoid this burst release is by selecting a different ligand,

instead of CTAB, to bind to the AuNRs that would promote a stronger electrostatic interaction with the fibrinogen. The nanorods used in this experiment had an L-LSPR around 759 nm, and the laser still prompted a change in the system, despite the particles being somewhat off resonance.

Ultimately, these tests show that using a handheld low power laser in the mW regime had sufficient power to trigger aggregation or agglomeration, and may be sufficient to yield a plasmon-based release of fibrinogen. A deeper understanding of the fibrinogen configuration and temperature studies of free fibrinogen and fibrinogen bound to AuNRs would be helpful to determine if the release of fibrinogen is sufficient and controllable for this application.



Figure 4.7 Nanoparticle Tracking Analysis size results showing size of AuNRs in various conditions calculated using the Stokes-Einstein equation. a) AuNR with and without fibrinogen, b) fibrinogen AuNR before and after laser irradiation, c) UV-Vis for AuNR incubated in fibrinogen before and after laser irradiation with a 100 mW laser, and d) fibrinogen AuNR at 20 °C, 37 °C, and 50 °C.

4.6 Experimental Determination of Local Temperature

around Nanoparticles

As it is possible to determine the temperature at the surface of spherical nanoparticles (and at distances away from the surface), we wanted to determine the local temperature around nanorods using surface enhanced Raman spectroscopy (SERS). It is known that the local temperature of a Raman active molecule can be determined using the Anti-Stokes and Stokes intensities of the same Raman mode by the equation 4.1, where A is the asymmetry factor, ω_L and ω_v are the frequencies of the laser and Raman wavenumber (for a specific mode), \hbar is Planck's constant, k_B is Boltzmann's constant,

 T_{Si} is the temperature of the silicon wafer (reference sample), and ΔT_{mode} is the temperature rise above the substrate the samples were on.⁶

$$\rho = \frac{I_{AS}}{I_S} = A \frac{(\omega_L + \omega_v)^4}{(\omega_L - \omega_v)^4} \exp\left(-\hbar \frac{\omega_v}{k_B (T_{Si} + \Delta T_{mode})}\right)$$

$$4.1$$

The asymmetry term arises because the Stokes cross section of a vibration and the Anti-Stokes cross section of vibration are not equidistant from the Rayleigh scattering line. In the referenced work, Rhodamine 6G was bound to silver nanospheres on a silicon substrate. A thermal stage was used to control the temperature of the sample. All of the values are known except for the asymmetry factor and the rise in the temperature of the sample (local temperature of the molecule), which need to be experimentally determined. When A is fixed, the temperature can be solved for. In the referenced work, the theoretical values for ρ are calculated by setting A=1 and ΔT_{mode} . Generally this does not correspond well to the experimental results, and a combination of A and ΔT_{mode} give the best fit to the data, using A values between 0.85 and 1.35 and ΔT_{mode} equal to 25 K for low power (0.5 mW), and A values between 0.4 and 1.35 and ΔT_{mode} equal to 96 K for high power (5 mW).

In this work, a Raman active molecule, 4-aminothiophenol (ATP), was bound to gold nanoparticles. This molecule is about 1 nm in length, and when covalently bound to the nanoparticles, it can be used as an effective Raman reporter to probe the SERS effect.

4.6.1 Synthesis of Particles and Ligand Exchange:

Gold nanorods were made using the seed mediated method by Nikoobakht and El-Sayed, and CTAB spheres were synthesized using a method by Huang et al.^{1,7} Following synthesis, the particles were purified several times to remove excess CTAB.
For each of the particles, 1 mL of particles were taken and incubated in 2 μ L of 1.625 M ATP prepared in ethanol. Particles were left on a shaker for 2 hours to facilitate ligand exchange of ATP and CTAB. The particles were centrifuged at 8500 g for 10 mins and the pellets were extracted. The particles were drop casted on glass slides cleaned with acetone and ethanol. The samples were dried completely before testing.

4.6.2 Experimental Conditions:

Raman experiments were performed using a Renishaw InVia Raman microscope with a notch filter equipped with a 633 nm laser, whose power is 10 mW at the source. Samples were placed on a thermal stage, which allowed for fine temperature control of the substrates between 25 and 100 °C with 25 °C intervals. A 50x long working distance objective was used, and the system was calibrated using a silicon wafer. Experiments were conducted on short nanorods, with 3 conditions shown in **Table 4.3**.

Table 4.3 Conditions for running SERS experiments on ATP bound to short nanorods on
a glass slide. There were 20 accumulations taken for each of the experiments, and 3
locations for each condition.

Condition	Laser Power (%)	Exposure Time (seconds)
1	10	10
2	10	60
3	50	10

Three locations were chosen for collection of Stokes and Anti-Stokes spectra, respectively. Static scans were taken centered at 1250 cm⁻¹ and -1250 cm⁻¹ wavenumbers, and the Raman mode chosen for the analysis was at 1079 cm⁻¹. With this set of experiments, one sample was used for several temperatures: 25, 50, 75, and 100 °C, with the final temperature of 25 °C, and a second samples was used to collect additional spectra at 75 °C for comparison to the first sample.

4.6.3 SERS Results

The spectral peaks in **Figure 4.8**a) are comparable to what is found in literature for ATP bound to Au films or AuNRs, and it can be seen in **Figure 4.8**b) that there is a large degree of electric field enhancement around the nanoparticle tips and in a hot spot between nanorods. ^{8,9} We decided to focus on the Raman peak at 1079 cm⁻¹, assignable to the a_1 mode C-S stretching vibration, from in-plane modes of the benzene ring. The intensity of the mode depends on the orientation (whether it is vertical or tilted) of the ATP molecule on the gold surface. ⁸⁻¹¹



Figure 4.8 a)Experimental Stokes intensities for short rods with ATP at 25°C for 3 locations. The maxima of the vibrational peak of interest varies in intensity demonstrating that orientation of the Raman molecule in/out of plane affects the scattered intensity collected by the detector. b) Theoretical calculations from literature showing the normalized electric field intensity at the tips of rods or in a hot spot, which would result in enhanced signals Raman signal if a molecule was located in these positions.¹⁰ Reproduced with permission.

The results in Figure 4.9 show that Condition 1 consistently has the lowest Stokes

and Anti-Stokes intensities while Conditions 2 and 3 yield higher Stokes and Anti-Stokes

intensities, consistent with the pumping conditions. The intensities (photon count) represent the populations of the electrons at ground and excited states Since Anti-Stokes scattering requires electrons to have a higher energy than ground state, it can be observed that increasing the temperature increases the number of electrons in the excited states, hence producing higher Anti-Stokes peak intensities with increasing temperature. Having taken into consideration that most scattering events are in form of Rayleigh (or elastic) scattering, Stokes scattering is generally more common as electrons are excited from the ground state, and the scattered photon is emitted at lower energy compared to the impinging one. Anti-Stokes scattering results from electrons from a higher energy state to an excited state, and back to the ground state, yielding a scattered photon with a higher energy. The populations of electrons can be described by Maxwell-Boltzmann statistics, ¹² which states that energy levels in solids can be distinguished and depend on the overall energy of the system. Maxwell-Boltzmann statistics relates the electron population in a microstate to the equilibrium temperature of the system. This suggests that at higher temperatures there will be a higher electron population at excited states.

Since there is always a higher population of electrons at the ground state, the Anti-Stokes intensities will always be significantly lower than the corresponding Stokes intensities. However, as the temperature of the system increases (in our case provided by the thermal stage), the Stokes intensities did not vary greatly, while the Anti-Stokes intensities increased, as predicted. This effect is due to the fact that, at higher temperature, higher energy levels become more populated than they were in the ground state. The second sample, whose temperature was fixed at 75 °C showed the same trend for Anti-Stokes bands, albeit of lower intensities, which may be due the sample being less concentrated. A curious find is that upon returning the thermal stage to 25 °C, both Stokes and Anti-Stokes scattering increased from the initial intensities at 25 °C. Even though they were brought back to room temperature over a period of 30 minutes or more (thus allowing sufficient time for thermal equilibration), the samples still retained high electron populations at the ground state and excited states. This could be due to the elevated temperature promoting binding of ATP to the NPs.



Figure 4.9 SERS Results for short rods with ATP at different temperatures controlled with a thermal stage a) Stokes intensities and b) Anti-Stokes intensities. Condition 1: Laser Power: 10%, Exposure Time: 10 seconds, Condition 2: Laser Power: 10%, Exposure Time: 60 seconds, Condition 3: Laser Power: 50%, Exposure Time: 10 seconds. The bar graph shows the average intensities with the standard deviation.

Figure 4.10 shows the Stokes and Anti-Stokes with the set values of the thermal stage. For the conditions of low laser power and low exposure time, the Anti-Stokes intensities show a steadily increasing trend as the temperature of the thermal stage is increased. The Stokes intensities fluctuate slightly over the temperature range, with relatively low standard deviations, thus these values could be used as a calibration curve to determine the temperature for the other conditions.



Figure 4.10 SERS Results for short rods with ATP at different temperatures controlled with a thermal stage Stokes intensities and Anti-Stokes intensities.

The local temperature was calculated in **Figure 4.11** according to equation 4.1, with a value for A equal to 0.574, so that the first Anti-Stokes /Stokes ratio is equivalent to the first temperature set on the thermal stage (25 °C). Although the temperature is not the exact value, we can use this value as a reference point for comparing the temperature

increase between the low and the higher power conditions for the rods. Further, we determined that the theoretical temperature increase for citrate spheres irradiated by a 100 mW laser is 0.000375 K, and the laser power being used for this experiment is less than 1 mW. When the fit is done for the low power condition, we can see deviations from the fit (which was calculated using equation 4.1 using A=0.574). The original point at 25 °C may not be correct which has an effect on the rest of the temperature values calculated in the fitted curve. There are 2 possible deviations from the fitted curve, and this is due to the range in intensities on both the Stokes and Anti-Stokes intensities due to:

1) Orientation of the Raman molecule on the gold nanoparticle in/out of plane which affects the scattered intensity collected by the detector.

