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Biomass Assisted Synthesis of Antibacterial Gold Nanoparticles and Commentary on its Future Potential and Applications in Medicine

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BIOMASS ASSISTED SYNTHESIS OF ANTIBACTERIAL GOLD NANOPARTICLES AND COMMENTARY ON ITS FUTURE POTENTIAL AND APPLICATIONS IN MEDICINE

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By

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ABSTRACT

Xylose is a natural monosaccharide found in biomass such as straw, pecan shells, cottonseed hulls, and corncobs. Using this monosaccharide, we report the green synthesis and characterization of biocompatible, biodegradable xylose encapsulated gold nanoparticles (Xyl-GNPs) with potential antibacterial activity. GNPs were synthesized using the bioreduction property of xylose on the chloroaurate anions in an aqueous solution at room temperature and at atmospheric pressure. The characterization of synthesized GNPs was examined by UV-vis spectroscopy; transmission electron microscopy (TEM), energy-dispersive x-ray spectroscopy (EDS) and fourier transform infrared spectroscopy (FTIR). Results indicate that the particles were stable; near spherical in shape with an average diameter of 15 ± 5 nm. Microbiological assay results showed the concentration dependent antibacterial activity of these particles against Escherichia coli. Thus the facile, environmentally friendly Xyl-GNPs have potential application in the biomedical field, particularly in the development of alternative antibacterial agents.

Key Words: Biomass, gold nanoparticles, green synthesis, antibacterial activity, microbiological assays, propidium iodide (PI).

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I would like to thank the many people that not only helped make this research possible, but also those that have had a positive and supportive influence in my life endeavors and encourage me to constantly set new goals and seek greater potential. First on this list is Dr. Rajalingam Dakshinamruthy. Without his steady, firm push and the immense insight he has in science and investigation, I would not have such a full and encompassing education in science.

Second, I would like to thank Mr. Vivek Badwaik for taking me under his wing to guide me into my research career and his immense patience along the way. I would also like to thank the other members of Dr. Dakshinamurthy's lab for every unique perspectives and the friendship.

Thirdly, I would like to thank the Honors College at WKU for the support and for providing an environment that encourages and believes that the potential of its students is limitless. Without the support and push from the enthusiastic Honors College Faculty, I would have been much too timid to go after and accomplish a majority of the goals I have set through my college career.

Finally, I would like to thank my family. Throughout my life, my father has expected nothing short of excellence in my education, and this has fostered my

expectations of myself. Without such high expectations I would not have found my full potential. My mother has always lovingly expressed how proud she is of me. She has also managed to raise not one but three stubborn, ostentatious boys, which is a feat of its own. The rest of my family has also provided nothing less than 100% support and encouragement to me throughout my life.

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PREFACE

> "Science is built up of facts, as a house is built of stones; but an accumulation of facts is no more a science than a heap of stones is a house." -Henri Poincaré, Science and Hypothesis, 1905

As an undergraduate, the research I have completed with Dr. Dakshinamurthy has been a valuable learning experience that has given me a more unique and alternative experience of science than the ordinary classroom lectures of most students. The hands-on experience, strategic problem-solving, and thought provoking results provide the fundamental backbone for what science is all about. Just as a heap of stone is not a house, an education in science cannot be complete without diving into the world of scientific investigation. This is why the research I have completed and am presenting truly is a Capstone Experience for my education at WKU.

Although I am very grateful for the research experience I have received, what excites me even more is the implications of what has been accomplished. As a future professional in the medical field, my underlying motivation for research was to positively influence the world of medicine. As you will soon read, my research has no patients, and no clinical trials of any sort. However, I hope the final result of this work will serve as a catalyst for future medicinal research.

Nanomedicine is a field that I believe shows tremendous potential. However, just with as with all technology, the field must overcome many barriers before benefits can be enjoyed. I hope that the evidence set forth in this research will remove some of those barriers to entry, as to allow the advancement of medicine for patients around the world.

PART I

CHAPTER 1

INTRODUCTION

Gold nanoparticles (GNPs) are becoming increasingly significant within the realm of medicine, pharmaceuticals and ecology.[1,2] Within these arenas, the prevention and treatment of various infections has been one of the biggest challenges that still continues today.[3,4] The potential benefits that GNPs can provide as an antimicrobial agent are exponentially growing the demand for GNPs.[5,6] This raises the need for an efficient and preferably environmentally friendly method to produce biocompatible GNPs on a large scale. Currently, to obtain GNPs, various wet chemical methods employing a number of polar and nonpolar solvents have been used. The most common method is the reduction of tetrachloroauric acid ($HAuCl_4$) using an excess of sodium borohydride ($NaBH_4$) or sodium citrate in the presence of stabilizing/capping ligands such as citrates, thiolates, hydroquinones, amines, phosphanes, carbonyls, dendrimers, or other surfactants.[7-9] However, the use of these chemicals leave the synthesized GNPs with trace amounts of detrimental organic solvents. These methods raise environmental concerns and also limit the biocompatibility and biomedical applicability of GNPs.

The need for a green method to synthesize biocompatible GNPs can be fulfilled by utilizing biomass. Biomass is a renewable energy source derived from living or previously living biological material.[10] The biomass used in this study was xylose, a six membered monosaccharide found in plant parts such as straw, pecan shells, cottonseed hulls, and corncobs. Xylose is the building block monosaccharide of xylan and other hemicelluloses that coat the crystalline cellulose cores of cell wall microfibrils, and constitutes roughly 30% of plant matter. Xylose is also one of the eight essential sugars needed for optimal health and functionality for the human body. Moreover, according to research, xylose may help prevent cancer in the digestive system and may also possess antifungal properties.[11,12] Xylose has the necessary reductive capabilities, due to the presence of aldehyde groups, which can be used to reduce free gold ions and form gold nanoparticles.[13]

In this study, using xylose, we have synthesized near spherical, near homogeneous xylose encapsulated gold nanoparticles (Xyl-GNPs) in aqueous media in a single step. Because of the rapid degradation and renewable nature of the xylose, this method is of no detriment to the environment and more economical than previous methods for synthesis of GNPs.

Also, the successful antimicrobial agent requires functional ingredients that have antimicrobial activity on the surface of nanoparticle, and accompanied by an ease of fabrication and low toxicity.[14,15] GNPs with xylose as a functional ingredient give the advantage of biocompatibility and an ecofriendly nature. Considering the

previously mentioned facts, we evaluated Xyl-GNPs for their antibacterial activity and found them to possess significant efficacy.

