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Inorganic Nanoparticle Nucleation on Polymer Matrices

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Inorganic Nanoparticle Nucleation on Polymer Matrices

Adrian John Kosteleski

Dissertation submitted to the
College of Engineering and Mineral Resources
at West Virginia University
in partial fulfillment of the requirements
for the degree of

Doctor of Philosophy
In
Chemical Engineering

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Morgantown, West Virginia
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Abstract

Inorganic Nanoparticle Nucleation on Polymer Matrices

Adrian J. Kosteleski

The introduction of inorganic nanoparticles into organic materials enhances both the mechanical and chemical properties of the material. Metallic nanoparticles, like silver and gold, have been introduced into polymers for use as antimicrobial coatings or dielectric materials, respectively. The challenge in creating these materials currently is the difficulty to homogeneously disperse the particles throughout the polymer matrix. The uneven dispersion of nanoparticles can lead to less than optimal quality and undesired properties. By creating a polymer nanocomposite material with well-controlled size inorganic materials that are evenly dispersed throughout the polymer matrix; we can improve the materials performance and properties. The objective for this research is to use polymer networks for the in situ mineralization of silver and other metallic materials to create intricate inorganic structures.

The work performed here studied the ability to nucleate silver nanoparticles using poly (acrylic acid) (PAA) as the templating agent. Ionic silver was chemically reduced by sodium borohydride (NaBH_4) in the presence of PAA. The effect of varying reactant concentrations of silver, NaBH_4 , and PAA on particle size was studied. Reaction conditions in terms of varying temperature and pH levels of the reaction solution were monitored to observe the effect of silver nanoparticle size, shape, and concentration. By monitoring the UV spectra over time the reaction mechanism of the silver reduction process was determined to be an autocatalytic process: a period of slow, continuous nucleation followed by rapid, autocatalytic growth. The reaction kinetics for this autocatalytic process is also reported.

PAA was crosslinked both chemically and physically to 3 biopolymers; ELP, an elastin like peptide, cotton fabrics, and calcium alginate hydrogels. Various compositions of PAA were physically crosslinked with calcium alginate gels to design an antimicrobial hydrogel for use in wound dressing applications. PAA's ability to nucleate nanoparticles in a solid matrix was displayed. Interestingly enough PAA retains its ability to nucleate nanoparticle even when its reactive functional groups are used in the crosslinking process. Silver nanoparticle composition and size on the solid polymer matrices was controlled by varying the composition of PAA. PAA and silver nanoparticles effect on the mechanical properties of the calcium alginate hydrogels were also studied. Physically crosslinking PAA with calcium alginate gels enables the development of intricate gel structures that are decorated with nucleated silver; yielding a composite biomaterial with improved and enhanced antimicrobial properties.

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Chapter 1. Introduction

The introduction of inorganic nanoparticles into organic materials enhances both the mechanical and chemical properties of the material. Metallic nanoparticles, like silver, have been introduced into polymers for use as antimicrobial coatings or dielectric materials. The problem with these materials currently is the inability to disperse these particles homogeneously throughout the polymer matrix. The inability to evenly disperse nanoparticles throughout the system can lead to less than optimal quality and undesired properties in said material. Therefore, the goal of this project is to create an alginate/polyacrylic acid/silver nanoparticle (ALG/PAA/Ag) composite that controlled nanoparticle size and improved antimicrobial activity. The central hypothesis is the distribution of PAA throughout an alginate-based gel matrix will allow for evenly distributed mineralization of silver into the composite material. To test this hypothesis and accomplish the overall goal, the follow objectives were established:

1.1 Objectives of Research

1.1.1 Objective 1- Investigate impact of reaction conditions on silver nucleation with poly (acrylic acid) structures.

This objective addresses the lack of information in the literature on the detailed study of the chemical reduction of silver in the presence of PAA. The chemical reducing agent used for this work is sodium borohydride (NaBH_4) a strong reducing agent which produced an ultra-small well controlled size distribution.[1] The equation for the reduction process is shown in Equation 1.

The addition of PAA should promote nucleation due to the presence of the carboxylic groups located on the polymer backbone (Figure 1). This objective can be organized by three tasks:

- The reaction conditions such as silver, NaBH_4 , and polymer concentration will be monitored to observe the effect on silver size and shape.
- An in-depth study of the reaction mechanism and the effect of PAA on reaction kinetics.

- The third task is devoted to studying the effect of the reaction environment during nucleation on silver nanoparticle size and shape.

The knowledge gained from this objective will be applied to Objective 2 in designing the composite material.

Figure 1. Chemical structure of poly (acrylic acid)

1.1.2 Objective 2- Development of Biologically Relevant Antimicrobial Scaffold/Structure

This objective focuses on nucleating silver nanoparticles onto solid phase dual polymer systems using PAA as a templating agent. The scope of this objective is to prove that using a templating agent such as PAA allows for size control and increases the amount of silver nucleated onto the polymer.

To accomplish this goal a silver nanoparticle-alginate hydrogel system for wound healing applications was chosen. Multiple studies have been performed involving *in situ* metallic nanoparticle nucleation onto to alginate hydrogels.[2-7] The novelty of the work performed in this objective is the addition of the templating agent, in this case PAA. Therefore, the tasks to achieve this objective are:

- Investigate the effect of PAA on the mechanical integrity and swelling properties of the alginate gels.
- Use various compositions of PAA within alginate gels to determine the effect on PAA on particle size.

- Monitor and antimicrobial effects of composite gels and compare with pure alginate with silver nanoparticles nucleated under the same conditions.

1.2 Significance of Research

The introduction of inorganic nanoparticles into a polymeric material can be beneficial, as inorganic nanoparticles are responsible for increasing the properties and performance of materials. The reason silver nanoparticles inclusion in bandages and dressings for burn and wound healing has garnered much attention is due to its ability to kill antibiotic resistant bacteria. [8] While the addition of these nanoparticles improves material properties, many challenges arise:

- Homogenous dispersion is a significant problem, nanoparticles properties stem from their high surface area to volume ratio; therefore, aggregation within a polymer poses a huge threat because aggregation leads to that ratio decreasing. [9-13]
- Nanoparticle diffusion into a solid matrix is difficult and time consuming. [14-16] Both *in situ* nucleation and blending polymers with premade nanoparticle require diffusion into a solid crosslinked matrix. It takes a higher mass transfer driving force to load nanoparticles into a crosslinked material, meaning a higher concentration of raw materials. Therefore, introducing a second polymer as a nucleation agent is beneficial.
- The ability to control nanoparticle size is a big issue in polymer nanocomposites as well. For example, smaller silver nanoparticles are more effective at killing microbes than larger particles. [8, 17-21] On the contrary, platinum nanoparticles are often used for electrodes on fuel cells and are more effective in the 40nm to 50nm range.[22, 23] Varying the chemical reduction process and the addition of a stabilizer like poly (vinyl alcohol) (PVA) has been utilized to effectively control size.[24-26] With similar functional groups as PVA, poly (acrylic acid) (PAA) also has the ability to nucleate polymers.

In this project, the strategy of incorporating PAA into alginate gels will address the issues of dispersion and nucleation, as the chemically relevant PAA will be dispersed within the alginate gel prior to nucleation. Composite hydrogels with various compositions of PAA will be prepared. These composite hydrogels will be immersed into silver nitrate and the reducing agent, sodium borohydrate, solutions at various concentrations. The effect of polymer, silver and reducing agent concentrations on silver nanoparticle size and antimicrobial activity will be monitored. Silver ions are coordinated by the carboxylic groups located along the PAA backbone (Figure 2).

Figure 2. Silver nucleation on negatively charged functionality groups located on polymer chains.

Silver ions reduced to metallic silver nanoparticles are coupled with the oxidized carboxylic acid groups on PAA. [27, 28] Due to the presence of the chemical bond between the AgNPs and PAA chains, silver nanoparticles are strongly attached to the matrix. [29] By dispersing PAA throughout a solid matrix, silver can be nucleated directly onto the carboxylic sites using *in situ* chemical reduction of a silver salt. Since PAA is well dispersed the nucleated silver will also be well dispersed throughout the material. The fact that PAA is hydrophilic means it should aid in the swelling capacity of the alginate hydrogels and it will attract the silver, subsequently lowering the driving force necessary and decreasing the amount of raw materials. PAA will improve composite performance since more silver nanoparticles will be nucleated, and silver will not leach off the structure due to its interaction with the carboxylic groups. By successfully nucleating silver into homogeneous morphologies, difficulties of particle dispersion into a material can be prevented, particle size can be tailored for specific applications, and additional functionalization will not be needed to combine the two materials.

Chapter 2. Literature Review

2.1 Classification of Nanomaterials

Nanomaterials are defined as particulate matter with at least one dimension that is less than 100nm in size. Nanoparticle sizes are compared to aggregates, colloids and other macromolecules as shown in Figure 3. These materials can be separated into two classes: fullerenes and nanoparticles. A fullerene is any molecule composed entirely of carbon, in the form of a hollow sphere, ellipsoid or tube. Spherical fullerenes are known as buckyballs; cylindrical fullerenes are called carbon nanotubes. [30] Nanoparticles are inorganic nanomaterials which include quantum dots, nanowires and nanorods.

Figure 3. Size domains of natural colloids and nanoparticles [31]

Nanoparticles garner much scientific interest because they bridge the gap between bulk materials and atomic or molecular structures. Bulk materials have constant physical properties regardless of their size, but when analyzing at the nano-scale, this is often not the case. [32] Small size dimension, controlled to only a few atomic layers in thickness, can be created with any type of material (metal, ceramic, polymer, glass or composite), and the physical properties of these materials can be very different from the macro-scale properties of the same substance. These

special and highly fascinating attributes can be accredited to the shape, spacing, confined sizes [33] and exceptionally large surface area to volume ratio which has been identified as the key reason for their unique properties that nanoparticles exhibit (Figure 4). [34] Size-dependent properties are observed such as quantum confinement in semiconductor particles, surface plasmon resonance in some metal particles and superparamagnetism in magnetic materials. [35]

Figure 4. Surface area compression of bulk materials to nanoparticles [36]

Nanoparticles have been a focus of research in the scientific community due to their interesting properties and potential in various applications. Several metallic nanoparticles being studied currently include gold, silver, iron oxide, palladium, and platinum. These particles have gained interest due to numerous applications which include but are not limited to: antimicrobial thin film coatings, enhancement of MRI imaging, hypothermic treatment for tumors, drug delivery, dielectric and optical applications, and catalytic activity. [37, 38]

2.2 Silver Nanoparticles

2.2.1 Synthesis – Chemical Reduction

Chemical reduction is the most frequently applied method for the preparation of silver nanoparticles (AgNPs) as stable, colloidal dispersions in water or organic solvents. This process involves adding a reducing agent to a metal salt solution. These reducing agents can vary in strength and therefore vary the type of particles produced. In a study varying reducing agents reported by Dong et al., 2mL of 1mM silver nitrate were added dropwise to a bath of 2mM sodium borohydride solution. [1] Particles formed by this strong reducing agent were characterized by TEM. Particles formed were strictly spherical in shape with a narrow size distribution between 1nm to 12nm with an average of 4nm. The same study used a process with sodium citrate, which is known as a mild reducing agent. [39-41] For these experiments, 5mL of 1% trisodium citrate were added to a boiling 50ml solution of 1mM silver nitrate. The particles synthesized by this process were irregularly shaped, but spherical in nature, with a wide size distribution ranging from 15nm to 60nm in size. Other weaker reducing agents that have been used in other research include ascorbates [42, 43] and elemental hydrogen. [44] The conclusion from this work is that stronger reducing agents produce a narrow distribution of nanoparticle size, which is why sodium borohydride will be used in the work described in this dissertation. This is advantageous for monitoring changes in particle size in relation to PAA composition for nucleation on a solid polymer matrix.

Biological methods for synthesizing silver using bacteria or fungi such as *Penicillium* fungi, *phanerochaete chrysosporium*, or vegetation such as *Desmodium* have garnered much attention. [45-47] Silver nitrate was introduced into the cell cultures of these various microorganisms leading to silver nanoparticle nucleation. The nucleation was driven by proteins produced during the growth process of these cell cultures. The nucleation of nanoparticles is almost instantaneous, similar to the chemical reduction method, as shown in a study by Ahmad et al. *Desmodium* plant extract was added to a solution of 0.025M silver nitrate. Using UV-Vis to track the evolution of the reaction over time, observations made at 5 minutes show a strong absorption peak around 400nm, which is the typical adsorption peak for metallic silver nanoclusters. [45] Special ion transportation systems in these microbial cells allow for a silver-ion reduction process, leading to

a precipitation of silver nanoparticles. The use of bacteria or plant life can allow for well-defined shapes and sizes of silver nanoparticles. These processes do not require any additional reducing agents or surfactants to stabilize and nucleate the nanoparticles.

A similar alternative to using a cell-based approach is to use an *in vitro* approach called biomimetic synthesis. This synthesis technique utilizes proteins or enzymes, which are known to nucleate when in cells or organisms, as templates for material synthesis. Naik et al. used a combinatorial phage display peptide library to isolate the silver binding peptides found in *Pseudomonas stutzeri* AG259. [48] They found three small chain peptide cloned sequences, designated as AG3, AG4, and AG5, which nucleated silver in the presence of aqueous silver nitrate which is predicted to occur as shown in the schematic in Figure 5.

Figure 5. Peptide induced silver nucleation. [48]

These peptide clones were incubated with 0.1mM silver nitrate solution for 24-48 hours. Silver nanoparticles with a variety of morphologies were produced.[48] Naik et al. determined that specific amino acid groups were responsible for the nucleation of silver in an aqueous-based system. They found that proline and hydroxyl containing amino acids play a key role in the silver nucleation process. Naik's group also noted that these peptides behaved as templating agents due to their negatively charged functional groups. Their claim is peptides that serve as templates for

inorganic deposition offer a way for spatially controlling the deposition of inorganic material into an ordered array.[48] Peptides were placed in micromoulded capillaries and using a mercury lamp to illuminate the silver nanoparticles they found that growth only occurred on the area covered with peptide. Templating agents controlling where metallic particles are located are useful for various applications. Using PAA as a templating agent will allow the homogeneous distribution of nanoparticles throughout the material, and will prevent particle aggregation improving performance.

Other similar biomimetic methods have been used, for example, a nitrate reductase mediated method which is a nicotinamide adenine dinucleotide phosphate (NADPH) dependent, enzymatic method for producing silver nanoparticles. [49] In a 0.2M phosphate buffer, 1mM of NADPH, which was purified from cell cultures of *Fusarium oxysporum*, was incubated with 1mM silver nitrate and 100µg of nitrate reductase for *in vitro* synthesis of silver nanoparticles. It was proven that without the NADPH, silver nucleation did not occur. This is because NADPH is a strong reducing agent; the main purpose of NADPH is to reduce reactive oxygen species. NADPH reduces silver ions when it enters the cell. The underlying reasoning for the research of biomolecules to nucleate silver nanoparticles is its potential for biocompatibility and reducing the need for intense purification for biomedical applications.

Alternatives to biological means, including the use of synthetic materials, have also been investigated for silver nucleation. By studying the peptides produced by silver nucleating microorganisms, researchers have used polymers with similar functional groups to its biological counterparts to nucleate silver. Two groups of polymers are involved when synthesizing nanoparticles. Polymers that nucleate and promote nanoparticle growth such as, poly (acrylic acid) (PAA) [50], poly (amidoamine) dendrimers [22] contain thiol, amine or carboxylic function groups. [51] These functional groups are responsible for promoting particle nucleation and aid in the growth step. An example of these functional groups acting as a templating agent is shown in the research performed by Wang et al. use poly-(styrene-methyl acrylic acid) (PSA) spheres as templates for hollow silver spheres. [50] Silver nitrate was used as the silver source with ascorbic acid as the reducing agent. The claim of this research group is that the carboxylic groups located on the PSA block copolymer were responsible for nucleating silver nanoparticles via templating

nucleation. The PSA/Ag composite material was heated at 150°C for three hours to anneal the silver nanoparticles into a solid silver shell. Tetrahydrofuran was used to dissolve away the PSA sphere leaving a hollow silver sphere 1 μ to 4 μ m in size. [50]

The second type of polymers used in the chemical reduction process are stabilizing polymer such as, poly (vinyl alcohol) (PVA) [25], poly (ethylene terephthalate) (PET)[52], poly(vinylpyrrolidone) (PVP) [53],and poly (ethylene glycol) (PEG) are absent of the aforementioned functional groups and all have been used to stabilize metallic particles or hollow spheres. Metallic nucleation for both types of polymer has been reported on both a solid polymer surface or in aqueous solutions. [22, 50, 53, 54]

2.2.2 Synthesis – Physical Approach

The nucleation of metallic nanoparticles can also be achieved by physical modification of bulk materials. These processes use energy to evaporate or deposit nanoparticles, which can be used in subsequent applications.