 Position of the Raman active molecule at the tips of rods or in a hot spot (junction between 2 or more nanoparticles) due to nanoparticle clustering, which shows a severely enhanced signal.

When comparing the low power experimental data to the fit, two points match up relatively well on the line, which means that either those calculated temperatures are correct, and the other two points and 50 °C and 100 °C have increased Raman scattering due to the second reason given above. The other explanation is that the first and third points are incorrect, and that in the area of the sample interacting with the laser, the ATP is oriented in a direction that scatters less photons to the detector.



Figure 4.11 Anti-Stokes/Stokes ratios for short rods with ATP with laser power 10% exposure time 10 seconds. a)The green values are the experimental data points for the ratios with the x axis being the set values of the thermal stage. The red and blue curves are the calculated temperature values based on the values selected for A, the asymmetry factor. The graph demonstrate how much of an effect A, plays a role on the calculated temperature of the molecule. b) The experimental data points are in red, and the fitted line is in blue, which was calculated by setting A=0.574, making the first data point in the series start at the first value set for the thermal stage.

Now that there is a fitted curve, the Anti-Stokes/Stokes ratios for the higher power

laser can be plotted to determine if there is a major temperature increase from increasing

the laser power to 50%. Figure 4.12 shows the fitted line with the experimental values.

Similarly to the low power experiment, there are a few deviations from the fitted line.



Figure 4.12 Anti-Stokes/Stokes ratios for short rods with ATP with laser power 50% exposure time 10 seconds. The green points are the experimental data points for the ratios with the x axis being the set values of the thermal stage. The fitted line is in blue, which was calculated by setting A=0.574 for the laser power 10% exposure time 10 seconds condition. In Tables x and y the Anti-Stokes/Stokes ratios are given for the fitted lines and the low and high power laser conditions as well as the calculated temperatures based on the fitted line, respectively.

Table 4.4 Anti-Stokes/Stokes	ratios for fi	tted line, l	low laser p	power, and	high lase	er power
conditions.						

	Thermal Stage values			
Condition	T=25 °C	Т=50 °С	T=75 °C	Т=100 °С
Fit to LP 10 ET 10	0.005476	0.008174	0.011540	0.015556
LP 10 ET 10	0.005476	0.012216	0.012102	0.018622
LP 10 ET 50	0.005681	0.007012	0.011988	0.017707

	Thermal Stage values			
Condition	T=25 °C	Т=50 °С	T=75 °C	Т=100 °С
Fit to LP 10 ET 10	25	50	75	100
LP 10 ET 10	25.0	79.4	78.7	116.8
LP 10 ET 50	27.1	39.9	77.9	112.0

Table 4.5 Calculated temperature values for low laser power and high laser power conditions by using the Anti-Stokes/Stokes ratios and comparing them to the fitted line. The temperatures for a few of the data points for each condition are within a few degrees of the fitted line.

As a follow up to the above experiments, additional experiments were conducted in an effort to determine an *absolute* temperature around the nanoparticles. The goal was to use a symmetric particle, such as spheres, so that the electric field is uniform around the particle, and could be used as a reference to calculate temperature of the molecules around nanorods. In the case of spheres, if the particles were well distributed on the substrate, this would eliminate differences in Raman intensities due to particle aggregation (Raman active molecules in the junction between two nanoparticles) and from the Raman active molecule experiencing increased intensity due to the lightning rod effect. There would still be differences in intensity due to the orientation of the Raman active molecule (in and out of plane), however collecting data from many locations averages out the relative intensities taking into account all the possible molecular orientations.

CTAB spheres, short rods, and long rods (for comparison) were all incubated in ATP, using the method described for short rods, and the LSPR before after incubation can be seen in **Figure 4.13**. The LSPRs are 524 nm, 653 nm, 748 nm for as synthesized particles and 523 nm, 652 nm, 752 nm, for ATP incubated particles, respectively. Ultimately it was determined that spheres would not make for a good reference point, nor

would long rods provide any other data about temperature surrounding the nanorods because the particles are off resonance with the 633 nm laser. In **Figure 4.14** and **Figure 4.15**, it is apparent that spheres and long rods do not follow the same trend as the short rods where the Anti-Stokes intensities increase with temperature while the Stokes intensities remain relatively constant. A better comparison would be to perform the same experiments on the spheres with a 532 nm laser, a laser that is near resonance of the particles. A comparison could be made between the short rods data and the long rods if a 785 nm laser was used for the long rods.



Figure 4.13 UV-Vis for particles before and after incubation in ATP.



Figure 4.14 Stokes intensities for particles with thermal stage set to 25 °C a) short rods, b) long rods, and c) spheres. Anti-Stokes intensities vs. wavenumbers for particles with thermal stage set to 25 °C d) short rods, e) long rods, and f) spheres. The three lines in each graph represent 3 locations on the same sample.



Figure 4.15 a) Average Stokes and Anti-Stokes Intensities and b) Anti-Stokes/Stokes ratios for various particles using the 633 nm laser at 3 locations per sample using 50% laser power, 10 second exposure times, and 20 acquisitions.

4.7 Conclusions

AuNPs (AuNS and AuNR) were synthesized using CTAB as the surfactant/capping agent. The purification and subsequent characterization with UV-Vis shows the role of the surfactant on the L-LSPR wavelength, with each purification step blueshifting the L-

LSPR. These results show the discrepancy between modeling bare AuNPs in CMP and the experimental L-LSPR. Upon incubating AuNPs in a fibrinogen solution, there is a slight shift in the plasmon resonance, and the protein corona formation is confirmed by zeta potential. Determining absolute temperatures around nanorods can be possible using SERS, by binding a Raman active molecule to AuNRs which is short enough to experience the electric field confined to the nanorod surface. ATP bound to short AuNRs and irradiated with a 633 nm laser (close resonance to the LSPR of the rods) at two different powers (10% and 50%) allows for a relative determination of the local temperature around the AuNRs by measuring the Stokes and Anti-Stokes intensities of ATP, whose Raman signal is amplified by the increased absorption and scattering of AuNRs.

The 10% laser power Anti-Stokes/Stokes ratios were used as a calibration curve assuming that the starting temperature around the nanorods was equal to room temperature, which determined an asymmetric value of 0.57 for the AuNRs that were studied. For 50% laser power, the same A value was used as before, and the starting temperature was higher, which is what was expected as the number of incident photons is higher. The fitted lines (calculating the Anti-Stokes/Stokes ratios) were calculated based on the fixed A value with a range of temperatures values from 25 to 100 °C. The discrepancy between the fitted line and the experimental values were most likely due to clustering of AuNPs which placed the Raman active molecule in the junction between two or more particles, which increased the Raman scattering or because the ATP was oriented on the AuNRs such that the detector did not collect the scattered photons. The former results in Anti-Stokes/Stokes ratios above the fitted line and the latter results in lower Anti-Stokes/Stokes ratios, and explain why some of the data points fell below the fitted line.

Finally, an absolute temperature around nanoparticles can most likely be determined by binding a Raman active molecule to a spherical particles, such as AuNS, and carrying out the same experiments to determine what the temperature is around the particles with the AuNS in resonance with the Raman laser (533 nm). Spheres are recommended to create a calibration curve because the electric field distribution is well known and the pattern is symmetric. Then the Anti-Stokes/Stokes data points for rods can be fit to the calibration curve to the spheres, to determine the local temperature of the nanorods.

4.8 Future work

We have demonstrated that the nanoparticle/fibrinogen system experiences a size change in response to laser irradiation. There needs to be more research to determine if and how the fibrinogen is being released from the AuNRs. As the final application of this work is to make a wound healing patch, the same experimental work in this chapter needs to be carried out with thrombin, as it is required to cleave fibrinogen to form fibrin. The next step would be to perform ligand exchange on different aspect ratio AuNRs to cap them with a negatively charged particle in order to electrostatically bind thrombin to the particles. Once this is complete the two aspect ratio AuNRs with their layers will be embedded into the biocompatible matrix, Matrigel. We will then test the patch *in vitro* to ensure that the theoretical work we have completed is valid. We will determine whether the laser irradiation can release the wound healing proteins in a timely manner and initiate the formation of fibrin into the ECM matrix of Matrigel.

4.9 References

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5 Polymer Nanocomposites

5.1 Introduction

Polymer nanocomposites are excellent alternatives to traditional polymer composites since well-dispersed nanofillers can produce enhanced physical properties with loading amounts of less than 5 vol% compared to 60 vol% with micron sized fillers. ¹ Due to the large surface area of the nanofiller, almost the entire volume of the polymer matrix is affected by the addition of the filler simply because of the vast number of interfaces present. Nanofillers have the benefit of preventing material failure due to cracking, and providing enhanced toughness and ductility, since the particles are significantly smaller than typical critical crack sizes.

Nanocomposites with a polymer matrix reinforced with nanoparticles, have the potential application for hard, tough, and strong materials that can endure large strains. A few toughening mechanisms nanoparticles provide in polymers are crack pinning, crack deflection, immobilized polymer, plastic void growth. Immobilized polymer refers to the surrounding polymer in close proximity to the nanoparticles being constricted. Due to the small interparticle distances between nanoparticles, even at low loading of NPs, a large volume of the matrix is affected by the NPs. Plastic void growth is a toughening mechanism that occurs due to debonding of the matrix from the nanoparticles. Debonding reduces the constraint at the crack tip, which allows the nanocomposite to deform plastically, absorbing some fracture energy.²

The main goal of our research on polymer nanocomposites was the design and fabrication of puncture resistant surgical gloves. We used a dip molding method to

produce polyisoprene (PI) films approximately 0.5 mm thick, with nanoparticles dispersed within the matrix. Along with nitrile and vinyl, PI is a common material used in surgical gloves.³ PI was chosen due to its low modulus at low strains, which corresponds to a high comfort level for gloves. Carbon based nanoparticles as well as gold nanoparticles were incorporated into a matured PI, and then tested for their puncture resistance. The films had to meet a tensile testing standard, which required the polymer to undergo 500% strain without failure.