Here, we provide the evidence to show how to form xylose encapsulated gold nanoparticles in single step with a completely green ecofriendly approach. The formed GNPs were characterized using various techniques such as UV-vis spectroscopy, transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive X-ray spectroscopy (EDS), and Fourier transform infrared spectroscopy (FTIR). The bactericidal properties of Xyl-GNPs were evaluated using various microbiological assays. The mechanism was confirmed by using TEM images and confocal fluorescence microscopy.

CHAPTER 2

MATERIALS AND METHODS

Reagents and Material: Chemicals including $KAuCl_4$/ $HAuCl_4$, D-(+)-Xylose, LB agar and dextrose were purchased from Sigma-Aldrich Company , St. Louis, MO*. E. coli* was purchased from Life Technology Company: Invitrogen, Carlsbad, CA. Analytical grade chemicals were typically used for the following experiments.

Synthesis of xylose reduced 15 nm gold nanoparticles: GNPs were prepared using xylose as both a reducing and capping agent employing a simple, single step environmentally friendly method. In this method, xylose was added to 10 mL of media (pH ~7± 0.2) in a test tube to make the final xylose concentration 100 mM. To this solution, 100 mM stock solution of pure potassium aurochlorate ($KAuCl_4$) was added to reach a final concentration of 0.5 mM. This solution is then kept in an incubator at 37 °C and shaken at 150 rpm. Within one hour the light yellow solution changes to a light red color, indicating the dissociation and reduction of $AuCl_4^-$ to Au^0. After 6 hours, the solution turns to a completely red color, indicating the formation of gold colloids. To obtain the pure nanoparticles, the samples were collected and centrifuged at 12,000 revolutions per minute for 4 minutes. The clear

supernatant was discarded and the pellet that formed was again suspended in nanopure water. This was repeated several times to get pure nanoparticles.

Characterization of GNPs: Surface plasmon resonance for the GNPs was observed, by UV-visible (UV-vis) spectroscopy using PerkinElmer's LAMBDA 45 UV-vis system. To further confirm the shape/size of GNPs, transmission electron microscopy (TEM) was performed using JEOL-TEM. For TEM sample preparation, 4µL of a 1:4 (GNP stock: water) dilution of the particle solution was added onto formvar coated copper grids, air-dried and then analyzed using 100kV. Energy dispersive X-ray spectroscopy (EDS) was performed using a JEOL JSM-5400 LV with a IXRF system to determine the elemental composition of GNPs. For performing EDS analysis, the thoroughly washed nanoparticles were placed onto the aluminum stub and dried completely in a desiccator. FT-IR spectroscopy study has been carried out with Perkin-Elmer Spectrum 100 FT-IR spectrometer with Fourier transformation in 4000 to 400 cm^{-1} frequency region using single reflection diamond ATR accessory. The samples for FTIR were thoroughly washed with nanopure water several times.

Antibacterial Activity of Xylose Encapsulated GNPs: The antibacterial activity of Xyl-GNPs was tested by performing turbidimetry and plate assay against *E. coli*. Turbidimetry was performed to measure the cell growth, and plating assays were performed to compare the viability of cells against different concentrations of Xyl-GNPs. [16,17] To determine the bacterial growth rate, the bacteria were grown in minimal liquid media (M9 media) with different concentration of GNPs (0.2 mg mL^{-}

^{1}, 0.4mg mL^{-1}, 0.8 mg mL^{-1}, 1.6 mg mL^{-1}, 3.2 mg mL^{-1}). The bacterial growth was monitored by measuring the optical density (OD) at 600 nm with respect to time. The OD was measured by using Thermo Scientific's SPECTRONIC spectrophotometer. The scattering due to nanoparticles was corrected by taking blank readings. The samples were made by adding miniscule amounts of bacteria from the overnight culture to the 5 mL of media into culture tubes containing different concentrations of Xyl-GNPs. The bacteria were allowed to grow for 12 hours at 37 °C in the incubator and shaken at 150 revolutions per minute. To determine the viability of cells, a separate set of experiments was performed in which the bacterial cells were grown in the liquid minimal media containing various concentrations of GNPs. After 12 hours of incubation time, the culture medium were diluted to 10^{-4} times and spread on agar plates. Percentages of viable cells were determined by counting the colonies and plotted against various concentrations of GNPs.

Morphology of untreated and treated bacterial cells: TEM images were observed to determine the morphological changes occurring in the bacterial cells treated with GNPs. 4 µL of both control, as well as GNP treated *E. coli* cultures, were collected at different time points and placed directly on the copper grid. These grids were then washed with 10 µL of water. The excess water from the washed sample was then removed using filter paper, and the dried sample was spotted under TEM at 80 kV.

Confocal laser scanning microscopic studies: To monitor the fluorescence from the GNP treated samples, confocal microscopic studies were performed on an

Olympus confocal laser scanning microscope.[18] Propidium iodide (PI) is a fluorescent dye that fluoresces only after binding with nucleic acid, but it is impermeable through normal *E. coli* cell walls and only dead cells will fluoresce with this dye. Therefore, PI was used to determine the relative proportion of living and dead cells. In a typical method, 1 ml of overnight (12 hours) grown GNP treated and untreated (control) bacterial cells were collected. The samples were then washed twice by 10 mM PBS buffer (pH~ 7.2). To these samples, 10 µL of 10 mM PI was added, gently mixed and incubated for 1 hour. This entire sampling process was carried out in a dark room. After the incubation, the samples were washed with PBS to get rid of excess PI dye and clear excess fluorescence in the background. 5 µL of the above mentioned suspensions were then placed on a glass slide, mounted with a coverslip, and analyzed under a confocal scanning microscope.

CHAPTER 3

RESULTS AND DISCUSSION

For the preparation of gold nanoparticles, a reducing agent is required to convert $AuCl_4^-$ ions into Au^0 nuclei, which then combine to form colloids. But to control the shape and size of these colloids, a capping agent is required.[19] People have been using various reducing and capping agents to get nanoparticles with controlled shapes and sizes, but the environmental hazards of these chemicals limit their applicability. Also, nanoparticles are widely used in nanomedicine, which requires biocompatibility of the nanoparticles, as well as the ligand molecule (capping agent). Due to the toxic nature of most organic ligands, their application is very limited. The application and sustainability of gold nanoparticles in medicine is dependent upon a completely green method utilizing biodegradable and biocompatible chemicals. In this report, we used xylose as a reducing element for the formation of nanoparticles. Xylose is a sugar, classified as a monosaccharide of the aldopentose type. It is one of the main components of plant biomass constituting roughly 30% of plant matter. Xylose can be easily absorbed in the small intestine, where it helps to increase the absorption of other nutrients effectively.[12] It is an

important sugar for optimal health and body function in humans. Xylose is clearly a promising candidate for applications in green chemistry and nanomedicine.