Silver nanoparticles can be synthesized by chemical vapor deposition (CVD). This evaporation–condensation process can be carried out using a tube furnace at atmospheric pressure. The source material within a boat centered at the furnace is vaporized into a carrier gas and deposits as nanoparticles. [44] This method has several drawbacks because a tube furnace takes up an immense amount of space, the system requires a large input of energy, and it takes a long time to reach thermal stability.

Laser ablation deposition (LAD) is a process in which a high-powered laser is applied to the bulk material in solution. The process efficiency and characteristics of the nanoparticles formed as a result of LAD ablation are dependent on the wavelength of the laser impinging the metallic target, the duration of the laser pulses (in the femto-, pico- and nanosecond regime), the laser influence, the ablation time duration and the effective liquid medium, with or without the presence of surfactants. [55, 56] Figure 6 shows a diagram of the LAD process.

Figure 6. Diagram of a LAD process to form nanoparticle solutions [57]

Nanoparticles can be modified in size and shape due to their further interaction with the laser light passing through the system. [44] Also, the formation of nanoparticles by laser ablation is terminated by the surfactant coating. The nanoparticles formed in a solution of high surfactant concentration are smaller than those formed in a solution of low surfactant concentration.[44] One advantage of laser ablation compared to other conventional methods including chemical and biological reduction is the absence of chemical reagents in solutions.

2.2.3 Silver Nanoparticles Applications

Silver nanoparticles have several biomedical applications that are being investigated. Diagnostic applications include biosensors and numerous assays where the silver nanoparticle materials can be used as biological tags for quantitative detection. [38] Silver nanoparticles also have antimicrobial properties, which are incorporated in apparel, footwear, paints, wound dressings, appliances, cosmetics, and plastics. [38] Research by Nam et al. has shown that genetically engineered biological scaffolds can mediate and reduce silver ions into nanoparticles by using short-chain peptides with carboxylic acid functional groups. [58] The results of Nam's research

showed these engineered peptides not only mediated the reduction of silver ions but also acted as templates for synthesis of nanostructures. [58] It is also suggested that attaching these peptides to a biological scaffold or another surface can enhance the reduction potential for nucleating silver nanoparticles.

Silver nanoparticles have been incorporated into a variety of material substrates to impart an antimicrobial effect. These materials range from simple fabrics and wound dressings to implanted materials such as bone scaffolds. [59-61] The work of Falletta et al. tested the antimicrobial effect of incorporating silver nanoparticles into wool, cotton and polyester fabric samples. Silver nanoparticles were nucleated by using PAA with two different molecular weights (1200 and 15000 g/mol). 125 mL of a 1% w/w AgNO₃ solution and 125 mL of a 10% w/w PAA solution were mixed and kept under vigorous stirring at room temperature for 10 min. The reduction with NaBH₄ was performed by adding 10 mL of a 0.1M NaBH₄ solution (refrigerated in an ice bath) to the solution containing PAA and silver ions, with an addition rate of 1 mL/min. Another reduction approach used in this study included UV reduction, which was performed by irradiating the solutions containing PAA and silver ions for 2 h. The fabric samples (cotton, wool, and polyester) were conditioned at constant relative humidity (RH 33%) and temperature (20 °C). Wool and cotton samples (5 cm × 5 cm) were soaked for 15 min at room temperature in the Ag nanoparticles dispersion under magnetic stirring. The samples were then squeezed to remove the excess dispersion, rinsed, and dried in an oven at 130°C for 15 min at atmospheric pressure (dry heat). [59] All three fabrics exhibited antimicrobial activity against both Gram-negative and Gram-positive bacteria. [59]

Other than simple fabrics, silver nanoparticles have been introduced into more complex biomaterials. In bone tissue engineering, bacterial infection is a major issue that determines success or failure of an orthopedic implant. Research has been done in this area to combine silver nanoparticles with a chitosan/hydroxyapatite composite material (CS/HA/nAg), which is commonly used in bone implants. [60] 100mg of CS was added to 10ml of 1% acetic acid and kept for constant stirring till a clear viscous solution was obtained. Secondly, 100mg of nHAp was added, and the stirring was kept till solidified mixture was obtained. The mixture was distributed in 24 well culture plates and were kept at -80°C overnight to freeze them. Finally, the

mixture was lyophilized in a freeze dryer until dried and a scaffold was achieved. To load this material with silver, the ion-exchange and reduction technique was applied by soaking the scaffold in 0.05M aqueous silver nitrate solution at 37°C for 8h in order to enrich the scaffold surface with a controlled amount of silver ions and the obtained scaffold was dried and stored in an air tight container. In an antimicrobial activity study, it was shown that the CS/HA/nAg composite had a zone of inhibition was almost three times greater for both Gram-positive and Gram-negative bacteria that compared to its CS/HA counterpart. [60] This added antimicrobial effect minimizes the risk for bacterial infection for patients and prevents implant failure.

Another biomedical application for silver is in a composite scaffold for wound dressings. Similar to bone implants, materials with antimicrobial properties are desirable when developing dressings for wounds and burn victims. Wound dressing scaffolds made of chitin, chitosan and collagen have been investigated to include silver as composite materials. [61, 62] The work of Kumar et al. has reported adding silver nanoparticles into a wound dressing comprised of chitin to make a composite material. A premade colloid of silver nanoparticles was prepared by 125 ml solution of 1 mM silver nitrate in water was made and was heated until it begins to boil. 5 ml of 1% sodium citrate (the reducing agent) solution was added as soon as boiling commences.[61] The scaffolds were prepared by 20 ml of 1 mM nanosilver solution was added to 40 g of chitin gel, stirred well for 15 min followed by lyophilization to obtain chitin/nanosilver composite scaffolds having 107 μ g silver nanoparticles per cm². [61] Cell death was achieved due to the release of silver nanoparticles. Silver release per cm² dressing was 4.4 $\pm$ 0.6 μ g at 2 h, increasing to 19.2 $\pm$ 2.5 μ g at 24h, to a maximum of 23.9 $\pm$ 2.1 μ g of dissolved silver by 48 h.[61] The silver nanoparticles were not chemically attached to the chitin matrix rather loaded by mass transfer diffusion. Highly crosslinked materials prohibit mass diffusion. The limitation of diffusion makes it beneficial to design an *in situ* nucleation protocol using a templating agent in a hydrogel matrix. The incorporation of silver into a material by an *in situ* process should be greater than relying on mass diffusion of prepared particles into a matrix.

The ability to nucleate silver nanoparticles directly onto cross-linked and other self-assembled structures would be more beneficial than introducing previously prepared silver nanoparticles into biomaterials via molecular diffusion. Nucleating silver allows for greater homogenous

dispersion and control in particle orientation. This technique will afford us the ability to better control the distribution of nanoparticles with specific sizes and shapes by manipulating reaction conditions. Having this control would enable the design of nanoparticle composite materials for specific applications.

2.3 Hydrogels

[63] Due to their hydrophilic nature and relative biocompatibility, hydrogels have garnered interest from biomaterial scientists for years in biomedical applications. Hydrogels can absorb up to thousands of times of their dry weight in water. They can be characterized into two main different groups: physically or chemically crosslinked gels. Physically crosslinked hydrogels are referred to as reversible; these networks are held together by molecular entanglements, and/or secondary forces including hydrogen bonding, ionic and hydrophobic forces. [64, 65] These types of gels are not homogenous due to the fact that they are clusters of ionic, hydrophobic domains. These gels are referred to as reversible gels because their structure can be disrupted by changes to the environment such as ionic strength, pH, temperature, an application of stress, or addition of specific solutes. Chemically crosslinked hydrogels are called “permanent” gels. These types of hydrogels are usually formed by covalently crosslinking water-soluble polymers to form a network. The equilibrium swelling level in an aqueous solution for these types of hydrogels depends greatly on the crosslinked density. Chemical hydrogels are not homogenous, and similar to physical hydrogels, they contain regions of low water swelling and clusters of regions with very high crosslinking density. [66] These defects are often a result of aggregation of hydrophobic crosslinking agents.

Hydrogels are used for biological applications due to their ability to retain water. These materials create a moist environment, which promotes wound healing and reduces friction making it less painful for application and removal of the material. One application for hydrogels is cell encapsulation, and synthetic and natural hydrogels have been used for cell encapsulation. Synthetic polymers include poly (ethylene glycol), polyvinyl alcohol, polyurethane, polypropylene and polyacrylate. Some examples of natural polymers used for this application are alginate, agarose, chitosan, cellulose and collagen. [67-69] The work of Lim and Sun was one of the first papers to use alginate for cell encapsulation.[70] Unwashed cell cultures were suspended

in 0.6% to 0.8% sodium alginate solution. This solution was added dropwise into a 1.5 percent calcium chloride solution immediately forming a gel and entrapping the cell cultures inside. After treatment, the alginate gels containing the cell cultures were allowed to grow for 3 to 4 days. The results showed the cell cultures could survive the encapsulation process. [70] This paper displayed that large molecules can be entrapped in an alginate solution and subsequently entrapped into alginate gels due to the instantaneous gelation of alginate when in the presence of calcium ions. The ability of alginate to crosslink within itself makes it a good candidate for design of the polymer scaffold described in this dissertation.

2.3.1 Alginate gels

Alginate is isolated from the cell walls of brown algae. The biological functions of alginates in the plant include preventing desiccation, maintaining the integrity of the cell and providing mechanical strength.

2.3.1.1 Gel formation process

Formation of an alginate gel is a result of specific binding of divalent cations to alginate leading to firm cohesion between alginate chains. [71] Alginate (ALG) is a polysaccharide made up of a family of unbranched binary copolymers with alternating regions of 2 blocks: β -D-mannuric acid (M) and α -L-guluronic acid (G). [3, 72] Multiple divalent cations can be used for gelation, producing varying gel properties. [2] For example, zinc binds with consecutive M blocks and alternating MG blocks; since zinc is less selective with its binding sites than other cations it creates a denser gel. [2] The most popular divalent cation for alginate gel formation is calcium, which binds with consecutive G block residues. This interaction is referred to as the “egg box model” as seen in Figure 7. [2, 71]

Figure 7. Schematic representation of calcium binding to guluronic acid sequences: 'egg box model' [2]

2.3.1.2 Alginate applications

Alginate gels have many advantageous properties: they provide protection for cells, fragile drugs and cell nutrients for delivery. [73] These gels are biocompatible, and can be easily modified for cell adhesion. In addition, due to the nature of its gelation process, alginate can be injected as a liquid *in vivo* and the gel structure can be molded and formed inside the body. [74] Alginate gelation occurs instantly when dropped into a divalent cation solution. [75] This instantaneous gelation makes alginate a prime candidate to entrap molecules of biological significance, including food products, microbes, enzymes, plant or animal cells. [75]

Because calcium alginate is a natural hemostat[76], alginate-based dressings are excellent for bleeding wounds. The gel forming property of alginate helps in removing the dressing without additional trauma and reduces the pain experienced by the patient during dressing changes. It provides a moist environment that leads to rapid granulation and reepithelialization. [76]

Alginate in some clinical trials has been proven to reduce healing time compared to patients using paraffin gauze. [76]

A few pitfalls to these materials include weak mechanical properties and a difficulty during sterilization. The difficulty to sterilize alginate gels is one reason why silver/alginate (Ag/ALG) composite gels have been the topic of so many studies. Adding silver nanoparticles helps keep the alginate gel microbe free; therefore, providing better care for patients. As a result, biomedical applications for alginate gels typically include drug delivery, tissue engineering and serving as a bulking agent. [73, 74]

2.4 Polymer Nanocomposites

Polymer nanocomposites can be synthesized by various methods, and the choice of the synthesis method depends on both the nanoparticle and the polymer being used. The methods can be separated into three different approaches. The first approach is the *in situ* preparation of the nanoparticles directly into the polymer matrix by reduction of metal salts. Another approach is the polymerization of the matrix around the nanoparticles. Third approach is the blending of pre-made nanoparticles into the polymer matrix.

The benefits of *in situ* nucleation is the ability to disperse nanoparticles to increase performance. The work of Vimala et al. developed chitosan-PVA silver nanocomposite material via an *in situ* nucleation. 100mg of AgNO₃ was added to separately into 50ml of various chitosan solutions (1%, 2%, 3%). Nanoparticles were formed by leaving the solution out in sunlight then 1% PVA was added to the reaction mixture and stirred for 1 hour. 2% glutaraldehyde was added to the solution to crosslink the materials. The solution was poured onto glass plates allowed to dry and crosslink. It was found that the composite material exhibited improved mechanical and antimicrobial strength with the addition of silver nanoparticles.

In nanocomposite systems, the nanomaterial enhances the mechanical, electrical, thermal, optical, electrochemical, and catalytic properties of the bulk material. Some of these bulk materials include ceramics, metals, colloids and polymers. Noble metals such as platinum, gold and silver nanoparticles are used as the nanomaterial component because of their applicability in

catalysis, imaging, sensing and antimicrobial activity. [23, 77] For example, gold nanoparticles have been investigated for therapeutic drug delivery. In the study from Owens et al., gold nanoparticles were made by a chemical reduction process of gold chloride and a citrate reducing agent. The particles were encapsulated into a polyacrylamide (PAAm)/poly(acrylic acid) interpenetrating networks via inverse emulsion polymerization. Then covalent grafting of a linear methoxy-PEG-N-hydroxysuccinimide (M.W. 3400) to the primary amine groups of the PAAm network was performed. This thermally responsive polymer/metal nanocomposite can be designed to have molecular recognition, biocompatibility and intelligent response. [78] Temperature responsive polymer networks attached with gold can encapsulate desired molecules for effective drug delivery. Gold nanoparticle cores can be tuned to absorb near infrared wavelengths of light (530nm-1200nm) depending on their size. [78] This absorbed light energy is converted to heat which can be transmitted to the surrounding polymer triggering a swelling response that releases the carried molecules or drugs. [79] The addition of gold nanoparticles to polymers can open up new applications and methods for drug delivery.

Carbon nanotubes display the ability to add bioactivity and mechanical strength to polymer scaffolds such as chitosan, hydroxyapatite, collagen and other synthetic polymers. [80-83] In the work from Wang et al. they used the 3rd type of process mentioned earlier in this section to mix functionalized multi walled nanotubes (MWNT) with chitosan to form a composite hydrogel. The MWNTs were functionalized with hydroxyl groups to promote the dispersion within the amine rich chitosan. [82] The preparation of these MWNT/chitosan thin films includes dissolve MWNTs into a suspension with adding 100ml of DI water. 1ml of acetic acid and 1g of chitosan was then added to the suspension. The mixture was mechanically homogenized by mixing at 18,000 rpm for 30 minutes. The solution was poured into plastic dish and heated to 50°C to produce 0.08mm thick thin films. This paper concluded that the adding functionalization to the MWNTs improved homogenous dispersion which in turn improved mechanical strength in the composite material. By adding 0.8 wt% MWNTs to the chitosan matrix the mechanical strength of the composite increases by 93% to 99%. Single walled carbon nanotubes (SWNT) have been used in bone tissue engineering applications specifically because of their ability to significantly reinforce a polymer such as poly(propylene fumarate) (PPF). [84] SWNT/polymer nanocomposites are readily injectable, can be thermally crosslinked, and are cytocompatible *in vitro*. [85, 86]

When blending nanomaterials into polymer matrix achieving homogenous dispersion is a concern; the nanomaterials need to be functionalized in order to achieve this dispersion to maintain the nanomaterials properties. The pitfalls to this strategy includes difficulty in functionalizing certain nanomaterials and this functionalization effect the nanomaterials properties and performance.

