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Review of Application of Tissue Nano transfection for Vascular Injuries and Traumas

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ABSTRACT

With vascular surgeons involved in increasing numbers of lower extremity arterial traumas, vascular injuries are a prevalent problem in today's society [16]. Vascular injuries are often causes of vascular diseases such as atherosclerosis and are separated into three categories: blunt, penetration, or a combination of the two.[1] These injuries can often lead to symptoms such as bleeding, swelling, lumps on the skin, and bruising. Tissue Nano Transfection (TNT) is a novel technology that uses nanotechnology-based chip hardware to deliver specific genes to reprogram one tissue type into another. TNT can improve vasculature by repairing damaged tissues. TNT has the benefits of not requiring laboratory-based procedures, quick treatment, and minimal invasiveness. However, the status quo of TNT calls for commercial, clinical, and biological needs, all of which are discussed in this paper. Although relatively novel, tissue nano-transfection, combined with practicality, could be used worldwide to help patients not only as a vascular injury treatment but also as a revolutionary form of cell therapy. All things considered, TNT has had numerous successes through non-viral gene delivery, an advantage over many current forms of cell therapy due to complications of doing viral gene delivery.

Introduction

Tissue Nano Transfection (TNT) is a novel nanotechnology-based chip hardware to deliver specific genes to reprogram one type of tissue into another by applying a focused electric field [6]. The usage of TNT is a novel research field in which a PubMed search yielded only 20 papers between 2017-2023 (Figure 1). While TNT is not available for public use as of yet, it is a promising form of cell therapy through its numerous successes in treating tumors, diabetic conditions, muscle therapy, and vascular injuries and traumas.

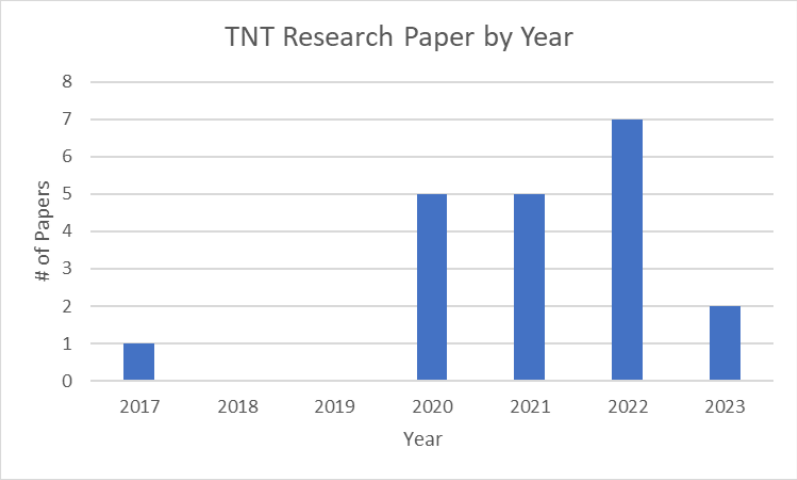

Figure 1. TNT Research Papers by Year. Number of PubMed research publications by year with search term "Tissue Nano Transfection."

One major injury group that needs better cell therapy treatment is vascular injury. In countries with well-developed medical systems, vascular injuries may not be the most prevalent type of trauma, but are certainly very deadly. In Australia, the number of patients with vascular injuries was only approximately 1.8%, yet it accounted for approximately 22% of all trauma-related deaths [13]. In a British trauma center, not only were the mortality rates high, but they also consumed many resources as well, highlighting the need for new, efficient forms of treatment.[22] Current standards of care for blunt vascular injuries or blunt cerebrovascular injuries (BCVI) include antithrombotic treatment and antiplatelet or anticoagulation therapy; however, surgical repair is still the most common form of treatment for vascular injuries. Although treatments are effective, many issues remain. Surgical repair of vascular injuries, for example, peripheral vascular bypass, undergo risk of wound infection, excessive bleeding, and nerve damage, making the treatment risky for patients with complicated health such as smoking and advanced age. Vascular injuries are identified primarily through computerized tomography (CT), ultrasound imaging, and angiography. With surgical repair being the primary form of care for all three categories, there are complications that come at risk, whether it is the patient's history or other serious conditions. It is also riskier when major blood vessels, arteries, or the chest undergo surgery. Complications include heart attack, stroke, infection, blood clot, and blood vessel/nerve injury. Other standards of care include endovascular treatment and endoprostheses. Endovascular treatment can result in postoperative hemorrhage, extracranial hemorrhage, and pseudoaneurysms. [2] Endoprosthesis also comes with several complications: wound necrosis, deep wound infections, joint instability, and prosthetic loosening.[2] In regards to cell therapies, they are riddled with problems; most of them are limited by cell sources and require tedious preparations[6]. Moreover, many of them require viral transfection, which could often lead to infections and adverse side effects [6]. TNT, on the other hand, is a non-viral cell therapy method that is not limited by the drawbacks of status quo cell therapies. Considering these factors, with its non-invasive property, Tissue Nano Transfection has immense potential to improve the current situation. TNT shows promising qualities that could benefit patients with vascular injuries or trauma. Although successful in most cases, TNT must consider many commercial, clinical, and biological needs to positively impact people's lives.