5.1.1 Carbon Based Materials

Hardness is an important material property for this type of application because it gives an indication of how much the material will resist indentation. A nanofiller that exhibits high hardness is graphene. Graphene is known for its Young's modulus of approximately 1 TPa (higher than that of diamond) and a theoretical strength of 130 GPa. ⁴ Single-layer graphene has shown to produce the best results so far, however it is difficult to synthesize compared to multi-layer graphene and it is quite expensive. An excellent alternative to graphene is reduced graphene oxide (rGO), a cheaper and more readily available material. Carbon nanotubes (CNTs) are another form of graphene that has been studied to enhance polymer matrices. The alignment, chirality, diameter, and length of the CNTs can significantly change the material properties but they are nonetheless the strongest material available. A low loading of graphene (around 1 wt%) has been shown to increase the Young's modulus of poly(methyl methacrylate) (PMMA) by 80% and its ultimate tensile strength by 20%.⁴ Even loading 0.1 wt% of SWCNTs, MWCNTs, and graphene platelets have enhanced the mechanical properties of epoxies, with the graphene platelets showing the best results in terms of Young's modulus,

ultimate tensile strength, fracture toughness, fracture energy, and resistance to fatigue crack propagation.⁵

GO micron sized flakes were purchased from graphene supermarket, however crushing using a mortar and pestle followed by sonication did not stabilize the particles well enough in water, as was described by literature. The flakes clumped up, and the samples were unusable. Polymer films consisting of PI with a fixed concentration of gold (7.26×10^{-6} wt% Au) and 0.0033, 0.0066, and 0.01 wt% CNTs were successful. The hybrid films were characterized as described below.

5.1.2 Gold Nanoparticles

Gold is a popular material with increased strength and hardness compared to PI that is easily employed to synthesize NPs. It has been reported that adding AuNS up to 5 nm in size in waterborne polyurethane improves the thermomechanical properties of the polymer matrix.⁶ The proposed mechanism for improved properties could be restriction of polymer chain movement along with gold acting as a nucleation site for enhanced crystallization of the polymer. We will be using larger diameter AuNS as well as a polymer coating to increase the stability in the matrix. This method of adding AuNS directly to a polymer, versus forming the AuNS in situ, is not very common, because it often results in forming gold aggregates, but we have proven steric stabilization of AuNS with the polymer coating, PEG, by successful incorporation into the PI at low loading. In general, gold is also ideal as synthetic procedures to produce NPs with it are widely available with the added benefit of long term stability and extended shelf life. Our group has vast expertise in the synthesis and blending of gold NPs with polymer films of less than 100 nm in thickness and we can produce gold NPs cheaply and with high reproducibility. Some other advantages of using AuNPs are their vast arrays of morphology and sizes (spheres, rods, cubes, pyramids, stars), their biocompatibility, and their ease of functionalization.

Thus far, we have synthesized 16 nm diameter AuNS using the Frens method (a modified version of the Turkevich method), in which the reducing and capping agent is sodium citrate. ⁷ In an effort to stabilize the AuNPs in the polymer matrix (and reduce aggregation), ligand exchange was completed to replace the citrate with PEG. UV-Visible spectroscopy and dynamic light scattering (DLS) were used as characterization methods to verify ligand exchange shown in **Figure 5.1**. We have prepared PI films with PEG capped AuNS into the polymer matrix with compositions 0.0033, 0.0066, and 0.01 wt% gold, with the last composition being unsuccessful perhaps due to very high loadings in the solution form. The last composition resulted in poor adhesion and film quality and could not be removed from the former intact. Injection molding of PI with dry NPs could be an option, but it would limit the applications to thicker films, the particles would need to be stable in the dry form and dispersed properly within the matrix in order to improve the properties. Without proper dispersion, the nanoparticles will aggregate and mimic the effect of incorporating micron sized fillers, with agglomerates comparable in size to critical crack sizes, causing early failure.¹



Figure 5.1 a) Experimental absorption spectra of citrate capped spheres and PEG capped spheres. Redshifting of the absorption curve for the PEG capped spheres indicates that ligand exchange did occur. The LSPR shift from 520 nm to 523 nm is due to the higher permittivity of the surrounding medium, PEG and b) Dynamic Light Scattering data showing that the hydrodynamic radius increased after the PEG addition.

5.2 Thermosets

5.2.1 Latex and Polyisoprene (PI)

Latex is extracted naturally from the *Hevea brasiliensis* tree, and consists of 33% cis-1,4-polyisoprene (PI), 2% resin, 65% water, and 18% protein. ⁸ The allergies associated with latex are irritant dermatitis, Type I allergy (due to the proteins in the natural rubber), and Type IV allergy (from the chemicals such as thiurams added during the vulcanization or curing process of the rubber). ⁹ Past attempts to remove proteins and produce a sulfur-free product have led to deteriorated mechanical properties that rendered latex unsuitable for glove production. Over a decade ago, a method was discovered to synthesize protein- and sulfur-free synthetic cis-1,4-polyisoprene which maintains the desired mechanical properties of latex. ⁹

5.2.2 Dip Molding Polyisoprene

Medical devices such as surgical gloves can be produced using PI in a dip-molding process, making this the ideal material for this application. This process involves dipping a former/mandrel, a tool that is used to form the shape of the glove, into a medium that has the liquefied polymer, removing the mandrel from the medium, drying the polymer to remove the solvent and form a film, and curing the polymer to its final form via vulcanization (with high energy radiation to promote crosslinking). Afterwards, the film is stripped from the mandrel and ready for its application. There are a few variations that use alternative approaches, such as for example a prevulcanization step, but these processes are similar to the method described above. However, this method requires multiple dips, six to be exact, and long drying times to remove the solvent. After subsequent dipping, it would be difficult to ensure uniformity of glove thickness.

A specific type of dip molding is coagulant dip molding. In this process, a former is first dipped into a coacervant. In our process, which is dry-coagulant dipping, calcium nitrate is used as the coacervant, which is dissolved in a solvent, in our case water. The coacervant also has calcium carbonate as a parting agent, which facilitates in removing the film from the former. The water is then evaporated in an oven, leaving only calcium carbonate and calcium nitrate on the mandrel. The former is then dipped into the polymer. We selected PI from Kraton Polymers Inc., from their Cariflex products (IR0401), which is composed of 92 wt% cis-1,4 polymer and the remaining wt% is from trans-1-4 and 3,4 polymer. This synthetic polymer is stabilized anionically, and polymerized using an alkyl-lithium initiator, and undergoes a 24 hour maturation period once a stabilizer and vulcanizing reagents are added. To make a polymer film, the former is vertically dipped into the latex (typically at a rate of 0.5-1 cm/sec), the polymer particles sense the high concentration of salt on the mandrel, and get destabilized, attaching themselves onto the mandrel. The former is then left inside the polymer to increase the thickness of the polymer film, and the total time inside the polymer is called the dwell time. The former is withdrawn at the same rate as it was dipped, and then inverted to provide uniform thickness (as the last part of the former has been in the polymer the longest it is the thickest region) before inverting. The polymer undergoes prevulcanization at an elevated temperature for one minute, followed by a leaching step in deionized water at room temperature for five minutes. Finally, the polymer is vulcanized at an elevated temperature for fifteen minutes. It is then cooled to room temperature and stripped off the mandrel. The benefit of using this technique is that it provides a thicker polymer film, with only one dip into the polymer.

5.3 Mechanical Testing

5.3.1 Puncture Testing

The most important common stresses the gloves will undergo that will lead to failure are tensile stresses from initial stretching of the glove onto the hand and indentation/punctures from sharp objects in contact with the gloves. The most relevant tests to ensure that the gloves have adequate mechanical properties are tensile testing and puncture testing. Since the material needs to withstand high stresses and needs flexibility while being stretched onto the hand, the elongation to failure will provide valuable information on how much strain the material can withstand upon failure. Puncture tests can be performed using ASTM F2878-10 "Standard Test Method for Protective Clothing Material Resistance to Hypodermic Needle Puncture". A standard that was developed

earlier was ASTM F1342 / F1342M – 05 "Standard Test Method for Protective Clothing Material Resistance to Puncture". The needle for the ASTM standard can be seen in **Figure 5.2**, as well as the testing apparatus, and the testing will be carried out using an MTS machine with 5 N and 100 N load cells for the puncture and tensile testing, respectively.



Figure 5.2 a) Puncture resistance test assembly, using a modified version of ASTM F2878-10 and b) 25 gauge needle used for puncture testing using ASTM F2878-10.

It is important to change the needle often during puncture testing to make sure the tip is sharp and has not deformed. **Figure 5.3** shows the effect of repeated needle usage. This results in a larger contact area of the tip with the polymer film and can lead to higher values of force at puncture. The normal testing speed for the ASTM F2878 standard is 500 mm/min, however Gauvin et al. used a slower testing speed of 100 mm/min, which resulted in a similar force at puncture for the calibration material, proving that the test speed can be changed without altering the results.



Figure 5.3 25 gauge hypodermic needles a) used three times and b) used at least 25 times. The extended use of the needle blunts the sharp tip.

Figure 5.4 shows the results from puncture testing using ASTM F2878. The nitrile gloves clearly show a lower force at puncture, but the results for the PI and PI with nanoparticles have too much variation to conclude whether the particles were able to improve or deteriorate the properties. A few things can be concluded from the testing, however. The needle was changed for each new set of materials, upon which it was used to puncture 9 samples. Each time the needle punctured a sample, the force upon puncture became higher and higher, indicating that the needle was becoming blunted. It is also possible that the plastic covering touched the needle tip, before it was used, which also results in a blunted tip. These are reasons why there is a large variation in the results, and therefore to eliminate as many variables as possible, for further testing, the needle needs to be changed for each sample, it is difficult to control the thickness to the nearest tenth of a millimeter. The polymer films' thicknesses varied between 0.45 and 0.55 mm.