Chapter 3. Experimental Methods

3.1 Materials

Silver nitrate (AgNO_3) and calcium chloride dehydrate (CaCl_2) were obtained from Acros Organic (Geel, Belgium). Sodium borohydride (NaBH_4) was obtained from MP Biomedicals (Solon, OH). Poly(acrylic acid) (PAA) was obtained from polymer source (Montreal, Canada). Hydrochloride acid, sodium hydroxide, sodium chloride and the required reagents for cell cultures (tryptone, yeast extract, and agar) were obtained from Fischer Scientific (Fairlawn, NJ). Alginic acid sodium salt from brown algae was obtained from Sigma-Aldrich (St. Louis, MO). TEM grids were purchased from Ted Pella (Redding, CA). DI water was provided by the WVU shared facilities.

3.2 Objective 1

3.2.1 Chemical Reduction of Silver

A flowchart giving the process for nanoparticle nucleation is shown in Figure 8. Silver nitrate and PAA were dissolved into purified water at various concentrations. The silver/PAA aqueous solution was mixed for an hour at room temperature under constant agitation. The reducing agent used in the silver nanoparticle reaction was NaBH_4 . A fresh solution of NaBH_4 was prepared by dissolution in purified water prior to each nucleation experiment. A volume of 2mL of silver/PAA incubated solution was added to 30mL of NaBH_4 solution. The silver nanoparticle solution was allowed to react under constant agitation overnight to equilibrate and stabilize (approximately 16 hours).

Figure 8. Flowchart of AgNP reaction process

Experiments were run to determine the impact of each component of the reaction, which required varying the concentration of one component when holding the concentrations of the other two constant. To study the impact of silver nitrate, the silver nitrate concentration was varied from 0.1mM to 50mM, while the concentrations of PAA and NaBH₄ were held constant at 1mg/mL and 1mM respectively. To investigate how the polymer impacted the reaction, the poly (acrylic acid) concentration was varied from 0.1mg/mL to 4mg/mL, with the concentrations of AgNO₃ and NaBH₄ kept constant at 1mM. Finally, the reducing agent concentration was varied from 1mM to 50mM while keeping the polymer and silver concentrations constant at 1mg/mL and 1mM respectively.

3.2.2 Rate of Reduction

To study the overall reaction rate of silver nucleation at varying concentrations of polymer, the UV-Vis spectrum was monitored over time. Rate of reduction and rate of nucleation will be used interchangeably through this dissertation as literature has demonstrated similar use of these terms. Using the silver reaction mentioned in the previous section, the concentration of AgNO₃ and NaBH₄ was kept constant at 1mM respectively; while the concentration of polymer was varied

from 0.1mg/mL to 3mg/mL. For the rate study, ultraviolet-visible light (UV-Vis) spectra were collected every minute at a constant wavelength of 395nm. This wavelength was the characteristic peak for the silver reaction when no polymer was present; therefore, it would be used to monitor the rate at which particles were forming. Monitoring peak development, a sigmoidal curve for the relationship between time and absorbance indicates the reaction was autocatalytic, and the rate equation in Equation 1 could be used to determine the rate of nucleation. [87]

$$K_a t + C = \ln\left(\frac{a}{1-a}\right) \quad (2)$$

In Equation 1, a is the ratio of the maximum absorbance at time t and time ∞ , K_a is the rate of reaction, and C is a constant. The rate was determined and reported by plotting the natural log expression as a function of time.

3.2.3 Reaction Mechanism

To determine the reaction mechanism two separate experiments were run. The silver complex study varied the amount of time the polymer and silver nitrate solutions were allowed to mix before the addition of the reduction agent. Four polymer concentrations were studied: 0.1mg/mL, 1mg/mL, 2mg/mL, and 3mg/mL. The concentration of silver and reducing agent were kept constant at 1mM. All reaction mixtures were made in a conical tube and allowed to react outside the UV-Vis sample holder. 1mL increments were taken over time for UV-Vis measurements. Step II of the flowchart in Figure 8 was varied from 1 minute to 60 minutes in 10 minute increments. UV-Vis measurements for all resulting solutions were recorded.

The second experiment was to monitor the same reaction conditions mentioned above over time with UV-Vis. The reaction (step III in Figure 8) was monitored for 30 minutes. The silver/PAA solution was allowed to react for an hour for each sample. The evolution of the UV-Vis spectra over time will reveal the mechanism of the silver nucleation reaction, revealing if silver is nucleated homogeneously or heterogeneously.

3.2.4 Reaction Environment

The effects of the reaction environmental conditions such as temperature and pH on particle nucleation were analyzed. Using the same chemical reduction of silver reaction discussed in the previous section, the reaction concentration of polymer, silver and reducing agent were all held constant at 0.1mg/mL, 1mM and 1mM respectively. Four pH values were tested; aqueous PAA solutions of 2.5, 4.5, 7.0 and 10 were prepared before it was introduced into the reaction. These four pH values were tested at two different reaction temperatures 4°C and 25°C. A run with chilled reducing agent (4°C) was also performed at 25°C. All samples were allowed to react for 24 hours.

Characterization of the particles formed by this nucleation process include UV-Vis and Dynamic Light Scattering (DLS). 1mL of solution was placed in a disposable cuvette for both UV-Vis and DLS analysis. Imaging of the nanoparticles was done by transmission electron microscopy (TEM) by dropping 5µL of solution onto a mesh copper grid and dried overnight.

A special acknowledgement to Paula Pacurari for running the experiments described in this section.

3.3 Objective 2

3.3.1 Copolymer System Determination

3.3.1.1 Elastin-Like Polypeptide and Poly(Glutamic Acid)

As a proof of concept that polymers with carboxylic groups can nucleate silver nanoparticle when in the solid phase, an elastin-like protein (ELP) was crosslinked with poly(glutamic acid) (PGA). PGA has carboxylic groups similar to PAA (Figure 9). This crosslinked material was not soluble in water so it was a good candidate for the proof of concept that polymers with carboxylic groups in their solid phase maintain their reactive ability to nucleate silver nanoparticles. Three different mass ratios of ELP-PGA crosslinked material were tested: Mass 4:1, 2:1, and 1:1 ELP: PGA. These materials were immersed in 1mM AgNO₃ for an hour. 30mL of 1mM NaBH₄ was added to 2mL of Ag/ELP-PGA solution. 5µL of the resulting solution was dropped on copper tape and imaged by SEM.

Figure 9. Structure of PGA

3.3.1.2 Poly(acrylic acid) grafted cotton fabrics

The purpose of these experiments was to observe if PAA retained its nucleation ability even when the carboxylic groups are reacted to graft the polymer onto a cotton fabric. PAA was grafted onto the cotton fabrics by an esterification reaction described by Martel et al.[88] Figure 10 shows the stepwise process for the esterification reaction, which can be applied to any polymer backbone with multiple carboxylic acid groups. In this reaction, hydroxyl groups on adjacent carboxylic acids form an ester under constant heating. The ester bonds then break and replace any hydroxyl group on the cellulose cotton fabric.

Figure 10. Chemical scheme showing the esterification reaction between PAA and cotton fabrics. [88]

A square piece of cotton weighting 271.8mg was soaked in a solution containing 2.5mg/mL PAA and 15mg/mL $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ the phosphorous catalyst. The fabric was air dried in a fumehood followed by heating in an oven at 190°C for 5 minutes. The fabric was rinsed with DI water to remove excess unreacted PAA and catalyst. The fabric was air dried and reweighed to observe any weight gain. Both fabrics with grafted PAA and without PAA were immersed in 1mM AgNO_3 for 1 hour. The fabrics were removed and dried again, then were immersed in 25mM

NaBH₄ and allowed to nucleate for 1 hour. Finally the fabrics were dried and mounted on a silicon wafer by carbon tape in preparation to be imaged by SEM.

3.3.1.3 Alginate-Poly(acrylic acid)

Sodium alginate (25mg/mL) dissolved in DI water was introduced into a bath of calcium chloride (100mM) drop wise by a syringe. Keeping the final concentration of sodium alginate constant; PAA was dissolved into the alginate solution at various concentrations (10wt%, 25wt%, 50wt%, 75wt%). Due to alginate immediately crosslinking and becoming a gel once in contact with the calcium chloride bath, PAA was physically entrapped within the gel beads. The beads were then washed 3 times with DI water to removed excess calcium chloride solution and were freeze dried overnight. Table 1 shows the reaction conditions tested for all gel materials. ALG beads were mounted onto silicon wafers by black carbon tape in preparation to be imaged under SEM. Hydrogel samples will be referred to their sample name shown in Table 1. ALG refers to pure alginate beads while 10% PAA refers to a composite gel with 10wt% PAA.

Table 1 Reaction Conditions Tested

Sample Name	AgNO ₃ Conc. (mM)	NaBH ₄ Conc. (1mM)
ALG	5	0.1 1 10
	10	
	20	
	40	
10% PAA	5	1
	10	
	20	
	40	
25% PAA	5	0.1 1 10
	10	
	20	
	40	
50% PAA	5	1
	10	
	20	
	40	
75% PAA	5	1
	10	
	20	
	40	

3.3.2 Nucleation on Composite ALG-PAA beads

Both pure alginate and ALG-PAA beads were used as scaffolds for nucleation. 3-5 beads (2-3mg per bead) were immersed into 10mL of aqueous AgNO₃ solution for three hours. The beads were removed from the silver solution and were air dried for 5 minutes. 10mL of NaBH₄ was freshly prepared and the beads were immersed in the reducing agent for 15 minutes. The reducing agent solution was removed and the beads were immersed in DI water and allowed to freeze dry overnight.

3.3.3 Polymer Nanocomposite Bead Characterization

3.3.3.1 Silver Composition Study – ICPU

Inductively coupled plasma mass spectrometry (ICP-MS) is a type of mass spectrometry which is capable of detecting metals and some non-metals at concentrations as low as one part per trillion. By ionizing the sample with inductively coupled plasma, a mass spectrometer can be used to separate and quantify those ions. Compared to atomic absorption techniques, ICP-MS

has greater speed, precision, and sensitivity; however, analysis by ICP-MS is also more susceptible to trace contaminants. Samples for this technique need an acidic solvent like hydrochloric acid or nitric acid and have a pH less than 1.

All 5 gel bead samples (ALG, 10% PAA, 25% PAA, 50% PAA, 75% PAA) were nucleated with silver under the same reaction conditions: 40mM AgNO_3 and 1mM NaBH_4 . Composites with the highest concentration of silver were used to ensure there was enough silver to meet the detection limit. Each gel sample was weighed then dissolved into at least 50mL of 1M HCL. ICPU detects the concentration of silver present in the acidic solution. The concentration was recorded and the samples were sent out to the National Research Center for Coal and Energy (NRCCE) building on campus at West Virginia University.

3.3.3.2 Rheology

To measure the change in mechanical properties when PAA and Ag nanoparticles are introduced into the alginate gel system rheology studies were done. 1mL of ALG and ALG-PAA composite solutions were dropped onto a glass microscope slide and evenly distributed throughout the surface of the slide. These slides were dipped into a bath of 100mM CaCl_2 creating a thin film of gels. A thin film of 50% PAA gel was loaded with silver by immersing it in 25mL of 1mM AgNO_3 for 3 hours. The gel was removed, lightly blotted dry, and immersed in 25mL of 1mM NaBH_4 .

3.3.3.3 Swelling Studies

The swelling of a hydrogel is one of its most important properties. In wound healing applications it is important to create a moist environment to promote quicker healing and reduce friction which in turn reduced pain during the removal process. To measure the change in swelling properties when PAA and Ag nanoparticles are introduced into the alginate gel system rheology studies were done. Multiple beads per sample were weighed before and after they were soaked in DI water. For the kinetics study, the swollen beads were removed periodically and weighed. The wet weight was determined by blotting them dry with kimwipes to remove excess moisture and

weighed immediately. The swelling kinetics experiment was done in triplicate with pure alginate beads. The time in which maximum swelling occurred was determined and used for all swelling experiments.

A set of dried ALG/PAA gel beads were held in DI water for 3 hours; the degree of swelling was determined by measuring both the dry weight before and wet weight after swelling. The swelling of ALG and 10%-75%PAA samples were measured. The average values of 5 samples were reported.

3.3.4 Antimicrobial Test

The antimicrobial activity of ALG-PAA composite materials in comparison to pure alginate beads was determined by using ASTM standard E2149-10, and images of the experiment are shown in Figure 11. Control samples of pure PAA, pure alginate bead and 50% PAA beads without silver were tested. Silver was nucleated on both pure alginate and 50% PAA beads by the process previously described and tested for its antimicrobial activity. The reaction conditions for nucleation were 1 mM for both AgNO_3 and NaBH_4 , respectively.

Figure 11. Dynamic Shake Flask Antimicrobial Test[89]

An overnight culture of *E. coli* was diluted with sterile buffer until an absorbance of 0.28 was reported at 475nm. The solution was then diluted 1000 times to get to the working concentration recommend by the standard. Each sample and control was placed into 50mL of dilution buffer and shaken for one hour at 37°C. Proper cell plating techniques were used for all serial dilutions. Plate counts were compared to an inoculum only sample to calculate percent reduction.

3.3.5 Ultra-microtome TEM preparation

ALG/PAA/Ag composite beads were embedded with Epon resin using the following process:

Procedure:	Time:
2.5% Glutaraldehyde	1 hour
PBS (3 times)	15 min each
30% EtOH	15 min
50% EtOH	15 min
70% EtOH	15 min
90% EtOH	15 min
100% EtOH (3 times)	15 min each
Propylene Oxide (2 times)	10 min each
1:1 Epon/ Prop. Oxide	Overnight in hood
100% Epon (3 times)	1 hour each
Curing at 37°C	24 hours
Curing at 60°C	48 hours

Epon resin was made using the following volume ratio: 48.44mL of Scipoxy 812, 30.89mL of Dodecenylsuccinic anhydride (DDSA), 20.67mL of Nadic methyl anhydride (NMA), and 2mL of DMP-30. The viscous mixture was desiccated overnight to remove all gases from the mixture. After the curing process samples were trimmed and cut by the Reichert-Jung Ultracut E Ultramicrotome. Samples for TEM were cut at two settings: 60nm thickness and 10nm thickness. A few thin slices were applied to mesh copper grids.

3.4 General Characterization Techniques

3.4.1 Fourier transform infrared spectroscopy (FTIR)

IR spectroscopy is one of the most common characterization techniques to identify molecular groups on polymers. It allows the researchers to identify functional groups present in the solid, liquid or gaseous sample. The sample is positioned in the path of the IR radiation beam and absorption measurement of different IR wavenumbers is taken. IR radiation can itself be divided into three smaller regions, near-IR ($13000\text{--}4000\text{ cm}^{-1}$), mid-IR ($4000\text{--}400\text{ cm}^{-1}$), and far-IR ($200\text{--}10\text{ cm}^{-1}$).

Infrared spectroscopy exploits the fact that all molecules have characteristic resonant frequencies when absorbing infrared light. These resonant frequencies are determined by the shape of the molecular potential energy surfaces, the masses of the atoms, and the associated bond coupling. In order for a vibrational mode in a molecule to be “IR active,” it must be associated with changes in the dipole. A permanent dipole is not necessary, as the rule requires only a change in dipole moment.[90] IR spectroscopy is often used to identify structures because functional groups give rise to characteristic bands both in terms of intensity and position (frequency). The wavenumber positions of these bands are summarized in Figure 12.

Figure 12. Infrared spectroscopy peaks of various functional groups.

The beam generated by FTIR is a broadband light source; one containing the full spectrum of wavelengths to be measured. The light shines into a Michelson interferometer which is a certain configuration of mirrors, one of which is moved by a motor. [91] A schematic can be seen in Figure 13. Mirrors have the ability to move, each wavelength of light in the beam is periodically

blocked, transmitted, blocked, transmitted, by the interferometer, due to wave interference. [91] Different wavelengths are modulated at different rates; the beam coming out of the interferometer has a different spectrum.

Figure 13. Schematic diagram of a Michelson interferometer, configured for FTIR.

Michelson interferometer has two advantages over other FTIR setups; the Michelson interferometer has an improvement in the signal to noise ratio that is gained when taking multiplexed measurements, and a throughput advantage called Jacquinot's advantage.[92] FTIR instruments do not require slits to achieve resolution; therefore, you get much higher throughput with an FTIR than you do with a dispersive instrument.