Regarding vascular injuries, TNT has the potential to improve vasculature by repairing damaged tissues. Past studies regarding TNT revolve around experimentations of mice *in vivo* and *in vitro*, and have shown increased vascularization by utilizing this technology. In an experiment on ischemic tissues, TNT treatment lasting only a few seconds led to increased angiogenesis, enhanced perfusion to the treated area, and the creation of superficial blood vessels. [6] TNT also allowed the direct delivery of demethylation to rescue the expression of EMT regulators, as well as advanced ischemic wound closure in mice. [19] Furthermore, TNT delivery of antisense oligonucleotides augmented physiological NPGPx expression by miR-29, which overcame the harmful effects of diabetes. [7] Other applications of TNT include tissue necrotization, muscle function, diabetes, and chronic wounds. Ultimately, however, TNT is a viable method of repairing vascular injury, but there are current limitations and lack of research that must be addressed.

Current Literature

Several experiments have been conducted using TNT and the majority of them have been successful and provide insight on its potential use for vascular injury treatment. TNT has been applied in numerous sectors of research not confined to vascular injuries and has some potential therapies like healing burns and treating damaged or diseased tissue. Previous approaches that tried to ameliorate diabetic peripheral neuropathy (DPN) such as pharmacologic therapies failed to proceed past phase II or III clinical trials due to a lack of efficacy and/or adverse side effects when continuously used [18]. Thus, an experiment utilized TNT to deliver *Ascl1*, *Brn2*, and *Myt1l* to convert skin fibroblasts into active induced nerve cells, causing neurotrophic enrichment of the skin stroma [18]. The induced neuronal cells

were also increased substantially in the dermis 4 weeks after topical TNT treatment. This experiment suggests that alternating tissue structure can serve as a form of therapy and treatment for diabetic conditions, giving a possible avenue of field that TNT can extend its reach to, giving evidence to support the fact that TNT could truly be effective for TNT treatments on humans. More recently, a study utilized TNT to deliver MyoD to injured tissue and was used as a therapy of volumetric muscle loss of mass and, as a result, rescued muscle function of the rat, suggesting potential of TNT in the realm of muscle therapy as well [5]. The experiment utilized TNT due to its nonviral gene delivery approach to achieve successful in vivo tissue reprogramming. And since blood vessels are constituted of smooth muscle cells, the same success can be correlated to treating vascular traumas or injuries.

The concept of using TNT for vascular injuries involves delivering specific factors, which are the genetic cargos, to target cells within the injured blood cells to stimulate regeneration and repair processes so that scientists can transform existing cell types within the wound into the desired cell type. Conditions of mice with tumors have also improved with topical utilization of TNT and even had improved survival rate, suggesting TNT could treat tumor tissue more effectively later on (Figure 2a). In regards to specifically TNT application of vascular injuries and trauma, an experiment entails employing TNT to rescue necrotizing tissues and whole limbs of mice [6]. Topical application of TNT on dorsal skin led to increased angiogenesis and vascularity as well as successful anastomosis of the parent circulatory system, ultimately counteracting tissue necrosis under ischaemic conditions, meaning that TNT can possible improve vascular structure through non-viral gene delivery [6]. TNT also led to increased blood flow and improved limb perfusion of rodents in only 7 days post-TNT treatment, fulfilling the sought concept of TNT on vascular injuries and trauma (Figure 2b). It should be noted that blood vessels other than capillaries are composed of tissues like smooth muscle, elastic tissue, and epithelial tissue. Thus, the success of TNT not only in increased angiogenesis and vascularity but also in restored tissue suggests that it will prove to be a promising source of treatment for humans with vascular injuries. Another experiment using TNT successfully transfected human dermal fibroblasts with anti-miR-200b oligonucleotide in mice, which exhibited blood vessel forming function *in vivo*, wound healing, and vasculogenic fibroblasts, meaning that vascular injuries can be treated through this treatment [17]. Overall, TNT shows a high success in treating vascular-related treatments From the current literature, TNT can be seen to be a promising, versatile form of treatment that could treat injuries outside the boundaries of vascular injuries and trauma.

