

# Application of Nanotechnology in Medicine as Treatment for Thrombotic Diseases

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PASS WITH MERIT

(230 words over limit)

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## ABSTRACT

As we dive headfirst into the technologies of the future, one of the most fascinating and rapidly developing scientific ventures of the 21<sup>st</sup> century is nanotechnology. In this paper we will be discussing one of the most life altering aspects of the nanotechnology revolution — nanomedicine. Defined as the “*intentional design, characterisation, production, and applications of materials, structures, devices, and systems by controlling their size and shape in the nanoscale range (1 to 100 nm)*”<sup>[1]</sup>, Nanomedicine it seems will completely revolutionise the way we look at modern day medicine by altering the body on a cellular level, demoting even the sharpest scalpel to the level of a blunt implement. In particular this report will be focused on improving thrombolytic therapy, targeting in particular deep venous thrombosis and atherosclerosis, through the application of nanoscale drug delivery systems (DDS).

## INTRODUCTION

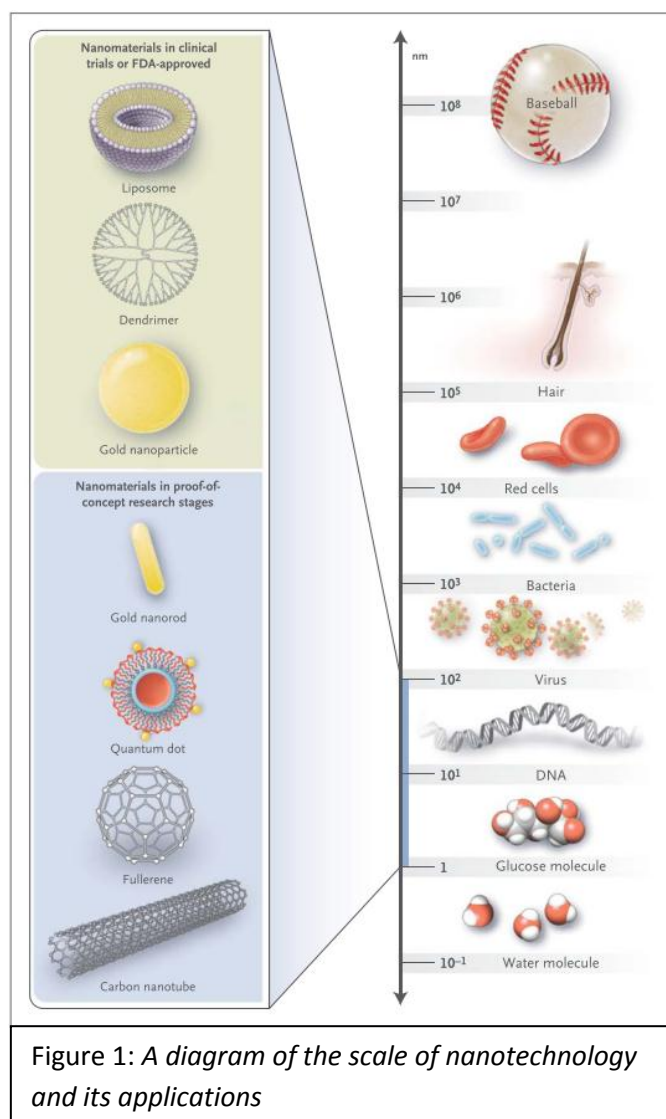
### 1.1 History

“*There’s plenty of room at the bottom.*”<sup>[2]</sup> These are the prophetic words of Richard Feynman, who on the 29<sup>th</sup> of December 1959 gave an impassioned talk at the California Institute of Physics and set down the first real foundations of nanotechnology (though it was not known as nanotechnology then). It was only later in 1974 that the term “*nanotechnology*” was coined by Professor Norio Taniguchi in a scientific research paper he wrote commenting on how materials could be broken down or made up one atom at a time.<sup>[3]</sup> It was later at Rice University that nanotechnology potential really exploded with the work done by Smalley et al. (1985) who discovered and named the Buckminsterfullerene molecule. Buckminsterfullerene or in other words Carbon – 60 was named after Buckminster Fuller who was the inventor of the geodesic dome — which the fullerene allotrope resembled. Also known as a “*Bucky Ball*” this fullerene molecule is 1nm in diameter ( $1 \times 10^{-9}$ m) and resembles a football — it can now be utilised in the delivery of certain drugs. The discovery of this molecule really was a landmark in the timeline of nanotechnology, leading to the development of structures such as the carbon nanotube.

