

Therapeutic Delivery Technology and its Economic Impact

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Abstract

Therapeutic delivery technology is a current area of high interest in both university and industrial settings. These technologies are being developed in order to deliver therapeutic agents, such as genes, proteins, and drugs, to patients more efficiently. Nanoscale delivery vehicles have proven to be useful for these applications; these vehicles may either be naturally produced or chemically synthesized. The physical properties of these nanomaterials must be characterized correctly using instrumentation that evaluates their size, morphology, and potential for agglomeration. These technologies represent a high-growth economic area that fosters entrepreneurship and innovation. Because of this innovative spirit, research and economic interest will continue to be focused on therapeutic delivery technologies.

Therapeutic Delivery and its Economic Impact

Introduction

The primary goal of modern therapeutic delivery research is to develop new ways to localize treatments to a region of the body. These therapies are highly popular among research groups focusing on diseases ranging from cancer to cardiovascular to gastrointestinal diseases (Serna 2018, O'Quinn 2018, Beitelshees 2017). In cancer research, chemotherapy is a common treatment for the elimination of tumors, but its effects are often not specific to the affected area. The effects of chemotherapy impact the whole body and is typically a painful process for those needing treatment. Localizing treatment can alleviate pain associated with systemic drug administration. Another important aspect of current drug delivery research is the design and engineering of controlled release mechanisms. These mechanisms prevent an excess of drug initially introduced to the system. Although it is typical that a majority of the drug is released upon administration, there is a sustained release of the remaining drug or therapeutic agent over-time. This controlled release allows continuous treatment to the localized areas. While the general principle of drug delivery remains the same, there are multiple designs or types of methods.

Gene Therapy

Several types of therapeutic agents can be used for treatment in a drug delivery system. Genes, proteins, and conventional drugs may all be used for treatment; however, the particular use of each depends on the disease. For disease with a genetic basis, gene therapy techniques are utilized. Genetically based diseases are the result of a mutation in

the genetic code, chromosomal mutations, or alteration of chromosomal ploidy. A mutation in the genetic code can be defined as the alteration of a single base (NIH 2019). In the context of inherited disease, this mutation would not be caused externally. The severity of these mutations varies significantly, from no direct impact to the production of a nonfunctional protein (NIH 2019). Chromosomal mutations refer to alterations in specific chromosomal regions. In comparison to genetic code mutations, chromosomal mutations involve many more bases since a whole chromosomal region is being altered (NIH 2019). There are several types of chromosomal mutations which include inversion, insertion, and deletion (NIH 2019). Gene delivery is commonly used with chromosomal deletions (NIH, Gene Therapy 2019).

Chromosomal deletions are being targeted as potential gene therapy treatment areas. For example, Wiskott-Aldrich syndrome (WAS), a disease resulting in small platelet size, thrombocytopenia, and recurrent bacterial and viral infections, is caused by partial deletion of the WAS gene (Chandra et al 2004). In total, the WAS gene is over 9000 bases, indicating that partial deletion involves hundreds or even thousands of bases (Chandra et al. 2004). To be treated using gene therapy, the deleted region of the WAS gene would be delivered to the cell and integrated into the cellular genome. The basis of these techniques is the delivery of a healthy DNA segment containing the mutated or missing portion of the gene. This “healthy” DNA segment is integrated into the cellular genome, and cellular proliferation occurs. Newly created cells do not have the mutation and are not defective. As cells reproduce and die, a larger percent of cells are now healthy and the damaged cells are gradually phased out. Gene therapy was first successfully used

to treat adenosine deaminase deficiency, a genetic defect in which the enzyme adenosine deaminase is deficient, in 1990 (NIH history). Since 1990, other diseases, such as hemophilia, WAS, and cancer, have been identified as potential gene therapy targets; however, acute lymphoblastic leukemia is the only currently FDA approved disease for gene therapy (Beitelshees 2017). Although gene therapies are still being explored, many treatments only reach clinical trial phase and are not granted FDA approval (Beitelshees 2017). While gene therapy has great promise, issues with cellular integration have hindered its widespread usage. Since DNA will not be directly integrated into the genome when introduced to the cell, its delivery method must be designed such that cellular integration occurs (NIH). Nanocarriers have recently proven to be an effective method of gene delivery; these delivery methods will be discussed later.

Peptide and Drug Delivery

This variation is a direct result of the greater breadth of protein function when compared to DNA. Protein functions include, but are not limited to, enzymatic, immunological, and transport activities. Another aspect of protein therapy that differs from gene therapy is the necessity of continued treatment. Since healthy proteins do not replicate and become incorporated into subsequent cell lines like DNA does, there must be continual protein application. For example, patients that require protein therapy have to be treated by medical professionals every few weeks to ensure sufficient protein levels. In this way, the protein is more similar to the case in which a traditional drug is administered. A key motivation of protein therapy design is cytotoxicity, for the purpose of eliminating tumors. Cytotoxicity is vital for the treatment of cancer and inflammatory