Figure 5.4 Results from puncture testing using nitrile gloves, PI, PI with 0.0033 wt% Au, PI with 0.0066 wt% Au, PI with 7.26×10^{-6} wt% Au and 0.0033 wt% CNTs, PI with 7.26×10^{-6} wt% Au and 0.0066 wt% CNTs, PI with 7.26×10^{-6} wt% Au and 0.01 wt% CNTs. The respective volume fractions for the Au and CNT composites are PI with 1.58×10^{-4} vol% Au and PI with 3.2×10^{-4} vol% Au, PI with 3.47×10^{-7} vol% Au and 1.45 × 10⁻³ vol% CNT, PI with 3.47×10^{-7} vol% Au and 4.36 × 10⁻³ vol% CNT.

Future work would involve production of more uniform film thicknesses. This could be done with a device that would control the dipping and rate of the mandrel more carefully. In addition, each needle should be closely examined with a light optical microscope to ensure that the tip is not blunted, and each needle should only be used one time. Although a torque wrench was used to secure the polymer films within the puncture testing apparatus, it would be ideal to have the polymer taut so there is no sagging of the polymer.

5.3.2 Tensile Testing

After the samples have been made using the dip molding process, cooled to room temperature, removed from the mandrels, and dusted with talc, the samples can be tested in accordance to ASTM D412-06a.¹⁰ It is important to note, however, that the

recommended die, die C in the ASTM standard, is meant for injection molded samples, however properties of rubber surgical gloves as well as other types of commercial gloves such as nitrile and polyvinyl chloride gloves are tested according to this standard. According to the standard, it is valid to use straight cut specimens; however, during the testing we conducted, the material would consistently fail within the grips, as seen in Figure 5.5a). When using a dumbbell specimen, the material broke within the grips, however if the testing setup had been improved, most likely would have occurred along the tear in the gauge length seen in Figure 5.5b). Dogbone specimens would require a die and punch in order to produce a smooth curve without stress concentrations. Ultimately, we decided not to use a die/punch to form the dumbbell samples due to cost and time of production, as well as the risk of introducing stress concentrations by not cutting out the samples properly, which could lead to premature failure. The consequence of using straight cuts specimens is that the stress on the polymer is highest within the grips, causing failure to occur within the grips instead of the gauge length it is not possible to calculate the ultimate tensile strength of the material. However, straight cut specimens will still allow for the tensile (stress, strain and modulus) comparison of samples, which is still valuable information for R&D purposes.

Figure 5.6 shows the stress vs. strain curve reported by Kraton Polymers, the company that produces the polymer, and our results using the dip molding method we have reported. Kraton also reports that they follow ASTM D412, however they do not report the dimensions of their sample, which could be why their stress at failure is higher than what we report. Other reasons for differences in stress results are the experimental setup and conditions, such as, for instance, the oven temperature stability.

The samples were cut into strips or straight cut specimens as per ASTM D412, with length of 5 cm and width of 2 cm. The thickness varied, but was generally around 0.5 mm; we recorded the exact values for reference. A major problem with tensile testing was that the sample kept slipping out of the grips. This was especially a problem when the grips needed to be tightened mechanically. Switching to pneumatic grips helped the strips stay within the grips for a longer duration of tensile testing. Adding a material to modify the grips so that it could provide some friction to keep the samples in place seemed like a good option. Unfortunately using grit paper resulted in early failure of the material. Ultimately, a flexible material polymer was used on the grips, which prevented the sample from slipping out.

Aligning the samples within the grips is essential to acquiring the tensile results that best represent the samples. The first part of aligning the samples is by centering the strip in the top grip, and closing the top grips. The sample hangs within the open bottom grips, and only if it appeared to be aligned properly, the bottom grips can be closed. The dip molding process produces thin and flexible polymers that require a preload for tensile testing in order to ensure the polymer taut before testing. Without a preload, the beginning part of the stress curve would be inaccurate due to the polymer straightening itself out and the results would not be reproducible. The testing procedure involved bringing the grips as close as possible until they almost touched, about 17 mm apart. The samples were inserted within the grips modified with the flexible material. The gauge length and load were reset. The sample was preloaded to 0.5 N. The gauge length varies after the preload, and is recorded for reference and for the strain calculation, but is between 17 and 18 mm. The gauge length and load are reset and then the test is started.

For tensile testing of other samples, one film was made for each composition, and from each sample three specimens were cut into strips, and tensile tested. For the stress vs. strain curves presented later in this chapter and the following chapter, the specimen that undergoes the highest stress (out of 3) was plotted and the other two specimens were represented on the stress vs. strain curve with a marker, either an open circle of a cross. The open circle markers indicate the end stress and strain values of a test for a strip that was tensile tested that did not fail but was elongated to the maximum limit of the machine, and the cross markers indicate the end of a test for a strip that ended in failure (breaking) of the strip. Only one specimen was plotted because misalignment in the grips, shown in **Figure 5.7**, and holes in the samples could be reasons for premature failure. ¹¹ By plotting the best sample, we evaluate the sample in the most ideal experimental conditions.



Figure 5.5 a) Straight cut specimens and the material response to tensile testing. Failure occurred within the tensile grips. b) Dogbone specimen that started to show failure along the gauge length.



Figure 5.6 a) Typical stress vs. strain results for a Cariflex IR0401 latex sample (from literature). Sample dimensions were not given. ¹² and b) results from testing with a straight cut specimen



Figure 5.7 Dogbone specimens that are aligned and misaligned within the tensile testing grips. ¹¹ Reproduced with permission.

5.4 Processing Parameters

5.4.1 Effects of Former Material and Finish

The former materials that are commonly used are glass, aluminum and its alloys, stainless steel, and porcelain, to name a few. We used commercially available alumina formers in the shape of an arm to dip into the latex as well as big and small 304 Stainless Steel (S/S) mandrels that were a quarter and an eighth inch in thickness, respectively, shown in **Figure 5.8**. When using the 304 S/S mandrels as received, the smaller ones had

a smoother finish, and the coacervant was not able to properly adhere to the mandrel. This resulted in poor quality latex films that were very thin and difficult to strip from the mandrel. Once all of the mandrels were sandblasted, to produce a rough yet uniform surface, the coacervant was able to adsorb to the mandrel surface and the polymer film quality improved, as shown in **Figure 5.8**. The alumina mandrels produced a thicker glove, which could be due to the type of material, surface roughness, and dwell time. Since it was the largest mandrel we used, its total dipping time, including the time of insertion and withdrawal, was the longest.



Figure 5.8 a) Alumina, small 304 S/S, and large 304 S/S mandrels (from left to right) and b) large 304 S/S as received on left and sand blasted on right shows the influence on surface finish on coacervant adhesion/polymer quality.

5.4.2 Temperature of Mandrel

To determine whether the temperature of the mandrel, while dipping the mandrel into PI, affects the mechanical properties, films were produced at two temperatures, 27°C and 60°C using stainless steel mandrels. The general protocol was to dipping of the mandrels into the coacervant and subsequently removed in a swirling fashion so that no

striations from the coacervant were visible. The coacervant was uniformly spread over the mandrel. The mandrels were then placed in a 130°C oven to evaporate the solvent (water). This process takes at least 9 minutes to occur. The mandrels were removed from the oven after drying the coacervant, and monitored with an IR temperature sensor until they reached the above temperatures.

Glass slides (used in the next chapter) were also dipped at 60°C. The glass slides were double thick glass with eased edges with dimensions 1.5 in x 4 in x 0.12 in, purchased from Metro Glass. The surface of one side of the glass was ground with 120 grit paper in order to create a roughened surface so that the coacervant would adhere, resulting in a uniform film. Without the roughened surface, the film was not uniform and the coacervant and polyisoprene did not adhere to parts of the slide.

Figure 5.9 shows the cooling profiles for both glass slides and stainless steel mandrels. The variation in initial temperature of each mandrel was due to the duration of time that the mandrels were left in the oven. **Figure 5.10** shows stress vs. strain curves for mandrels dipped at 60°C and 27°C, with the lower temperature (27°C) mandrels producing films that appear more stiff than the mandrels dipped at 60°C. With the variation in results, it is difficult to conclude whether temperature does play a role in the film properties. Variation in results could be due to improper alignment of the sample within the grips. Given that the samples are flexible (because they are thin and not injection molded) and there is a backing material to prevent the samples from sliding, the samples are difficult to align properly. Once again, for consistency, all of the samples produced in the later experiments used a mandrel temperature of 60°C because ideal

processing conditions would allow the mandrels to be dipped into the polyisoprene soon after being removed from the oven.



Figure 5.9 Cooling profiles of mandrels (stainless steel and glass) that were used for dip molding polyisoprene. Temperatures were collected using an IR temperature sensor every minute.



Figure 5.10 Stress vs. Strain curves for polyisoprene films created via a dip molding process using stainless steel mandrels. The mandrel temperatures were 60°C and 27°C.

5. Conclusions

The procedure to produce coagulant dip molded PI gloves and the puncture testing setup are in place, while the tensile testing setup is being optimized. This initial setup of designing and manufacturing the mechanical testing equipment was time consuming, and the dip molding procedure took time to perfect. Further steps required to conclude this work are to incorporate different types of nanoparticles in the polymer matrix. A different approach will be taken to incorporate AuNPs which will focus on understanding the surface chemistry to predict how the NPs react with the polymer matrix. For example, the AuNPs could be coated with a different capping agent instead of PEG to allow the polymer on the nanoparticle to bond with the polymer in the matrix, thus producing a stronger material.

5.5 References

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6 Polymer-Matrix Interactions

6.1 Introduction

In an effort to understand the interaction between polymers and the surrounding matrix, the goal of this chapter is to determine the effect of the capping agent of the particle and how the shape of the nanoparticles may play a role in the mechanical properties of the composite. It has been shown by Carretero-González et al. that adding nanoclay to natural rubber (PI) produces strain induced crystallization (SIC) because the nanoparticles are small enough to be distributed *homogeneously* (compared to micron sized fillers) and the anisotropic particles possessing large interfacial surfaces of the nanoclay particles facilitate the chain orientation of the PI under uniaxial stress seen in **Figure 6.1**.¹ The crystallization of the PI forms a microfibrillar structure which produces a higher modulus. Hence, addition of anisotropic particles, such as AuNR in PI may provide the same mechanism for SIC that the nanoclay provided for PI. Continuing work from Chapter 4, AuNPs were selected to investigate the properties of polymer nanocomposites due to reproducibility in morphology, long term stability, and ease of functionalization.



