Investigating a Hybrid Nanoparticle System for Treatment and Detection of Deep Vein Thrombosis

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INVESTIGATING A HYBRID NANOPARTICLE DESIGN FOR DEEP VEIN THROMBOSIS TREATMENT AND DETECTION

Abstract

This review focuses on additions to an already established treatment for Deep Vein Thrombosis (DVT). Nanoparticles with the addition of PPACK ((Phe[D]-Pro-Arg-Chloromethylketone), an efficient thrombin inhibitor, attached to their cores have demonstrated a capacity for outperforming unmodified nanoparticles. Furthermore, this study will look into how P-selectin, a cell adhesion molecule, has shown evidence of being a risk factor in the development of DVT. A systematic review was conducted on studies examining the effect of nanoparticles modified with PPACK on occlusion time, as well as those exploring early thrombus detection through various biomarkers. Biomarker concentrations were measured in vivo and separated into two groups: positive for DVT and negative for DVT. Studies that were analyzed included tests on both mice and records of humans. PPACK-nanoparticle efficacy was demonstrated by experimentation on mice through laser injury of the carotid artery. Of the studies evaluated, P-selectin consistently located acute thrombus formation which was demonstrated by an observed increase in P-selectin concentration for positive DVT groups with exception of one study. PPACK NPs also demonstrated more efficient capability of delaying occlusion time and removing thrombus site. PPACK NPs have an advantage of removing thrombus site due to PPACK being an effective thrombin inhibitor. Furthermore, an increase in P-selectin concentrations has shown promises of predicting acute thrombus formation.

Key Terms: PPACK, deep vein thrombosis, anticoagulant, P-selectin, nanoparticles, thrombus

Introduction

It has been estimated that deep vein thrombosis (DVT) affects nearly two million people in the United States (Wadajkar, 2013). Approximately 60,000 to 100,000 Americans will die due to illness relating to venous thromboembolism; however there are up to 900,000 cases of DVT and pulmonary embolisms (PE), blockage of arteries connected to lungs, each year (Ramacciotti, 2016). DVT is a serious threat because many of the symptoms, including swelling, pain, and discoloration, are often overlooked, allowing mortality to be completely undetected. Typically, 10% to 30% of patients will die within one month of diagnosis. Furthermore, people who have already experienced DVT will oftentimes experience long-term complications, known as post-thrombotic syndrome (PTS), where increased pressure in the patient's vein due to a former blood clot can have long-lasting effects. These complications are also very similar to symptoms such as chronic leg pain, swelling, or varicose veins (CDC, 2015).

Wadajkar et al (2013) explains more of who is affected by DVT and also the status of the possible applications nanoparticles can have as a treatment option. Multiple methods of locating thrombus formation include venography, impedance plethysmography (IP) magnetic resonance imaging (MRI) venous scintigraphy (VS), and ultrasound. Venography is a procedure of x-raying the veins after an injection of radiopaque fluid. They also show how nanomedicine has high potential in dealing with DVT due to its accuracy in drug delivery. Research in the field of nanotechnology has focused on the development of non-invasive and accurate diagnostics, such as ultrasound enhanced techniques and molecular imaging methods to assess thrombus locations and its treatment course in a more effective manner.

Deep vein thrombosis describes a blood clot formed in a vein deep inside a person's body. Areas prone to this include the lower leg, thigh, and pelvis, although thrombosis can also occur in other places in the body as well. A blood clot has the potential to travel from the original site of the clot to another part of the body, possibly leading to severe organ damage, heart attack, stroke, or kidney failure if not diagnosed immediately.

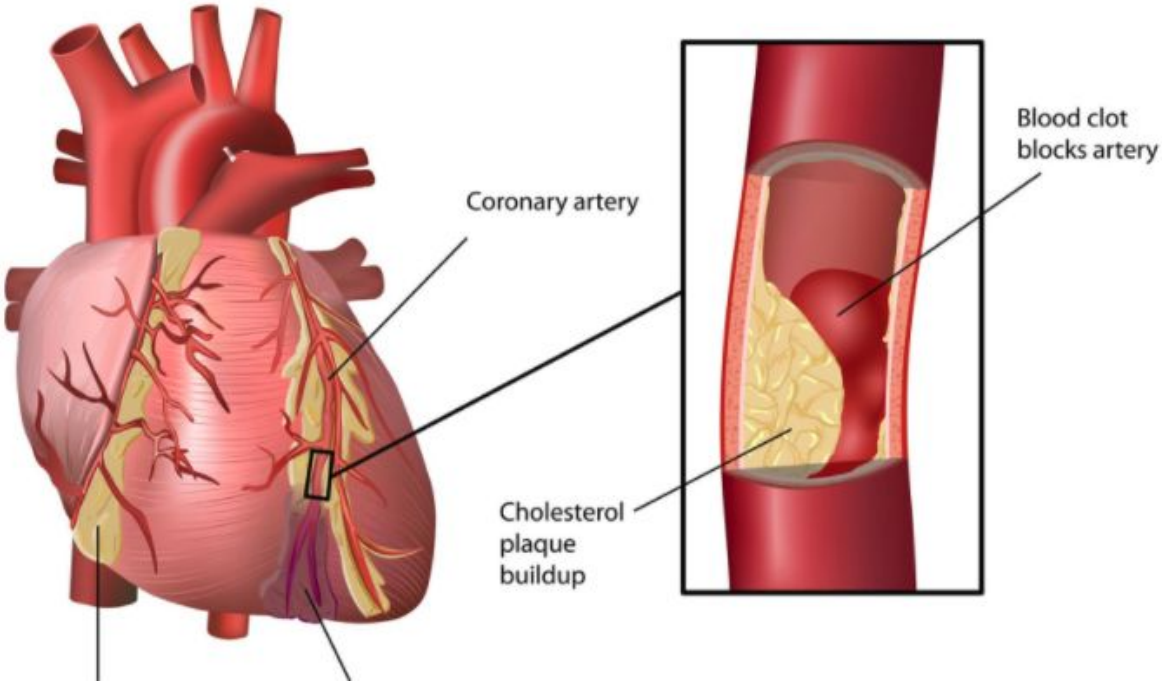

Figure 1. Illustration of a thrombus formation within the major artery of the heart. This blockage formed due to high cholesterol in the blood vessels, causing inflammation to occur. The left side of the heart depicts a health heart muscle while the right displays a dead heart muscle. If the blockage becomes unattached to the wall of the artery, it can travel further into the heart or to other vital organs. The blockage restricts the blood and oxygen supply to the heart since it severely narrows the coronary blood vessels. (Disease and Conditions, 2018)