diseases (Serna et al. 2018). Although cytotoxic cancer treatment may be effective by reducing cancerous tissue, the side-effects of non-localized therapies are detrimental. Cytotoxic treatments, whether by peptide or drug, by nature are poisonous. If their effects are not reduced to areas of interest, such as tumors, the global effects on the body will be harmful (Serna et al. 2018). Several key side effects of non-localized cytotoxic therapies include kidney failure, cardiotoxicity, and immunosuppression (Serna et al. 2018). While beneficial long-term, there is a clear motivation for improvement of these treatments with regards to localization within the body. Targeted nanocarrier-protein complexes are highly promising treatment localization methods. Similarly, targeted nanoscale delivery vehicles are effective for drug delivery as well. The key difference between protein delivery and drug delivery is molecule classification. If the delivered molecule is a peptide, then the treatment would be considered protein therapy. Alternatively, if a non-peptide molecule is being delivered, the method is considered drug delivery. Cytotoxic cancer treatments apply to traditional drug-methods as well. The type of treatment dictates what drug delivery method should be used. Many of these technologies are on the nanoscale to ensure efficient delivery; if the size of the deliverer is too big, it could be harmful. Based on blood lifetimes and bioavailability, the ideal size range for nanoparticles is between 10-200 nm; the properties of the nanoparticles are highly variable within this size range (Goldberg 2007). Natural and synthetic nanocarriers are used for therapeutic delivery.

Nanoscale Vehicles for Therapeutic Delivery

Viral vectors are effective vehicles for gene therapy, while polymeric nanocarriers and polymer conjugates are the most common peptide and drug delivery vehicles.

Polymeric vehicles must be synthesized. In preparation for gene therapy, virus particles must be grown in culture (Warnock et al. 2006). The species used varies and is dependent on viral properties such as size, toxicity, and the molecular composition of the viral genome. The size of the virus naturally affects the amount of genetic information that can be integrated into the viral genome. Adenovirus (90-100nm) and adeno-associated virus (20 nm) integrate up to 38 kilobases (kb) and 4.8 kb of DNA, respectively (Nayerossadat et al. 2012). This disparity in deliverable DNA is directly related to particle size. Viral gene delivery vehicles utilize normal virus-host cell interactions, in which the viral genome and the host-cell genome are integrated (Huang et al. 2011). While this normal physiological action is useful, viral genome editing must be done to prevent infection (Huang et al. 2011). Vectors must not promote an immune response (Howarth et al. 2009). For example, Herpes simplex virus (HSV) naturally causes cold sores or genital warts in humans; however, if its replicative genes are deleted or mutated, no symptoms occur (Berto et al. 2005). In regards to the viral genome molecular composition, viruses can either have genomes composed of DNA or RNA. This difference is manifested upon nucleic acid uptake by the host-cell. In the case of DNA viruses, the injected DNA will be directly inserted into the host cell genome. Alternatively, RNA-viruses, or retroviruses, make use of reverse-transcriptases, which convert RNA to DNA; this newly synthesized DNA can then be integrated into the host genome (Youngsuk et al. 2011).

Polymeric Nanoparticles

Polymeric nanoparticles are a common type of synthetic vectors, or vehicles, for therapeutic delivery. There are several varieties of polymeric nanoparticles. These nanoparticles are comprised of a polymeric core. The characteristic polymer must be biocompatible in order to prevent toxic side-effects; biodegradability is also an important nanoparticle quality. (Masood et al. 2016). If the nanoparticles were not biodegradable, they would not be able to be broken down. This buildup of residual material has the potential to cause side-effects even though the polymer itself may not be intrinsically harmful. Several biocompatible and biodegradable polymers used as nanoparticle cores are poly(lactic-*co*-glycolic acid) (PLGA), poly(aspartic acid), and polylactic acid (PLA) (Masood 2016, Crucho et al. 2017). PLGA is currently the only FDA approved polymer for drug delivery purposes (Elsabahy 2012). In addition to toxicity concerns, drug-polymer compatibility must be considered when selecting the polymer comprising the therapeutic vehicle. For example, cancer drugs such as paclitaxel and doxorubicin are not water soluble. If these drugs were desired to be used via drug delivery vehicles, a hydrophobic polymer core would be optimal; for this reason, PLGA and PLA nanocarriers are currently being investigated as deliverers of paclitaxel and doxorubicin (Masood 2016). Also, polymeric nanoparticles act as nucleic acid or drug protection. Nucleic acids are easily degraded, so their protection is necessary for treatment action (Amreddy et al. 2017). Premature degradation or release inhibits therapeutic effectiveness, especially when targeting is crucial to treatment rationale (Amreddy et al. 2017). A targeted cytotoxic drug, such as PLGA-paclitaxel nanoparticles, that does not have a protective vehicle should not be any less detrimental to the patient than using an

uncomplexed form. Modification of PLGA and PLA nanoparticles is necessary to add durability as well as to water solubilize them.

Nanoparticles composed of PLGA or PLA need to be altered so they will be hydrophilic; an unmodified, hydrophobic nanoparticle will not mix well in the body's aqueous environment. This problem is solved by attaching a hydrophilic polymer to the nanoparticle exterior (Elsabahy 2012). Polyethylene glycol (PEG) is currently the only FDA-approved polymer for this alteration (Elsabahy 2012). PEG is an effective polymer for this role because of the single hydroxyl groups at each terminal. These hydroxyl groups promote interaction with both the body's aqueous environment and functionalization for polymerization with PLGA or PLA. PEG essentially acts as a coating for polymeric nanoparticles, offering protection and enhanced pharmacological properties. Nanoparticles with a PEG exterior have a hydrated radius due to hydrogen bonding between PEG and water molecules; this hydration prevents premature degradation by enzymes (Makadia et al. 2011). PEG must be functionalized in order to polymerize with PLGA or PLA. For PLGA-PEG copolymer synthesis, PEG may be carboxylated and added to an N-hydroxysuccinimide derivatized PLGA (Cheng et al. 2006). Another older method causes PEGylation of PLGA by first polymerizing PEG-PLA and then subsequently PEG-PLGA by opening the cyclic lactide and glycolide (Li et al. 2001). These modifications are preceded by synthesis of the nanoparticle core.