Figure 6.1 PI with nanoclay incorporated in the matrix in ideal conditions (top) and real conditions (bottom) where the sulfur cross-links the PI chains inhomogeneously. The anisotropic particles facilitate the alignment of the PI chains.² Reproduced with permission.

It is known from literature that the mechanical properties of a nanocomposite can be improved by strong bonding between the nanoparticles and the surrounding matrix.³ In the previous chapter, Chapter 4, PEG-capped AuNS and carbon based nanomaterials were incorporated into PI, and puncture testing was done to characterize the composite. In this chapter we will study the interaction between AuNPs and the surrounding matrix using tensile testing and nanoindentation. Although both methods allow for determination of mechanical properties of thin films, tensile testing assesses the bulk properties of the polymer and monitors the mechanical properties during stretching of the polymer chains, while nanoindentation deforms the material locally and studies surface effects.

To determine what role the capping agent has on the polymer's interaction with the matrix, ligand exchange was performed on citrate spheres to replace citrate with two molecular weight thiolated Polystyrene (PS). The PS-capped particles were then incorporated into a PS to determine if the PS on the NPs would interact with the PS in the matrix. The goal for the latter study was to determine how the molecular weight of the
polymer influences the mechanical properties of the composite. PS was chosen for this work for several reasons. PS is a widely used polymer both as a homopolymer and a copolymer with PI. As a capping agent, it is also easy to perform ligand exchange for the NPs because different molecular weight thiolated PS can be purchased. For consistency, it would have been ideal to cap the particles with PI, but this would have required synthesis of the thiolated ligand, as it is not commercially available. Chuayjuljit et al. have shown increased mechanical properties of PI by incorporating PS-capped silica nanoparticles.⁴ For comparison, to determine if citrate capped spheres would improve or degrade the mechanical properties of PS, citrate AuNS in water were added to PS in a solution form (using THF, but they became unstable).

As we have already studied PI before, we introduced citrate capped spheres into PI. The citrate capping provides no favorable interaction between the nanoparticles and the matrix, in analogy to the case of carbon-based materials. Although the application is no longer limited to producing puncture resistant surgical gloves, we selected PI as one of the polymer matrices, as we were able to optimize the dip molding process to produce thin uniform films and had already acquired a good understanding on the physical and mechanical behavior of this polymer.

Even though the NPs were incorporated into two different polymers, it is still possible to determine whether the capping agent improved or degraded the properties of the original (neat) polymer. PI, however, is a thermoplastic, and PS is a thermoset, thus they need to be processed differently. After reviewing the results from the experiments with citrate AuNS in PI, we can verify the effect of not having a capping agent on nanoparticles in PI, and determine how the presence of a capping agent on nanoparticles can favor the interaction with the polymer matrix (PS). Taking these results into account, we sought to improve the original system, with PI as the matrix, investigating how the shape of AuNPs and the MW of the capping agent PS play a role in the system. This ultimately allows for the investigation of the NP/matrix interactions between PI and the AuNPs.

6.2 Thermosets – Polystyrene

Thermosets are the most widely used polymers and desirable materials to use as they can be processed simply by heating or dissolving in a solvent, allowing them to be processed more than once. PS is composed of long chains of hydrocarbons. At room temperature, PS is a rigid material whose properties are based on van der waals attractions between the polymer chains. Upon heating, the polymer chains can slide past each other and the material can flow and be processed.

6.3 Block Copolymers - SIS

Block copolymers are composed of two or more homopolymers. Typically a triblock copolymer has the structure A-B-A or A-B-C, where A, B, and C represent different homopolymers. The block copolymer used in this work is SIS, with polystyrene, hard segments, as the terminal groups and polyisoprene soft segments in the middle. Due to the incompatibility between the two polymers, the homopolymers segregate in short range order, forming microscopic phases. These phases can have lamellar, cylindrical, and spherical morphologies as the volume percent of one of the blocks increases, respectively.

When block copolymers are at the interface between two homopolymers, the different blocks will segregate to their respective homopolymers. Addition of block

copolymers to join homopolymers is an ideal method to improve the adhesion between the homopolymers, as there is a covalent chain used in the process. Choosing selective solvents to dissolve the blocks of the block copolmer can lead to formation of micellar structures in solution.

Styrene-Isoprene-Styrene (SIS) block copolymers are widely studied, and fit into the category of thermoplastic elastomers, providing ease of processing from the thermoplastic entity and the physical properties from the elastomer, which is physically crosslinked. The addition of SIS into the PI matrix would facilitate the dispersion of nanoparticles and create a bond between the NPs and the BCP incorporated into the matrix. PS and PI are immiscible, thus incorporating PS-capped NPs into the PI matrix alone will not provide good dispersion and matrix-particle interaction; however the microphase segregation of SIS will allow the particles to be dispersed in the SIS. This thermoplastic elastomer can be processed by heating it to form a processable fluid and subsequently cooled (after forming) or by dissolving it in a solvent, followed by evaporation of the solvent to produce a strong elastic solid. ⁵

This material gets its strength from the hard phase, and acts similarly to the sulfur crosslinks that give vulcanized rubber its strength, but is termed physical crosslinking. The benefit of using a thermoplastic elastomer is that it does not require vulcanization to get its strength, and can thus be processed as many times as desired. Generally, as the weight percent of the hard phase is increased, the material behaves more like a thermoplastic, thus becoming harder and stiffer.

6.4 Functionalization of AuNPs with PS

6.4.1 Purpose

NPs in a composite need to be able to successfully transfer the stress from the matrix to the nanofiller. To ensure that the AuNPs will facilitate the alignment of the PI chains, the particles cannot debond from the matrix. The AuNPs were capped with PS to enable van der waals bonding between PS on the NPs and PS blocks in the SIS BCP. The dimensions of the particles are given in **Table 6.1**. As mentioned in the beginning of Chapter 6, the mechanism for increased modulus of PI is due to SIC under uniaxial tension, which forms a microfibrillar structure when the crystallites are formed. AuNS and AuNRs were chosen for this experiment to determine if incorporating anisotropic particles would enhance the mechanical properties of the nanocomposite, by alignment of the PI chains under strain, as shown in literature. Thus far, many carbon-based materials have been incorporated into PI and various polymers, but using AuNPs allows for the ability to study the interface between the nanoparticles and fillers and the effect of the nanoparticle shape, while keeping the filler material constant.⁶⁻⁸

	Leng	gth (nm)	Width or Di		
Particles	Average (nm)	Standard deviation (nm)	Average (nm)	Standard deviation (nm)	Aspect Ratio
AuNS	N/A	N/A	15.63	1.31	1.00
AuNR	53.18	5.71	14.36	1.99	3.70

Table 6.1 Dimensions of AuNS and AuNR as synthesized and purified.

6.4.2 AuNR Functionalization

The AuNRs were purified via centrifugation several times at 8000 g for 40 mins to remove excess CTAB and byproducts of the synthesis. A 0.5 mM stock solution of polystyrene was made by dissolving 11000 MW PS in THF. The AuNS solution and stock solution were mixed with a 1:1 ratio together scintillation vial. Upon capping, PSfunctionalized Au nanospheres precipitated out of solution, and the particles were centrifuged. The pellet was resuspended in toluene and centrifuged at 8000 g for 40 mins. This process was repeated several times to remove excess CTAB and PS. Particles were dried and stored in a dessicator to remove solvent.

6.4.3 AuNS Functionalization

Citrate AuNS were synthesized using the Frens method (a modified version of the Turkevich method), in which the reducing and capping agent in this synthesis is sodium citrate. A modified version of Yockell-Lelièvre's protocol was used to functionalize and purify the particles. A stock solution was made of 20 mg PS (Mn 11000 and Mn 5000) in 100 mL acetone. The spheres were added drop wise to the PS stock solution at room temperature and left stirring for at least 5 minutes. The particles were centrifuged at 8500 g for 10 mins at which point they were stuck to the centrifuge tube. The supernatant was

removed and acetone was added. The particles were then resuspended via ultrasonication. The purification process was optimized by adding ethanol to acetone. Ethanol and acetone are miscible, however the particles are not soluble in ethanol, thus more easily forming a pellet when centrifuged with ethanol. The absorbance for PS capped AuNS washed using different ratios of acetone/ethanol mixtures (10%, 40%, 70%, and 100% acetone (remaining ethanol)) can be seen in **Figure 6.2**. As absorbance can be used to calculate the concentration of particles, the graph can be used to determine which ratio of acetone to ethanol gives the best yield, with 70% acetone 30% ethanol mixture being the best. The average interparticle distance between the 5000 MW PS and 11000 MW AuNS, respectively are 6.52 nm and 8.99 nm, using the TEM micrographs shown in **Figure 6.3**. In **Figure 6.3**a) it is possible to see an outline around a small group of particles, indicating that PS capping may result in clustering of NPs.



Figure 6.2 Absorbance for PS capped AuNS that were purified in mixtures of acetone and ethanol. Using a mixture of 70% acetone and 30% ethanol gave the best yield.



Figure 6.3 TEM micrographs of PS capped AuNS using a) 5000 MW PS and b) 11000 MW PS.

6.5 Citrate AuNS in PI and PS AuNS in PS

6.5.1 Sample Preparation

The citrate AuNS were simply added to the PI and stirred to distribute and disperse the AuNS. The PI is water based, and the citrate particles are stable in water, thus the particles remained stable. The PS capped AuNS were dissolved in acetone (as this was the solvent used for purification), and was added to a PS solution and mixed. The weight percent of the particles can be seen in **Table 6.2** and **Table 6.3**. The polystyrene was dissolved at 7.59 wt% PS in THF, and had varying concentrations of PS capped AuNS. The wt% of PS-AuNS in PS was calculated such that the wt% of particles in solids were equivalent to the wt% of citrate AuNS in PI. However, due to low loading of PS in THF, 7.59 wt%, compared to wt% solids in PI, 60-65 wt%, additional samples of PS-AuNS were made. After the AuNS were well incorporated, small volumes were drop casted on double thick glass slides and the solvent was allowed to evaporate at room temperature.