Conventional treatments for DVT include basic lifestyle modifications, such as monitoring vitamin K intake, exercising, and applying mechanical compression. Other treatments can involve more serious measures including 1. anticoagulant therapy (administration of anticoagulants, agents to prevent formation of blood clots), 2. inferior vena cava filter (implantation of a vascular filter to prevent

life-threatening PE), or 3. thrombolysis/thrombectomy (breakdown process of blood clots within blood vessels though medication). However, these treatment options often have side effects ranging from improper clot clearance to an increased risk of hemorrhage occurrence, the escape of blood from a ruptured blood vessel. Thrombus formation continues despite the use of aggressive regimens such as anticoagulant and antiplatelet agents administered orally or intravenously. Other drawbacks to these forms of treatments are the potential of bleeding in organs or tissues, severe back pain, or fainting (Wadajkar, 2013). Nanotechnology has demonstrated good prospects for filling this role as it offers a safer and more efficacious option for the detection and removal of the thrombus site.

This study is an evaluation of whether a multifunctional nanoparticle system can eliminate blood clots more efficiently in the human body and also assist in the detection of early thrombus formation. Nanoparticles can be categorized by shape, size, and material components. However, the use of nanoparticles for DVT are typically designed to have only one specific function. Many studies have focused on the individual tasks nanoparticles can serve, such as localizing the site of thrombi or increasing the efficiency and/or accuracy in drug delivery to the targeted site. The results of these studies allow for cross-examination between the basic functions of individual nanoparticle designs so that the functions may be effectively combined into hybrid designs. Nanotechnology has a significant number of medical applications; however a design for a hybrid nanoparticle system with the addition of PPACK that aids in both the detection and treatment of DVT through the identification of P-selectin is absent. Thus the research goal in this project is to determine if a multifunctional nanoparticle which has all of these features can be used specifically for DVT.

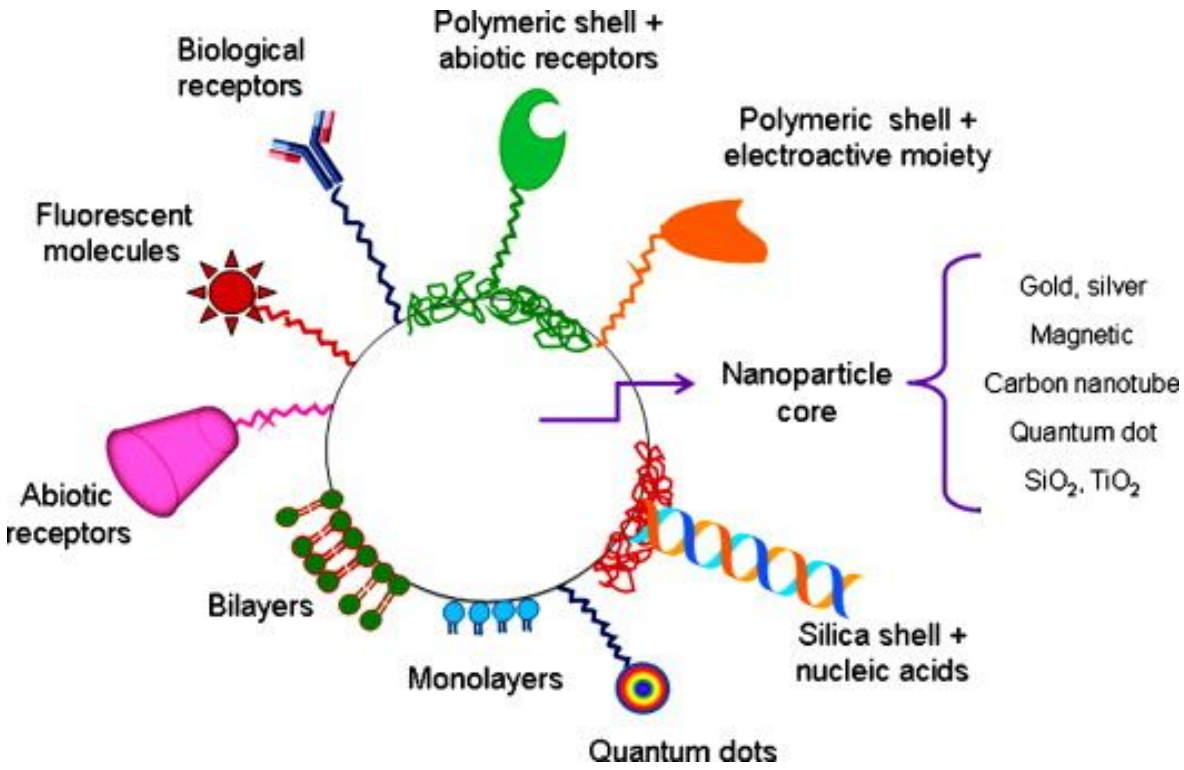

Figure 2. Structure and composition of a multifunctional nanoparticles (MFNP). Nanoparticle core can be doped with different nanoparticle and/or dye. MFNPs have the ability to achieve a mixed effect since these systems use variable strategies to attain a combination of optimized optical-, electrical or magnetic properties as well as analysis capability. (Simón de Dios, 2010)

Nanoparticles offer a better diagnosis and treatment option for those suffering with DVT because of the advantage of size. These particle have the ability to travel through microscopic blood vessels to the predicted site of thrombosis, visualize an area through ultrasound, or be used as a more specific targeted drug delivery system. Unlike traditional methods of treating DVT, nanotechnology is able to perform tasks at an atomic, molecular, and macromolecular level in the nanometer range. It can lead to an early detection of thrombi, and subsequently prevent the blockage from accumulating. The accuracy and efficiency of nanoparticles at the microscopic level has key advantages over common DVT treatments as the rapid development of nanotechnology has the potential to treat diseases more accurately and efficiently. It has the edge in size and speciality in their functions compared to conventional treatments.