Synthetic Methods of Polymeric Nanoparticles

Emulsion methods are the most commonly used polymeric nanoparticle synthetic methods. They can be classified as either single or double emulsion. An emulsion

consists of droplets suspended in a solvent; the droplets and solvent are immiscible. Single emulsions are classified as water in oil (w/o) or oil in water (o/w) emulsions. A w/o emulsion is one that has water soluble droplets suspended in a hydrophobic solvent, while o/w has hydrophobic components suspended in an aqueous or polar environment (Rao 2011). The term “water” in the context of emulsions specifies a hydrophilic environment and does not necessarily imply the presence of water. A surfactant, or stabilizer, is present in any emulsion in order to prevent particle aggregation. Common surfactants include poly(vinyl) alcohol (PVA), poly(ethylene) oxide (PEO), and polyvinyl pyrrolidone (PVP) (Heinz 2017). The solvent evaporation method is an older, but commonly used o/w emulsion method to synthesize polymeric nanoparticles. In this method, a hydrophobic polymer is dissolved in a solvent that is miscible with water; this solution is mixed with water and surfactant (Murakami 1999). As these two solutions mix, the water miscible solvent mixes with the water; however, the hydrophobic polymer cannot, and nanoparticle formation is promoted (Murakami 1999). After this dispersion, the polymer solvent is evaporated and the particles are subsequently resuspended (Murakami 1999). These nanoparticles are then dried for storage. Lipid nanoparticles have also been prepared using a solvent evaporation method (Trotta et al. 2003). In order to load therapeutics onto the nanoparticles, the desired therapeutic is added to the polymer or hydrophilic solution, depending on drug polarity. A double emulsion version (w/o/w or o/w/o) of the solvent evaporation method is similar to the single emulsion method; however, an additional dispersion step is done. Once the initial w/o or o/w dispersion is done, it is dispersed in a solution of the opposite phase. Although this

additional step may seem inconsequential, it affects the drug encapsulation properties of the nanoparticles (Iqbal 2015). Single emulsion and double emulsions differ in the properties of the synthesized nanoparticles, properties which include their ability to encapsulate hydrophobic or hydrophilic drugs and nanoparticle polydispersity.

Hydrophobic drugs can easily and effectively be loaded into nanoparticles using o/w or w/o single emulsions (Ramalho 2016). This method does not, however, encapsulate hydrophilic drugs.

Nanoprecipitation is a faster and simpler way to form nanoparticles than emulsion and other methods. A hydrophobic polymer is dissolved in a water-miscible solvent, which is often acetonitrile (Iqbal 2015, Fessi 1989). This solution is then mixed with water, and nanoparticles are formed; the synthesized nanoparticles are collected once the solvent is evaporated (Fessi 1989, Iqbal 2015). This method, first discovered in 1989, resembles the solvent evaporation method; however, nanoprecipitation is only efficient for hydrophobic drug encapsulation, while traditional emulsion methods may be used to encapsulate hydrophilic or hydrophobic drugs (Iqbal 2015).

Nanoparticle Targeting

Although treatment using nanoparticles is useful because of their sustained release properties, an important motivation for using them is their targeting properties. Targeting these nanoparticles can be done through ligand attachment (Duskey et al. 2014).

A ligand is added to the nanoparticle surface to localize a drug's effect. Ligands are designed specifically to target certain disease indicators. Cancer biomarkers represent

a large area of ligand usage (Bahrami et al. 2017). Antibodies are common nanoparticle targeting ligands for cancer cells (Bahrami 2017, Friedman 2013). Antigens, which are proteinaceous cellular recognition sites, are present on all cell membranes and act as antibody binding sites. These antigens are cell-type specific; this specificity enables effective targeting by a corresponding antibody. Tumor-specific antigens present due to cancer-associated genetic mutations (Escors 2014). Tyrosinase-related protein 1 (TRP 1) is a cell surface antigen characteristic of cutaneous melanomas; they do not express regularly with other types of cancer (Ghanem 2011). TRP 1 is currently a candidate for antibody targeted therapeutic delivery (Ghanem 2011). Non-small-cell lung cancer, another common cancer type, also has potential for antibody targeted therapy. In this type of lung cancer, epidermal growth factor receptor (EGFR) manifests itself in cancerous lung tissue (Karra et al. 2013). One study used EGFR targeted, paclitaxel loaded nanoparticles to specifically kill tumorous lung cells (Karra et al. 2013). This study also revealed improved cytotoxicity towards cancerous tissue using antibody targeted nanoparticles (Karra et al. 2013). While specific localization is a reason for this improved cytotoxicity, enhanced permeability and retention (EPR) may be an additional factor. EPR is a passive targeting mechanism in which nanoparticles agglomerate in bodily regions near tumors (Carter et al. 2016). Specific targeting, in conjunction with EPR, would logically increase delivery of cytotoxic drug, such as paclitaxel, as well as maintaining cell-localization. Antibody ligands are popular because of their widespread availability (Friedman et al. 2013). Large scale commercialization is due to already existing antibody use in common molecular research techniques. Antibody targeting

ligands are widely used for cancer treatment and are also being further investigated (Friedman 2013).