Samula	Composition (Weight Percent)								
Sample	Α	В	С	D	Ε	F	G	Н	
Citrate	2.91	5.82	8.73						
AuNS in PI	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$						
PS 5000	2.91	5.82	8.73	2.91	2.91	7.27	2.91	1 45	
AuNS in PS	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	×10 ⁻³	$\times 10^{-2}$	$\times 10^{-2}$	$\times 10^{-1}$	1.45	
PS 11000	2.91	5.82	8.73	2.91	2.91	7.27	2.91	1 45	
AuNS in PS	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-5}$	×10 ⁻³	$\times 10^{-2}$	$\times 10^{-2}$	$\times 10^{-1}$	1.45	

Table 6.2 Concentrations of Citrate and PS capped AuNS in PI and PS respectively, by weight percent.

Table 6.3 Concentrations of Citrate and PS capped AuNS in PI and PS, respectively by volume fraction.

Sampla	Composition (Volume Fraction)							
Sample	Α	В	С	D	Е	F	G	Н
Citrate AuNS	3.16	6.32	9.49					
in PI	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-8}$					
PS 5000	2.51	5.02	7.52	2.51	2.51	6.27	2.51	1.25
AuNS in PS	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-4}$	×10 ⁻³
PS 11000	2.51	5.02	7.52	2.51	2.51	6.27	2.51	1.25
AuNS in PS	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-8}$	$\times 10^{-6}$	$\times 10^{-5}$	$\times 10^{-5}$	$\times 10^{-4}$	×10 ⁻³

6.5.2 Tensile Testing

For tensile testing, the same approach for plotting of the stress strain curves was taken from Chapter 4. For each sample, three specimens were cut out into straight cut specimens. All samples were tested, while only the stress vs. strain curves for the best samples are plotted. The other samples are represented with an x indicating the sample failing at the specific stress and strain values, or with an o, representing a sample that was tested to the maximum length of the Instron. For citrate spheres, the stress vs. strain curves can be seen in **Figure 6.4**. PI has the highest modulus values at various strains. In particular, it is interesting to analyze the modulus at two sections along the stress strain curves, at low strains and high strains where the moduli greatly differ. The values chosen to calculate the moduli are 750% to 1250% and 2250% to 2750% strains, respectively.

The moduli plotted in the bar graphs in **Figure 6.4** are averages for all the specimens for each sample with the standard deviation. From analyzing the tensile testing results, it is evident that PI without any nanoparticles show the highest stress values at any strain shown in **Table 6.4**. The lowest two concentrations of citrate AuNS in PI had the lowest stress values, however, none of the tests ended in failure. The third concentration has two samples to test for reproducibility. These samples had higher moduli than the first concentrations, but some of these ended in failure. An explanation for the increased modulus could be due to addition of stiff nanoparticles that prevent the main chains from moving, also observed by et al. who incorporated PS-capped silica particles in PI.⁴ An interesting follow up study would be to increase the concentration of citrate AuNS in PI and observe if the stress values exceed PI.



Figure 6.4 a)Stress vs. Strain curves for PI and PI with citrate AuNS and modulus values for b) 750% to 1250% strain and c) 2250% to 2750% strain.

	Stress at 500% Strain (MPa)		Stress at Strain (1500% MPa)	Stress at 2500% Strain (MPa)	
Sample	Avg	Stdev	Avg	Stdev	Avg	Stdev
PI	0.656	0.021	1.303	0.072	3.110	0.303
Composition A	0.558	0.030	0.935	0.041	1.755	0.066
Composition B	0.526	0.024	0.860	0.065	1.603	0.166
Composition C – Sample 1	0.559	0.004	1.038	0.012	2.169	0.122
Composition C – Sample 2	0.606	0.031	1.148	0.079	2.430	0.271

Table 6.4 Stress values for PI and PI with citrate AuNS at various strain values.

6.5.3 Nanoindentation

Nanoindentation is a technique suitable for thin films which allows for the calculation of mechanical properties. It allows for the extraction of modulus and hardness by indenting the sample with a small tip and locally deforming the material. Nanoindentation is very sensitive to the substrate that the sample is mounted on. For example, if the sample is placed on a material that deforms easily (such as wax), the mechanical properties will be a convolution of the material properties of the actual sample and what is beneath the substrate. The samples must be flat on the nanoindenter stage. The dip molded samples were prepared directly on the substrate that was then used for indentation, which is why the glass former was chosen for the dip molded samples that were being nanoindented.

For sample preparation of the PI with citrate AuNS, the samples were dip molded according to the normal dip molding protocol. As the film was covering all sides of the glass slide, the glass slides were epoxied onto other glass slides to form a flat base for the nanoindenter stage. The dip molded samples were brought to a displacement of 3 μ m over a period of 30 seconds with a 1 second hold and a 30 second unload to zero displacement.

The PS-capped AuNSs in PS were drop casted onto the slides. Due to the solution processing of this material, not injection molding, the polymer dried in a pattern such that the center of the drop casted area appeared thinner than the edge. This effect is observed by Datta et al. who studied drying patterns of PS in toluene deposited on Si substrates.⁹ They attribute this effect due to solvent evaporating on the periphery faster than the middle, because of the curvature of the droplets on the substrate from incomplete wetting

of the surface. The solute (PS) molecules move to the edge to compensate for loss of the solvent, which results in a coffee-ring structure. For nanoindentation, both the centers and the edges were tested at 2500 and 500 μ N with a 10 second hold at the maximum load to observe substrate effects, if present. If the depth of the load depth curve increases during the hold, this indicates creep, and shows that the tip is contained within the polymer film, as glass does not have the same behavior. A lower load would be better for containing the indent within the polymer films, especially when the samples are so thin, but the higher load is better for calculations, since the tip can penetrate the sample further and the contact area of the tip is well known. Ultimately 500 μ N loads were used to ensure that the tip did not penetrate into the glass slide.

The results from nanoindentation of PI with citrate spheres seen **Figure 6.5** reinforce the findings from tensile testing, which shows that that PI without any nanoparticles has higher modulus and hardness compared to samples with citrate AuNS. Interestingly, the addition of nanoparticles shows a different trend from the results from tensile testing, which is deteriorated mechanical properties as the nanoparticle concentration is increased. The degradation of the mechanical properties is most likely due to the lack of bonding between nanoparticles and matrix since citrate does not bond to the PI matrix.

Nanoindentation of PS-capped AuNS in PS can be seen in **Figure 6.6**. **Figure 6.6**b) and d) show the modulus and hardness results from indenting in the center of the drop where the samples appeared to have a very thin polymer layer. **Figure 6.6**a) and c) show the modulus and hardness results from indenting at the edge of the drop. For each location (center and edge), 5 indents were done for each sample. The results do not vary

greatly for the center results, for any of the samples (with a low standard deviation) showing that the sample is mostly affected by the substrate due to the sample being too thin.¹⁰ The edge results show improved mechanical properties for the composite compared to the neat polymer. Overall, for the 5000 MW PS-capped AuNS, there is not a drastic difference in the average modulus and hardness compared to neat PS, however the samples with the highest modulus and hardness are marked with an asterisk, and these values are generally higher than the neat polymer. An explanation for these properties could be due to the short chain length having mobility to slide past PS chains in the matrix under local deformation. For the 11000 MW PS-capped AuNS, there is a noticeable improvement in the modulus and hardness at low concentrations. An explanation for these improved properties could be that the longer chains on the particle allow for a higher degree of interaction between the ligand and the matrix through van der waals bonding of the PS chains. Another possibility is that the grafting density on the particles is lower for the higher MW PS, which allows the polymer brush to be wetted by the polymer matrix, as seen by Corbierre et al.¹¹



Figure 6.5 Modulus and hardness results for PI and PI with citrate AuNS.



Figure 6.6 Nanoindentation results for PS and PS with PS-AuNS: a) edge and b) center modulus and c) edge and d) center hardness. The highest values are marked with an asterisk.

6.6 PI and PS-AuNP Interactions

The results from incorporating PS-AuNS in PS and citrate AuNS in PI suggest that nanoparticle matrix interactions of our original system, PI, can be improved by capping the particles with PS and incorporating PS into the system. SIS BCPs were chosen as the method to incorporate PS. The hypothesis is that the PS blocks interact with the PS on the nanoparticles and PI blocks interact with the PI in the matrix, which allows for the distribution of the particles based on literature that shows that PS-AuNS and PI-AuNS added to PI and PS lamellar structures phase segregate to their respective homopolymers. ¹² SIS BCPs for 14%, 17%, and 22 wt% were dissolved in a solvent before introducing the BCP to the PI matrix. The matured PI (emulsion) rejected all of the following

solvents (even without BCP present) by clumping upon addition: THF, toluene, chloroform, and acetone. It was apparent that the block copolymer and nanoparticles needed to be incorporated in a different method. Instead of making a blended material with the SIS and PI, we prepared a layered material which involved forming the PI film and then dipping into a SIS with the nanoparticles. Since the SIS will be added early in the curing process of the PI, it may be possible for the PI blocks to cross-link with the PI matrix with sulfur in the matured PI as the initiator. If cross-linking is not possible, perhaps there will still be some sort of bonding between the PI and the SIS due to diffusion at elevated temperatures.