Synthesis and characterization of xylose encapsulated gold nanoparticles:

Xylose possesses free hydroxyl and carbonyl groups, which provide its reducing nature.[13] In this method, the reducing nature of xylose was used to reduce free Au^{3+} into Au^{0}. For the preparation of nanoparticles, a 300 mM solution of xylose in phosphate buffer (pH~ 7.2) was made and the appropriate amount of $KAuCl_4$ stock solution was added to make the final concentration 0.5 mM. This reaction was placed in a test tube and shaken at 150 revolutions per minute at 37 °C in an incubator while under atmospheric pressure. Within one hour the color of the solution changed from light yellow to light pink, indicating the reduction of free gold ions. As the reaction proceeded, the shade of the solution gradually darkened to a dark pink color upon completion (12 hours). The surface plasmon resonance studies using UV-vis showed the λ_{max} at 541 which is typical for spherical nanoparticles in the range of 10-40 nm (Fig. 1A). To confirm the morphology, the samples were observed under TEM, which showed particles that were spherical in shape with an average diameter of 15 ± 5 nm (Fig. 1B).

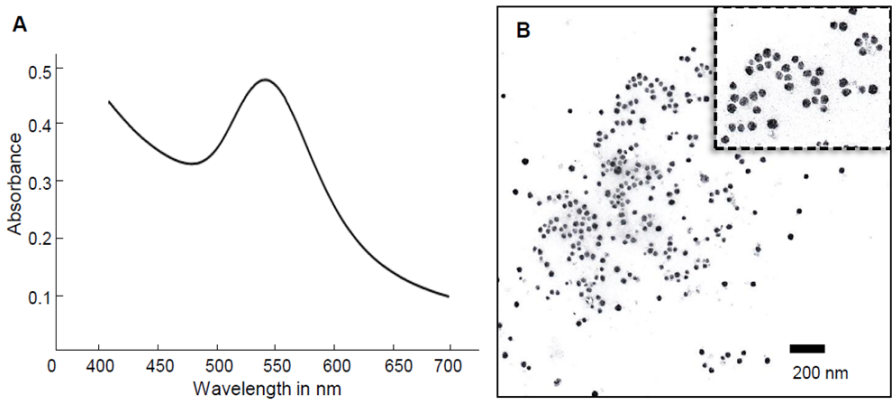

Figure 1: (A) Typical TEM image of xylose encapsulated gold nanoparticles. The nanoparticles formed were spherical with the size 15± 5 nm (scale bars correspond to 200 nm); (B) The surface-plasmon absorbance spectrum of Xyl-Au nanoparticles formed in the aqueous xylose dispersion. (λmax = 541nm)

Thus, the surface plasmon resonance results were in agreement with the TEM observations.

Elemental analysis was done using EDS, and showed the presence of ~85% metallic gold and ~15% of carbon, which confirms the presence of sugar on the surface of the GNPs (Fig. 2A). FT-IR spectra of Xyl-GNPs were different from FT-IR spectra for the pure xylose solution. The absorption bands characteristic for stretching and deformation of O-H bonds in hydroxl groups and water molecules appear at 3269 cm^{-1} for pure xylose solution (Fig. 2B-I), while that shifts to a lower wavelength of 3324 cm^{-1} for Xyl-GNPs (Fig. 2B-I). This hypochromic shift confirms the interaction of xylose molecules with the nanoparticle surface through weak electrostatic interactions.