ALG and all compositions of the composite ALG-PAA beads were crushed into fine powders and measured directly using a FTIR spectrophotometry. The samples placed on a gold substrate

and were measured in reflectance mode with a mid-IR Duraglow source (7,900 – 375 cm^{-1}). A UMA 600 detector using a Potassium Bromide (KBr) beam splitter. (Digilab FTS 7000)

3.4.2 Ultraviolet Visible Spectroscopy (UV-Vis)

UV-Vis spectroscopy is in many ways similar to the IR spectroscopy, the main difference being the use of photons in the UV- visible region of the spectra (200nm-800nm). An optical spectrometer records both the wavelengths and the degree to produce a graph of absorbance vs. wavelength. The concentration of particles affects the maximum absorbance. Silver nanoparticles display interesting optical properties in the UV-Vis range due to their ability to efficiently absorb and scatter light. The strong interaction of the silver nanoparticles with light occurs because the conduction electrons on the metal surface undergo a collective oscillation when excited by light at specific wavelengths (Figure 14). Known as a surface plasmon resonance (SPR), this oscillation results in unusually strong scattering and absorption properties. [38] This aspect of silver can be utilized by UV-Vis to both sense the presence of silver in a colloidal solution as well as monitor changes in sizes and shapes.

Figure 14. Surface plasmon resonance where the free electrons in the metal nanoparticle are driven into oscillation due to a strong coupling with a specific wavelength of incident light [38]

Although other techniques such as TEM, SEM and DLS are more accurate in measuring size of silver nanoparticles, UV-Vis can be used as a complementary technique. Size changes of

spherical silver nanoparticle leads to different wavelengths in which surface plasmon resonance is achieved, which is shown in Figure 15. Because of this phenomenon, UV-Vis can be used to monitor silver size and concentration over time to determine reaction kinetics.

Figure 15. (Left) Extinction (scattering + absorption) spectra of silver nanoparticles with diameters ranging from 10-100 nm at mass concentrations of 0.02 mg/mL. (Right) Extinction spectra of silver nanoparticles after the addition of a destabilizing salt solution. [38]

Silver nanoparticle shapes can also be monitored by UV-Vis as shown in Figure 16.

Figure 16. Color change and maximum absorbance peaks of various silver shapes [63]

All UV-Vis extinction spectra were recorded at room temperature with the Thermo Scientific Evolution 300 spectrophotometer. Absorbance measurements were taken at various wavelengths ranging from 300nm-800nm for each sample at a scan rate of 240nm/min. Measurements were taken in low volume dispensable plastic cuvettes. 1mL of colloidal silver per sample was measured.

3.4.3 Dynamic Light Scattering (DLS)

DLS is a characterization technique used to determine size distribution profiles for small particles or polymers in a suspension/solution. When light hits small particles the light scatters in all directions (Figure 17), known as Raleigh scattering, as long as the particles are relatively small (less than 250nm). If the light source is a laser, the scattering intensity fluctuates over time. This fluctuation is due to the fact that the small molecules in solutions are undergoing Brownian motion, and the scattering distance in the solution is constantly changing with time. [93] Analysis of these intensity fluctuations yields the velocity of the Brownian motion and the particle size using the Stokes-Einstein relationship. [94] DLS does not require any sample information beyond the solvent viscosity and refractive index of the solvent.

Figure 17. Dynamic Light Scattering of large compared to smaller particles.

DLS measurements were taken by the Malvern Zetasizer Nano ZS. All measurements were taken in a low volume disposable plastic cuvette at room temperature. Intensity size measurements were taken using water as the dispersant and silver having a refractive index of 1.3. Three replicates for each sample were taken to ensure accuracy in the results.

3.4.4 Transmission Electron Microscopy (TEM)

TEM is a characterization technique in which a magnetic field directs and focuses an electron beam as it passes through an ultra-thin sample. Lower density or lower atomic weight molecules allow for the electron beam to easily pass through, while the higher density or atomic weight molecules do not, thus appearing darker on the TEM phosphorus viewing screen. With the aid of high resolution cameras TEM has the ability to image single metallic nanoparticles and sometimes it has enough resolution to image single atoms. TEM can be used for both imaging and diffraction of a material. Diffraction patterns aid in determining atomic organization with a particle. TEM allows for more resolution than SEM due to an electron beam that has a well-defined de Broglie wavelength.

The samples for TEM were prepared by drop casting small drops of each colloidal sample onto carbon coated copper grids. For gel samples, gels were mechanically ground by hand with a mortar and pestle. Ground beads were immersed in DI water (2mL of water per bead) for 24 hours to allow particles to resuspend in solution. The drops were allowed to dry slowly at room temperature in a fume hood. The TEM images were taken using a JEOL TEM-2100 operating at 200kV.

3.4.5 Scanning Electron Microscopy (SEM)

SEM is similar to TEM in the fact that it uses a focused beam of electrons to scan the surface of a sample, but instead of detecting electrons passing through a sample SEM detects the electron beams that reflect off the substrate. Three essential signals are detected from the substrate: back scattered electrons, secondary electrons, and X-rays (Figure 18). Back-scattered electrons are primary beam electrons, which have been elastically scattered by nuclei in the sample and escape from the surface. Using detectors, the topographic contrast can be obtained, but at low resolution. Atomically heavier and denser atoms show up brighter on backscatter due to more electrons

being reflected. Using this mode, heavier metallic nanoparticles can be distinguished from lighter polymer molecules in polymer nanocomposites. Secondary electrons produced by the primary beam produce high-resolution topography images. Other sources of secondary electrons may degrade the image. X-rays are used for species detection.

Figure 18. Three different signals detected in SEM from the electron beam interactions with a sample: Secondary electrons, Backscattered electrons and X-rays.[95]

Colloidal samples were drop cast onto copper tape and allowed to dry overnight at room temperature in a fume hood. Solid gel/fabric samples were freeze dried or air dried. Hydrogel samples were cut in half with a razorblade. One half of the sample was placed with the cross-section placed up for analysis of inside of the gel; while the other sample's cross-section was placed face down to image the surface of the material. The tape was transferred onto silicon wafers and was imaged with a Hitachi S-4700 Scanning Electron Microscope (SEM) with a cold field emission electron source. Samples were coated with gold to avoid charging effects before

analysis. The instrument was operated by the Chemical Engineering Department Research Assistants Adrienne McGraw and Jeremy Hardinger.

Chapter 4. Objective 1 – Investigate impact of reaction conditions on silver nucleation with Poly (acrylic acid) (PAA) Structures

In this objective, PAA will be used as a nucleating agent to synthesize silver nanoparticles. Reaction conditions were changed to monitor its effect on particle size; concentrations of PAA, AgNO_3 and NaBH_4 were varied. The reaction mechanism of the PAA/Ag system and the reaction kinetics for various concentrations of polymer is reported. The effects of the reaction environment (pH, and temperature) were varied to see the effect on particle size. The results and understanding of the effects of these changes to the reaction will be used when developing the biological scaffold.

4.1 Reaction Conditions

Carboxylic acid functional groups in both synthetic polymers and biological molecules are used to stabilize and nucleate nanoparticles like gold and silver. To develop a better understanding of the nucleation process with PAA in the silver nucleation process, three parameters were varied: the concentration of silver, the concentration of PAA, and the concentration of reducing agent. To confirm the formation of silver nanoparticles, the colloidal solution was subjected to UV-Vis spectroscopy. The UV-Vis results shown in Figure 19 are for a silver colloidal solution with a silver concentration of 1 mM and polymer concentration of 1mg/mL. The reactants at the same concentrations were plotted on the graph as well. The silver nanoparticle solutions display a clear peak around 400nm while the reactants show very little absorbance response to the UV spectra.

Figure 19 UV-Vis spectra of silver nanoparticles, reaction conditions: 1mM AgNO₃ and 1mg/mL PAA

Silver nanoparticles display clear UV peaks between 400nm-450nm due to a physical phenomenon known as surface plasmon resonance (SPR). SPR occurs at specific wavelengths which induce the free metal surface electrons to oscillate in silver nanoparticles. [96] The variation of these peaks indicates a change in particle size and morphology. The result from the UV-Vis spectrum displays a clear peak at around 400nm therefore confirming the presence of silver nanoparticles in solution. Peaks around 400nm indicate silver cubes and spheres ranging from 5nm-15nm in size.[96]

The reduction of silver salts in solution will result in colloidal silver nanoparticle formation regardless of the presence of polymer; therefore, DLS was used to determine if PAA was promoting nucleation of silver within the solution. Figure 20 shows intensity as a function of size for nucleation without PAA (Figure 20A) and with 0.1mg/mL of PAA (Figure 20B). The experiment was run in triplicate, which are the three curves displayed on each graph. These results indicate a higher concentration of particles in the order of 100nm for a solution with PAA present opposed to the sample without PAA. The colloidal solution without PAA shows a larger distribution of particles spanning from 10nm to 1000nm. The dramatic shift of the intensity graphs for DLS shows that, when PAA is present, larger silver nanoparticles are present in solution. Therefore, it can be concluded that PAA particulates are larger and less disperse in comparison to samples with no PAA.

Figure 20. A) is the intensity plot from DLS with no PAA present in the reaction. B) is an intensity plot generated by DLS with a PAA concentration of 0.1mg/mL

DLS and UV-Vis techniques were used to confirm the nucleation of silver in solution. To further characterize the nanoparticles, TEM imaging was used. Figure 21 shows two images of the same particle. Figure 21A shows an image of silver nucleated in the presence of PAA with a polymer concentration of 1mg/mL, AgNO_3 concentration of 1mM and NaBH_4 concentration of 1mM. The cubic polymer structure observed is enveloping the silver nanoparticles. The size of the particles located in the polymer structures ranged from 5nm-10nm, while particles formed without the presence of PAA were about 1nm or less. Figure 21B is the same sample after the electron beam on the TEM was used to burn away the polymer structure. Degrading the polymer reveals a silver nanoparticle nucleating in the center of the structure, with additional smaller silver nanoparticles attached to the surface of the polymer structure. Due to inaccuracy of the DLS from sample to sample it will not be used for the rest of the project.

Figure 21. TEM Images of 1mg/mL PAA with a scale bar of 50nm.

4.1.1 Effect of silver concentration

The silver concentration of the nucleation reaction was varied while keeping the concentration of polymer (1mg/mL) and NaBH_4 (1mM) constant. Figure 22 shows an image of colloidal silver nanoparticles with silver concentrations of 1mM and 5mM and 50mM. The 50mM silver concentration shows black silver aggregates and the solution is clear, indicating the silver particles precipitating out of solution. At higher concentrations of silver (around 30mM and higher) particle size increases to the point where the nanoparticles are not stable in solution and begin precipitate out as silver aggregates.

Figure 22 Silver nanoparticle solution of 1mM, 5mM, and 50mM of AgNO_3 from left to right respectively.

Figure 23 displays the UV-Vis spectra for both the lower concentrations of silver, ranging from 0.1-0.75mM (Figure 23A), and higher concentrations ranging from 10mM-50mM (Figure 23B).

Figure 23. UV-Vis spectrum of various AgNO_3 concentrations

For the higher range of silver concentrations displayed in Figure 23B, the UV-Vis spectra indicates that at silver concentrations of 10 mM or higher no longer form nanoparticles but form silver aggregates. It should also be noted that at higher concentrations, silver starts to visibly aggregate out of solution (see Figure 22). and UV-Vis readings did not change if the solution

was mixed. Lower absorbance values are seen for higher concentrations of silver; this can be explained due to the aggregation of silver at these higher concentrations. The aggregation of silver lowers the concentration of nanoparticles suspended in solution; therefore, the dilution of nanoparticles contributes to the lower absorbance readings. In addition, DLS results indicated silver particle size slightly increased with higher concentrations but still were on the order of 100nm (not shown).

4.1.2 Effect of PAA concentration

The polymer concentration was varied for this set of experiments while the keeping the concentration of both the silver and NaBH_4 constant at 1mM. Figure 24 shows samples with varying concentrations of PAA. There is a noticeable color change of the colloidal solutions; at 1mg/mL PAA concentrations the solution is yellow/gold but increasing the polymer concentration to 20mg/mL the colloidal solution displays a pink tint. The color changes indicate a change in size or shape, which is confirmed by the UV-Vis results shown in Figure 25.

Figure 24 Samples with PAA concentrations of 1mg/mL, 10mg/mL, and 20mg/mL from left to right respectively

Figure 25. UV-vis spectra of various polymer concentrations

The UV-Vis results show new peaks at higher wavelengths with polymer concentrations higher than 2mg/mL. The additional peaks seen around 450nm for the samples ranging from 2mg/ml-4mg/mL indicate a shift in size and morphology in the silver nanoparticles being formed which is consistent with the work of Sun and Xai.[96]

Using the TEM to image samples of polymer concentrations of 0.1mg/mL, 1mg/mL, and 2mg/mL, changes in the polymer structure can be seen during silver nanoparticle nucleation. Figure 26 shows representative images of the polymer structures at these various PAA concentrations.

Figure 26. TEM images of silver nanoparticle samples with polymer concentrations: a) 0.1mg/mL b) 1mg/mL c) 2mg/mL. Scale bar is 50nm

Figure 26 shows the various polymer formations at different concentrations of polymer. The results from the 0.1mg/mL samples showed very little order and structure surrounding the nucleated silver nanoparticle. The polymer cubes were formed in the 1mg/mL solution samples producing a silver nanoparticle with similar size and shape to the 0.1mg/mL. The 2mg/mL samples displayed a polymer structure in the shape of a cross. Within this polymer shape there are more particles that are larger than the previous samples.

4.1.3 Effect of reducing agent concentration

The reducing agent concentrations were varied while keeping the concentrations of AgNO_3 and PAA constant at 1mM and 1mg/mL respectively. At higher concentrations (25mM and 50mM) of reducing agent provides a more narrow distribution of particle sizes. The results of these experiments have shown that the reducing agent concentration has the greatest effect on particle size; particle sizes for 1mM NaBH_4 ranged from 5nm-10nm while at 50mM NaBH_4 the particle size ranged from 25nm-50nm in size as shown in Figure 27.

Figure 27. Silver nucleation with 1mg/mL PAA 1mM AgNO₃ and A) 1mM NaBH₄ scale bar = 20nm and B) 50mM NaBH₄ scale bar = 50nm.

More representative images of varying polymer, silver and PAA concentrations can be found in Appendix A.

4.2 Reaction Kinetics

Time scale experiments were run to measure the stability and monitor the growth of the nanoparticles over several days. Several samples at various times spanning from a few hours to a few weeks were taken over a set of reaction conditions in order to determine the effect of reaction time on the size of the particles. Techniques used to characterize the particles formed by this nucleation process include UV-Vis and DLS. Imaging of the nanoparticles was performed using SEM and TEM.

In previous experiments, samples reacted for approximately 18 hours prior to characterization. The purpose of this study was to observe the formation of silver nanoparticles size and shape over time; therefore, a time scale study which ranged from 12 hours – 5 days. It was observed that reaction times longer than 18 hours led to larger particles. After 5 days, particles were noticeable bigger within the square/rectangular polymer matrices seen in Figure 26. Figure 28 is an image of particles formed under the following reaction conditions: 1mM AgNO₃ and NaBH₄, and PAA concentration of 10mg/mL. Larger sized particles are observed nucleating within the cubic polymer structure. These particles found were much larger ranging from 20-50 nm in size, compared to 5nm-10nm sized particles previously imaged after 18 hours of reaction. In this

sample, there are also several more nucleation sites compared to the sample in Figure 26, which only has one.

Figure 28. TEM image of a 1mg/mL PAA allowed to react for 5 days

4.2.1 Determination of Nucleation Mechanism

4.2.1.1 PAA-Silver Complexation Studies

Berhrens et al. suggested two mechanisms for nucleation [97]:

- I. Conjugates of polymer template and silver seeds homogenously nucleated in solution
- II. Heterogeneous nucleation of silver particles on the microtubule surface due to silver ion adsorption

To study the nucleation mechanism for this project, the effect of silver complex formation of the polymer in the silver ion source was studied and the growth of silver nanoparticles immediately after the reducing agent was added was tracked using UV-Vis. The incubation time of silver with PAA prior to forming the PAA/AgNP complex was varied.