Emergence and Characteristics of Nanoparticles

Nanoparticles can be categorized into two major types. Those that are based off of organic molecules including carbon nanotubes, liposomes, and dendrimers, and also particles containing inorganic elements. Inorganic particles' cores consist mainly of metals and metal oxides (Simón de Dios & Díaz-García, 2010).

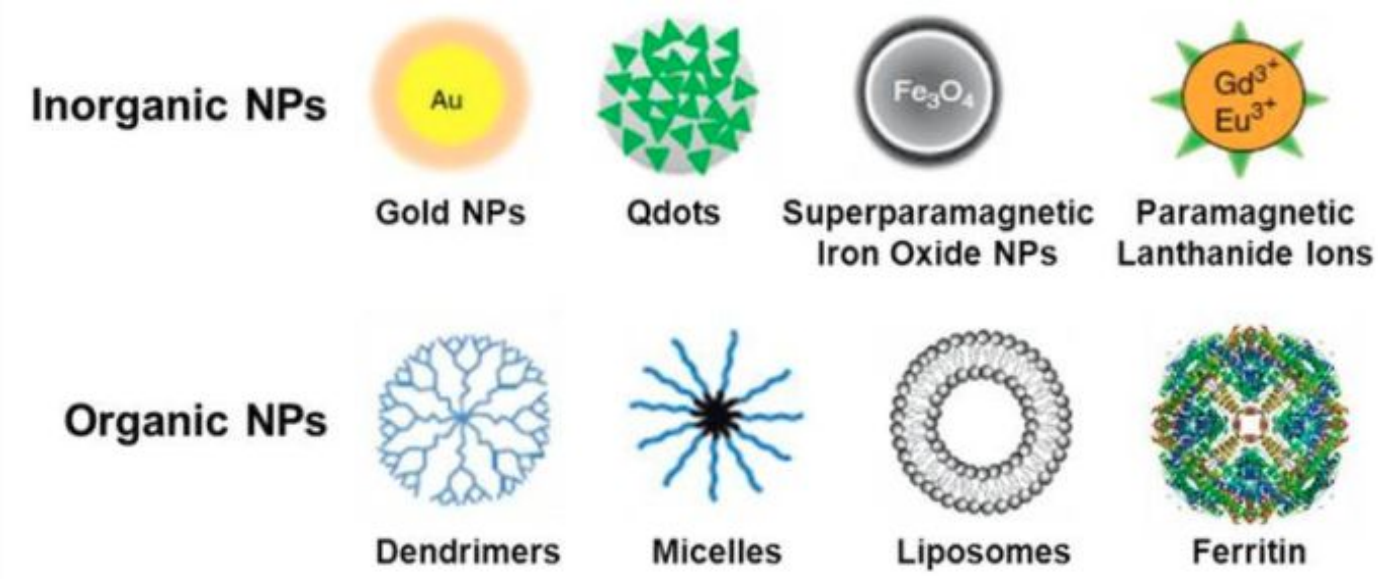

Figure 3. Display of organic and inorganic nanoparticle and a schematic representation of typical nano cores for the construction of radiolabeled nanoparticles. Liposomes are phospholipid vesicles (50–100 nm) that have a bilayer membrane structure similar to that of biological membranes and an internal aqueous phase. (Xing, Zhao, Kai Chen, 2014).

Nanoparticles allow more accurate and noninvasive detection of inflammation and the detection of new blood vessels. The "size effect" of nanoparticles has led to the development of advanced nanoparticle probes designed for biomedical imaging and drug delivery (Wickline et al, 2006). Wickline states the benefits of nanoparticles: their ability to function at the nanoscale within cells or even individual organelles. Furthermore, the study introduces the common categories of nanoparticles. These

include liposomes, 50 to 700 nm, emulsions, 200 to 400 nm, polymers, 40 to 200 nm, or iron oxide nanoparticles (15 to 60 nm).

These are categorized based on their distinctive design and function. McCarthy and Weissleder (2008) add to the possibile potential of nanoparticles. However, there is an introduction of another variable: multifunctionality. Nanoparticles have a large surface area to volume ratio which allows the attachments of other ligands. Nanoparticles offer a method for locating the site of early thrombosis and also serve as a more accurate platform for localizing antithrombotic actions. Multiple copies of these receptors can translate to an increase in binding of particles for the cell. When nanoparticles are equipped with different ligands, they are termed multifunctional since they have multiple imaging and therapeutic capabilities. Ahmad & Lone (2017) explain the properties and structural characteristics that make up nanoparticles, specifically lutetium ferrite ones.

The interest in multiferroic materials grew due to their ferromagnetic and ferroelectric properties. These materials are fairly resistant to high magnetization which allows for applications in transducers and valves. Ahmad and Lone also synthesized a monophasic nanocrystalline orthorhombic Lutetium ferrite particle with a size of 90 nm and SA of 214m^2g^{-1}. This demonstrated the actual construction of a multifunctional nanoparticle and the process in which they created it. Simon de Dios and Diaz-Garcia (2010) take a more broad view and inform all the possible applications of multifunctional nanoparticles including biosensing, bioassays, catalysis, and separations. However, in their paper they only reviewed nanoparticles composed of magnetic, quantum dots, gold nanoparticles, carbon and inorganic nanotubes, and sicilia. Nanoparticles are essential in detection of diseases, bioagent threats, chemical or infectious diseases that are not detected through conventional methods. They also undergo another boundary: the wide range of size distribution.