Pharmacodynamics and Pharmacokinetics of Therapeutic Delivery Vehicles

While these methods of drug delivery are all used because of their size, several properties, such as biocompatibility, biodegradability, encapsulation efficiency, and drug-release kinetics, have made some methods more favorable. These properties comprise the pharmacodynamic and pharmacokinetic properties of therapeutic nanoparticles.

Pharmacodynamics is the study of how the drug is altered by the body, whereas the pharmacokinetics of a drug are its effects on the body (Shargel et al. 2012). These two principles are material dependent, as different compounds naturally cause diverse body-drug interactions. Although these properties will be described briefly, further reading is recommended for deeper understanding

Biocompatible compounds are not toxic to the body. Biodegradable compounds are those the body can break down. Nanoparticles for therapeutic use should be both biocompatible and biodegradable. PLGA and PLA are two compounds used because of their biocompatibility and biodegradability. In aqueous environments, these polymers are degraded; the products formed are lactic acid and glycolic acid or just lactic acid for PLGA and PLA, respectively (Mahapuro et al. 2011). The degradation products are natural metabolites, so they are not inherently toxic (Mahapuro et al. 2011). Many genome-edited viral vectors satisfy these requirements; however, there are still health and safety concerns.

Besides targeted therapy, a prime motivation of nanoscale drug delivery is enhanced drug-release rates. Nanocarriers release their drugs over time; this sustained release is particularly important for cancer drug administration (Iqbal et al. 2015). Continual release prevents a large influx of cytotoxic cancer drugs and diminishes harmful chemotherapy side-effects. Dialysis is the key method for evaluating nanoparticle drug-release kinetics (D'Souza, 2014). In dialysis, the sample is injected into dialysis tubing or a dialysis cassette; release media is added, and the prepared sample is placed into a container of release media (D'Souza, 2014). The tubing or cassette membrane is selectively permeable by size (Iqbal 2015). Because of this size permeability, molecules below the size cutoff are free to diffuse across the membrane into the outer release media. An ideal in vitro release media would be a buffer that mimics biological conditions, such as phosphate buffered saline at pH 7.4 (PBS) (D'Souza 2014). Aliquots of the outer release media should be taken to evaluate the amount of released drug; this quantity can be measured spectrophotometrically or fluorometrically using a drug-compatible dye. Encapsulation efficiency can be extrapolated from the drug-release kinetics profile. Encapsulation efficiency is the percentage of drug loaded onto a nanoparticle (Wallace et al. 2012). A good encapsulation efficiency is vital to the ability of a nanoparticle to deliver sufficient drug amount. In addition to the aforementioned biologically relevant characteristics of therapeutic nanoparticles, characterization of their physical properties is important to determine their effectiveness.

Characterization

Instrumentation for nanoparticle characterization is vital for effective therapeutic effect. The nanoparticles must be within a certain size and stability range; the zeta potential of a nanoparticle is indicative of its stability. Therapeutically effective nanoparticles have a size range between 70-200 nm (Goldberg et al. 2007). Nanoparticles have a surface charge; because they are charged, there is a charged layer surrounding them called the Stern layer (Xu 2007). For example, a negatively charged nanoparticle would have a layer of positively charged ions surrounding it. The potential between this layer and the external medium is the zeta potential (Xu 2007). A nanoparticle suspension having a zeta potential greater than or about ± 20 mV are stable enough to be used for drug delivery applications; the larger the zeta potential, the more stable the nanoparticle suspension is (Honary et al. 2013). Zeta potentials below ± 10 mV promote nanoparticle aggregation because the electrostatic attraction between one nanoparticle's surface charge and another's Stern layer is greater than the repulsion due to the surface charge of each nanoparticle (Honary et al. 2013). Zeta potential is dependent on pH, ionic strength, and concentration; it is a size independent property (Bhattacharjee 2016). Each nanoparticle has an isoelectric point when pH is varied, a point where aggregation is favored; zeta potential is directly and indirectly related to concentration and ionic strength, respectively (Bhattacharjee 2016). Size measurements are typically done by Dynamic Light Scattering (DLS), but Nanoparticle Tracking Analysis (NTA) is another effective method. Stability measurements can be done by electrophoretic light scattering (ELS) or phase analysis light scattering (PALS). If an electric field is applied, NTA also measures zeta potential. The theory behind these techniques is similar in their analysis of scattered light, but they

differ in how they process the information. Zeta potential measurements require the Smoluchowski-Einstein equation, which relates a particle's velocity in solution when an electric field is applied. Particle shape and morphology can be evaluated using transmission electron microscopy (TEM) (Slocik et al. 2005). Particle size is measured through examining each particle's Brownian motion through a diffusional coefficient calculated by the Stokes-Einstein equation.

Dynamic Light Scattering

Dynamic light scattering (DLS) measures the average nanoparticle size based on its diffusion due to Brownian motion and the resultant elastic light scattering in a colloidal suspension. Doppler broadening of the scattered light occurs because of the particles' characteristic Brownian motion. This diffusion is only possible if nanoparticles are dispersed since they have an intrinsic tendency to aggregate (Xu 2007). Its hydrodynamic radius is then extrapolated from the Stokes-Einstein equation (Ito et al. 2004).

$$D = \frac{k_B T}{6\pi\eta r_h}$$

Stokes-Einstein Equation where D is diffusion coefficient, k_B is Boltzmann's constant, T is temperature, η is solution viscosity, and r_h is particle radius (Schulze et al. 2014).