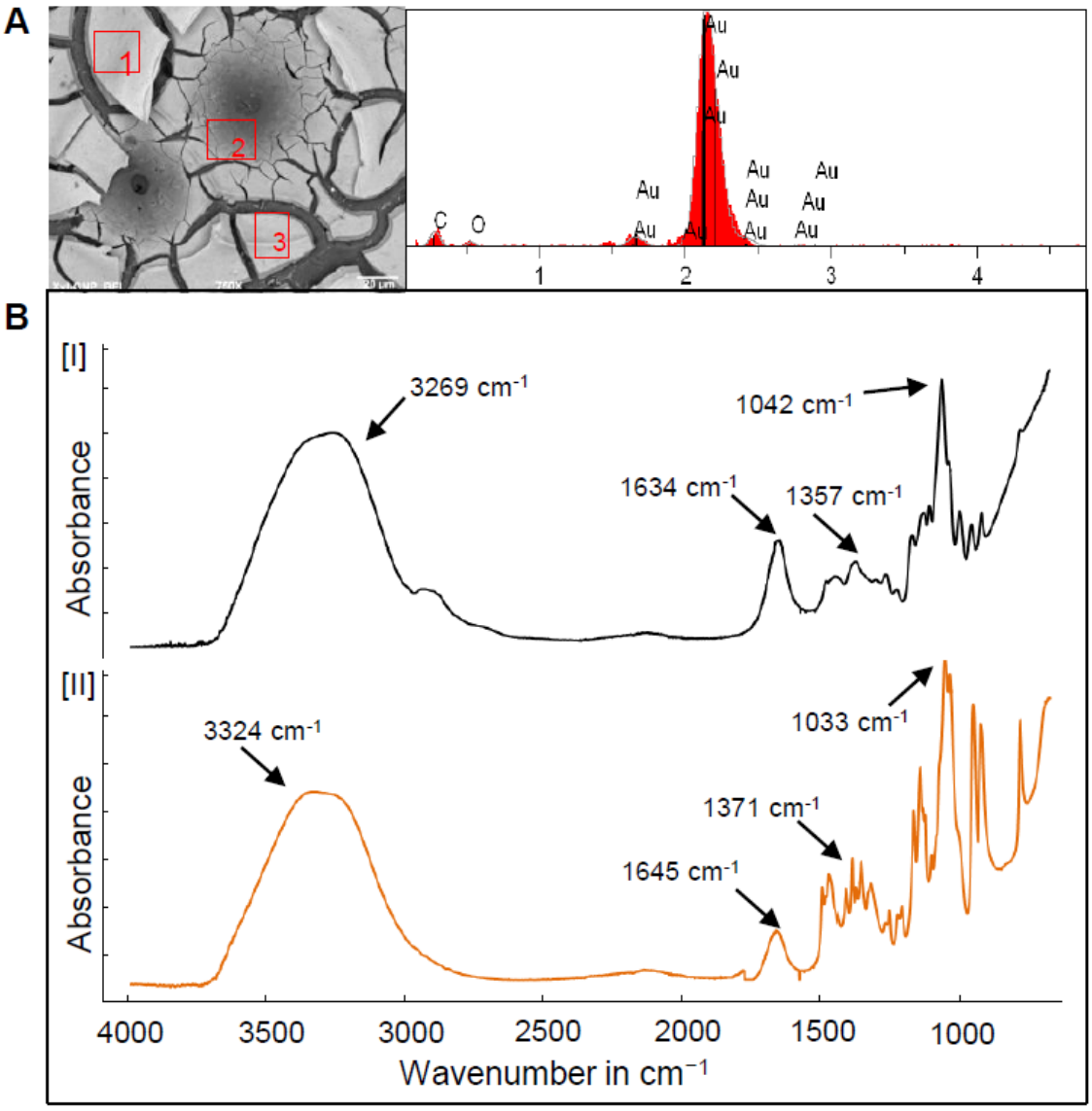

Figure 2: (A) EDS spectrum for Xyl-GNPs for region 3 showing 85% of Au element, which is to be expected for gold nanoparticles and 15% carbon confirming the presence of a sugar coating on the GNPs; (B) TGA data recorded from carefully weighed powders of purified Xyl-GNPs (rigid), and pure xylose (broken).

The biomedical applications of nanoparticles demand high salt concentration resistant GNPs.[20] To test Xyl-GNP applicabily in this context, the thoroughly washed

GNPs were suspended into 10 mM PBS (pH~ 7.2), and their stability was monitored with respect to time. The stability of the Xyl-GNPs was determined by examining the effects of 10 mM PBS (pH~7.2) on their shape and size using TEM, and surface plasmon resonance using UV-vis spectroscopy. The results were monitored for more than 96 hours. Results suggested that the GNPs were relatively stable with no significant color change and no significant bathochromic shift. TEM analysis also showed no difference in the shape and size of GNPs (Fig. 3A, B). Overall results suggest that Xyl-GNPs are stable in a highly saline environment.

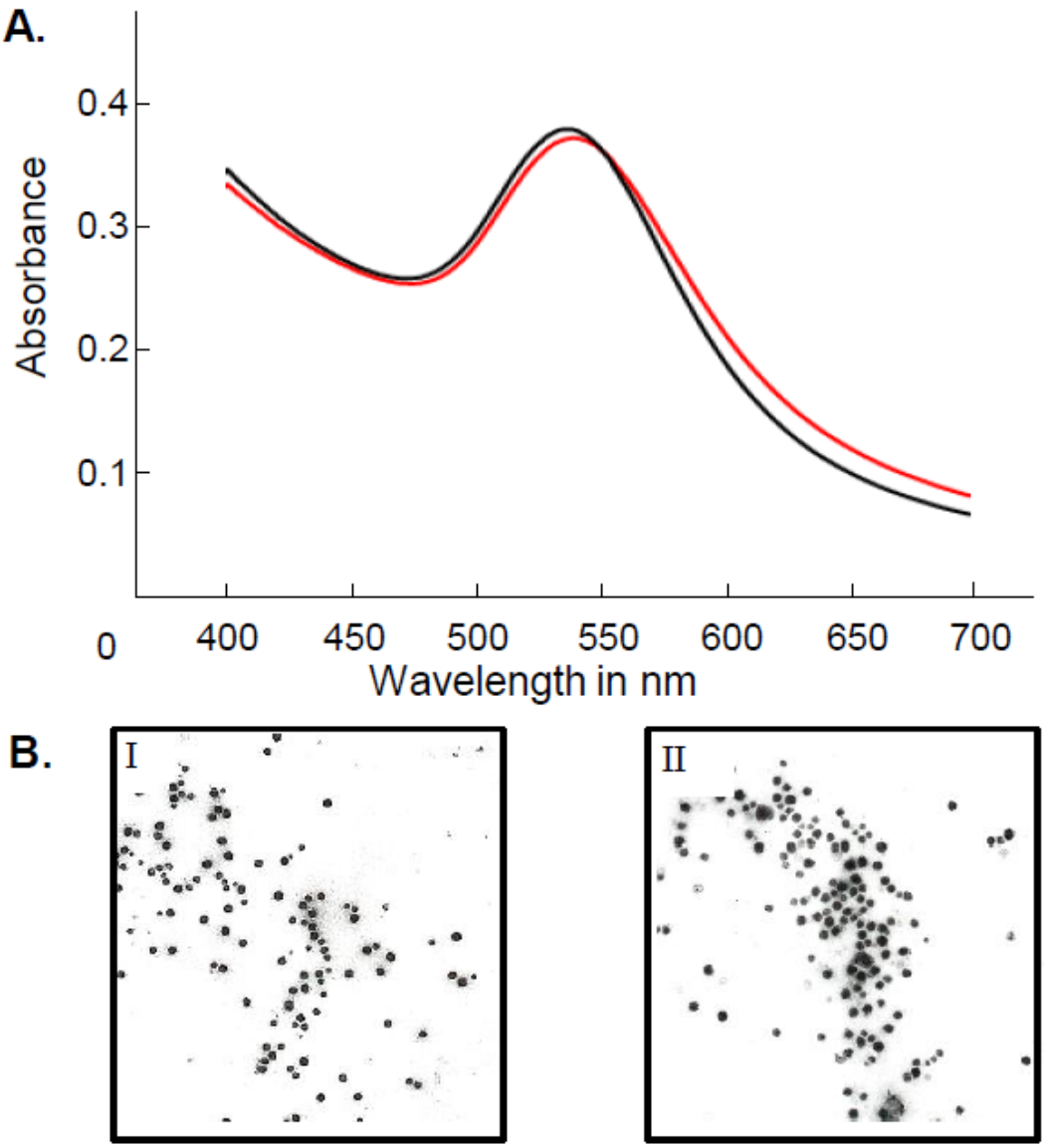

Figure 3: (A) UV-vis spectra for Xyl-GNPs suspended in distilled H_2O (black) and PBS with pH~7.2 (red); (B)The GNPs were suspended in the respective solution for more than 96 hrs; (B-I) TEM images of Xyl-GNPs in PBS before and (B-II) after 96 hrs.