Bourigua et al (2010) focused on the improvement of biosensors which led to a miniaturization system, using microelectrodes to develop microsensors and nanosensors. Bouriga's team of researchers

took a more narrow approach. They designed an impedimetric immunosensor based on a carbon nanotube, aimed at increasing the detection and dynamic range. Overall, the paper introduced a new method for fabrication of a miniaturized impedimetric immunosensors for D-dimer detection. The modified microelectrodes can lead to 4000 times lower detection limit and five times higher saturation limit. They also showed how this can be easily reproduced as the biosensor exhibited a short response time of only ten minutes.

Myerson and his colleagues (2011) demonstrate the application of nanoparticles on thrombosis. This study provided an alternative strategy to using perfluorocarbon nanoparticles as an option for magnetic resonance imaging to detect acute thrombosis. Perfluorocarbon (PFC) itself, can act as an antithrombotic molecule by preventing thrombin-absorbing surfaces to the vascular injury site (Myerson et al, 2011). This led to the cooperation of PPACK Phe[D]-Pro-Arg-Chloromethylketone, an effective irreversible thrombin inhibitor and PFC nanoparticles. PPACK was attached to the perfluorocarbon-core of this nanoparticle which exceeded the performance of common anticoagulants such as Heparin. It acted as a highly effective irreversible thrombin inhibitor.

Toxic Potential

However, it's important to note the possible dangers of introducing such an experimental treatment into the medical world despite its wide variety of benefits. One of the biggest setbacks of implementing a hybrid nanoparticle system to detect and treat DVT is the potential to cause harm to patients due to its toxic qualities. Xia, Li, & Nel (2009) expand on this hesitation by stating more possible obstacles with the use of this new technology in the medical field. Unlike other reviews, it takes more of a cautious stance. One major drawback is nanotoxicology which could initiate many biological responses. Xia and her colleagues discuss this issue by comparing the possible injury mechanisms and adverse health

effects of engineered and ambient ultrafine particles. It provides a scientific basis for understanding the toxic potential of these said materials.

Table 1. Comparison of ambient ultrafine particles (UFPs) and nanoparticles (NPs). The table displays how UFPs and engineered NPS may differ in a variety of aspects including sources, composition, homo- or heterogeneity, size distribution, oxidant potential, and potential routes of exposure (Xia T, et al. 2009). Abbreviations: ROS: Reactive oxygen species

Particle types	UFPs	NPs
Source	Incidental (combustion)	Engineered (controlled synthesis)
Surface area/volume	High	High
Uniformity	Low	High (size, shape, functionality)
Organic chemical content	High	Low
Metal impurities	High	Varies
ROS generation	Yes	Varies
Exposure route	Inhalation	Inhalation, skin, ingestion, injection
Adverse health effects	Yes	Unknown

This review also informs how nanoparticles have the potential to cause drastic pulmonary and respiratory effects from engineered ultrafine particles (UFPs). However, they briefly provide a solution to this issue: using an oxidative stress paradigm as a screening assay for nanomaterial toxicity. Using an oxidative stress paradigm as a screening method for nanomaterial toxicity allows a way to test for responses to cellular injury. The amount of toxic material contained in nanoparticles is unknown since the effects it can have on the human body is not thoroughly tested. The characteristics of nanoparticles should be considered including size, shape, purity, surface area, charge, hydrophobicity, state of aggregation, crystallinity, and an electronic state.