### 1.2 Current Nanomedicine

Although, this may seem fairly obvious, evident from the term “*nanotechnology*” itself (which employs the Greek prefix “*nano-*” meaning dwarf), but nanotechnology allows us to influence and effect the body on a previously inaccessible level. The precision of nanoparticles stems from their small size range: from 1 – 100nm. Nanomaterials are now being designed which can aid the transport of medicinal drugs or diagnostic agents through barriers and into the cells themselves. These pathogens are only nanometres in size and can now be protected against by biological barriers such as nuclear pores which have a diameter of only 9 nm.<sup>[4]</sup> Whilst in the past, treating these sorts of disorders would have been impossible, nanomedical trials are now being conducted on rats and sheep, which allow these size challenges to be overcome.

One major initial drawback of nanotechnology was how the size of the particles meant that only very small masses of drugs could be “loaded” into the nanoparticles. However with the production of nanoparticles such as unilamellar liposomes, [9] polymeric micelles and carbon nanotubes, these impediments were easily overcome as the surface area of these particles is so much greater than regular molecules. All nanomaterials have the distinct advantage of a huge surface area to volume ratio. “Cutting a 1-cm cube into  $10^{21}$  cubes that are each 1 nm on a side will result in the same overall volume and mass, but the surface area will be increased by a factor of 10 million.”[4] This enables scientists to coat tiny nanoparticles with not only a large and effective amount of the necessary drug, but also allows them to load particles with many different types of drug. Along with targeted drug delivery as outlined above, there are a few other key aspects of medicine where nanotechnology is playing a key role. These include implantable devices, diagnostic tools and surgical aids.



In this report we will be focusing on the biopharmaceutics of nanomedicines and how controlled drug delivery will be able to vastly increase the efficiency of treating both thrombolytic and thromboembolic (the combination of thrombi and a resulting complication embolism) conditions. Our discussion will include ways in which we can speed up movement of nanoparticles inside the body, using external electromagnets and magnetic nanoparticles, [5] decreasing the time taken for fibrinolytic drugs to reach and dissolve a thrombus. We will also discuss how we can greatly decrease risk of haemorrhage inside a patient who is being treated with antiplatelet, anticoagulating and other thrombolytic drugs by not allowing these drugs to rampage around the body, but rather to concentrate the drugs at the exact places they are needed.

### **1.3 Examples of Thrombotic Diseases and their Mechanisms**

The three diseases we will focus on are deep vein thrombosis, myocardial infarction (heart attack) and stroke. These diseases are a result of a blood clot or thrombus — hence the namesake of the umbrella term “*Thrombotic Disease,*” pertaining to those caused by thrombi.

Deep vein thrombosis, which also known as DVT or deep venous thrombosis is the formation of a blood clot in a deep vein, most commonly the femoral or popliteal. It leads to venous

inflammation, as well as swelling of the afflicted extremity, as a result of the blood clot. Thrombi in themselves are the final product of coagulation, in which platelet adhesion or aggregation is accomplished, with fibrinogen being the connecting agent in the process. According to Virchow's Triad, the three mechanisms of vascular thrombosis are: blood vessel trauma; changes in blood flow rate and composition as well as a tendency for the blood to clot (hypercoagulability).<sup>[10]</sup> Decreased blood flow or stagnation of the blood allows for clots to form in the veins — which can be induced by long periods of limited movement: this can include long-haul flights or bus journeys (which have brought DVT into public view); hospitalisation and orthopaedic casts.<sup>[10]</sup> However, the real risk that DVT poses is the detaching of the clot from the vein, which is known as embolism. The mass can be carried by the circulatory system, with the capability of blocking blood vessels, the most serious complication being pulmonary embolism.