Antibacterial activity of xylose encapsulated nanoparticles

We performed various microbiological assays against *E. coli* to evaluate whether the Xyl-GNPs possess antibacterial activity or not. The results of the liquid broth assay i.e., culture turbidimetry, showed significant inhibition of culture growth in the presence of Xyl-GNPs (Fig.4).

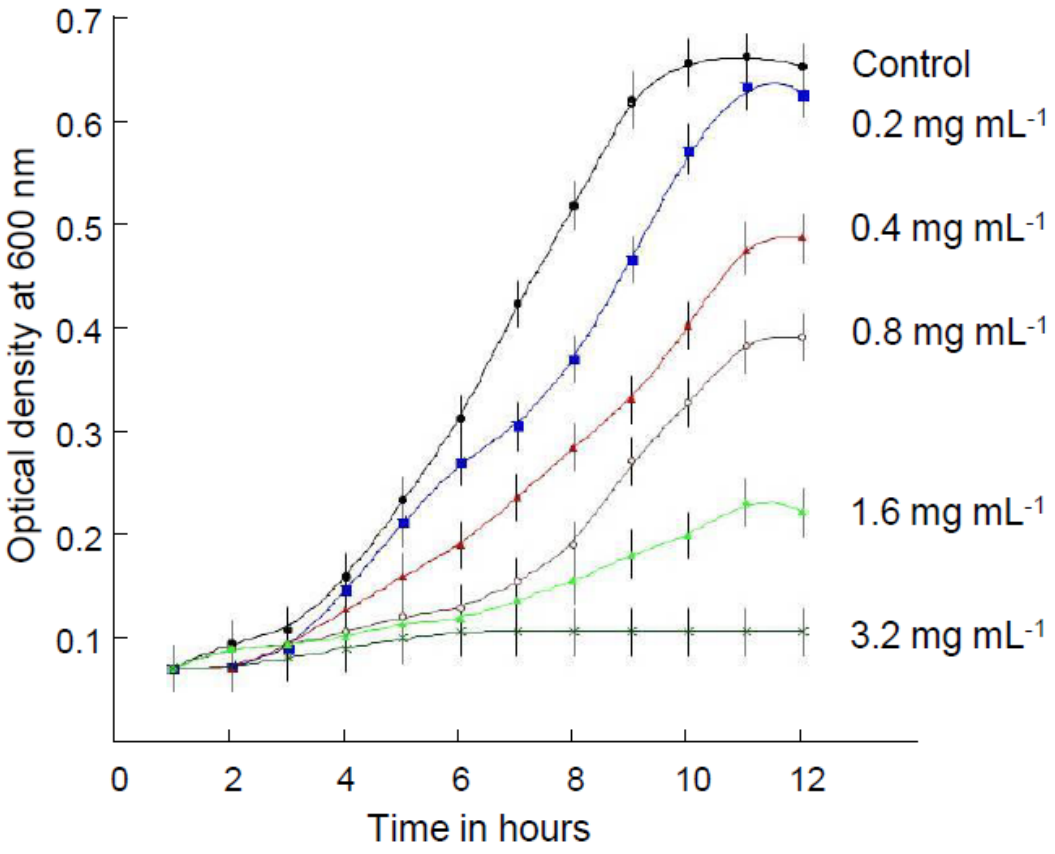

Figure 4: Effect of different concentrations of Xyl-GNPs on the Growth of *E. coli*. Growth analysis curves were measured by monitoring the optical density (OD) at 600 nm.

The effect was dose dependent and the minimum inhibition concentration (MIC) was found to be 3.2 mg mL^{-1}.

In order to verify the viability of cells treated with Xyl-GNPs, a separate experiment was performed. In this experiment, the bacterial cells were grown in the presence of various concentrations of GNPs and after 12 hours the cultures were placed on agar plates. The percent of viable cells were determined by counting the number colonies and this was compared across cultures treated with differing concentrations of Xyl-GNPs(Fig. 5A, B).

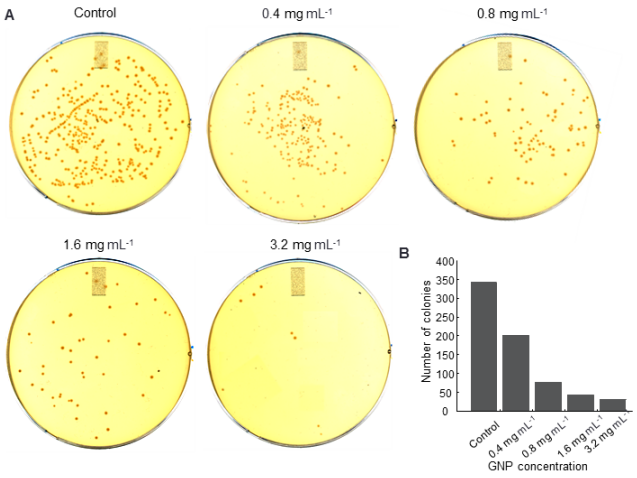

Figure 5: (A) Plate assays showing the number of viable cells recovered after the treatment of E. coli without (control) and with different concentrations of Xyl-GNPs. The bacterial cells were grown in the presence of various concentrations of GNPs and after 12h cultures were diluted to 10^{-7} times and placed on agar plates. (B) Plot showing the percentage of viable cells against the Xyl-GNP concentration the cultures were treated with. Percentage of viable cells was determined by counting the colonies.

The results were in agreement with the turbidimetry results, which showed the significant decrease in number of recovered viable cells with an increasing doses of

Xyl-GNPs. Also, the viable cell count for 1.6 mg mL^{-1} was found to be less than 1%, as compared to that of the control plate.