The diffusion coefficient measured by this method is characteristic of the whole suspension, rather than that of an individual nanoparticle; the average nanoparticle

hydrodynamic radius for the entire suspension is calculated (Xu 2007). Due to the tendency of the nanoparticle to aggregate, solution viscosity must also be minimized to increase the diffusion coefficient. A particle's hydrodynamic radius is its theoretical size; its true size is impossible to measure due to limitations relating to its shape and matrix interactions; this theoretical size assumes the globular particle would have the same velocity as a spherical particle of the same size (Malvern). A particle size distribution is generated and exhibits broadening because of such limitations. Ensemble averaging must be applied to this data to produce an average size estimate (Ito et al. 2004). When applied, ensemble averaging generates a better signal to noise ratio as well as a smoother curve. For non-spherical particles, such as nanorods, DLS produces a bimodal size distribution; this bimodal distribution occurs because of the detected translational and rotational movement of the nanorod (Liu et al. 2012). While it is important to note the differences between spherical and non-spherical nanoparticle distributions, DLS for spherical particles will be considered here.

Instrumentation for DLS instruments contain a laser, sample cell, and a detector. The laser used in DLS acts as the light source. This laser has historically been a gas laser, but many instrument manufacturers are switching to laser diodes, which are more powerful, 30 mW compared to 10 mW, and cheaper (Xu 2007). Laser diodes also have several operating wavelengths, accommodating for a potentially necessary change in source intensity or fluorometric nanoparticle analysis. Sample cells are typically cuvettes. Because of solution viscosity considerations, the sample should be mixed homogeneously before placement into the sample cell. A photomultiplier tube is the most commonly used

detector. It is necessary to use a photomultiplier tube because weak scattering signals need to be amplified to attain a sufficient signal to noise ratio. Detectors for DLS must be at a fixed angle to the source and sample to ensure that scattered light can be detected over a wide range of angles since scattering occurs at many angles (Nemoto et al. 1981). While sizing is accomplished by DLS, zeta potential measurements are an indication of nanoparticle stability.

Zeta Potential

Nanoparticles are charged molecules and have oppositely charged ions, or counter-ions associated with them. These counter-ions have electrostatic interactions with the surrounding solution, forming what is known as the shear plane. The potential arising from these interactions is the zeta potential (Cho et al. 2012). Zeta potential is a measure of nanoparticle stability. The most common technique to measure zeta potential is electrophoretic light scattering (ELS), also called phase analysis light scattering. ELS probes the surface charge response when an external electrical field is applied to the suspension (Zhang et al. 2008). Similarly to DLS, there is a frequency shift due to nanoparticle motion, which leads to Doppler broadening (Ito et al. 2004). Nanoparticle motion during ELS is not Brownian motion, as in DLS, because it is affected by the electrical field. Electrophoretic mobility of the particle is measured, and its zeta potential is extrapolated from the Smoluchowski Equation:

$$v = \zeta (\epsilon E / \eta)$$

where v is electrophoretic velocity, η is solvent viscosity, ϵ is electrical permittivity, or dielectric constant, of solution, and electric field strength (Zhang et al. 2008). The ionic strength of the solvent is an important consideration when evaluating zeta potential; the relative strength of ions in the solution affects how and to what extent they interact with nanoparticles (Codday et al. 2014). The solution's pH also affects the nanoparticles and their counter-ions (Ito et al. 2004). This association is directly proportional to the electrophoretic velocity of the nanoparticles. Electrical permittivity is the resistance of the solution when an electric field is applied. It is measured as a ratio of solution field strength to the initial electric field strength. Viscosity must be minimized for the same reason as it is for DLS. For biological applicability, nanoparticles must be negatively charged. Negatively charged nanoparticles will have positive counter-ions and will then interact with negatively charged molecules. This positive charge is important in biological systems because cell membranes carry a net negative charge (Cho et al. 2012). Repulsion would occur between positively charged nanoparticles not because of its inherent charge, but because of the counter-ions associated with it. Much of the instrumentation for ELS has similarities with DLS instrumentation.

A laser source is oriented perpendicularly to the sample cell and detector instead of just the detector like DLS. Since the source is orthogonal to the sample cell, mirrors must be utilized (Kaszuba et al. 2010). While the sample cell and detector are perpendicular to the source, they are not in line with each other to account for scattered light from the suspension; an optimal angle between the sample and detector is 13° (Kaszuba et al. 2010). An electrode is used to apply the external electrical field. A

photomultiplier tube is also used in ELS. The Malvern Nanosight NS500 can measure nanoparticle size and zeta potential using nanoparticle tracking analysis (NTA) and ELS.