When more than 75% of the lumen's surface area is occupied by a blood clot, the supplied tissues are at risk of hypoxia (oxygen deprivation) as well as the agglomeration of lactic acid and other metabolic products. If this figure were to increase to 90% as a result of the build-up of the clot, cell death due to anoxia becomes almost certain.<sup>[11]</sup> Arterial thrombi induced cell hypoxia is the process which takes place in the case of myocardial infarction (heart attack), in which the coronary artery (the main provider of blood to the heart) is blocked off by an atheroma. And, since the first 24 hours are critical for a heart attack patient, it is important to begin thrombolysis (breakdown of the thrombi) as soon as possible. Of all the diseases actuated by thrombi, heart attack is by far the greatest killer; being according to Robert Beaglehole, et al. (2004) the leading cause of death for both men and women worldwide.<sup>[12]</sup>

Similar to myocardial infarction, stroke is another arterial thrombotic disease, which can be a result of either: haemorrhage; or ischemia (lack of blood flow) caused by thrombi or arterial embolism. It is the latter, stroke triggered by blockage, which we will focus on. Stroke must truly be treated quickly, due to the rapid loss of brain functions due to the blockage. The permanent neurological damage actuated by stroke and drastically lowers a patient's quality of life. Therefore doctors must thrombolyse a suspected stroke patient quickly with thrombolytic agents.

## DISCUSSION

### 2.1 Current Thrombolytic Therapy

The current thrombolytic therapy administered to the body in case of severe deep vein thrombosis or even in other thrombotic cases, is an immediate cocktail of drugs including anticoagulants to prevent the blood from clotting, antiplatelet drugs which prevent the aggregation of platelets, fibrinolytic agents such as streptokinase and tPA (tissue plasminogen activator) which dissolve fibrin. Each drug has a specific role:

#### **Anticoagulants – Warfarin, Enoxaparin**

<b>Name of Drug</b>	<b>Action in the Body</b>	<b>Positives</b>	<b>Negatives</b>
Enoxaparin (Clexane) "low molecular weight Heparin"	Work as thrombin inhibitors. Thrombin inactivation prevents and slows the formation of fibrin.	Takes immediate effect when injected into the body and effectively clears blood clots in conjunction with	Must be injected. The site of injection of Clexane can become sore and irritable with dangers of necrosis. Uncontrollable haemorrhaging is also a risk, meaning that Clexane too must

		fibrinolysis.	be closely monitored
Warfarin	Slows down the production of a Vitamin K, which plays a key role in the production of prothrombin (the zymogen of thrombin). <sup>[14]</sup>	It is a painless oral drug that can be taken in tablet form and is quite cheap to produce.	Due to poly-pharmacy, the effects of warfarin can be easily altered in the presence of other drugs or even food and alcohol. Needs to be closely monitored to prevent haemorrhage. Acts slowly.

### Antiplatelets – Aspirin, Clopidogrel.

These act to disperse the platelets which are aggregated together. Antiplatelet drugs work by inhibiting the effect of adenosine disphosphate, which is a substance produced in the body which enhances the clumping of the platelets. As ADP concentration in the blood lowers, platelets lose the ability to clump together and form thrombi. However anti platelets also increase the risk of haemorrhaging.

### Fibrinolytic Agents – Streptokinase

Streptokinase is a peptide produced by haemolytic streptococci, which works to break down clots by dissolving the fibrin mesh at the site of a clot. Streptokinase stimulates the production of plasmin from plasminogen, breaking down fibrin into fibrin degradation products.

However it can lead to often fatal haemorrhaging and the drug becomes less effective on second time of use. Urokinase can be a good alternative.<sup>[7]</sup>

Note: Anticoagulants work to curb the “*growth*” of a clot, whilst fibrinolytic/thrombolytic agents work to *reduce* the size of a clot.<sup>[7]</sup> Therefore in emergency cases (i.e. myocardial infarction, stroke or pulmonary embolism) thrombolytic agents should be used, whereas for preventative measures (i.e. to stop the *development* of DVT) anticoagulants are used.