Mechanism of antibacterial activity

The morphology of bacterial cells treated with GNPs in liquid media were examined at different points in time and compared to the untreated or control cells. For the control cells it was found that the cell structure remained intact at different times even after the stationary phase (18 hours-Fig. 6A). But the examination of nanoparticle treated cells showed significant morphological changes. At the time of initial mixture of GNPs with *E. coli*, the nanoparticles were observed to interact by adhering to the surface of *E. coli* (Fig. 6B). After 6 hours of treatment the GNPs were able to perforate into the cell wall (Fig. 6C). Subsequently, these perforations cause lysis of the cell wall leading to complete disruption of the bacterial cell eventually (Fig. 6D). [21, 22]

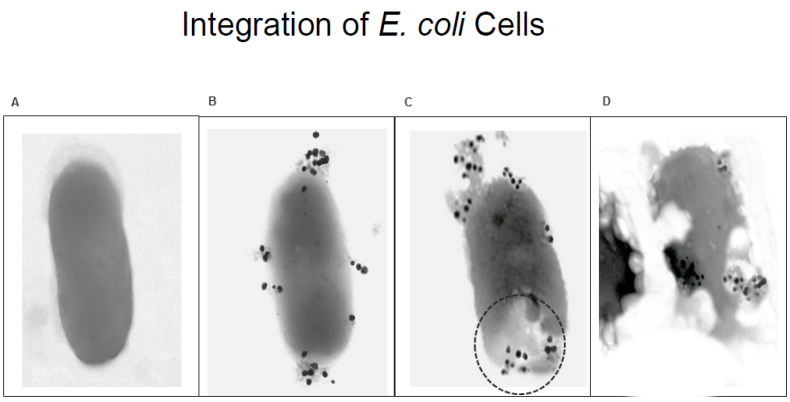

Figure 6: Visualizing GNP induced morphological changes of cell membranes via TEM. (A) Morphology of the untreated *E. coli* cell; (B) Morphology of the GNP treated *E. coli* cell at the time of initial mixture of GNPs with bacterium showing the interaction of GNPs with the *E. coli* cell membrane; (C) treated *E. coli* cell after 6hr depicting the initiation of perforations in the cell membrane and cell disruption; (D) complete cellular disruption occurred after 12 hr of interaction of *E. coli* with Xyl-GNPs

To further confirm the bactericidal action of GNPs, confocal laser scanning microscopy was used. Propidium iodide (PI) is a fluorescent dye that fluoresces only after binding with nucleic acid. This dye is impermeable through normal *E. coli* cell walls and can only pass through permeable (dead) cell walls. Thus, fluorescence due to PI represents the permeable cell walls, hence the relative proportion of living and dead cells. The results suggested a relatively increasing amount of permeable cells with increasing concentrations of Xyl-GNPs (Fig. 7A). The permeable cell percentage for nanoparticle concentrations of 3.2 mg mL^{-1} was found to be about 90% (Fig. 7B). The control, i.e. untreated cells, did not show any fluorescence. Thus, experimental

results clearly illustrate the bactericidal action of GNPs through cell wall lysis.

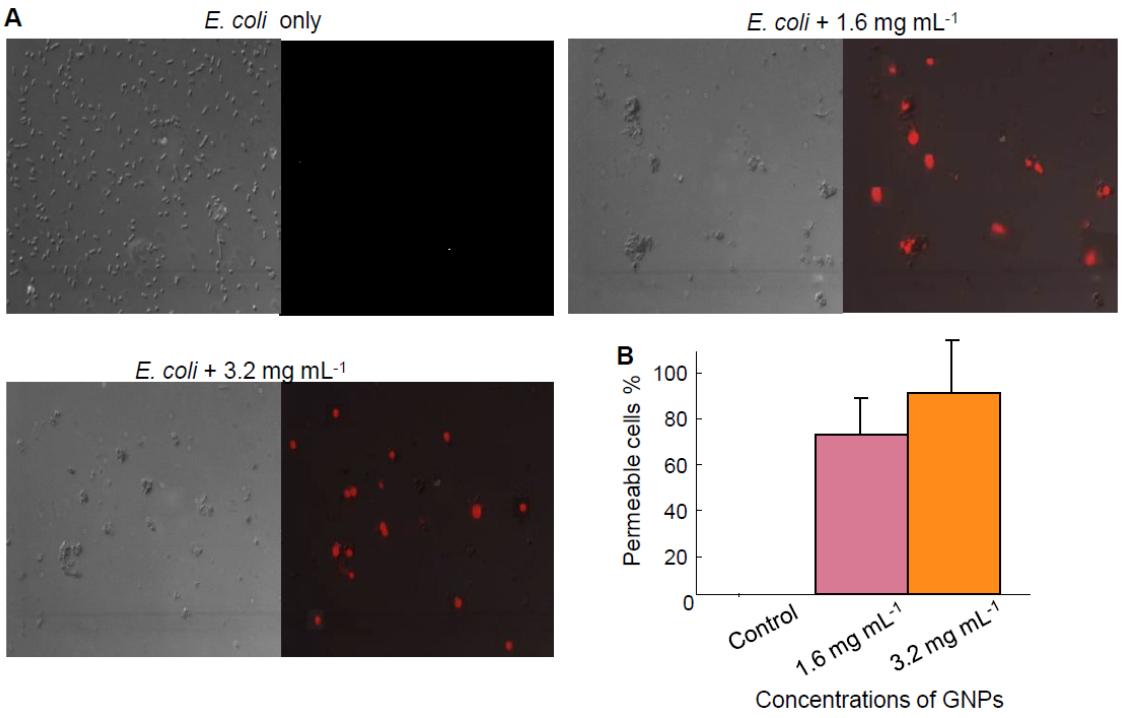

Figure 7: Monitoring Xyl-GNPs induced permeability of *E. coli* cell membranes and leakage of nucleic acids via propidium iodide fluorescence. (A) For each image, the left half (grey) shows an image in the differential interference contrast mode, while the right half shows the corresponding fluorescence image. (B) The percentage of cells with permeable membranes was measured from five or more fields of view from two independent experiments.

CHAPTER 5

CONCLUSION

This report shows the formation of spherical, highly monodispersed gold nanoparticles with a size range of 15 ± 5 nm, using xylose as both a reducing and capping agent. The method was shown to be a completely green, single step, single phase process performed at room temperature and atmospheric pressure. The nanoparticles were biocompatible and stable at high salt concentration. Xyl-GNPs have been shown to possess significant dose dependent antibacterial activity. The bactericidal action was found to be due to lysis of the prokaryotic cell wall, which eventually leads to cellular disruption. In brief, we report a completely green synthesis of biocompatible, spherical, highly monodispersed gold nanoparticles having significant antibacterial activity.

PART II

CHAPTER 6

NANOMEDICINE

In scientific terms, the prefix nano- means one-billionth of something. Scientists put this jargon to work in front of anything from seconds to liters, but for the purpose of my research, I am concerned with lengths. A nanometer is one-billionth of a meter. To put this in perspective, a single human hair is approximately 50,000 nanometers in width.[23] The scales of magnitude are unimaginably small, but that is one of the main reasons for the vast potential of nanotechnology that I will discuss later.