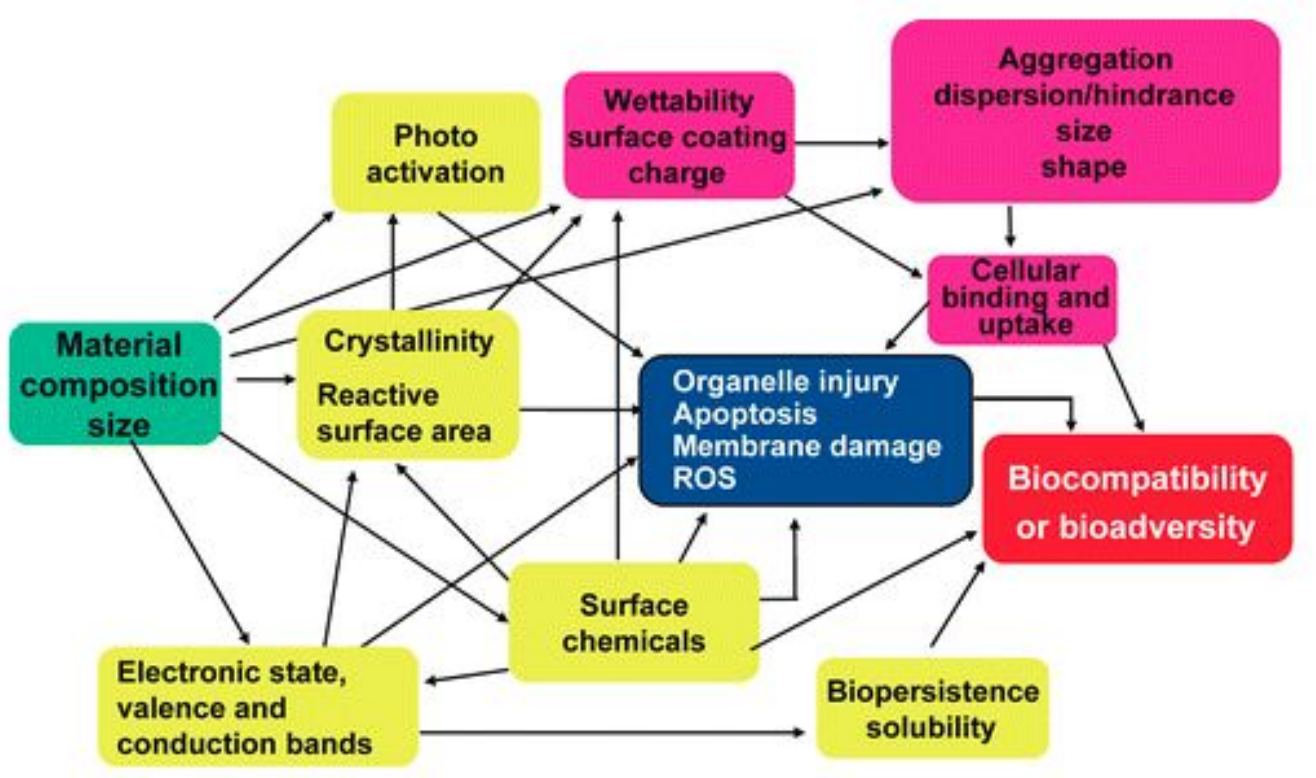

Figure 4. Overlapping module displaying the structure-activity relationship as a way to determine biocompatibility or toxicity. Physicochemical characteristics of NPs can be divided into three sets of material characteristics. 1) determines surface reactivity 2) determines subcellular localization and cellular uptake 3) determine interaction with specific cellular components or processes. Through these integrated modules the linking of NM properties and biological outcomes can possible be determined (Xia T, et al. 2009)

Having all these properties in check may allow an easier evaluation to determine any potential toxicity in nanomaterials. Figure 4 is a visualization of structure-activity relationship connecting nanoparticle properties to possible biological responses. It is still essential to further understand the toxicological potential that nanoparticles due to the interactions taking place at the nano-bio interface, as this recognition can allow preemptive screening paradigms for nanomaterial toxicity.

Correlation of P-selectin and thrombi formation

It is essential to investigate the major causes of DVT, one being the P-selectin protein. P-selectin is an adhesion glycoprotein which is present in a-granules and endothelial cell Weibel--Palade bodies which are storage granules that make up the inner linings of the blood vessels and the heart (Wakefield, et al. 2011). The role of P-selectin in the body is to mediate the interaction of activated endothelial cells with leukocytes, a cell that is part of the immune system for fighting foreign substances. P-selectin is a marker for platelet activation and a direct inducer of procoagulant state (Hameed, et al. 2017). The activation of platelets leads to the exposure of this particular protein. While this is occurring, endothelial cells degranulate due to the massive movement of P-selectin to the plasma membrane which shows how it has a role in the accumulation of tissue and generation of fibrin within the platelet thrombus. Once P-selectin is redistributed back onto the surface of activated endothelial cells, it initiates an inflammatory cascade (Barnes, et al. 2008). The significance in this is that detecting P-selectin can possibly allow diagnosis of acute thrombosis, thus preventing an increase in thrombi blockage severity. Wang and his colleagues (2010) elaborate on how one of the major causes of DVT is P-selectin. They go more in depth with this relationship by explaining that this protein is responsible for the activation of platelets. As endothelial cells degranulate, an accumulation of tissue and generation of protein fibrin within the platelet thrombus occur. As a result, this study suggests the development of a nanoparticle that can provide accurate detection and localization of P-selectin, which may lead to early identification of microthrombi so early signs of thrombosis can be achieved.

Purpose

As of now, many nanoparticles designed for this disease have one specific task. However, the objective in this project is to research if an engineered device at a molecular level can assist in more efficient thrombin removal and provide a more accurate location of the affected site. Although the introduction of nanomedicine is not new, there are currently no available well functioning hybrid nanoparticles designed to aid in both the diagnosis and treatment of deep vein thrombosis. The research question is investigating whether a hybrid nanoparticle system can be applicable to treating and detecting DVT.

Research Question

How can a hybrid nanoparticle system with addition of PPACK and detection of P-selectin be applied for DVT diagnosis and treatment?

Hypothesis

A design of a multifunctional nanoparticle that can provide early diagnosis and a more efficient and accurate drug delivery system, specifically for DVT is feasible.

Null Hypothesis

Due to the high potential of toxicity of nanoparticles (such as high content of redox cycling organic chemicals and possible generation of reactive oxygen species), a hybrid design aimed at treating DVT will not be pursued because of too much risk.

Methods

The research design was conducted via secondary data analysis. A few sources used to retrieve information regarding this topic that were capable of reaching were Ebscohost, CSUCI's library databases, or PubMed. However, there was no restriction to only use these sources. Other databases that were

available include ScienceDirect, ResearchGate, or PUBMED-NCBI. The availability of data was not a concerning issue since a wide range of reliable databases are provided. These sources contained many studies about nanoparticle applications in the medical field and also the background of DVT and its experimental treatments. The majority of these articles were up to date since the concept of nanoparticles is still a recent development. Most studies were taken between the years 2006-2017. This research design method was most appropriate for this study because it allowed a more analyzation process rather than a "hands-on" experimentation route. Through systematic review, different designs of nanoparticles specific for DVT were reviewed and various biomarkers were studied to understand the effectiveness. This allows an easier development of a hypothesis of whether the research goal was plausible.

Data was obtained by analyzing studies testing the occlusion time of three different forms of treatment. These include negative control, NPs without addition of PPACK, and NPs with PPACK attached to NP core. The efficiency of PPACK NPs was determined by comparing the delayed occlusion time in vivo. Mice were used through all studies and were given each form of treatment option in separate groups. Systemic effects of PPACK NPs were determined based on various times after injection. Carotid artery experiments were conducted either through acute photochemical injury or laser injury to assess effects of PPACK NPs.

Evaluation of the accuracy on P-selectin predicting thrombus formation was conducted by comparing patients with DVT and patients without DVT. Data also included other biomarkers for diagnosing DVT, including the most common clinical marker, D-dimer. Differences in pre and post P-selectin concentrations were measured to determine the biomarker accuracy. Unit conversions were applied to all necessary studies to allow proper comparisons. Statistical analysis was performed t-test using two tail distribution with $p < 0.05$ considered statistically significant.