To review, the dip molding process is as follows:

- Dip mandrels into coacervant
- Heat in oven at 130°C until dry (9 minutes)
- Cool mandrel until 60°C
- Dip into PI
- Prevulcanize film for 1 minute
- Leach out salts
- Cure for 15 minutes at 130°C
- Cool to room temperature and strip film from mandrel

Thus far, BCPs have mostly been used alone or with a homopolymer (in a blend). As we have created a layered composite, we wanted to determine the optimal time in the dip molding process to introduce the SIS. With this in mind, two sets of samples were made by incorporating the SIS at two stages of the dip molding process after the bolded steps above, which we refer to as Step 1) and Step 2): Step 1) Dipping the PI film into SIS solution after dipping into PI and air drying the film for 5 minutes

Step 2) Dipping the PI film into SIS solution after prevulcanization and leaching step

6.6.1 SIS Solution Preparation

SIS block copolymers with 14, 17, and 22 wt% PS were purchased from Sigma Aldrich. The block copolymer pellets were dissolved in THF at two concentrations, 5 and 10 wt% solids in THF. The solutions were sonicated until the block copolymers had completely dissolved. Results from literature show that below 16 wt% PS a sea island structure is formed, and SIS BCPs with higher PS content forms a cylindrical structure. ¹³ As the inter- and intra-molecular bonding between PI and PS chains is determined by the morphology of the dried SIS, a quick method to verify the morphology of the SIS is Raman spectroscopy. The structural properties of 17 and 22 wt% PS SIS did not vary much, shown in **Figure 6.7**, where the most intense Raman peak around 996 cm⁻¹ is characteristic of cis-1,4 PI. ¹⁴ Only 14 and 22 wt% PS SIS were used for initial experiments to determine which block copolymer had the best interaction with PI.



Figure 6.7 Raman spectra for SIS block copolymers.

6.6.2 Tensile Testing of PI Dipped into SIS

Our hypothesis was that the 22 wt% SIS block copolymer would provide a higher strength to PI, due to the higher content of the rigid thermoplastic and by comparing stress vs. strain curves for 18 and 16 wt% PS SIS from literature ¹³; however this was not the case seen in **Figure 6.8**. An explanation for this could be that the since the PI block is encased between two PS blocks, the 22 wt% SIS PS blocks may prevent the interaction between the PI blocks and the PI matrix, compared to a PS-PI diblock copolymer where the PI block could freely interact with the PI matrix. Dadmun et al. demonstrate how the length of the block determines the entanglement of a block with its respective homopolymer, and show that there is a critical block length that will allow sufficient entanglement between the block copolymer and a homopolymer. ¹⁵ Perhaps the higher wt% PS SIS BCP did not allow enough entanglement of the PI chain with the PI matrix, which might be why the mechanical properties were not significantly enhanced and a lot of the specimens experienced early failure.

The upturn in the stress strain curves may indicate the onset of strain-induced crystallization of PI, reported by Hsiao et al, in which they discuss how at high strains most of the PI chains remained amorphous and unoriented, while a small percentage (5%) of amorphous chains are oriented, and 20% of the chains were crystalline. They believe that the alignment of the amorphous chains is a precursor for SIC, and that the strain-induced crystallites have a microfibrillar structure that dominates the mechanical behavior of the PI.² Based on these results, only the 14 wt% PS SIS was used for the remaining experiments, in which the nanoparticles were incorporated into the block copolymer solution, and for ease of processing, 5 wt% SIS in THF was used.



Figure 6.8 PI dipped into SIS block copolymers a)14 wt% PS and b) 22 wt% PS dipped into 5 and 10 wt% SIS in THF. Within each graph there are 2 lines for each SIS solution, where the SIS was added Step 1 and when the SIS was added after Step 1.

6.6.3 Nanoindentation of PI Dipped into SIS

Since a layered structure was created by dipping into the PI and BCP, the bonding between the PI and SIS was very important. To determine how well the SIS layer adhered to the PI, nanoindentation was done on PI and PI dipped in 14 and 22 wt% PS BCP. The load vs. depth curves are shown in **Figure 6.9**. The load vs. depth curve of PI is very noisy, however, ultimately it is apparent from **Figure 6.9**b) that the hardness and modulus for the PI with 14 wt% PS SIS are higher than plain PI and the 22 wt% PS SIS. Hameed et al. report that a diblock compolymer (PDMS-PGMA) which was solvent cast onto a cross-linked epoxy matrix formed covalent bonds with the matrix.¹⁶ These results support our hypothesis that the PI blocks from the BCP form a strong bond with the PI in the matrix from either cross-linking or entanglement of the PI chains.



Figure 6.9 a)Load vs. depth curves of PI dipped into SIS block copolymers and b)moduli and hardness values.

6.6.4 Nanoparticles Incorporated into SIS

To determine how the MW of the capping agent and the shape of the nanoparticles play a role in the mechanical properties of the nanocomposite, CTAB capped AuNRs were ligand-exchanged with 11000 MW thiolated PS, and citrate spheres were ligand exchanged with 5000 and 11000 MW thiolated PS. The concentrations can be seen in **Table 6.5** for AuNP in 14 wt% SIS. The final concentrations of AuNP in the entire composite (Au, SIS, and PI) are given in **Table 6.6**. The weight percent and volume fraction of SIS in PI is 13%, as the density of the two materials are equivalent. It is more difficult achieve high yields with the AuNR synthesis, and the purification process is more complicated, which is why the concentrations of spheres are higher. The dimensions of the AuNRs were 14.4 nm and 53.2 nm in width and length, respectively.

Despite the differences in compositions between the nanocomposites, a comparison can be made between composition B of the rods and composition A of the spheres samples, since they weight percents are comparable.

Sampla	Composition (wt%)						
Sample	Α	В	С	D	Ε		
PS 11000 AuNR in SIS	7.13×10 ⁻⁷	2.38×10 ⁻⁶	4.76×10 ⁻⁶	7.13×10 ⁻⁶			
PS 11000 AuNS in SIS	2.69×10 ⁻⁶	8.97×10 ⁻⁶	1.79×10 ⁻⁵	2.69×10 ⁻⁵	1.34×10 ⁻		
PS 5000 AuNS in SIS	2.59×10 ⁻⁶	8.63×10 ⁻⁶	1.73×10 ⁻⁵	2.59×10 ⁻⁵	1.30×10 ⁻		

Table 6.5 Weight percent of PS capped AuNR and AuNS in 14 wt% PS SIS.

Table 6.6 Concentrations of AuNP in composite (AuNP, SIS, and PI), by volume fraction.

Sampla	Composition (Volume Fraction)						
Sample	Α	В	С	D	Ε		
PS 11000 AuNR in PI+SIS	4.39×10 ⁻⁹	1.46×10 ⁻⁸	2.93×10 ⁻⁸	4.39×10 ⁻⁸			
PS 11000 AuNS in PI+SIS	1.66×10 ⁻⁸	5.52×10 ⁻⁸	1.10×10 ⁻⁷	1.66×10 ⁻⁷	8.25×10 ⁻⁷		
PS 5000 AuNS in PI+SIS	1.59×10 ⁻⁸	5.31×10 ⁻⁸	1.06×10 ⁻⁷	1.59×10 ⁻⁷	8.00×10 ⁻⁷		

6.6.5 Tensile Testing of PI Dipped into SIS with Nanoparticles

Since a new batch of PI was used for these experiments, the experiments for PI and PI dipped in14 wt% PS SIS solution were repeated. The results in **Figure 6.10** show that the PI with SIS has higher stress and any given strain than PI alone, and the results for dipping the PI in the SIS at two different steps (after air drying PI and after the prevulcanization step) do not show a significant difference.

Upon analysis of the PI dipped into SIS containing PS-AuNS, it appears from the stress vs. strain curves in Figure 6.11 and Figure 6.12 that for both 5000 MW PS and 11000 MW PS, generally introducing SIS at step 1 had higher stress values at various strains. We attribute the increase in modulus to introducing the particles and SIS at a time before cross linking of the PI matrix had occurred. This allowed SIS and the nanoparticles to diffuse into the PI network. Many of the samples that which had SIS introduced at step 2 had lower strain values than PI with BCP alone. We believe the deteriorated mechanical properties are due to the PI matrix being cross linked from the prevulcanization step. The PI blocks from the SIS have a high affinity to the PI matrix and may debond from the PS-capped particles, or the PI from the SIS blocks are not able to The modulus results from **Figure 6.13** show that the low strain moduli have slight variations in the modulus with the samples containing the SIS with AuNS being slightly lower than the PI with the SIS. The high strain moduli show more noticeable differences between the samples. For the 5000 MW PS-AuNS, the samples with SIS incorporated at step 1 had improved in mechanical properties compared to PI with the SIS alone. The samples with SIS incorporated at step 2 were slightly lower in the high strain modulus compared to the PI with SIS alone. For the high strain modulus, the 11000 PS AuNS had higher moduli for samples with SIS incorporated at step 1, while the samples with SIS incorporated at step 2 had lower moduli at all concentrations that decreased as the nanoparticle concentration increased.



Figure 6.10 PI dipped into 14 wt% SIS BCP solutions.



Figure 6.11 Stress vs. strain curves for PI dipped into 14 wt% SIS BCP solutions containing 5000 MW PS-AuNS added at a) Step 1 and b) Step 2.



Figure 6.12 Stress vs. strain curves for PI dipped into 14 wt% SIS BCP solutions containing 11000 MW PS-AuNS added at a) Step 1 and b) Step 2.



b)

2

1

0

Plain Pl

PI with SIS



А

В

Composition

С

D

Е

Incorporating 11000 MW PS-AuNRs into the SIS had a more noticeable effect on the tensile properties of the PI (seen in **Figure 6.14**), compared to the spheres, even while incorporating at much lower concentrations, thus confirming our initial hypothesis. The samples that were dipped into SIS with particles at step 2 only had higher stress value at various strains for the highest compositions. The samples that were dipped into the SIS with particles at step 1 had improved mechanical properties, higher stress values than PI and PI with SIS at all strains. The modulus at high and low strains also showed and increasing trend in modulus with increasing concentration of rods, seen in **Figure 6.15**.