There are several realms of the nano world that pertain to both my research and my interest in medicine. The first of these is "nanomaterials." The working definition for a nanomaterial is anything that has at least one dimension within the scale of 1 to 100 nanometers.[24] This means that a nanomaterial could be nano in one, two, or three dimensions. A one-dimensional nanomaterial would be analogous to a piece of paper, having large measurements of length and width but extremely thin. A two-dimensional nanomaterial can be compared to a single hair that can have a large length but is extremely thin on all other sides. The final

possibility would include only particles that are less than 100 nanometers on all sides. This can includes nano-spheres, cube, pyramids, etc.

Nanotechnology is a second area that must be explained in order to gain perspective on this research. Nanotechnology is the manipulation of materials at the nanoscale.[24] It can be inferred from this definition that it takes nanotechnology to make and use the nanomaterials mentioned above.

Nanotechnology is different than other technology because of the size of the objects that are used. With most technology we are able to build things by altering materials we already have at hand, like wood or steel or plastics. These materials can be easily cut, bent, or molded into whatever form is needed for their function.[25] However, with nanomaterials, individual atoms must be manipulated into precise locations for the material to work correctly. There is physically no way that this can be accomplished because even "touching" atoms would bring interactions that would make it difficult to "let them go," much less move them to a location that must be accurate within nanometers in order to work properly.

Chemistry, thus steps in to take over where physical manipulation fails. The solution is "self-assembly."[25] This technique brings chemistry into the equation to create an environment where atoms are attracted toward specific locations where they can bond (or be locked into position) permanently in a location that is necessary for the structure as a whole to function. More simply, it is the ability to mix chemicals in a certain way so they gravitate into the position needed to build what we want. This is the primary premise that my research addresses.

The final field to be discussed, that should not be confused with the other two, is "nanomedicine." According to *The New England Journal of Medicine* "nanomedicine aims to use the properties and physical characteristics of nanomaterials for the diagnosis and treatment of diseases at the molecular level."[25] This definition requires that both nanomaterials and nanotechnology be researched and understood in order to optimize everything that nanomedicine has to offer.

It is important to realize that the abilities offered by applying nanomaterials and nanotechnology to medicine cannot be offered by any other medium. This is because nanomedicine is unique in a number of ways.

CHAPTER 7

UNIQUE POTENTIAL

One of the unique qualities in the field of nanomedicine is due to the size of the materials being used. The properties that materials have in a 'nano' form are much different than they are when in a bulk substance. The most common and simple example of this is within the realm of my research, gold. It was found centuries ago that gold dissolved into a liquid had different properties than the gold that you and I think typically think of today.[26] At the nanoscale, gold physically changes color from its shiny finish and gains magnetic and semiconductive properties as well. Because particles like gold act differently as a nanomaterial, it opens up an immense number of options that were not previously available. Before steel had ever been a possibility in construction, we were limited to building homes and structures from bricks as long as they did not crumble under their own weight. Because steel is became an option we expanded our limits many times further. This example may be a stretch but it gets the point across that nanomedicine opens up an immense number of options that were not previously known. I have been studying gold for two years, but there are a number of other possibilities with perhaps different utilities based on their characteristics.

A second quality unique to nanomedicine is an enormous surface area to volume ratio. This may seem preposterous and insignificant, but in biology, the ratio of these two measurements are extremely significant.[25] To show the importance a comparison can be drawn between a biological cell and a steak. While it may sound crazy, I can clarify its relevance. If there are three steaks, one cut thick and two cut half as thick as the first, and they are all put on a grill at the same time, which steaks will cook the quickest? Obviously the two smaller ones will be done first. If four steaks that were each one-fourth as thick were cooked, or eight steaks that were one-eighth as thick, then the difference in time would continue to increase between the original larger steak. The reason this is so is because as the steaks become smaller, the exposure of the interior increases, and in turn the interior is exposed to heat much more quickly. Nanomaterials take this phenomenon to the extreme. They are so small that the exposure is increased orders of magnitude, making them highly reactive with the environment around them.[25] This property can only be accomplished with nanomedicinal techniques because it requires such small size. No other route can be utilized to take advantage of such high reactivity.

A third quality making nanomedicine inimitable is the nature of biology and life itself. Nature utilizes very small structures to accomplish the task at hand. Infectious agents and pathogens are minute structures that are nanometers in scale.[25] By manipulating materials on the same scale as pathogens, we can mimic nature to fight disease. The difference between fighting disease with nanomedicine instead of surgical treatment can be immense. There is a greater opportunity to

fight disease by leveling the playing field and fighting viruses and carcinogens by mimicking their behavior. This possibility extends the toolkit that doctors have at hand in order to more effectively treat their patients.

The advantages of nanomedicine due to the qualities of the materials can be summed up by: differing characteristics of nanomaterials over their bulk counterparts, high reactivity because of the surface area to volume ratio, and mimicry of nature on the molecular scale. All of these require that technology and materials of extremely small proportions be investigated and understood.

CHAPTER 8

APPLICATIONS

Nanomedicine is inimitable not only because of the qualities it offers, but the applications it has shown to be effective in were not conceivable with conventional medicine, as well. Previously one of the only uses of nanomaterials was as labels for electron microscopy purposes. It has been only recently that the enthusiasm is building over their medicinal potential.[26]

A significant application that nanoparticles can be utilized for is drug delivery. Conventional medicine deals with drug delivery by prescribing medicine that is in some way or another (e.g. orally, topically, injections) absorbed into the body and diffuses throughout the body without direction. Eventually, a small fraction of the drug reaches the tissue it was meant for. Nanoparticles can offer something better. One of the key features of nanoparticles is the relative ease of their modification.[27] This allows for the ability to simply stick drug particles onto the surface of the nanoparticles. Because of the enormous surface area to volume ratio, millions of drug particles can be placed on any single nanoparticle.[26] By attaching drugs to nanoparticles, the drugs travel as a pack and are much more

effective than they would be when left by themselves to diffuse throughout the body.