Nanoparticle Tracking Analysis

NTA is similar to DLS. It uses light scattering to analyze particle size in a suspension. While DLS analyses nanoparticles as a total distribution, NTA examines individual particle size; this individual particle sizing is possible as a result of a microscope detector (Malvern 2015). Even though NTA can observe individual particle sizes, a distribution is still generated. The microscope images the colloid suspension in real time and the particles can be visualized based on their scattered light. One of the main differences between NTA and DLS is the sample introduction method. Since particle motion is necessary for evaluation of electrophoretic mobility, the suspension is pumped into the sample cell. The sample colloidal system is first diluted if necessary, due to concentration and viscosity considerations. The solvent is allowed to flow through the system to eliminate potentially interfering residual materials. Water is a common solvent. The flow rate is controlled by a peristaltic pump that causes aspiration of the solution. A tube starting at the sample leads to the pump, which generates suction. After passage through the pump, the sample then flows through the sample cell. This sample cell is irradiated with a laser; the scattered light is analyzed by the microscope detector, which is orientated nearly perpendicularly (Malvern 2015). This nearly orthogonal orientation may be necessary for optimal scattering. If positioned at 90° , less scattered light would be detected by the microscope and measurements could contain significant error. An electric field is applied to this sample by electrodes (Malvern 2015). This electric field is

necessary to determine zeta potential. Electrophoretic velocity is measured similarly to that of an instrument dedicated to ELS. While NTA is an effective method of determining nanoparticle size and stability, DLS and ELS, when combined are more common methods for nanoparticle characterization. Morphological characterization is necessary to confirm particle shape. This type of characterization is commonly done using transmission electron microscopy (TEM).

Transmission Electron Microscopy

TEM is similar, in theory, to standard light microscopy; however, electrons are used instead of visible light. Electrons are transmitted through the sample and an image is generated based on beam interactions with a detector; electromagnetic lenses must be used instead of optical lenses to detect transmitted electrons. An electron has a shorter wavelength than visible light, so resolution is enhanced. TEM has been shown to resolve platinum nanoparticles 2 nm in diameter (House et al. 2016). While nanoparticles of this size and composition are not commonly used for biomedical applications, the resolving power demonstrates the usefulness of the TEM for nanoscale imaging. Currently, benchtop TEM's, or low-voltage electron microscopes (LVEM) are sometimes used to obtain a similar result as TEM (Bell et al. 2014). LVEM's have lower resolving power, but typically can be operated on a much shorter timescale than a TEM. LVEM are also much cheaper and smaller than a standard TEM (DeLong) TEMs are also large and generally take up a whole room or research space; LVEM's offer an advantage in this regard as well. An image taken using a TEM or LVEM is in grayscale and resembles an image seen on a light microscope, as seen in Figure 1. While this picture is blurry due to

instrument miscalibration, it demonstrates how TEM or LVEM can be used to analyze nanoparticles size and morphology.

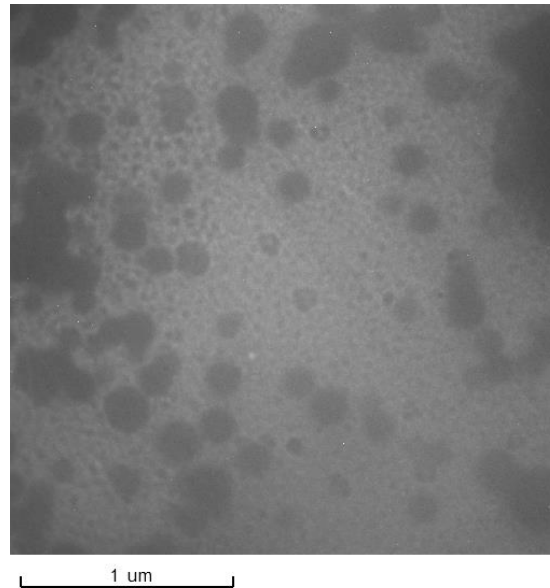


Figure 1. PLGA Nanoparticles Imaged Using Low Voltage Electron Microscopy (LVEM). Synthesized PLGA nanoparticle morphologies were characterized using LVEM. Nanoparticles were spherical and had an average diameter of about 125 nm. These nanoparticles were synthesized using microfluidic technology and the image was taken by the author.

Characterization of therapeutic nanoparticles is necessary to determine their functional use. Use in drug delivery methods is the most common application for these nanoparticles; however, they are only useful if they meet certain specifications of size and stability. Size measurements are effectively completed using DLS or NTA; the largest difference between these two methods is their detectors, which are a photomultiplier tube and microscope, respectively. Stability is determined by measuring

particles' electrophoretic mobility and relating it to their zeta potential, a clear indicator of nanoparticle stability. ELS is a proven method for zeta potential measurements.

Morphological characterization is done primarily by TEM; LVEM is becoming more reliable, but operates on the same principles as TEM.

Biotechnology across Disciplines

Scientific contributions are primarily made from chemical, materials, and biomolecular engineering; however, mechanical and electrical engineers have also developed new technologies to this always increasing field of study. Engineering principles allowed the development of controlled release mechanisms, as well as instrumentation design. Characterization of drug delivery materials may involve mechanical stability considerations, depending on the target function of the material. For example, polyethylene glycol (PEG) hydrogels coated neural implants have been shown to effectively reduce scarring of glial cells; however, the mechanical stress between the implant and glial cells is important to neural functionality (Spencer et al. 2017). The stress, in this instance, was evaluated using atomic force microscopy and Hertzian analysis, which is a mathematical technique used to evaluate contact stress (Spencer et al. 2017). Electrical engineering principles have been key in the design of new medical devices aimed at drug delivery. For applications to the gastrointestinal (GI) tract diseases or infections, the patient ingests the device; this device resides transiently in the patient while releasing drug until it is passed (Zhang, S et al. 2017). Since these electronic devices are continuously turned on, they may run out of power. A potential solution to this problem is wireless charging (Abid, A. et al. 2017). By focusing an electromagnetic

field on the device, the device can be recharged via wireless power coupling (Abid, A. et al. 2017). These engineering principles and approaches would likely not have developed from the perspective of a pure biologist or chemist and imply the necessity and importance of engineering collaboration in biotechnology development. Based on the extensive advances made by both scientists and engineers, a multi-disciplinary approach to solving drug-delivery problems is more effective than a one dimensional approach. Scientists that understand the system in question will often work with engineers to design an effective delivery and targeting mechanism. This expansion of health sciences into engineering has greatly advanced technology and has also increased the economic impact by the inclusion of more STEM fields.