Angioplasty is a technique, whereby blood vessels are widened by the insertion of a collapsed balloon into the artery, which inflate to crush the fatty deposits, increasing lumen size. This treatment was devised as a method to prevent having to use so many high risk drugs inside a patient, however angioplasty is far more invasive and time consuming than any drug based methods of treating thrombosis. This is where our idea in using magnetic nanoparticles to enhance drug delivery comes into play — and eliminating the risk factors associated with thrombolytic therapy. The problem with current chemical methods of thrombolysis is that treatment is non-specific; rather the drugs are distributed systemically. This would otherwise be of little importance, but the propensity of thrombolytic drugs to cause haemorrhages, means that targeted thrombolysis could drastically reduce the chances of haemorrhage in other parts of the body, by administering lower but more accurately targeted doses of drugs.

## 2.2 Coagulation Cascade

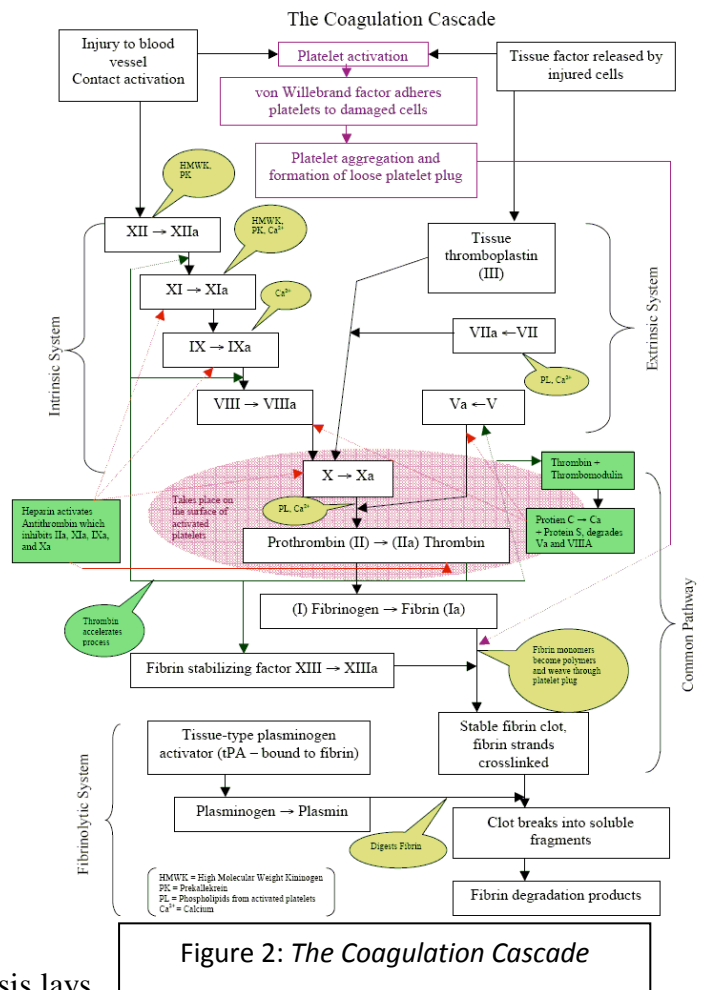
Coagulation and anticoagulation are the complex biochemical processes of forming and breaking clots. Clots are produced and dispersed constantly throughout the body and these reactions, expressed in the coagulation cascade are at the backbone of understanding thrombotic diseases. The coagulation cascade is almost like a biological chain-reaction, in which zymogens (inactive enzyme precursors) are converted to the active clotting factor, with factors encouraging each other to become active. Clots are platelet and fibrin composed “*plugs*,” whose purpose is to prevent blood loss from a damaged blood vessel. However, this is not the only stage in preventing blood loss — the process of forming clots and then allowing them to dissolve to enable tissue repair is called haemostasis. The stages of haemostasis are as follows:

1. Initial vascular constriction (limits blood flow to the inflicted blood vessel)

2. Thrombin activates platelet aggregation or adhesion, so that they can clump to the collagen, exposed by the trauma and form a temporary platelet “plug.” *Primary Haemostasis*. Platelet adhesion also stimulates other clotting factors, which start the cascade.
3. As a result of the clotting factors a fibrin mesh (also known as the clot) encapsulates the plug. *Secondary Haemostasis*.
4. The effect of plasmin dissolves the clot, so that tissue repair can continue. [13]

At the confluence of the intrinsic and extrinsic pathways, one of the most important factors in coagulation is produced. Thrombin (IIa) is enzymatically cleaved from its zymogen prothrombin (II) by factor (Xa). Once produced thrombin converts factor XI to XIa, VIII to VIIIa, V to Va, and fibrinogen to fibrin. [14] The lattermost being very significant, as fibrin is the mesh, to which the platelets are wrapped round and as such converting soluble fibrinogen to insoluble fibrin is a key process in the cascade.

To allow for healthy tissue repair the clot must be broken down by the process of fibrinolysis. This is the critical aspect of our project as in combating thrombotic diseases; we will have to focus heavily on fibrinolysis and its application to nanotechnology. In fact, fibrinolysis lays its roots, early in coagulation cascade. Plasminogen, the inactive zymogen precursor to plasmin binds to fibrin, thereby integrating itself with the cross-linked fibrin strand clot. [13] However, it is only when tissue plasminogen activator (tPA) found in the endothelium comes into contact with the plasminogen, that fibrinolysis begins. Tissue plasminogen activator cleaves the zymogen plasminogen into two chains connected by a disulphide bond, forming the active enzyme plasmin. Fibrin is a cofactor to plasmin, as plasmin does not attack the clot from the outside, rather breaks the clot up from the inside, when influenced by tPA. Fibrin is broken down into soluble products, known as Fibrin degradation products. [13] [14]



### 2.3 Magnetic Nanotherapy and our Proposal

Constructing Nanoparticles out of magnetic materials such as magnetite (Fe<sub>3</sub>O<sub>4</sub>) [21] and maghemite (γ-Fe<sub>2</sub>O<sub>3</sub>) will be the basis of our nanomedical proposal. These two materials have come under intense scrutiny and are being experimented with heavily due to the way in which they have the potential to revolutionise drug delivery systems (DDS). The main advantages of magnetic drug targeting in terms of thrombolysis lie in local drug action and how by directing magnetic nanoparticles to exactly the areas where they need to be, side effects (in this case excessive haemorrhage around the body) can be reduced. In particular

Iron Oxide nanoparticles have the advantage of having very low toxicity meaning that they will not affect the body in a negative way. The iron oxide based nanoparticles will have a “core shell structure” with an iron oxide core and usually a polymer based shell such as silica or metals such as gold to which functional groups can be attached using cross linkers, the polymer shell also prevents aggregation in the body of nanoparticles and increases stability of the molecule. The shell structure is more commonly known as an artificial liposome - an artificial vesicle which can be used to transport drugs around the body. Scientists have now also begun to use gold and cobalt nanoparticles in which to make this core-shell structure. Bao and Krishnan (2005) produced Cobalt nanoparticles in the size range of 5nm-25nm; the major advantage of these particles is that they have almost double the magnetic moment of iron oxides which means they can be controlled more easily in the body. Magnetic moment plays a crucial role in magnetic nanomedicine as magnetic force exerted on the particles must overcome the hydrodynamic drag force produced by the blood flow in the body. In addition to this, the magnetic field produced by the nanoparticles degrades rapidly as the particles get deeper into the body. As the magnetic force is highly dependent on the size of the particle, the nanoparticle produced should be an adequate size enough to be controlled by external magnets, but small enough so that they don't obstruct capillaries in the body [20]. With core-shell nanoparticles, the particles are functionalised, this allows drugs to be embedded into the polymer matrix surround the core. The nanoparticles themselves can be coated in the drug in a number of ways, “washing” the nanoparticles numerous times for example can achieve this effect. [19]