Another advantage of this relatively easy modification process is that nanomaterials lend themselves to be easily masked from the immune system.[27] Any foreign substance ingested into our body besides nutritional molecules are often attacked and broken apart by the "soldiers" produced by our immune system. These defenses include antibodies produced by our immune system, white blood cells that engulf intruders, and lysosomes that release toxins into foreign bodies. The drugs we ingest are subject to these attacks as well. Conventional medicine has squelched this problem by simply overloading the dosage of drugs in hopes that a fraction of the treatment will make it through the mêlée. Treatment with nanomedicine can solve the problem entirely by fooling the body that the drugs are not foreign. The same "ID tags" that are on every cell of the body that let immune system know they are not a threat can be fused to nanoparticles, along with drug particles, to fool your immune system into believing the particles are part of your body. This increases the amount of therapeutic drugs that make it their destination, reducing the amount that must be prescribed.[26]

Nanoporous technology can be utilized to create canals in individual cells, limiting the amount of drugs that can pass through at any point in time.[28] This can allow a time restraint on drug delivery, which can be an advantage in areas that do not have immediate access to healthcare or drug supplies. Instead of having to frequently refill prescriptions, patients in rural areas or third world countries can be

given a drug dosage that can be timely and effectively administered throughout the body over a period of months instead of weeks. All of these benefits make the pharmaceutical process many times more efficient. By reducing the amount and frequency of drugs needed to treat a disease, the pharmaceutical costs of treatment can be reduced by a large factor, opening the door for modern medical care to be accessible to a larger percentage of the population.

However, this is only the tip of the iceberg with nanomaterials for pharmaceutical purposes. The same tactic that can be used to mask particles from the immune system can also be modified to give drug-covered nanoparticles a greater affinity for cancerous cells. Just as healthy tissues have "ID tags," unhealthy cells have this same component. Nanoparticles can be used for specifically and effectively targeting and unloading drugs at cancerous sites, and also avoiding spreading symptoms and toxins to healthy tissues.[25] This feat is something that chemotherapy and radiation treatments have yet to refine, so this is a clear win-win scenario for all parties.

Conventional medicine can often times be highly invasive and burdensome. One way to forgo this is by using nanomedical devices. These devices are easily ingested because of their small size and can often be even easier to remove. Research is being done using magnesium and iron nanomaterials to form nanostructures that are biodegradable to utilize for temporary purposes.[28] This solves the problem of having to frequently make appointments to get orthopedic screws and plates replaced. Instead, it will be simpler to ingest the needed

nanostructures and allow them to continually build and wear down to coincide with each time treatment is ingested. Nanosurgery can also be made possible by creating chemically modified target structures within cells without damaging the cell itself.[23] Since speed is often an issue in emergency, the small size and functionality of nanomaterials allows them to be administered topically to perform nanosurgeries at the site of injuries.[23] This would drastically reduce the cost, invasiveness, and relative imprecision that surgery imposes over nanomedical treatment.

Nanomedicine can also be used to improve medical diagnostics. Gold nanoparticles have been used in MRI applications to enhance imaging sensity by three fold and specificity by ten percent.[25] For doctors this can be analogous to the difference between seeing an X-ray without glasses versus having them on. Increased resolution is necessary to pinpoint and treat problems effectively. Never before have doctors been able to see tumors with such accuracy while still making the diagnostics process cheaper than beforehand.[25] Not only will this enhance visibility of malignant tumors, but treatment as well. Once gold nanoparticles are embedded within tumors, light can be shone upon them. Gold nanoparticles only absorb light, which in turn causes them to heat up and cauterize the tumor. Because the nanoparticles are absorbed by the tumor and not healthy tissue, only the tumor is obliterated.[27]

This is why the authors of *What is Nanotechnology and Why Does It Matter?* proclaim that "Cancer is one arena in which nanotechnology is likely to have the biggest impact, and this impact will come in terms of both improved diagnostics and

improved treatment."[23] Cancer has been stumping modern medicine for centuries and nanomedicine has given promise for a cure. This fact alone is reason enough to give nanomedicine and the nanotechnology revolution a chance to succeed. If we can pursue an option that would give cancer patients an opportunity to not only survive, but thrive, the vision is worth the venture.

This is why individuals, corporations, and governments are pouring money into research to understand how we can maximize the potential of nanomaterials. The advantages nanomedicine has for cancer treatment is enough to ignite enthusiasm in the medical arena, not to mention the effectiveness it has shown to have in stem cell research, hepatitis, tuberculosis, gene therapy, and antibacterial agents.

Another exciting application of nanomedicine concerns Alzheimer's. This degenerative disease has been outmaneuvering doctors until it was found that gold nanoparticles can be used to detect proteins in very low concentrations that are accepted markers for Alzheimer's onset.[27] Not only will this technology allow early detection of the disease, but nanoparticles can be used as treatment. The brain is covered by a highly guarded membrane that is very selective in terms of what is allowed to pass through. Nanoparticles are small enough to penetrate this barrier and attack the problems that Alzheimer's creates at their source without the invasiveness of surgery.[23] This quality of nanomedicine is opening up an entirely new realm of research in the field of psychiatry. Patients with chemical imbalances

in the brain were previously restricted to options of surgery or drugs that would merely patch over their existing symptoms instead of attacking the problem.

A final major advantage that nanomedicine has in its arsenal is in the realm of sexually transmitted diseases. Sources of this include *The New England Journal of Medicine*, *Chemical Society Reviews*, and *Angewandte Chemie International Edition*, and they have all published studies that prove gold nanoparticle effectiveness in inhibiting transmission of HIV and disrupting the virus in effected individuals to lessen the symptoms. This is a huge step in the study of HIV that can be used to help the millions of people worldwide who suffer with the virus, and keep millions more from becoming infected. This is another product of the nanotechnology revolution that should be kept in consideration when determining its potential in the future.

All of these advantages, ranging from drug delivery, cancer ablation, Alzheimer's treatment, and STD prevention, are nothing to scoff at. They all show how nanomedicine provides advancement that was unforeseeable beforehand. The last several decades have been minimally productive while cancer, HIV, and other ailments have remained steadfast and out of reach. It is befuddling to see why not to use nanomedicine to launch us forward, putting these targets within range of our capabilities.

CHAPTER 9

CONCLUSION

While none of the previous examples of nanomedicinal applications directly pertains to the research I have completed, medicine remains my endeavor and my motivation. As an undergraduate who has been thrown into this booming field of research, I am very proud and excited to be a part of something that can have an impact on future patients for years to come. I hope that this small contribution toward the understanding of nanotechnology can play an integral role to allow future advancement of care. The previous applications speak for themselves. The advancement of nanotechnology can allow researchers and physicians to take steps towards conquering diseases that have been perplexing them for decades. I hope that just as penicillin revolutionized patient care nearly a hundred years ago, the improvements that come with nanomedicine will be just as extraordinary.

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