Economic Impact

The biotechnology industry affects nearly every facet of modern life. In total, the biotech industry makes up 1.67% of the United States GDP, which is equivalent to \$324 billion (Carlson 2016). While only a small percentage of the GDP, this sector is rapidly expanding at rates exceeding 10% since 2006 (Carlson 2016). Drug delivery technology for cancer treatment research and development alone made up \$4.31B of US GDP in 2016 and was estimated to grow 22.9% by 2025, according to a Grandview Research study. The instrumentation sector, although smaller than drug delivery, contributed \$314.3M in 2017; it was predicted to grow by 5.2% to \$405.1M by 2022 (Markets and Markets). This rapid growth should be an indication to investors that biotech has potential for profitability both now and in the future. The drug delivery and instrumentation growth should be regarded as especially important as the main thrusts of biotech research heavily

involve both of these fields. Health science biotech companies are not limited to large, publicly traded pharmacy companies such as Johnson & Johnson or Pfizer, but incorporates many start-up companies and mid-size businesses. While there is no funding shortage for big pharmaceutical companies, money is often an issue for start-up companies. Biotech startups, on many occasions, begin from university sponsored research. The number of startups originating from academia in the United States has increased from 61 in 2014 to 76 in 2016 (Huggett 2015, 2017). This increase further indicates the growth of the biotech sector. Although the success of these new companies has not been determined, biotech startups typically have a low success rate. Low success rates can be attributed to long research periods (8-12 years) and unpredictable results of this research and development process (Tsai, Erikson 2006). Additionally, large amounts of federal regulation may contribute to inhibiting startups' success. If a new treatment does not fit the Federal Drug Administration's (FDA) risk policy, the treatment will not be approved. While some regulation is beneficial to ensure drugs that are more detrimental do not reach the market. Funding for these start-ups is attained either through venture capitalist (VC) funding, angel investors, or research grants. While it is not necessary to repay research grants, they are highly competitive and it is difficult for a grant proposal to be accepted. VC funding and angel investors are different than grants as they require a share of the profit since they become partners with the founders. VC are investors that target early-stage companies.

Venture capitalists provide funding to high-growth companies. A high-growth company is one that has great potential to quickly generate profit or is currently

accelerating its rate of return. Because of their high rate of return, VC firms are attracted to these types of companies; however, high-growth companies, like biotech startups, are riskier investments. There is potential for a large return, but the risk of losing money is also greater. Technology company investments, in general, are particularly uncertain due to substantial research and development (R&D) dependence (Gompers 2001). If the new technology is not developed into a profitable product, money is lost. VC funding, while not explicitly limited to geography, is heavily influenced by company location (Gompers 2001). Location considerations are important due to travel costs and area-familiarity (Fritsch 2008). These two factors are reasons why VC hubs, such as Silicon Valley and Boston, are so popular for startup companies. Another reason why VC firms invest locally is because startups require a large time-investment (Alvarez-Garrido 2014). It would be much harder for venture capital firms and funded companies to actively engage with each other over large distances. While funding may be more competitive in these locations, there are also more VC firms to use. VC firms may be either independently or corporately owned (Alvarez-Garrido 2014).

Apart from difference in ownership structure, these VC firms are different in their investment approach and return rates (Alvarez-Garrido 2014). Independent VC firms are the traditional, historical version of VC. These firms are privately owned and have company partners or associates who make investments. These partners are usually well-versed in investment strategies and the targeted industry (Alvarez-Garrido 2014). For example, a well-qualified partner or associate at a biotechnology-focused VC firm would be someone who has experience investing with life-science technologies. An ideal

candidate could also be a biology, biochemistry, chemistry, or biomedical engineering PhD who has entrepreneurial experience. Venture capitalists provide funding in exchange for company equity. A typical equity rate is around 20% (Fried 1998). Coinciding with long R&D periods for biotech companies, VC firms usually stay involved with biotech companies for 7-12 years (Alvarez-Garrido 2014). This long involvement is a large risk for venture capitalists as it represents an extended period of making minimal or no returns. Because of the long investment timeline of the biotech industry, the VC investment landscape has been altered. Traditional VC investment strategies have changed with the growth of the biotech industry; in many cases, independent VC has completely been eliminated (Ford, Nelsen 2013).

Lack of VC success is the largest contributor to this trend (Ford, Nelsen 2013). Long-investment timelines may have also caused a shift from independent VC firms to corporately sponsored funding. Pharmaceutical and larger biotech companies two examples of corporate backed VC. Large pharmaceutical companies provide research and development funding for small companies in the form of a licensing agreement (Ford, Nielsen 2013). Although the company is not objectively any more stable, there is overall less investment risk for the corporate VC. While these smaller companies operate autonomously in their normal day to day operations, the product is sold under the pharmaceutical company's product line. One motivation for this strategy is the reduction of in-house research funding by pharmaceutical companies; by licensing new innovations from startups, larger companies may end up saving money long-term (Ford, Nielsen 2013). Startups also gain access to the larger company's sales pipeline and contacts

(Alvarez-Garrido 2014). By utilizing these connections, the drug becomes widespread more easily. Angel investors are another avenue through which startup companies can attain funding. These investors are established businesspersons that may have a close connection with the startup owners or the geographical location of the business (Morrissette 2007).