Selection Criteria

The studies were selected in the systematic review based on the following criteria:

1. Only studies recording pre and post values of common DVT biomarkers concentrations were included
2. Only studies that included evaluation of soluble P-selectin, D-dimer, C-reactive protein, and Wells Score were used
3. Study must include the addition of PPACK NPs on occlusion time
4. For studies measuring occlusion time on various forms of treatments, only ones with negative control group were included
5. Studies were conducted post 2001

Results

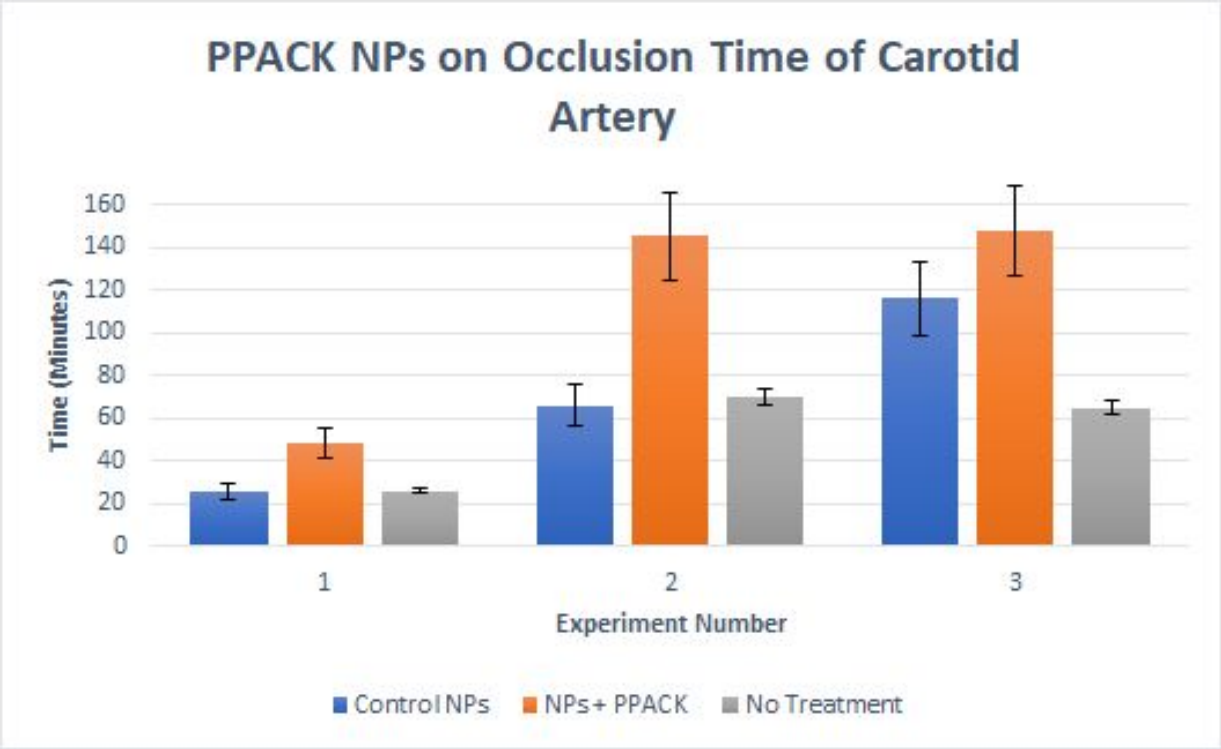

Figure 5A. Comparison on the effectiveness of NPs w/o PPACK, NPs w/ PPACK, and no treatment on occlusion time. Through all studies, NPs with the addition of PPACK demonstrated best results for delaying the blockage of the blood vessel (Myerson et al., 2012 & Palekar et al., 2017).