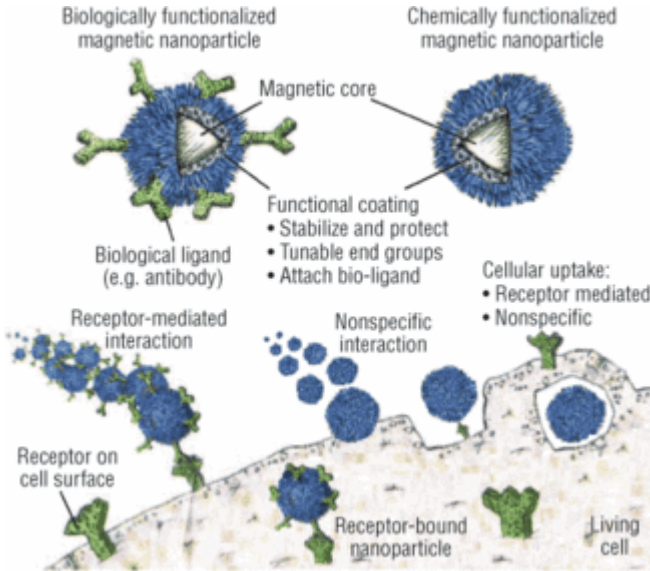


Figure 3: a magnetic nanoparticle

Magnetic targeting is based on the attraction between nanoparticles and an external magnetic field; in the presence of an external magnetic gradient (a Maxwell coil electromagnet) [21], the nanoparticles can effectively be trapped at the point where they are needed, and this reduces the risk of unwanted haemorrhaging around the rest of the body. The nanoparticles would be injected in to the body so that we can introduce the drug to the area of clotting directly. Also taking such a drug orally may cause harm as the particles themselves contain a mixture of drugs including streptokinase for immediate thrombolysis as well as anticoagulants and antiplatelets. The nanoparticles can be described as “paramagnetic” meaning that they are only magnetic in the presence of an external magnetic gradient so when the drugs have had chance to take effect, the magnetic field can be released, allowing the now empty nanoparticles to be filtered out of the blood. A major advantage of our nanoparticles is the time taken for the nanoparticle to reach the clot is heavily reduced as movement can be controlled.

For the thrombolytic diseases we are concentrating on in particular, the effects of magnetic nanotherpay could be truly amazing. In the cases of emergency thrombosis i.e. a myocardial infarction, stroke or pulmonary embolism — magnetically targeted drugs could reach the

thrombi a lot faster, reducing cell death in arterial thrombotic diseases and quickly breaking up embolism. But, paramagnetic nanoparticles could also be used as preventative measures in the case of DVT, stopping thrombi from forming, whilst still reducing the risk of haemorrhage and drug doses needed. We plan to use similar chemicals to before, but just improve drug delivery systems (DDS) with the aid of nanotechnology.

## **2.4 The Ethics of Nanomedicine**

As is most often the case, the advent of a new technology will ultimately result in the polarising of opinions. Both chimerical idealism and staunch cynicism are destructive for the development of nanomedicine and its therapeutic application. According to Gordijn (2005): *“Optimistic visionaries predict truly utopian states of affairs. Pessimistic thinkers present all manner of apocalyptic visions...These radically opposing evaluations hold the risk of conflicts and unwanted backlashes.”* <sup>[15]</sup> In order to combat premature and mendacious judgements, scientists will have to strive to tackle the nanotechnology “hype” and educate the masses to what nanotechnology can *actually* achieve and *possible* developments. Positive “hype” lacks sharp focus on the “now” but rather lingers in the world of ambiguity and hypothetical eventualities. And, whilst futurists predict nanotechnology spawning nanorobots, with the potential of enhancing our immune response or providing a constant monitor of our internal biochemistry, such developments are yet to be made. <sup>[16]</sup> This illustrates the important of informing the public of the actual uses of nanomedicine. On the other hand, negative “hype” is fixated on the unknown. And, this is definitely a huge cause for concern — as we really don’t know the *in vivo* effects of nanoparticles, however rigorous drug testing programmes should account for this with even more testing.