Early-stage biotech companies should seek out funding from angel investors because of their heavy involvement with the company and their more relaxed timeline, as opposed to VC. Angel investors typically invest early in a company's lifetime. Being involved in a company's beginnings stages allows the angels to maintain a more hands-on role as well as being able to shape the future of the company easier. A hands-on mentality of the angel investor is most likely due to his or her business background. These investors are typically successful and are entrepreneurs themselves (Morrissette 2007). This early, heavy involvement, by nature, requires a close relationship with company executives. The angel investor can be more impactful within the company if he or she is involved when the company is in its infancy (Ramadani 2012). Because of the close relationship between angel investors and executives, the entrepreneur's personality and drive are large factors in angel investing criteria (Sudek 2006). These criteria are intuitive since people who get along are more likely to work together better than those who do not. While the entrepreneur's profile is important for venture capital investment, it is perhaps even more important for an angel since he or she takes a more involved company role. Another difference between angel investors and VC is the investment size. Angels typically invest amounts less than \$100,000, while VC invest \$4 million; this

difference is attributed to the financial source (Morrissette 2007). VC firms use money they have gained from many sources, but angels use their own money. Since angels are investing their own money, it is understandable why there is a disparity between the average amount of funding provided by VC and angels. Angel investors and VC also have different investment timelines. Venture capitalists want to minimize their company-involvement time. This desire to reduce their time-commitment is so they can transition to new opportunities (Morrissette 2007). It also minimizes the risk of losing the investment. Angels' investment timeline is more flexible and would probably be longer than VC investors (Fenstel 2011). While VC and angel investors require a return on their investments, research grants provide funds for research groups or companies without any equity.

Research grants are often federally funded. Agencies that award grants are the National Institute of Health (NIH) and the National Science Foundation (NSF). The NSF typically awards grants to non-biomedical investigators. The NIH is involved in biomedical research, ranging from basic science to translational medicine, as well as biomedical engineering. In 2018, the NIH provided nearly \$21B in research funding; this funding includes research expenditures and staff and facility support (NIH 2018). While the NIH does not have a shortage of funding, grants are highly competitive; many research teams apply for a limited amount of money. The grant-awarding mechanism also plays into how competitive grants are. Once a grant is given approval status, it is ranked among other approved grants. The NIH then awards money in ranked order; however, since the NIH does not have funding for all research groups, the agency awards money

until it runs out. Two NIH research grants that startups should target are Small Business Technology Transfer (STTR) or Small Business Innovation Research (SBIR) grants.

STTR grants were started in 1994 as a way to increase technological advancement and economic activity from federal, university, and non-profit sponsored research (Baron 1993). By funding high-growth companies or technology with great potential, the United States would become economically more competitive. This grant highlights research that could be spun-off into a new company with large potential for success (Baron 1993). These grants also aim to bridge a divide between profit-driven corporate research and innovation-driven university research (Ford 2008). SBIR grants have a similar motivation as STTR's, but focus on women and minority researchers. The first SBIR program was started in 1977 at the NSF but later spread to other government agencies such as the Department of Defense, Defense Advanced Research Program Agency, and NIH (Audretsch 2002, Link 2008). This targeting towards underrepresented groups in science was initially implemented because of apparent discrimination towards women investigators (Link 2008). Although there may have been some preference towards men in the past, these grants should help eliminate any non-merit based bias that occurs. Statistical analysis has shown that female owned companies exhibit a similar level of commercialization for their product compared to male owned companies (Link 2008). This data supports the alleged bias towards women in grant funding. Both STTR and SBIR grants should be attractive to early-stage biotech companies looking for funding. Biotech companies involved in drug delivery and nanomedicine is not only a large

portion of the health sciences field, but it also greatly impacts many engineering disciplines as well.

Therapeutic delivery technology is a central focus in biomedical science related areas. These technologies include treatment mechanisms, as well as the delivery vehicle. The goal of these technologies is to produce more effective treatments for diseases such as cancer, atrial fibrillation, and numerous genetically based diseases (Serna 2018, O'Quinn 2018, Beitelshes 2017). Gene therapy, protein therapy, and drug delivery are three treatment methods currently being investigated. Gene therapy restores mutated genes to a healthy condition. By fixing the mutated gene, healthy gene products will be produced and disease symptoms will be mitigated. Protein therapy involves the delivery of a peptide-based therapeutic in order to treat a disease. Drug delivery technologies introduce non-peptide-based therapeutics into the patient. Just as important to the treatment as the drug is the delivery vehicle. Currently popular and effective vehicles are viral vectors, liposomal, or polymeric nanoparticles; these nanoparticles promote enhanced therapeutic payload. Due to the microscopic qualities of these particles, characterization instrumentation such as DLS, ELS, TEM, and SEM are necessary to determine their physical properties. Therapeutic delivery technology is not only a large research interest within the scientific community but is also a significant economic component. Economic impacts from this sector range from small startups to large biotech and pharmaceutical companies. These large monetary investments, as well as scientific interest, are key signs that biotechnology will be the focus of biomedical research and entrepreneurship for the foreseeable future.

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