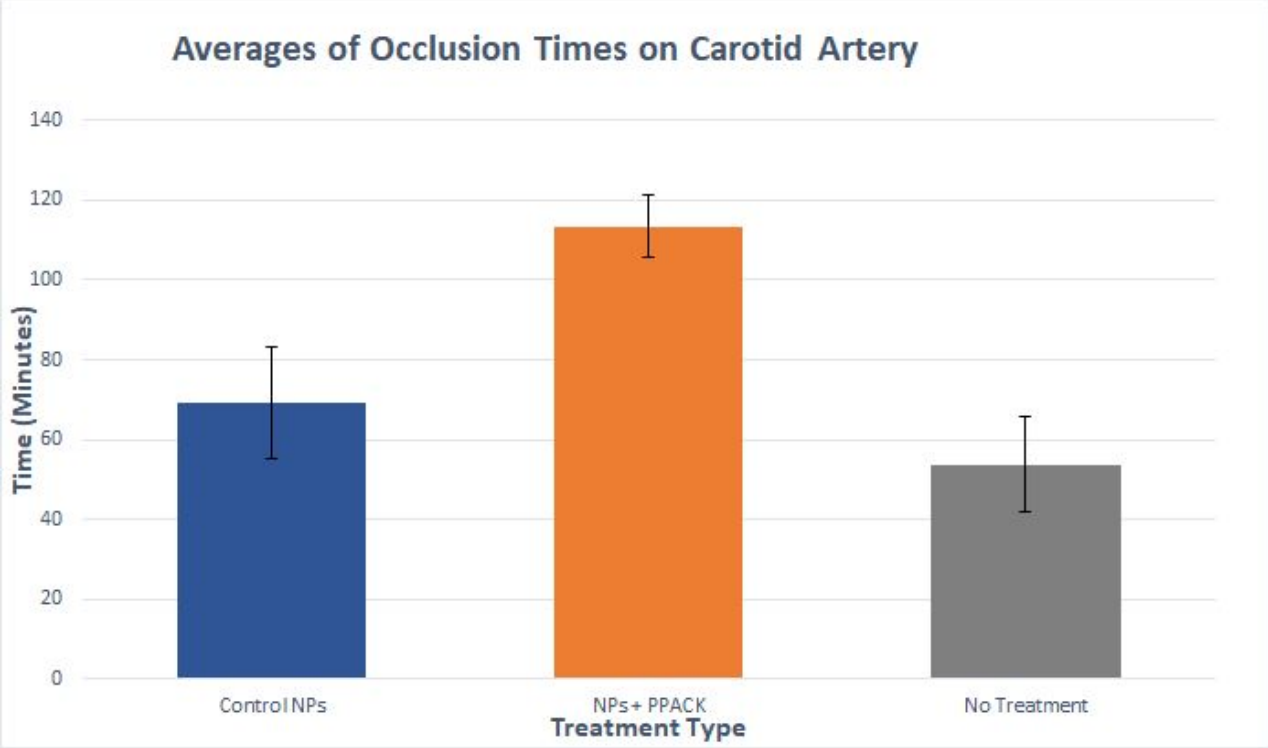

Figure 5B. Displays the averages of the previous graph on the multiple methods of treatments on occlusion time. It is evident the NPs with PPACK had greatest effect of preventing complete blockage of the blood vessel (Myerson et al., 2012 & Palekar et al., 2017).

Table 2a. Soluble P-selectin being used as a biomarker for determining thrombus formation. Concentrations were measured in vivo comparing groups positive for DVT and control group negative for DVT.

Biomarker (ng/mL)	Negative for DVT (95% CI)	Positive for DVT (95% CI)	Study Analyzed
Soluble P-selectin	40.5	51.8	Gremmel et al. (2011)
Soluble P-selectin	53.4	87.3	Ramacciotti et al. (2011)
Soluble P-selectin	54.5	77.2	Vandy et al. (2014)
Soluble P-selectin	50.0	77.4	Angelini et al. (2014)
Soluble P-selectin	14.18	93.06	Saadeldin et al. (2018)
Soluble P-selectin	48.9	31.6	Jungbauer et al. (2007)
Soluble P-selectin	42.1	45.9	Ay et al. 2007)

Soluble P-selectin	9.16	51.46	Hameed et al. (2017)
Soluble P-selectin	11.37	12.5	Entezari-Maleki et al. (2013)
Soluble P-selectin	53.2	86.70	Sood et al (2012)
Standard Deviation	18.66	26.9	

Table 2b. D-dimer test being used as a biomarker for determining thrombi occurrence. There was separation of two groups: one positive for DVT and one negative for DVT.

Biomarker ng/mL	Negative for DVT (95% CI)	Positive for DVT (95% CI)	Study Analyzed
D-dimer	1860	420	Gremmel et al. (2011)
D-dimer	5800	2100	Ramacciotti et al. (2011)
D-dimer	1040	5670	Vandy et al. (2014)
D-dimer	1155.9	5707.1	Angelini et al. (2014)
D-dimer	3190	7570	Rectenwald et al. (2005)
D-dimer	2350	6310	Sood et al. (2012)
Standard Deviation	1772.58	2750.74	

Table 2c. C-reactive protein being used as a biomarker for determining thrombi occurrence. There was separation of two groups: one positive for DVT and one negative for DVT.

Biomarker µg/mL	Negative for DVT (95% CI)	Positive for DVT (95% CI)	Study Analyzed
C-reactive Protein	16.5	1.2	Gremmel et al. (2011)
C-reactive Protein	3.2	2.2	Ramacciotti et al. (2011)
C-reactive Protein	1.51	5.29	Vandy et al. (2014)
C-reactive protein	1.9	5.4	Angelini et al. (2014)
C-reactive protein	2.86	4.87	Sood et al. (2012)

C-reactive protein	3	12.0	Saadeldin et al. (2018)
C-reactive protein	24	36.7	Entezari-Maleki et al. (2013)
Standard Deviation	8.95	12.41	

Table 2d. Compilation of studies using Wells Score as a biomarker. There was a separation of two groups: one positive for DVT and one negative for DVT.

Biomarker	Negative for DVT (95% CI)	Positive for DVT (95% CI)	Study Analyzed
Wells Score	2	3.2	Ramacciotti et al. (2011)
Wells Score	1.72	2.88	Vandy et al. (2014)
Wells Score	1.87	2.96	Angelini et al. (2014)
Wells Score	1	2	Modi et al. (2016)
Wells Score	2.80	3.53	Sood et al. (2012)
Standard Deviation	0.64	0.57	

A total of 2798 patients were analyzed in 12 studies containing both a negative DVT group and positive DVT group. Each table displays a common DVT biomarker, a measurable substance in vivo which is indicative for disease, infection, or environmental exposure, specifically for detecting thrombus formation within the body. Concentrations of sPsel (soluble P-selectin), C-reactive protein, and D-dimer were recorded as well as the Wells score being interpreted in both negative and positive DVT groups. A difference in these two values allowed for an evaluation of the accuracy on these biomarkers. Typically, Wells score < 1 is low probability for DVT, scores between 1-2 is moderate probability, and scores > 2 are high probability.

Average concentrations for the negative DVT groups include sPsel: 37.731 (ng/mL), D-dimer: 2565.983 (ng/mL), and C-reactive protein: 7.567 (μg/mL). Negative DVT through Wells score had an average of 1.878. The average concentrations for positive DVT include sPsel: 61.492 (ng/mL), D-dimer: 4629.516 (ng/mL), and C-reactive protein: 9.665 (μg/mL). Positive DVT through Wells score average was 2.914. P values for sPsel, D-dimer, C-reactive protein, and Wells Score were determined to be 0.034, 0.153, 0.723, and 0.086 respectively. Standard deviations for P-selectin are 18.66 for negative DVT group and 26.9 for positive DVT group. D-dimer standard deviation includes 1772.58 for negative DVT and 2750.72 for positive DVT group. C-reactive protein standard deviations include 8.95 for positive DVT group and 12.41 for negative DVT group. The Wells Score standard deviation has values of 0.64 for negative DVT as well as 0.57 for positive DVT.