Using four different polymer concentrations, samples were prepared with 1 mM AgNO_3 , and the effect of incubation time was monitored. Figure 29 shows the UV-Vis results of these

experiments. The spectra show a change in absorbance intensity, indicating a change in AgNP concentration, rather than a change in the peak position, which would indicate a change in particle size. These results demonstrate that incubation time does not have an effect on particle size and nucleation.

Figure 29. Incubation time over various polymer concentrations

4.2.1.2 Reaction Kinetics Studies

Continuing the study on the possible reaction mechanisms; the next group of experiments was to examine the UV-vis results of the reaction kinetics immediately after the nucleation process started. The results of these kinetic studies are shown in Figure 30, using the four same polymer concentrations and same silver concentration as in the complex studies. UV-Vis measurements were taken for over 30 minutes after the reaction started. For 0.1 mg/mL concentration the peak position did not vary with time right after the initial start of the reaction. This indicates that at this concentration silver nucleation was behaving as reaction mechanism I proposed by Berhrens et al. While a clear peak position shift was noticed for concentrations greater than 1 mg/mL,

which indicates that at these polymer concentrations, the silver nucleation process follows mechanism II proposed by Berhrens et al.

Figure 30. UV-Vis results of the silver nucleation reaction kinetics varying the polymer concentration

4.2.3 Kinetics analysis of PAA-AgNP system

The kinetics of particle formation can be followed by a variety of experimental setups using UV-Vis spectroscopy. According to Watzky and Fink, continuous nucleation is followed by autocatalytic surface growth which results in monodisperse size distribution. [87] This mechanism suggests that growth is a result of already formed particle nuclei that are bombarded by freshly reduced metal ions; as opposed to two nuclei joining together. [98] The final size of the particle is determined by the relative rates of nucleation and growth.

UV-Vis spectroscopy is applied to the characterization of metal and semiconducting nanoparticles due to the plasmon resonance lines which occur in the visible spectra. [99]

Henglein et al. monitored stepwise growth of silver nanoclusters by spectroscopy methods. [53] A similar method to characterize the particles is described here. The effect of PAA concentration on the reduction rate of silver nanoparticle nucleation was studied. These rates were obtained by monitoring the UV-Vis spectra over time.

Figure 31. UV-Vis of 1mg/mL polymer solutions over time

Absorption spectra for a silver solution with 0.1mg/mL PAA were collected every 5 minutes for 30 minutes and are shown in Figure 31. The wavelength for maximum absorption peak ($\lambda = 395\text{nm}$) does not change over time. Figure 32 shows absorbance spectra for varying PAA concentrations 30mins after the reaction has started. This Figure shows the maximum absorption peak increases at higher levels of polymer concentration. The broad distribution of the peak is caused by aggregation of the silver nanoparticles. For polymer concentrations lower than 1mg/mL, the max absorption peak is constant at 395nm. Higher concentrations of polymer start to develop a second peak indicating a bimodal distribution of particles. PAA is a nucleating agent and these UV-Vis results suggest that the reaction is a two-step process. The first involves reducing the silver ions initially into small silver nanoparticles. The second step is a growth step

in which the particles that are in contact with the polymer continue to attract more silver ions due to the negatively charged functional groups. These ions continue to be reduced and attach to the already formed particle to promote growth.

Figure 32. UV-Vis of various polymer concentrations at 30 minutes

4.2.4 Determination of the Rate of Reduction

The kinetics study measured the absorbance with time for several polymer concentrations at the 395nm wavelength. The 395nm peak was used since this wavelength was a result of the particles being formed without the presence of a polymer substrate. The aggregation of the silver due to the growth step of the reaction was ignored for the calculation of the rate of reduction. Figure 33 shows the absorbance at 395nm over 30 minutes for PAA concentrations of 1mg/mL, 2mg/mL, and 3mg/mL.

Figure 33. Absorbance of various polymer concentrations at 395 over time

The sigmoidal curve for all 3 polymer concentrations in Figure 33 indicates an autocatalytic reaction. [87, 98, 99] Kinetic rate constants for autocatalytic reactions can be found by plotting $\ln(a/(1-a))$ vs. time. [87] The absorbance at long times can be determined from Figure 33 as the constant absorbance value when the curve levels off.

The apparent rate constant of the autocatalytic process (K_a) was determined from the slope of Figure 34. The results for varying polymer concentrations are displayed in Table 2. Plots for the other polymer concentrations can be found in Appendix B. The rate of reduction increases with polymer concentration. This is due to PAA's ability to nucleate polymers. After reduction, the particles in contact with PAA quickly begin to grow which is why after 30 minutes a second peak is formed indicating bigger particles are present in the solution. Since this reaction is lowering the concentration of the initial smaller particles formed, the rate of the reduction increases to maintain equilibrium. This is significant because it is opposite to the effect of stabilizers like PVA. It is reported in the literature that PVA decreases the rate of reduction. [53] This is due to PVA stabilizing particles initially after the reduction phase instead of promoting a secondary growth reaction.

Figure 34. Plots of $\ln(a/(1-a))$ as a function of reaction time

The rate of reduction without polymer is initially higher than the various polymer concentrations reported values in Table 2 because of steric hindrance caused by the polymer. Adding a bulky molecule into the reaction is thought to inhibit silver/ NaBH_4 agent interactions. This effect was overcome as more nucleation sites are added and by the increasing amount of reaction sites that induce particle growth.

Table 2 Rate constant for the rate of reduction at various polymer concentrations

PAA Concentration	$k_a \times 10^3 \text{ (s}^{-1}\text{)}$
0	5.6
0.1mg/mL	1.3
0.25mg/mL	2.3
0.5mg/mL	2.8
0.75mg/mL	3.3

1mg/mL	3.6
2mg/mL	5.8
3mg/mL	9.9

4.2.5 Particle Growth

Polymer concentrations higher than 2mg/mL have shown broader peaks ranging from 400-500nm due to Ostwald ripening and particle aggregation. The TEM image shown in Figure 35 displays the result of Ostwald ripening for silver nanoparticles; these particles are around 40nm-50nm in size. While spherical particles around 40-50nm display UV-Vis peaks slightly above 400nm, these particles are not spherical in nature, and the elongation shown in Figure 35 is responsible for the peaks at higher wavelengths. [100] From these TEM and UV-Vis results, it can be concluded that increasing concentration of polymer promotes growth and larger particle sizes.

Figure 35. TEM image (scale bar 20nm) and UV-Vis of 2mg/mL polymer sample

4.3 Reaction Environment

In addition to changing the concentration of the reactants; nucleation in different temperature and pH environments will be studied in future experiments. Once the proper reaction conditions for nucleation are identified similar conditions will be needed to nucleate onto a dual polymer matrix consisting of PAA and a biomolecule such as proteins or polysaccharides. Figure 36 is the UV-Vis spectra for silver nucleated at various pH values (2.5, 4.5, 7, 10). These pH values were

chosen because the pK_a of PAA is reported as 4.5. [101] The carboxylic groups located on the PAA backbone become deprotonated at pH levels above the pK_a , which is the functional group that reacts to form the silver complex and is responsible for nucleation of silver nanoparticles. In Figure 36, it is observed at pH 4.5 and 2.5 that the maximum absorbance for these peaks decreases in comparison to pH values of 7 and 10. This indicates a lower concentration of particles as the pH drops below the pK_a value. As pH values decrease, more carboxylic groups on the polymer are not deprotonated leading to less nucleation.

Figure 36. Various pH absorbances of Silver Nucleated at 25 °C

Another observation made from Figure 36 is that with lower pH values a second peak begins to form and the distribution of particles becomes greater. A red shift in the second peak also occurs, suggesting with less nucleation sites at the same silver concentration, bigger particles are forming. Due to the nature of the peak tailing off at higher wavelength for the pH 2.5 spectrum, the UV-Vis results suggest these particles are becoming less uniform in shape and are becoming elongated.

The environmental temperature of the reaction was decreased to 4°C and the UV-Vis results can be seen in Figure 37. For this experiment, the reactants were mixed initially at room temperature and subsequently transferred into a 4°C refrigerator. In comparison to the same reaction conditions run at 25°C, the peaks are less broad and have a slightly higher intensity indicating

less particle size distribution and a higher concentration of particles. In addition, NaBH_4 is a strong reducing agent and overall very reactive with water, and reducing the temperature also decreases the rate of this side reaction with water. The decrease in side reaction kinetics leads to more NaBH_4 present to react producing a higher concentration of silver nanoparticles. The decrease in the primary reaction kinetics for silver nucleation prevents larger particles from forming thus narrowing the distribution.[18] The results at pH 2.5 were relatively the same at both temperatures because the nucleation is less likely when the polymer molecule is protonated, regardless of the temperature.

Figure 36. Various pH absorbance of Silver Nucleated at 4 °C

The next experiment was the same reaction conditions being run at 25°C with the reducing agent chilled to 4°C, and the UV-Vis spectra are shown in Figure 37. The distribution is narrower than when the reaction was just being run at 4°C without a reducing agent being chilled. This suggests that making the reducing agent at a lower temperature has more effect on slowing the reaction kinetics. Logically, these results indicate NaBH_4 reaction with water is instantaneous since a cold reducing agent at the start lowered the reaction kinetics more than just changing the reaction temperature after the solution was premade at room temperature.

Figure 37. Various pH absorbances of Silver Nucleated at 25 °C with chilled NaBH₄

Figure 38 shows TEM images of the chilled reducing agent at all four pH values. Particles synthesized at pH 2.5 (Figure 38A) show large single particles over 100nm in size as predicted by the UV-Vis results. These particles also were not spherical in nature rather they were rectangular. Particles made at pH 4.5 (Figure 38B) and pH 7.0 (Figure 38C) are relatively similar with a wide distribution of generally spherical particles; with the largest measuring around 50nm while the smaller particles were around 10nm. The pH 10 results (Figure 38D) displayed the smallest particle sizes ranged from 5nm-25nm. The numbers of particles overall coincided with the UV-Vis results as big single particles were seen for pH 2.5 while the highest number of particles were seen for pH 10.

Figure 38. TEM images of silver nanoparticles nucleated at 4°C with chilled NaBH₄. A) pH 2.5, B) pH 4.5, C) pH 7.0, D) pH 10.0 scale bar is 50nm for each image.

Overall decreasing the temperature of the reaction, and more importantly the reducing agent, allowed for a more narrow size distribution with more uniform particle shape. The pH studies showed that PAA has impact on the nucleation process. At the lower pH values (less than the pK_a of PAA), PAA was protonated and the number of particles produced by the silver reaction decreased significantly. The particles formed at this low pH were very large and irregularly shaped. When PAA was deprotonated, it provided reaction sites for the silver nucleation reaction which led to a high number of relatively spherical particles.

In objective 1 it was shown that the presence of polymer in the silver chemical reduction reaction increases the silver nanoparticle size. The reaction conditions were monitored and a bimodal distribution was observed with increasing amounts of PAA suggesting that silver ions that are

both in contact and not interacting with the polymer are being reduced. Kinetics data are reported for the rate of reduction over various concentrations of polymer. The effects of the reaction environment (pH and temperature) were also monitored. Increasing the pH decreased the particle size due to polyacrylic acid propensity for protonation. Decreasing the reaction temperature increases particle size because it limits the sodium borohydride water side reaction.

Chapter 5. Objective 2 – Development of Biologically Relevant Antimicrobial Scaffold/Structure.

Silver nanoparticles have antimicrobial properties which can be used in various biomedical materials such as wound dressings, bone implants and other simple fabrics. Research in this area has attempted to combine biological scaffolds with previously prepared silver nanoparticles via diffusion or *in situ* nucleation. One problem with these composite materials is the ability to disperse the nanoparticles evenly throughout the material. The goal of this objective is to prove that polymers with negatively charged functional groups maintain their nucleation potential in the solid phase helping with particle dispersion and increasing the number of particles nucleated onto the polymer matrix. Another goal of this objective is to prove that nanoparticle size can be controlled by varying the composition of the nucleation polymer in the solid phase composite material. The ability to control size opens the door for a wide variety of applications of these nanocomposite materials.

To accomplish our goals this objective will design a silver nanoparticle/calcium alginate nanocomposite intended for wound healing applications using PAA as a nucleation template. This objective assembles PAA into a calcium alginate gel and nucleate silver into the scaffold using similar chemical routes stated in Objective 1. Additionally, the effect of PAA and silver nanoparticles effect on the mechanical and swelling properties of the calcium alginate hydrogels will be monitored. The antimicrobial effects of the prepared composite scaffolds will be measured.

5.1 PGA-ELP nucleation system

Poly(glutamic acid) (PGA) cross-linked with ELP was used as the first polymer nanocomposite system for the silver nucleation protocol. PGA is a prime case study material because it contains carboxylic acid groups on its polymer backbone, similar to PAA. PGA-ELP cross-linked materials did not completely dissolve in an aqueous solution; however, the silver nucleation reaction was performed with concentrations of AgNO_3 and NaBH_4 at 1mM while the concentration of the cross-linked polymer was 0.2mg/mL.

Three different mass ratios of polymers were used in this study; 4:1, 4:2, and 4:4mg of ELP to mg of PGA. Figure 39 shows SEM images of silver nucleated in the presence of the PGA-ELP structures at the aforementioned various mass ratios. In Backscatter SEM images elements with a heavier atomic weight display brighter due to the fact that they deflect more electrons. The brighter areas on these images indicate the presence of silver nanoparticles. The background material is the carbon polymer backbone. Qualitatively more silver nanoparticles appear as the ratio of PGA increases. This case study shows that polymers with carboxylic groups retain their ability for silver nucleation even after cross-linking with another polymer.

Figure 39. SEM images of a) 4:1 b) 4:2 c) 4:4 mass ratios of crosslink ELP-PGA

5.2 Nucleation on PAA grafted cotton fabrics

The PGA case study proved that polymers with carboxylic acids retain their ability to nucleate in solution when the end chains are crosslinked with another polymer. In these three experiments want to test the ability of PAA to retain its nucleation ability even when the carboxylic groups are reacted and grafted onto the cotton fabrics. PAA was grafted onto cotton fabrics by an esterification reaction detailed in Martel et al. [88] The cotton fabrics were soaked in PAA and a

phosphorous catalyst solution and dried. The reaction takes place at 190⁰C to graft the carboxylic groups with the various hydroxide groups located on cotton fabrics. FTIR confirmed the new fabrics contained both cotton and PAA, the FTIR results are in Appendix C.

Figure 40. Cotton fabrics grafted with PAA (left) and without PAA (right) nucleated with silver

Figure 40 shows silver nucleation on a cotton fabric grafted with PAA (left) and without PAA (right). The concentrations of silver and reducing agent were 1mM. These fabrics were white before the nucleation process and after the fabrics grafted with PAA were a darker color than its counterpart indicating that silver particles nucleated on the PAA fabrics were larger. Using SEM (Figure 41) silver nucleation was confirmed for both materials using energy dispersive X-ray (EDX). Cotton fabrics grafted with PAA (right) showed a higher concentration of larger particles of silver with the presence of PAA. It can, therefore, be concluded that PAA maintains its ability to nucleate silver even with its carboxylic functional groups being used in the crosslinking process to another material.

Figure 41. SEM images of silver nucleated onto cotton fabrics without PAA (left) and grafted with PAA (right)

5.3 Alginate Material Development

Sodium alginate powders have the ability to form hydrogels when in the presence of a divalent cation like calcium chloride. Alginate is used often for biomedical wound dressings due to its biocompatibility and its ability to be removed with limited pain and discomfort. Alginate dressings with silver nanoparticles are already being sold today due to the fact that silver provides antimicrobial properties aiding in recovery by preventing infection. The introduction of PAA into the alginate hydrogel matrix allows for a more effective *in situ* nucleation. Providing a material with higher amounts of silver nanoparticles and the ability to control silver size simply by modifying the PAA content. Silver wound dressings have garnered much attention recently due to their ability to kill antibiotic-resistant bacteria.