At the present time, nano-based therapies are more expensive than their counterparts. However it has been suggested that the substantial expense of nanomedicine is caused by patent laws and intellectual property (IP). <sup>[17]</sup> Thus the question arises: are the efforts of drug companies to develop nanomedicines truly the corollary of altruism? Many believe that patents are an obstacle to benevolence and they highlight the very relevant issues of social injustice and inequality. Until the price of treatment drops due to intercompany competition, the lifesaving treatments, which are offered by nanotechnology, may be out of reach for the poorer people in society. Although this may not be the case of our NHS, as treatment is distributed evenly — in the current financial climate, the NHS may not be able to afford such new nanomedical therapy. *“Patent monopoly”* <sup>[17]</sup> isolates enhanced treatment from the masses, not only on a national scale but also internationally. Bawa and Johnson (2008) suggest that *“patent laws and intellectual property (IP) are the products of a new form of western colonialism designed to deny the developing world access to common goods.”* <sup>[17]</sup> Such allegations emphasise that real ethical considerations have to be taken into account when pushing nanomedical treatment onwards. Healthcare shouldn’t be a luxury — and therefore cannot be deprived to those who need it most.

Bawa and Johnson (2008) also state, quite shrewdly, that it is difficult for ethicists to foresee the possible outcomes of nanomedical research, when the science is in relative infancy. <sup>[17]</sup> Of course, should nano-sized computer chips ever monitor us, issues such as privacy and access to information will come into light. Even, patient autonomy will become increasing significant, should diagnostics improve to the point that nanochips will have the ability to determine susceptibility to diseases (and the decision has to be made whether patients deserve the “right-to-know” and “right-not-to-know”). <sup>[16]</sup> However, till such technologies *even* come into being, it is both difficult and wasteful to discuss pertaining ethical dilemma. Even

the European Commission's paper *European Technology Platform on NanoMedicine - Nanotechnology for Health (2005)* points out, through an inexplicit discussion on ethics that until nanotechnology makes greater developments into the future, ethical debate cannot take place effectively.

## CONCLUSION

As with any new treatment, our proposal will have to be examined by PESTLE (**P**olitical, **E**conomic, **S**ocial, **T**echnological, **L**egal and **E**nvironmental) analysis. Commonly used by pharmaceutical companies for their investigations into potential drugs and therapies, PESTLE analysis provides a sound grounding to the advantageous aspects of a treatment and its limitations.

From a political standpoint, the expense of the treatment could mean that paramagnetic nanoparticles may only be available in a select few hospitals in the country as specialist treatment. However, with time we foresee that our idea could be integrated into NHS trusts all over the country to aid the stroke and cardiology departments. Also, owing to the seriousness and prevalence of thrombotic diseases, the NHS may put more emphasis on our idea, as a less harmful alternative therapy. However, on a global scale our therapy may be too expensive for developing countries and in nations without a national health service; paramagnetic nanoparticles may be out of the reach of those in the lowest socioeconomic rungs. As was discussed under "*Ethics*" nanotherapy can exacerbate the wealth gap between upper and working class, developed and developing nations — here we can see the convergence of political and social issues. On a social level, promoting our therapy could save lives and soften the blow of severe thrombotic diseases.

Despite being a theoretically "*correct*" proposal, if we analyse our project from a technological perspective, we can see our idea has little basis to go out into the medical world and actually save lives — all we have is an almost puerile proposal without medical testing, employing a callow field of medicine. But, our idea does have potential, as it targets some of the most costly diseases to society today, with up to 50% of pulmonary embolism cases ending in fatality and 50% of hospitalised patients developing venous thromboembolism without prophylaxis. <sup>[18]</sup> The legal aspects are obsolete for this project, because we need to have proper pharmaceutical tests to prove that our treatment can be used. And, looking from an environmental stance, nanotechnology is shrouded in the unknown. We do not know the effects of the nanoparticles if they get into the environment, or whether they will even get that far. But, we do know that our method does not require as much of the thrombolytic agents, reducing the amounts needed to be synthesised.

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