Discussion

The results of this systematic review demonstrate how PPACK nanoparticles outperformed conventional NPs. This shows how PPACK has a major influence on determining the nanoparticle-thrombin relationship. PPACK is beneficial when it is covalently secured on the surface of perfluorocarbon-core nanoparticle structures. When attached to nanoparticles, they have a kinetic advantage rather than their free roam state on thrombin activity, allowing PPACK NPs to present thrombin-inhibiting surfaces to early thrombi formation. The results from the various studies testing the average occlusion time with control nanoparticles show that those with the addition of PPACK and then without either PPACK or control NPs demonstrate the advantage of PPACK NPs. As demonstrated in the Figure 5a, nanoparticles with PPACK had the longest delay of the blockage of a blood vessel.

Furthermore, sPsel has shown evidence of being a highly accurate biomarker for deep vein thrombosis. Compared to all previous biomarkers, sPsel has demonstrated the most consistent correlation through the studies analyzed on acute thrombus formation. One cause of sPsel's correlation

with DVT is its role in platelet related activation. Procoagulant microparticles form due to the interaction it has with P-selectin glycoprotein ligand 1 (PSGL-1): main counter receptor on leukocytes. This interaction increases expression of tissue factor, the main activator of coagulation in vivo, which is physically separated by the endothelium. This increase in expression essentially causes a coagulation cascade as sPsel increases surface-dependent thrombin generation on monocytes and induces phospholipid (phosphatidylserine) which plays a key role in cell signaling (Shi et al. 2014). Table 2a supports the correlation of sPsel presence and thrombus formation as an increase in sPsel. An increase in P-selectin leads to the development of DVT. With a $p = 0.017$, the results of sPsel as a DVT biomarker are considered statistically significant.

What is surprising is the low p value received for Wells score. However, a possible reason for this low p value could be due to the number of studies evaluated. There was a limitation of experiments available to record as many left out detection of DVT through Wells score. The Wells score is based off a point system which increases or decreases points based on various factors. These factors include the following: active cancer, pre-documented DVT, swelling, and collateral superficial veins. All of these add a point to the Wells score. Due to the limitation of studies record through Wells score, the p value generated cannot be deemed accurate.

Other biomarkers including C-reactive protein and D-dimer were also evaluated to determine its accuracy in detecting early thrombus formation. However, the results in these studies do not display the same relationship for DVT occurrence as compared to using soluble P-selectin as a biomarker. C-reactive protein is produced from the liver in responses to inflammation. Hence, a high level of this substance in the blood can be an indicator for inflammation. Low levels of C-reactive protein (CRP) typically indicates less inflammation within the body. The results of Table 2c have multiple outliers in the collected data which explains the large p value. D-dimer is a fibrin degradation product which is a small fragment of protein in the blood after a blood clot is degraded (Adam et al. 2009). Of all biomarkers evaluated in this

study, D-dimer method is the most commonly used for the detection of DVT. However, the results in the studies recorded had a p value of 0.153, much higher than sPsel's p value of 0.034. D-dimer tests can show high levels of fibrin degradation products yet it does not determine the actual thrombus location. This shows how soluble P-selectin can act as a better biomarker for early thrombus formation compared to the common D-dimer test method.

Conclusion

Currently, the common medical and surgical treatments that are administered to treat DVT are often ineffective. One major side effect is the risk of bleeding as blood thinners can lead to many bleeding episodes. For example, two common blood thinners, warfarin and heparin, can be over-anticoagulated. A non-invasive and accurate diagnosis is the main objective in this field. Nanotechnology offers a promising solution. It is estimated that DVT is the cause of death to 60,000-100,000 Americans. However, it affects nearly all ethnicities, age groups, and both sexes. Furthermore, those that survive DVT often will have long term complications that include swelling, pain, discoloration, and often scaling in the affected body part. Nanotechnology has an advantage compared to other conventional treatments for DVT because these devices can perform their intended tasks at a molecular level.

Already, 10% to 30% of those diagnosed will die within a month. An early diagnosis is imperative for a person's overall health. Early development of thrombosis is the primary cause of coronary and carotid artery occlusion which can lead to heart attacks and strokes. This is often countered with an abundant, and often times excessive, amount of anticoagulants and antiplatelet agents that are administered either intravenously or orally. Despite such daring treatment options, thrombus formation continues unpredictably (Myerson et al, 2009). Mortality is largely due to the blood clot leaving its original site and traveling to other body regions. This can be prevented if thrombi formation is detected

in its early stages so it can be expunged before causing further damage. The chance of thrombus formation reoccuring is also high; about 33% of those who experienced DVT will have recurrence within 10 years. Nanoparticles offer a more safe and effective treatment option for DVT.

Further Work

The major risk of implementing nanoparticles into the medical field is due to DEPs and UFPs. These particles can cause have pulmonary and respiratory effects or cardiovascular effects. Furthermore, other particles are unintentionally produced including TiO_2, carbon black, zinc oxide, and other metal oxides. These particles can then be entered into the air and be inhaled. Nanoparticles have the capability of generating pro-inflammation and may induce oxidative stress, possible factors which could lead to respiratory pathology. Another issue can be examined by looking at the use of carbon nanotubes. These nanotubes cannot be destroyed from the phagosomes in the macrophages. Another potential hazard is phagocytosis. These fibers can puncture the cell wall which will result in frustrated phagocytosis. This ultimately can lead to chronic granulomatous inflammation, which may precede mesothelioma. The ROS production and the production of oxidative stress are the two main concerns and largest contributors to material toxicity. More research should look into the risk of nanoparticle toxicity when inserted into human tissue. A method of dealing with this issue can be using an oxidative stress paradigm as screening for nanomaterial toxicity. It can test for any cellular injury responses. It can be a preemptive measure to the possibility if this sort of scenario ever occurs in an individual's body. The studies done on the potential hazard of carbon nanotubes were administered on mice. However, there has been no confirmation whether the results will apply to humans.

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