5.3.1 Chemical crosslinking with esterification reaction

With the success of coupling PAA to the cotton fabric, the first method of producing an alginate/PAA material was to attempt the same esterification reaction. PAA and sodium alginate were dropped as a thin film onto a glass slide at various compositions, and attempts were made to chemically couple the materials. After the reaction, the films were removed, resuspended into solution, and dropped into 100mM calcium chloride. When the coupled alginate and PAA solution was dropped into the 100mM calcium chloride solution no beads were formed. This was due to alginate degrading at the reaction temperature (190°C).

5.3.2 Determination of Silver Nucleation Protocol

Nucleation of silver nanoparticles onto alginate beads has been studied by multiple researchers, all of whom had slightly different methods for *in situ* nucleating.[4, 6, 102] Three methods have been observed from the literature. Samples of the 50% PAA composite gel were used in all three methods of silver nucleation to determine the best method for nucleation in this system. Backscatter SEM images were taken for each gel to determine which method yielded the most nucleation.

5.3.2.1 Method 1

Freshly made 25% PAA gel beads were removed from the CaCl_2 solution and soaked in 5mL of 10mM AgNO_3 solution for 18 hours. The beads were removed, dried and immersed in 10mL of 10mM NaBH_4 for an hour. The beads were then freeze dried in preparation of SEM, an Image of the prepared sample are shown in Figure 42.

Figure 42. Method 1 SEM image of 50% PAA 10mM AgNO_3 and 10mM NaBH_4

Method 1 yielded very little silver nucleation. Figure 42 is representative of a back-scatter scan of the 25% PAA bead. No silver was found on these samples; the only metal found was calcium, which is the divalent metal responsible for alginate gelation.

5.3.2.2 Method 2

Method 2 included freeze drying the freshly made 25% PAA gel beads before addition into the 5mL of the 10mM AgNO_3 for an hour. Then the gels are immersed into 10mL of 10mM NaBH_4 solution for 30 minutes. The beads changed color from pale white to dark yellow/gold. Similar to method 1 the final beads were freeze dried in preparation for SEM. A representative image of the resulting bead is shown in Figure 43.

Figure 43. Method 2 SEM image of 50% PAA 10mM AgNO_3 and 10mM NaBH_4

The Back-scattering image showed an abundance of silver nucleated onto the surface of the alginate gel. Elemental analysis was done by EDX to confirm these particles as silver (Figure 44). In backscatter the atomically heavier particle emanates a brighter image. The silver identified is not AgCl (an insoluble compound when in water) because the alginate beads were washed with DI water several times before the freeze drying process removing a majority of the chloride ions. The bigger nanocluster right above the analyzed particle was confirmed to be

calcium. Calcium, which holds the gel together, is easy to distinguish from silver since it is a lighter element and does not emanate as brightly as silver particles. Silver particle sizes ranged from 100-1000nm which is too high for effective antimicrobial activity; therefore, the concentration of both silver and reducing agent needs to be optimized for antimicrobial activity.

Figure 44. Method 2 EDX confirmation of silver for 50% PAA 10mM AgNO₃ and 10mM NaBH₄.

5.3.2.3 Method 3

When making the 25% PAA beads, PAA was entrapped with silver at the same concentrations as the other methods, 10mM AgNO₃. Alginate will gel instantly when it comes into contact with CaCl₂; therefore, it can entrap both polymer and silver ions. The freshly made beads were immersed in 10mM NaBH₄. All samples were freeze dried in preparation for SEM. As seen in the SEM image of Figure 45, small amounts of silver particles formed on the outer edges of the sample as seen at the top of this image.

Figure 45. Method 3 SEM image of 50% PAA 10mM AgNO₃ and 10mM NaBH₄

While method 1 produced no silver and method 3 produced very little nucleation; method 2 clearly nucleated the most silver throughout the hydrogel samples. This is due to the fact that after the gels were formed all of the water was removed by freeze drying. Freeze drying allowed the gel to dry without its structure collapsing on itself. Then the gels could be loaded with silver up to full swelling capacity allowing for more silver intake. Method 1 including loading silver into a hydrogel bead which was already swollen, forcing the ions to diffuse into the gel and reducing the amount of silver intake. Method 3 had silver loaded in an aqueous solution with alginate and PAA. When silver comes in contact with chlorine it forms insoluble precipitate and it is likely that silver ions were being lost to this side reaction during the gelation process.

5.3.2 Rheology results

The scope of this project is to develop a biomaterial suitable for wound healing. Mechanical strength is important for hydrogels in biomedical applications because they have to replicate the properties of native tissue. It is important to monitor the properties of the composite gels being made to make sure they are not losing structural integrity and mechanical strength. To ensure the introduction of PAA and silver nanoparticles into the alginate gel matrix does not change the properties of the system, a rheology analysis was performed on the composites. Various

compositions of PAA/alginate composite gels were investigated to measure the effect of PAA concentration on gel mechanical strength. The viscoelastic properties determine the mechanical performance of a material.

In the linear viscoelastic region, the structure of a material can be characterized by using a frequency sweep. This mode provides more information about the effect of colloidal forces, the interactions due to multiple phases in a material. [103] A strain sweep was performed at a frequency of 6.2 rad/s to find the viscoelastic region; which was determined to be at strains greater than 5%. Therefore, the frequency sweep was run at 10% strain to get the storage modulus (G') and the loss modulus (G'') curves shown in Figure 46.

Figure 46. Frequency sweep of various composite ALG-PAA gels

Analyzing the results from the frequency sweep, the storage (or elastic) modulus is dominant over the entire frequency sweep for the pure alginate (0% PAA) and 10% PAA samples. At a higher concentration of PAA, the loss (or viscous) modulus starts to dominate the response. 25% PAA samples show a crossover point at around 58 rad/s. At lower frequencies G'' was dominant, and after this observed crossover point at higher frequencies G' is dominant. When the loss modulus is dominant, the system has little internal network and is easily disturbed by the frequency oscillations. This indicates the network is not as strong. With both the 50% PAA and 75% PAA there is no crossover point and the loss modulus is dominant over the entire sweep. When PAA is introduced in the gel system, the alginate network begins to become disturbed and with increasing amounts of PAA, the bulky PAA molecule interferes with the crosslinking of the alginate network.

The results of the frequency sweep with 50% PAA gels nucleated with silver nanoparticles were compared to the analogous gel without silver nanoparticles (Figure 47). For this gel the storage modulus is dominant over the entire frequency range, which is the opposite of the results seen with 50% PAA gel without silver nanoparticles. The result indicates that the addition of silver nanoparticles strengthens the gel matrix. Despite not being reported for silver nanoparticles, it is not uncommon for nanoparticles such as silicon oxide, titanium oxide and carbon nanotubes to strengthen gel matrices. [104-106]

Figure 47. Frequency sweep of 50% PAA sample with and without Ag nanoparticles

Winter et al. experimentally established a power law relationship to relate angular frequency (ω) in a frequency sweep to gel properties, which are given in Equations 2 and 3, [107]

$$G' \propto \omega^{n'} \quad (3)$$

$$G'' \propto \omega^{n''} \quad (4)$$

where G' indicates the elastic modulus, and G'' is the loss modulus. The more liquid a sample is the more n' and n'' will approach 2 and 1, respectively. As these values are closer to zero the sample is more solid-like. [108] The relationship for n can be solved by plotting the raw data on

a log-log scale and finding the slope of the curves. Figure 48 and 49 shows the log-log relation for both G' and G'' , respectively.

Figure 48. G' frequency sweeps on a log-log scale

Figure 49. G'' frequency sweep data on a log-log scale

No trend can be seen in terms of intensity of both G' and G'' with gel composition. This can be attributed to inconsistency in gel thickness from sample to sample, which was due to processing issues in making a thin film of alginate gel. Regardless of thickness, the slopes of all these curves were calculated and a trend can still be seen in terms of the n' and n'' values, shown in Table 3.

Table 3 Power law relationship over various PAA compositions

Sample	n'	n''
0% PAA	0.137	0.128
10% PAA	0.163	0.099
25% PAA	0.276	0.172
50% PAA	0.244	0.195
75% PAA	0.221	0.175
50% PAA/Ag	0.079	0.095

The power law constants increase with increasing amounts of PAA until they hit a maximum at the 50% PAA sample. Increasing power law constants means more flexibility in those gels; therefore, as PAA is introduced into the gel matrix, the gel is more flexible due to less ionic binding, which would be interrupted by the cumulative effect of PAA. 75% PAA samples begin to behave more like a solid because of the presence of excess PAA. The gel begins to exhibit the properties of PAA, which is more rigid than hydrogels. The sample loaded with silver nanoparticles is more rigid than the rest of the samples overall; indicating that the presence of silver nanoparticles makes the gel more rigid.

As the amount of PAA in the hydrogel matrix increases, the network is disturbed and the PAA interferes with the amount of crosslinking within the alginate material. This leads to a more flexible material, which behaves more liquid like with increasing amounts of PAA; with exception to the 75% PAA sample in which the stiffness of the PAA and density of material starts to make the system stiffer. The introduction of silver nanoparticles in the gel matrix strengthens the gel and makes it less rigid, slightly improving the properties of the gel. Overall, the changes in the power law relationship quantified in Table 3 did not change enough to affect the hydrogel performance for antimicrobial applications.

5.3.3 Swelling Properties

The swelling of a hydrogel is one of its unique and most important functions. Creating a moist environment for wound healing is beneficial for both decreasing healing time and patient comfort. [73, 76] Additionally, monitoring the swelling capacity of gels affects the amount of silver that can be loaded into these composite materials.

The time for a hydrogel to reach its maximum swelling capacity depends on the degree of crosslinking in a hydrogel and the solvent with which it interacts. Figure 50 shows the swelling of three samples of the pure alginate beads that were monitored over time to determine the rate of swelling and the time in which maximum water retention was achieved. By plotting the wet weight of the alginate gels over time, the maximum swelling capacity for alginate beads in DI water can be determined. The results show that the longest it takes for the ALG beads to reach its maximum swelling capacity is three hours.

Figure 50. Swelling kinetics of pure alginate beads

The swelling capacity of a gel can be monitored by two metrics: degree of swelling (DS) and water content (WC). [7] These values can be defined in Equation 4 and 5.

$$DS = \frac{(Wet\ Weight - Dry\ Weight)}{Dry\ Weight} \times 100 \quad (5)$$

$$WC = \frac{Wet\ Weight}{Dry\ Weight} \times 100 \quad (6)$$

A number of gel beads were weighed before and after they were immersed in DI water for three hours. The DS and WC for all 5 composite gels were calculated shown in Table 4. Each calculation was the sum of 5 separate runs. Each run included a group of beads, which were weighed together before and after swelling. Raw data can be found in Appendix D.

Table 4 Degree of swelling and Water Content for various composite gels.

Sample	Dry Weight (mg)	Wet Weight (mg)	DS	WC
ALG	119.3	716.9	500.9	600.9
10% PAA	82.3	601.8	631.2	731.2
25% PAA	61.4	529.5	762.4	862.4
50% PAA	76.7	687.6	796.5	896.5
75% PAA	26.4	146.8	456.1	556.1

The degree of swelling hits a maximum at the 50% PAA composite gels; analogous to the results seen in the rheology study. PAA is very hydrophilic; therefore, it is understandable with increasing amounts of PAA the degree of swelling would also increase. But this effect is at a maximum at 50% PAA. At higher amounts of PAA, there is an increase in gel density of the network leaving smaller free spaces causing the gel to absorb less solvent. [109, 110]

Swelling tests were performed on composite gels loaded with silver via the previous mentioned *in situ* reaction. Table 5 shows the degree of swelling and water content for the silver loaded samples. With the exception of the 25% PAA sample, the silver loaded samples showed an order of magnitude higher degree of swelling than its non-silver counterparts. This can be attributed to

the hydrogels undergoing an ion exchange due to the presence of NaBH_4 in the silver reaction process. As sodium replaces calcium in the gel matrix the amount of crosslinking decreases; allowing for more free space therefore absorbing more solvent.

Table 5 Degree of swelling and Water Content for various AgNp loaded composite gels.

Sample	Dry Weight (mg)	Wet Weight (mg)	DS	WC
10% PAA	23.2	451.3	1845.3	1945.3
25% PAA	14.2	88.1	520.4	620.4
50% PAA	6.7	174.7	2507.5	2607.5
75% PAA	6.5	256.1	3840.0	3940.0

5.3.4 FTIR

To confirm that PAA is being entrapped in the composite gels, FTIR analysis was performed. The spectrum ranging from $4000 - 700 \text{ cm}^{-1}$ of alginate beads (Figure 51) can be seen with various concentrations of PAA gels. Three distinctive peaks can be observed that indicate the presence of PAA. Acyl C-O groups (the single bonded C-O group in a COOH functional group) show a sharp peak between $1300\text{-}1400 \text{ cm}^{-1}$. A pronounced peak ranging from $1680\text{-}1700 \text{ cm}^{-1}$ can be seen with hydrogel samples that contain PAA. This peak is absent from the pure alginate samples. This peak indicates carbonyl stretching by a saturated carbonyl group which is characteristic for PAA. [24]

Another set of new peaks located around $2500\text{-}3400 \text{ cm}^{-1}$ starts to form with increasing amounts of PAA. FTIR peaks in this region tend to be broad and not well defined. It is not uncommon in this region for multiple peaks to arise with increasing amounts of O-H functions groups. The ALG sample has its first peak seen at 2400 cm^{-1} tailing off around 2900 cm^{-1} . When PAA is introduced into the sample, this first peak becomes much wider and tails off at around 3100 cm^{-1} ; with multiple peaks in between $3200\text{-}2500 \text{ cm}^{-1}$, peaks that are not seen in the ALG sample. It is observed that these bands represent OH stretching vibration bands for organic acids. With increasing PAA, the OH stretching peak becomes both more intense and wider indicating gels continue to be enriched with PAA. No general trends in peak shifting are observed which is to be

expected due to the fact that PAA is physically crosslinked. Only chemical crosslinking would result in trends of peak shifting.

Figure 51. FT-IR results of ALG and Composite beads

5.4 Nucleation on Composite ALG-PAA beads

5.4.1 Ultramicrotome TEM preparation

An alternative technique, called the ultramicrotome, was attempted for TEM sample prep in order to observe the placement of the silver particles within the gel bead. The Reichert-Jung Ultracut E Ultramicrotome was used to produce nano-thin slices of material for the purpose of electron microscopy. Using a glass knife and mounting the sample to a cutting wheel, the sample was rotated, moving in small increments (nano and micro-scale) against the glass knife to cut ultra-thin slices. Samples were embedded in an Epon resin which solidifies after one day of

curing at 37°C followed by 48 hours of curing 60°C. The ultramicrotome has two setting the micro-scale (10µm-90µm) and nano-scale (10nm-90nm). Samples were shaved to have a clean smooth surface by the micro cutting setting. The nano-scale setting was then used for cutting the TEM samples. Two nano-scale settings were used for TEM analysis 60nm and 10nm.

The purpose of this method was to obtain TEM images of the silver throughout the polymer matrix. Figure 52 shows some representative TEM images of samples cut at the 60nm setting. The images show the sample being too thick for TEM analysis. Picture quality of the particles was poor due to the inability to see clearly through the thicker parts of the polymer and the polymer would move due to the effects of the electron beam.

Figure 52. Ultramicrotome samples cut at 60nm setting nucleated at 1mM AgNO₃ and 10mM NaBH₄. Scale bar = 500nm.

The next setting tried was 10nm thick shown in Figure 53. The sample nucleated at higher concentrations of silver and sodium borohydride started to grow into 40-50nm in size. Nanoparticle diameter was becoming larger than the sample thickness, and very few nanoparticles were being transferred onto the TEM grids. Another issue with these thinner sections of polymer is the movement of the sample under the electron beam. These polymers tend to move and fold when the electron beam is focused on the sample at higher magnifications. This movement leads to issues when taking images with the high-resolution camera.

Figure 53. Ultramicrotome samples cut at 10nm nucleated at A) 1mM AgNO_3 0.1 NaBH_4 and B) 1mM AgNO_3 and 10 NaBH_4 .

Due to issues with polymer thickness and limited number of nanoparticles over various nucleation conditions, the ultramicrotome method was no longer used for TEM sample prep. Instead composite gels were ground by a mortar and pestle and the particles were redissolved in DI water.