Carretero-González et al. explain the tensile behavior of anisotropic nanoclay particles in PI as a two step process, in which at lower strains the particles get aligned and in the second step they are completely aligned in the tensile direction and form a physical network that favors the alignment of the PI chains which allows the SIC to increase.¹ They also discuss how strong adhesion between the nanoparticles and matrix results in an earlier crystallization and a higher degree of crystallization of PI chains under uniaxial tension. The AuNRs do have strong adhesion with the PI matrix and the nanocomposites exhibit a similar behavior to the nanoclay in which the modulus at high and low strains are increased most likely due to the alignment of the rods at low strains and then promoting alignment of the PI chains a high strains.¹

The shape effects of the particles on the nanocomposite's mechanical properties can be seen in **Figure 6.16**, where the capping agent (11000 MW PS) was kept constant on the particles, so the direct effect of the shape of the particles can be seen. It is apparent that incorporating AuNR has a drastic effect on the tensile properties of the nanocomposite, and possesses the highest modulus. This supports our hypothesis that AuNR within the PI matrix increases the modulus of PI as the particles align and the SIC is increased.



Figure 6.14 Stress vs. strain curves for PI dipped into 14 wt% SIS solutions containing 11000 MW PS-AuNR added at a) Step 1 and b) Step 2.



b)



Figure 6.15 PI dipped into 14 wt% SIS solutions containing 11000 MW PS-AuNR a)low strain (750% to 1250%) modulus and b) high strain (2250% to 2750%) modulus.



Figure 6.16 Stress vs. strain curves for PI dipped into 14 wt% SIS solutions containing 11000 MW PS-AuNR (Composition B) and 11000 MW PS-AuNS (Composition A) at similar concentrations incorporating the SIS and NPs after Step 1.

6.6.6 Nanoindentation of PI Dipped into SIS with Nanoparticles

Figure 6.17 shows the values for modulus and hardness for the PI dipped in SIS with the highest concentrations of AuNPs. Compared to the PI dipped in SIS alone, the nanoparticles incorporated either improved the modulus of the nanocomposite or stayed constant. Interestingly, incorporation of rods did not provide the best properties to the nanocomposite like they did for tensile testing, seen in **Figure 6.17**, where the highest concentrations of NPs in SIS were nanoindented. This reinforces the hypothesis that the rods tend to align the PI chains during tensile deformation, but since the mechanism of deformation is more local in nanoindentation, the shape of the nanoparticles does not influence the mechanical properties as significantly. Since the AuNRs were incorporated

at a lower wt% than the AuNS there was less PS introduced to the system, which provided less of a stiff phase, which could also be the reason for lower modulus and hardness results compared to the AuNS. The variation in results for nanoindentation may be from the tip interacting with hard phases (aggregates) in the system (PS and AuNPs) or partial delamination of the material from the substrate. Typically the samples that had the SIS incorporated at step 2 had higher moduli compared to the air dried samples which shows that the bonding between the nanoparticles and the matrix was sufficient but also indicates that the SIS and NPs did not diffuse into the PI matrix as well as incorporating SIS at step 1. The hardness values need to be revisited because the values are much lower than the hardness values from the original test and have large standard deviations. An explanation for these deteriorated properties is that it is possible that the PI aged over time and delaminated from the substrate (glass slide).



Figure 6.17 Modulus and hardness values of PI dipped into 14 wt% SIS solutions containing the highest volumes of PS-AuNPs.

6.7 Conclusions

Dip molded PI with citrate AuNS at low concentrations showed deteriorated mechanical properties of the nanocomposite in comparison to PI. As the concentration of AuNS was incorporated in the matrix was increased, the mechanical properties approached that of plain PI. As citrate has no favorable interaction with the matrix, the mechanical properties were not expected to surpass that of the neat PI. When a capping agent, PS, was chosen to cap AuNS in PS, the ligand and matrix were compatible, showing equal or improved hardness and modulus results of the nanocomposite. When introducing AuNPs in SIS and forming a layered structure with dip molded PI, the AuNS showed little to no improvement in mechanical properties, with the air dried samples showing the most improved results in most cases. The incorporation of AuNR even at low concentrations had noticeable changes under uniaxial stress. The explanation for improved mechanical properties is most likely due to strain induced crystallization of the PI. Although more studies need to be conducted to understand the deformation and strengthening mechanisms of the layered nanocomposite, the results confirm our hypothesis that the incorporation of anisotropic nanoparticles improves the mechanical properties of PI. Thus far AuNPs have been used in applications that harness their optical properties. Our work shows that it is possible to have a deeper understanding of nanoparticle matrix interactions by using Au as the reinforcement material via straightforward ligand exchange.

Nanoindentation shows that the shape of the NPs does not play a big role in the mechanical properties as the mechanism for deformation is more local and does not involve uniaxial stretching on the bonds (in tension). Much research is being conducted on BCP blends with a homopolymer and the effect of BCP diffusion within the matrix, but we show that it is possible to create layered nanocomposites. We are able to create composite materials that encompass the high strain of PI with increased stiffness from incorporating SIS and NPs. In the future, the mechanisms for strengthening could be investigated further.

6.8 References

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7 Future Directions

This work has looked in detail at applications of AuNPs that leverage their optical and mechanical properties. We have demonstrated the reproducible syntheses for various morphologies of AuNRs and their relatively easy functionalization via thiolated ligands. AuNPs will continue to attract attention for biomedical applications due to their absorption in the NIR, the transparent biological window, which allows them to be used for applications such as photothermal therapy. Their SERS enhancement enables AuNPs to be used for single molecule detection, which can be harnessed to detect diseases at early stages. In this work, we successfully formed protein coronas around AuNRs using fibrinogen with the goal of studying its heat-dependent release for the preparation of wound healing patches.

Realistically, the surfactant used in the synthesis of AuNRs, CTAB, should be completely replaced by a different capping agent with positive charge, for improved uptake. The removal of CTAB would also improve the biocompatibility of the system. Fibrinogen also needs to be studied in the presence and absence of AuNRs to determine the response of these particles to laser irradiation and to determine their aggregation dynamics. There also need to be more studies on how fibrinogen-AuNRs complexes respond to temperature variations, with characterization methods that can determine the size and shape of the system in real time. A second set of AuNRs will need to be synthesized that absorb at different wavelength, resonant with commercially available NIR low power laser. Ligand exchange will be performed on these particles to induce a negative charge on the surface of the AuNRs so that thrombin can electrostatically bind forming protein coronas. The laser release study will need to be conducted at body temperature to determine if the proteins can be released from the particles even after the initial change in morphology/aggregation of proteins from body temperature alone. Protein release needs to be quantified using an ELISA kit, which can detect ng of fibrinogen.

The two sets of AuNRs will be embedded in a flexible patch, which includes Matrigel, a commercially available gel-like protein mixture containing the components of the ECM, which could potentially facilitate the wound healing process since the role of ECM is to support and maintain cell growth. As Matrigel gellates at body temperature, the diffusion properties of AuNPs need to be studied as well as the optical properties, to ensure that the laser light reaches the AuNRs without attenuation and to determine if the AuNRs still possess an L-LSPR in the NIR. Other considerations include how quickly Matrigel gellates to determine if it is feasible to release AuNRs to the site of the wound where they are needed.

As AuNPs become popular in biomedical applications because of their unique plasmonic properties, their heat generation could be a potential concern. We have shown that, for AuNS, a low laser power (5 mW and 100 mW) minimally raises the local temperature around the nanoparticle (by less than hundredths of a degree). We believe that the methodology outlined in the SERS experiments for AuNRs and AuNS can be employed to determine absolute temperatures around AuNPs of all morphologies. Proper modeling of our NP and matrix system using CMP would greatly aid in understanding the correlation between heat losses and temperature around NPs. Thus far groups have performed 2-D simulations with AuNPs, but none have shown temperature distributions around NPs, to the best of our knowledge. This information about local heating and overall heating of a system over a period of time is critical information for plasmonic particles in biomedical applications.

Typically carbon-based or ceramic materials have been used as reinforcement materials in polymer nanocomposites. Although these materials have been shown to drastically improve the mechanical properties of polymers (such as modulus or hardness) when polymers have been grafted to the NPs, the individual studies do not provide a complete picture of the role of the nanoparticles and their capping agents interacting with the surrounding matrix. There have not been studies that directly compare the results of maintaining a set nanofiller material and altering the shape of the nanofiller as well as the polymer grafting material, possibly because of the intrinsic difficulrty of accomplishing the study with only one class of materials. Although more studies need to be conducted to determine the grafting density on AuNPs, and how the NPs interact with PI to promote SIC, our work provides the foundation for using a single class of nanomaterials, i.e. metallic nanoparticles, to understand nanoparticle/matrix interactions at a fundamental level.

Our use of a SIS BCP was unconventional as we used it to form a layered structure instead of a blended material with one of the homopolymers (PI or PS). The dip molding method still allowed for the strong bonding between the interface of the SIS and the PI as well as the successful incorporation of the AuNPs. Our work shows how the method of deformation (uniaxial tension or nanoindentation) provides different responses by the material due to stretching of PI chains and probing surface effects of the composite, respectively. Although the final application of this work was not intended for puncture resistant gloves, it could potentially be used for such an application, and is actually ideal if the outer layer (with the SIS and stiff nanoparticles) indeed provides increased puncture resistance.

We have demonstrated how we can tune the bonding between the nanofillers and surrounding matrix via ligand exchange using variable molecular weight thiolated PS to cap AuNPs. We have shown how incorporating AuNR into PI/SIS have drastically improved the properties of the composite materials, compared to incorporating AuNS. Our characterization methods of nanoindentation and tensile testing has allowed us to gain an understanding of how the deformation of the composites in uniaxial tension allows the shape effects of the nanofillers to play a major role in the enhanced modulus of PI.

A layered structure could benefit from an application such as gloves because the tensile properties of the nanocomposite that still possesses low modulus at low strains, which indicate a high comfort level to the glove wearer, while the outer layer provides a surface that resists deformation. In addition, the SIS layer appeared to be hydrophobic compared to PI, which could lead to the use of the material in applications that require water resistant surfaces with a rubbery material on one or both sides.

Our work has shown how AuNPs can be incorporated in biological and synthetic polymers for biomedical applications such as wounding and in nanocomposites to understand the mechanism of reinforcement provided by nanofillers. As nanotechnology gains more attention, we believe that our work has opened up new applications of AuNPs.