5.4.2 Effect of NaBH_4 Concentration

To determine the appropriate reducing agent concentration for future experiments, 50% PAA composite beads were compared to the pure ALG beads while varying the concentration of reducing agent from 0.1mM-10mM. In preparation for TEM the composite beads were ground by a mortar and pestle and soaked in DI water overnight to allow for the silver nanoparticles to resuspend into solution. This solved the issue with any silver chloride contaminating the TEM samples since AgCl is not soluble in water.

Figure 54 shows TEM images for both 0.1mM and 1mM NaBH_4 for each gel. Particle size increases for the 50% PAA samples in comparison to ALG beads. For these experiments the silver concentration is kept constant at 1mM for both the 50% PAA and ALG samples.

Figure 54. TEM images A) ALG 0.1mM NaBH₄, B) 50% PAA 0.1mM NaBH₄, C) ALG 1mM NaBH₄, d) 50% PAA 1mM NaBH₄, scale bar = 200nm.

The use of the Image J image analysis software to determine particle size is almost impossible due to particle aggregation and the presence of the polymer in the sample. As a result, particle size was approximated by visual observation. Nanoparticles nucleated on the pure ALG beads (A) and (C) had nanoparticle sizes ranging from 10-20nm while the 50% PAA samples (B) and (D) had particles ranging from 20-40nm.

Smaller-sized silver nanoparticles are more efficient at killing bacteria [6]; therefore; it is necessary to determine a reducing agent concentration that is useful for both TEM imaging (larger sizes needed) while effective in the antimicrobial experiments (smaller sizes needed). The results from Objective 1 determined that the concentration of reducing agent greatly effects particle size. Consequently, all samples with 10mM NaBH₄ were not considered for future experiments due to particle sizes seen in the TEM images shown in Figure 55. Images displayed

particle aggregation with many particles sizing at 25nm or larger. As a result, 1mM NaBH₄ concentration was selected for all future experiments.

Figure 55. TEM image for 50% PAA gel nucleated with silver at 1mM AgNO₃ and 10mM NaBH₄

5.4.3 Effect of PAA and AgNO₃ Concentration

All compositions of the composite ALG-PAA gels underwent the silver nucleation reaction with varying the concentration of AgNO₃ from 5 mM to 40mM, while keeping the concentration of reducing agent constant at 1mM. After nucleation the beads were dried; mechanically crushed and resuspended into DI water for both UV-Vis and TEM analysis. UV-Vis results for various gels nucleated with 5mM AgNO₃ is shown in Figure 56.

Figure 56. UV-Vis results of various composite ALG-PAA nucleated at 5mM AgNO_3

Peak values experience both higher intensity and a red shift (peaks are located at higher wavelengths) with increasing amounts of PAA (with exception to 75% PAA sample which actually starts to develop a second very broad peak between 500 and 600nm) according to the UV-Vis results. Peaks increasing in absorbance indicate an increase in particle size, while higher intensities indicate higher concentrations of silver. Due to the nature of the sample preparation process, UV-Vis intensities are a function of bead size and the effectiveness of grinding. Since bead size varies due to gelation process variables and grinding preparation varies between samples, the silver content needs to be quantified by another characterization method, described in Section 4.4.3. Analysis of the UV-Vis data does give information about the peak position and the full-width, half-maximum (FWHM), which are given in Table 6.

Table 6 Peak and FWHM values reported by UV-Vis for each composite material.

Sample	AgNO₃ Conc. (mM)	UV-Vis Peak (nm)	FWHM (nm)
ALG	5	388	132
	10	407	N/A
	20	410	141
	40	394	208
10% PAA	5	416	152
	10	428	141
	20	402	363
	40	420	218
25% PAA	5	420	125
	10	426	151
	20	400	122
	40	410	167
50% PAA	5	422	117
	10	414	178
	20	416	149
	40	428	150
75% PAA	5	409	354
	10	430	228
	20	420	145
	40	390, 544	468

With increasing concentrations of both silver and PAA, there is a general trend with both increasing peak and FWHM. This indicates larger particle sizes with greater distributions as reaction concentrations increase. Increasing amounts of PAA in the composite gel allows for more reactive sites in the material. More reactive sites generate more nanoparticles, which in turn, leads to Oswald ripening and aggregation similar to the results seen in the PAA studies of aqueous reactions described in Objective 1. These results are confirmed by TEM seen in Figure 57.

Figure 57. TEM images of Silver nanoparticles nucleated at 5mM AgNO_3 and 1mM NaBH_4 for various composite gels: a) ALG, b) 10% PAA, c) 25% PAA, d) 50% PAA, e) 75% PAA. Scale bar for all images is set at 50nm.

Representative images for each composite gel sample formed at the same reaction conditions (5mM AgNO_3 and 1mM NaBH_4) are shown along with the corresponding UV-Vis spectra in

Figure 57. Seen in this side-by-side comparison, increasing amounts of PAA results in an increase in the size of particles. Figure 57A shows the results of the silver reaction for pure ALG beads; yielding particles between 5nm-25nm. The 10% PAA sample (Figure 57B) generally has a higher number of particles in the 25nm range than the previous sample, which explains the shift in the UV-Vis peak from 388nm for the ALG sample to 416nm. Particle size continues to increase with both the 25% PAA (Figure 57C) and 50% PAA (Figure 57D) samples. This is reflected in the UV-Vis spectra as well as peak 420nm and 422nm respectively. Also, for these two samples, it is observed that the particles begin to exhibit a bimodal distribution with a smaller set of particles measuring less than 15nm in size and a bigger set of particles ranging from 25nm-50nm. In Figure 57D, the smaller particles can be seen on top of the larger particles. This is due to a drying effect of the TEM prep process. These particles can be seen in other TEM images located in Appendix E. This bimodal distribution continues to develop and can be observed in the UV-Vis results for 75% PAA samples. Figure 57E shows particles continue to grow to over 50nm and become irregularly shaped. As noted in the kinetics analysis of the AgNO_3 -PAA aqueous system, particle elongation in these larger particles is responsible for the UV-Vis peak shift into the 500nm range.

This bimodal distribution becomes more prevalent when the concentration of silver is increased from 5mM to 40mM. Figure 58 shows three representative TEM images of the 75% PAA gels nucleated with 40mM AgNO_3 as well as the UV-Vis spectra for that sample. The UV-Vis results show clear peaks at 390nm and 544nm. The smaller particles (5nm-15nm some are shown in a black box) are spherical in shape, which is responsible for the peak at 390nm. The peaks located at 544nm are due to particle elongation and aggregation shown by the white arrows.

Figure 58. TEM and UV-Vis spectra of 75% PAA nucleated with silver at 40mM. Scale bar for all images are set at 50nm.

As PAA composition increases nucleation is more prominent within the hydrogel network and larger silver particles are synthesized. Generally the size of silver can be controlled by varying the PAA content in the alginate gels. A bimodal distribution is developed with higher amounts of polymer and becomes more evident when the silver concentration is increased. The results from the UV-Vis spectra are confirmed by the TEM imaging.

5.4.5 Silver Composition Experiment

All hydrogel samples were nucleated with 40mM AgNO₃ and 1mM NaBH₄. All composite beads were dissolved into 50mL of HCL to dissolve the gel and silver into solution. ICPU results reported the concentration of silver in each sample (Table 7). ICPU reports silver concentration directly in parts per million (mg/L). Total composite bead weight and HCL volume were recorded to calculate the total sample concentration. The silver concentration and sample concentration can be used to determine the weight percentage of silver in each sample.

With the exception of one point of data (50% PAA) silver content increases with PAA content. It is believed that this point is an outlier due to the weighing scale's measurement error. The concentrations the total sample weight heavily effects data calculations. The margin of error for the weighing scale is 0.1-0.3mg, which explains for the outlying data point.

Table 7 Ag Composition ICU results

Sample	Solvent	Ag Concentration (mg/L)	Sample weight (mg)	1M HCL Volume (mL)	Sample Concentration (mg/mL)	Ag wt%
ALG	water	5.072	6.7	55.25	0.1213	0.0418
10% PAA	water	36.925	14.6	55.50	0.2631	0.1404
25% PAA	water	5.177	1.5	53.25	0.0282	0.1838
50% PAA	water	6.492	2.2	54.33	0.0405	0.1603
75% PAA	water	7.433	2.0	55.67	0.0359	0.2069

Figure 59 shows a graphical representation of the silver content within the gel compared to the increasing PAA content. The presence of PAA clearly impacts the amount of silver nucleated onto the gel as the 10% PAA sample is 235% higher than the control gel without PAA. The trend is not as strong when more PAA is added into the system; the 75% PAA gel only contains 47% increase over the 10% PAA sample. This means a 65% increase of PAA gel into the system only yields 47% more silver.

Figure 59. Silver content vs. PAA content

The addition of PAA into the matrix promotes nucleation for two reasons. First, the polymer is hydrophilic and allows for more water, or silver, to be absorbed as seen in the swelling studies. Second, the number of reactive sites increases with the addition of PAA, increasing the number of silver complexes that form.

5.5 Antimicrobial Tests

The antimicrobial effect of the silver composite gels was measured using ASTM standard E2149-10. The purpose of this study is to compare the performance of the composite ALG/PAA/Ag gels to the pure alginate gels loaded with silver. 50% PAA composite gels were loaded with silver nanoparticles. The reaction conditions for the silver reaction were 1mM AgNO_3 and NaBH_4 respectively. The control samples were pure PAA; pure alginate beads (ALG), 50% PAA composite gel without silver (ALG/PAA), and ALG/Ag composite gels were tested under the same reaction conditions as the control sample.

In order for this standard to be valid, a leaching test was first conducted. 5mL of sterile working buffer was loaded with Ag/ALG/PAA bead and shaken for 1 hour at 37°C. After boring an 8 mm hole in the center of an agar plate 100µL of sterile buffer was transferred and incubated overnight. No zone of inhibition formed which meant the samples were not leaching silver nanoparticles when put into solution.

To calculate cell reduction, cells were grown in an overnight culture and diluted in a sterile buffer into a cell count of $1.5-3.0 \times 10^5$ Colony-Forming Unit (CFU)/mL. Each sample was immersed in 50mL of sterile buffer solution. A separate flask with 50mL of sterile buffer solution with no samples or controls was prepared labeled “inoculum only”. All flasks were shaken in concert at 37°C for one hour. Serial dilutions were run for each flask and allowed to incubate overnight at 37°C. The count for the inoculum only run was used as the basis for the percent reduction in Equation 6, where B is the cell count for the inoculum only sample and A is the sample count.

$$Reduction \% \left(CFU/ml \right) = \frac{B-A}{B} \times 100 \quad (7)$$

Figure 60 shows the average percent reduction for each sample and control. Each sample was run in triplicate or more. The raw data can be found in the Appendix F. For the PAA and ALG controls only one sample showed a cell reduction. The other three samples showed no cell growth and are responsible for the large standard deviations seen in Figure 60. It is expected that pure alginate samples do not exhibit antimicrobial properties; therefore, this one data point is considered to be a result of experimental error. ALG/PAA/Ag composite materials saw an average of 96% cell reduction compared to only 8.4% cell reduction in the ALG/Ag sample. PAA when combined with another polymer in a solid matrix can display some antimicrobial ability.[111] This explains why the PAA/ALG composite showed a 27.8% cell reduction. Assuming pure alginate beads do not exhibit antimicrobial properties, the 59.8% increase in cell death for the ALG/PAA/Ag samples is due to the increase in the amount of silver nanoparticles nucleated onto the material. Despite nucleating silver under the same reaction conditions for both

samples the presence of PAA promotes more nucleation and greatly enhances antimicrobial activity.

Figure 60 Cell reduction results

5.5.1 Composite Optimization

The antimicrobial test results show that 50% PAA composite gels nucleated with silver at 1mM AgNO_3 and 1mM NaBH_4 are responsible for 96% cell reduction. Pure alginate gels with nucleated silver nanoparticles synthesized from the same reaction conditions only display an 8.4% cell reduction. The ICPU results show a 235 wt% increase in the amount of silver nanoparticles nucleated for 10% PAA beads from their pure alginate counterparts. In comparison the 50% PAA composite gels, the composites that were used for the antimicrobial test, only showed a 14 wt% increase in silver nanoparticle nucleation in comparison to the 10% composite gels. Therefore for practical applications of this alginate/PAA/Ag composite materials the previous results suggest that there is an optimal point between pure alginate gels and the 10% PAA composite gels. For this optimization the weight percent of PAA was varied from 0-10 wt% in increments of 1 wt%. ICPU was performed to detect the composition of silver nucleated on these composite gels. Silver nanoparticles were nucleated with 1mM AgNO_3 and 1mM NaBH_4 . Antimicrobial tests using the same ASTM standards mentioned earlier in this section were

performed on all composite samples as well to monitor the lowest amount of PAA needed to achieve the most cell reduction. Each run alternated between odd wt% and even wt% samples.

Table 8 shows the cell reduction for all composite materials tested in the optimization. The controls used were pure alginate beads designated as ALG without silver nucleated onto the matrix and pure PAA. 5mg of beads for each sample were used per run for each test. 10% PAA composite materials showed 100% cell reduction. For this set of experiments the pure alginate/silver composite reported a 3.7% cell reduction, while adding 1 wt% PAA into the composite system increased cell reduction by approximately 29%. As soon as PAA is introduced into the composite materials the amount of silver nanoparticles that are nucleated greatly increase causing more cell reduction.

Table 8 Cell % reduction Optimization

Run	ALG	PAA	0%	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
1	3	75.81	0	-	52	-	64.8	-	64	-	76	-	100
2	0	18	-	21.2	-	62.9	-	72	-	85.5	-	96.9	-
3	0	0	0	-	21.1	-	39.39	-	57.6	-	63.6	-	100
4	0	26.9	-	38.5	-	61.5	-	76.9	-	84.6	-	92.3	-
5	15.5	0	11.11	-	64.5	-	77.8	-	82.2	-	91.1	-	100
6	0	38.5	-	37	-	66.7	-	74	-	88.8	-	83	-
AVG	3.1±5.2	26.5±24	3.7±4.5	32.2±6.8	45.8±15.8	63.7±1.9	60.7±13.8	74.3±1.7	67.9±9	86.3±1.6	76.9±9.7	90.7±5	100±0

Both PAA and ALG samples have outliers which skew the average and standard deviation results shown in both Figure 60 and Table 8. To access the statistical significance of each sample a t-test was performed. For a one sample test the t value can be found by using equation 8:

$$t = \frac{x - \Delta}{\frac{s}{\sqrt{n}}} \quad (8)$$

Where x is the mean of the sample, Δ is null hypothesis which in this case is the data point in question, s is the standard deviation and n is the sample size. When a t value is determined it is compared to the t values found in a student's t-distribution table. If the t value calculated is less than those found in the table then it can be concluded that the data point is insignificant therefore

it can be removed from the data set. Every data point for both the control and test samples were subjected to this test and the data points that were insignificant can be found highlighted in red on Table 9. The first 3 runs reported in this table was the first antimicrobial test mentioned earlier in this section and can be found in Appendix G, the rest of the runs were from the optimization experiments. The average and standard deviation reported in this table does not include the insignificant values.

Table 9 t-test results of control samples

Run	PAA	ALG
1	0	0
2	0	61.9
3	100	0
4	0	0
5	75.81	3
6	18	0
7	0	0
8	26.9	0
9	0	15.5
10	38.5	0
AVG	10.4	2.06
STD	14.4	4.84

Figure 61 shows a graphical representation of the antimicrobial results of the composite optimization experiments without the insignificant results determined by the t-test. The cell reduction increased with increasing PAA content in the composite gel. The 10% PAA composite gels displayed a 100% cell reduction and therefore is the optimal point for this study.

Figure 61 Cell reduction optimization of PAA content in the composite material

The silver composition of the various composite beads for this optimization experiment is shown in Table 10. Silver was nucleated with 40mM AgNO_3 and 1mM NaBH_4 to avoid the minimum detection limit. Also the silver content in 1 sample from the 0% PAA, 5% PAA and 10% PAA composite beads that was used in the antimicrobial test. Beads from before and after were sent for analysis by ICPU to determine if silver directly leaves the composite and enter the cell wall during cell death.

Table 10 Silver composition of optimization experiments.

Sample	Ag Concentration (mg/L)	Sample weight (mg)	1M HCL Volume (mL)	Sample Concentration (mg/mL)	Ag wt%
1% 40mM	29.95	31	50	0.620	0.0483
2% 40mM	32.09	33.3	50	0.666	0.0482
3% 40mM	24.63	24	50	0.480	0.0513
4% 40mM	32.5	31.2	50	0.624	0.0521
5% 40mM	33.1	29.1	50	0.582	0.0569
6% 40mM	32.18	19.9	50	0.398	0.0808
7% 40mM	4.76	2.7	50	0.054	0.0881
8% 40mM	30.1	12.1	50	0.242	0.124
9% 40mM	32.68	17.9	50	0.358	0.0913
10% 40mM	33.12	15.8	50	0.316	0.105
0% Before	0.293	3.8	50	0.076	0.00386
0% After	0.164	2.3	50	0.046	0.00357
5% Before	1.256	12.4	50	0.248	0.00507
5% After	0.080	1.5	50	0.018	0.00267
10% Before	0.230	2.2	50	0.044	0.00523
10% After	0.195	8.5	50	0.170	0.00115

The silver content increases progressively from 0.0483 to 0.105 wt% as the composition of the composite ALG/PAA gels increase from 1 to 10% PAA; from 1 to 10 % the composition of silver nanoparticles more than doubles, increasing by 117%. The increasing amount of silver nanoparticle being nucleated suggests that more silver ions are being loaded into the gel matrix. This is due to the increasing amount of negatively charged functional groups located on the polymer backbone of PAA. These negatively charged functional groups form a complex with the silver ions and become strongly attached to the matrix. [29] The more functional groups located in the hydrogel matrix allows for more silver complexes to form, after the reducing agent is added more silver nanoparticles are nucleated due to the presence of these complexes. 10% PAA beads have over 0.0523 wt. % of silver nanoparticles, which is the minimum amount of silver needed in the ALG/PAA/Ag composite system to kill $1.5-3.0 \times 10^5$ CFU/ml when exposed to the inoculum solution for an hour.

For the comparison of the silver content in the composite beads before and after the antimicrobial test, from the same batch of freshly nucleated composite beads samples both used and not used in the antimicrobial test. Composites with higher amounts of PAA contain more silver and experience more silver loss than their counterparts that contain less PAA. The more silver being released into the inoculum solution is responsible for the increase in cell death during the antimicrobial shake test. Microbes are killed not by the actual silver nanoparticles but by the slow release of silver ions leached from the particle into the surroundings. [112] More silver nanoparticles provide a larger concentration gradient which in turn leads to a greater driving force for mass transfer. This phenomenon describes why more silver is being used up from the composite and leading to higher rates of cell reduction.

5.6 Objective 2 Summary

Two case studies were performed showing that carboxylic acid groups are responsible for silver nanoparticles nucleation. For the case of grafting polyacrylic acid to the cotton fabrics it was shown that even when the carboxylic groups are reacted they still maintain its nucleation ability. Alginate hydrogels were loaded with PAA at various compositions. Increasing PAA content in these gels yielded more Ag particles with larger sizes. A bimodal distribution with higher concentrations of silver nanoparticles was seen for the 75% PAA hydrogels similar to higher concentrations of PAA results in objective 1.

Chapter 6. Conclusions and Future Work

6.1 Conclusions

This project investigated the ability to use PAA as a templating agent to nucleate silver nanoparticles directly in a biological scaffold. Negatively charged function groups such as thiols, amines and carboxylic groups have the ability to nucleate metallic nanoparticles. PAA being rich in carboxylic groups was a perfect candidate for these studies. The novelty of this work shows that for an *in situ* chemical reduction process nanoparticle size is dependent on the composition of the templating agent, which in this case was PAA. Also, the introduction of PAA into the solid dual polymer matrix improved composite performance by increasing the amount of silver nanoparticles nucleated onto the composite material. By adding only 10 wt% PAA into the alginate gels allowed for over a 200% increase in silver nucleation for the composite materials. Performing these studies a few conclusions can be made.

An indepth study of the effect of PAA in the presence of the chemical reduction of a silver salt was performed. UV-Vis and DLS confirmed that PAA nucleates silver nanoparticles with larger average size. Varying the concentration of the silver salts only changes the concentration of nanoparticles, not particle size. Increasing the concentration of PAA creates a bimodal distribution of particle size. This is caused by the fact that not all silver ions come into contact with the PAA functional groups. Silver ions that interact with PAA form bigger particles and the shape starts to change. This change occurs when the originally spherical particles start to elongate and form different shapes. Increasing the reducing agent concentration increases the size of all particles.

Also reported in this study was the reaction mechanism and kinetic data, which has not been reported for silver nucleation on PAA in the literature. By monitoring UV-Vis over time the reaction mechanism was identified as heterogeneous nucleation of silver particles on the polymer surface due to silver ion adsorption. The rate of reduction is also reported, with increasing polymer concentrations the rate of reduction increases which is the opposite of the stabilizing polymer like poly (vinyl alcohol) (PVA). This is due to the fact that PVA stabilizes the

nanoparticles after reduction while PAA has a second reaction, which causes the particles to grow.

Changes to the reaction environment like pH and temperature effect silver nucleation. The pH of a solution greatly affects the protonation of the carboxylic groups on the PAA backbone. Lower pH yields a lower concentration of bigger particles while raising the pH has the opposite effect. Lower temperature increases yield of silver nanoparticles. This is mostly due to limiting the side reactions of the reducing agent, NaBH_4 which is very reactive with water.

The second objective focused on nucleating PAA in a solid phase polymer matrix, with two case studies. ELP was coupled with PGA, another polymer with carboxylic functional groups, and silver was nucleated onto these structures. The second case study compared silver nucleation on pure cotton fabrics to cotton fabrics that had PAA grafted onto it by an esterification reaction. The grafting process reacted carboxylic groups located on the PAA backbone to the cotton fabric. SEM images revealed that the samples with PAA nucleated more particles which were larger than the pure cotton control sample. This proved that even when the negatively charged functional groups are reacted they still have the ability to nucleate nanoparticles.

The esterification reaction was attempted to chemical crosslink sodium alginate and PAA. Due to the high temperatures required for this reaction the alginate degraded losing its gelation ability. Therefore, PAA was physically entrapped into the alginate gels by ionic crosslinking with CaCl_2 . The PAA composition of these gels were varied from 10%-75% PAA. Rheology studies showed that with increasing amounts of PAA, the gel becomes more liquid like and less rigid until reaching a concentration of 75% PAA. 75% PAA gels were more rigid due to increasing gel density. Swelling studies are analogous to the rheology results, with increasing PAA content in the gels the more swelling occurs, due to PAA's hydrophilic nature. At 75% PAA, the degree of swelling decreases due to gel loading and increasing gel density. Adding silver nanoparticles to these gels improved mechanical strength and swelling capacity both of which are important factors for a biological scaffold.

Several methods were reviewed to nucleate silver nanoparticles onto the composite ALG/PAA gels. The method that produced the most nucleation included freeze drying the hydrogels after the crosslinking process, and re-swelling the composite materials in silver salt solutions. UV-Vis results of the nucleation in an ALG/PAA gel show a bimodal distribution again, similar to the results of varying PAA in Objective 1. The particle size was able to be controlled; increases in PAA content resulted in an increase in silver nanoparticle size. Increasing the silver concentration makes the bimodal distribution more prominent. Using the same concentrations of silver and NaBH_4 , increasing amounts of PAA increase the nucleated silver content on the composite material this was confirmed by ICPU. The increases in silver content lead to a composite that showed better performance in antimicrobial tests. The ALG/PAA/Ag composite material recorded a cell reduction of 96% while the ALG/Ag material only recorded a 8.4% cell reduction. These results indicate the in situ formation of silver nanoparticles on biologically relevant materials may be used with success in wound healing applications.

6.2 Future Directions

6.2.1 PAA Polymer/Ag Content Optimization

The objective of this work was to determine if PAA content had an effect on particle nucleation throughout the solid gel matrix. Section 4.4.3 studying the effect of PAA content under the same reaction conditions showed that increasing amounts of PAA leads to nucleation of bigger particles and the UV-Vis results suggest that a higher concentration of particles are generated as well. This is confirmed from in section 4.4.1 as the composition of silver increases with PAA. The antimicrobial studies showed that ALG/PAA/Ag composite materials had over a 1000% increase in cell reduction than its ALG/Ag counterpart. The composite material was made up of 50 wt% of PAA. It is suspected that the amount of PAA in these gels can greatly be reduced without losing performance. Various gels with PAA content lower than 50% (possible even lower than 10%) should be tested under the same reaction conditions to find an optimal point between PAA composition and cell reduction.

6.2.2 PAA Polymer Square Phenomenon

Multiple samples both in Objective 1 and Objective 2 have showed polymer squares (or cubes) with several silver nanoparticles inside. Figure 61 shows representative images of the polymer formations found in Objective 1 (Figure 61A), Objective 2 (Figure 61B), silver nanoparticles nucleated with poly (styrenesulfate) (Figure 61C), and even in the nucleation of platinum nanoparticles (Figure 61D). These polymer structures were observed for different polymers and different metal nucleation. Metal nanoparticles are within these polymer structures, which can vary from one particle to multiple.

Figure 62. A) 1mg/mL PAA, 1mM AgNO₃, 1mM NaBH₄. B) 50% PAA composite 5mM AgNO₃, 1mM NaBH₄. C) 1mg/mL PSS, 1mM AgNO₃, 1mM NaBH₄. D) 1mg/mL PAA, 1mM K₂PtCl₆, 1mM NaBH₄.

To our knowledge these shapes have not been reported in literature. Further investigation is needed to determine if this is a drying phenomenon due to the TEM prep process or if they form shapes which are normally found for polymers have an impact on the process of silver nucleation. Insight on this topic could lead to a better physical understanding of how nanoparticles are nucleated by these polymers.

6.2.3 Diblock Copolymer Studies

Diblock copolymers are well known for their self-assembly properties either triggered by being dissolved in various solvents or in solid thin films. The morphology of the copolymers can be manipulated by changing the polymer ratio and the block lengths [113]. These polymer orientations are useful because silver nucleation can occur on these structures. This is useful for energy applications such as solar cells because silver nanoparticles has been proven to boost electrical current generation when introduced into plastic [114]. Silver has the ability to absorb light and conduct electricity, and this ability is enhanced when silver is assembled in regular mosaic patterns. Nucleation of silver onto well-defined block copolymer structures could provide these regular patterns needed to enhance the efficiency of solar cells.

6.2.4 Alternate Inorganic Nanoparticle Nucleation with PAA

The ability to assemble nanoparticles into well-defined configurations is crucial for electronic devices. Platinum, when paired up with polyaniline (PAN), is often used for electrodes on fuel cells.[115] A polymer nanocomposite is used instead of pure platinum because it enhances performance. Pure platinum is inefficient in conducting electricity due to aggregation issues and lowering the surface to area ratio of the material.

Carboxylic groups have the ability to nucleate other metals including by not limited to platinum, palladium, gold, and zinc.[23, 56, 96, 116] The ability to assemble nanoparticles into well-defined configurations is crucial for electronic devices. One example of other metals that can be explored is platinum nanoparticles. When paired up with polyaniline (PAN) the composite material is often used for electrodes on fuel cells.[115] PAA can be used to nucleate platinum and reduce aggregation in the PAN matrix. PAA has the ability to nucleate noble metals other than silver as shown in Figure 62. This Figure is a TEM image of platinum particles that were

nucleated with PAA in an aqueous solution. Similar to the silver reduction used in this project; 1mL of a 1mM hexachloroplatinate and 1mg/mL PAA solution was allowed to react for 1 hour. Then 15mL of 1mM NaBH₄ was added to the Pt/PAA solution to reduce the platinum ions, the reaction was allowed to run overnight. Particles synthesized by this reaction were measured to be around 30-40nm in size.

Figure 63. Pt Nanoparticles nucleated with 1mg/mL PAA, 1mM NaBH₄. Scale bar is 200nm.

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Appendix A: TEM images of Chemical Reduction of Silver

Representative TEM images of various concentration of the chemical reduction of silver:

Figure A 1 . A) 1mM AgNO₃, 1mM NaBH₄, 1mg/mL PAA. B) 1mM AgNO₃, 1mM NaBH₄, 2mg/mL PAA. C) 1mM AgNO₃, 1mM NaBH₄, 3mg/mL PAA. D) 5mM AgNO₃, 1mM NaBH₄, 1mg/mL PAA. E) 1mM AgNO₃, 50mM NaBH₄, 1mg/mL PAA. F) 1mM AgNO₃, 25mM NaBH₄, 1mg/mL PAA.

Appendix B: Rate of Reduction Calculations

$\ln(a/(1-a))$ plots of various polymer concentrations used to determine reduction rate constant K_a :

Figure B 1. 0.1mg/mL Plot of $\ln(a/(1-a))$ as a function of reaction time

Figure B 2. 0.25mg/mL Plots of $\ln(a/(1-a))$ as a function of reaction time

Figure B 3. 0.5mg/mL Plots of $\ln(a/(1-a))$ as a function of reaction time

Figure B 4. 0.75mg/mL Plots of $\ln(a/(1-a))$ as a function of reaction time

Figure B 5. 2mg/mL Plots of $\ln(a/(1-a))$ as a function of reaction time

Figure B 6. 3mg/mL Plots of $\ln(a/(1-a))$ as a function of reaction time

Appendix C: FTIR confirmation of Cotton-PAA

FTIR results showing the presence of PAA grafted onto cotton fabrics:

Figure C 1. FTIR confirmation of PAA grafted onto cotton

Appendix D: Swelling Studies Raw Data

Raw data of swelling tests done on various hydrogel compositions:

Sample	ALG		10%PAA		25% PAA		50% PAA		75% PAA		AgNPs 50/50	
	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW	DW	WW
1	33.9	197.3	7.9	40	6.5	27.4	9.2	80.8	7.6	38.2	0.9	15.6
2	30	101.2	12.6	52.7	19.9	235.8	7.8	59.2	9.2	61.3	0.6	9.3
3	34.2	362.6	9.4	26.5	17.5	162.2	17.9	140.2	9.6	47.3	0.4	5.2
4	9.1	24.5	23.9	199.9	7.9	44.5	17.2	194.5			0.3	1.5
5	12.1	31.3	28.5	282.7	9.6	59.6	24.6	212.9				

Figure D 1. Raw data for swelling tests

Appendix E: TEM images of Composite Nucleation

Representative TEM images of various concentrations of the silver nanoparticles nucleated on composite hydrogels:

Figure E 1. TEM images of Silver nanoparticles nucleated at 10mM AgNO₃ and 1mM NaBH₄ for various composite gels: a) 10% PAA, b) 25% PAA, c) 50% PAA, d) 75% PAA. Scale bar for all images is set at 50nm.

Figure E 2. TEM images of Silver nanoparticles nucleated at 20mM AgNO₃ and 1mM NaBH₄ for various composite gels: a) ALG, b) 10% PAA, c) 25% PAA, d) 50% PAA, e) 75% PAA. Scale bar for all images is set at 50nm.

Figure E 3. TEM images of Silver nanoparticles nucleated at 40mM AgNO₃ and 1mM NaBH₄ for various composite gels: a) ALG, b) 10% PAA, c) 25% PAA, d) 50% PAA, e) 75% PAA. Scale bar for all images is set at 50nm.

Appendix F: Antimicrobial Raw Data

Raw data of every run of the antimicrobial tests:

% Cell reduction					
Run	PAA	ALG	PAA/ALG	ALG/Ag	ALG/PAA/Ag
1	0	0	11	8.69	100
2	0	61.9	33.3	14.3	85.7
3	100	0	39.1	2.2	97.8
4	0	0	-	-	100

Figure G 1. Results from all antimicrobial tests performed

Sample	3 plate counts (colonies)	AVG (colonies)	% Cell Reduction
Inoculum 0 time	122,64,62	82	-
Inoculum	67,60,57	61	-
ALG	100,0,100	66	0
PAA	300,100,100	167	0
ALG/PAA/Ag	0,0,0	0	100
ALG/PAA	50,63,49	54	11
ALG/Ag	61,50,57	56	8.69

Figure G 2. Run 1 Raw Data

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