Other therapies have also shown some possibility of treating vascular injuries. Some say that gene therapies or small molecular approaches show promise, but they have, at the moment, largely failed with no significant successes [12]. Stem cell therapy of pluripotent embryonic stem cells show promise due to their replicative ability, but are fraught with ethical and immunological issues [12]. Adult cell stem therapies are limited and “in the very patients that they are needed, they are rare and often dysfunctional” [12]. Stem cell therapies in general are limited by the lack of complete control of stem cells to harness them for therapies. Therefore, while other forms of cell therapies may show promise, they are restrained by lack of understanding, viral delivery, and quantity, making TNT a better alternative.

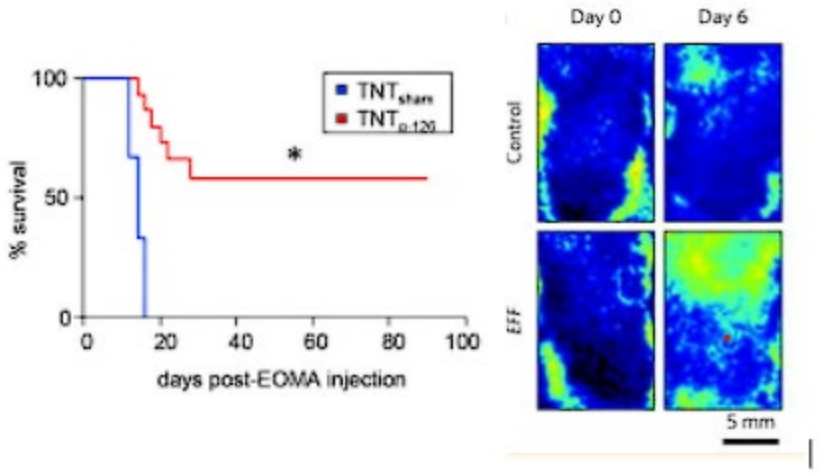

Figure 2 a. Survival Rate. Survival rate of murine that underwent topical *in vivo* TNT treatment of sham and anti-miR shows tumor-free survival for the anti-mir TNT group[Source: 8]. **b. Blood Flow.** Depiction of increased blood flow to murine tissue treated thorough TNT (Source: 6).

Biological Limitations

Currently, there are obscurities to how TNT will be compatible for treatment in humans. While it may have helped mice in numerous aspects, such as vascular injuries or chronic wounds, there is still no definitive answer to which patients with vascular trauma could benefit from the technology. First, the interfollicular epidermis and dermis are significantly thicker in humans than in mice [10]. Second, the boundary between the epidermis and dermis was different from that of mice (Figure 3). These two qualities make it difficult to correlate the effects of TNT in mice with those in humans. This concern is heightened by the fact that TNT requires contact with the skin to conduct its treatment, meaning that the skin is the medium at which the treatment is carried out. In essence, with the difference in the qualities of human and mouse skin, it is uncertain whether the exact effects of TNT on mice can be replicated in humans. To address this issue, when conducting clinical trials, if clinical results in humans show degrees of similarity, then it can be concluded that the trials conducted in mice can be correlated to those in humans. Some hypothesize, on the other hand, that TNT could be applied to tissues in the human body because TNT has shown its capability of achieving tissue reprogramming in immunocompetent settings through in vivo electroporation [11].

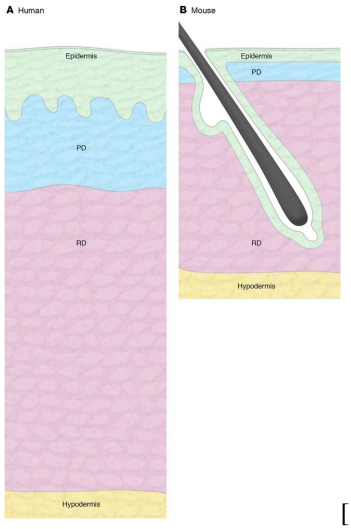

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Figure 3. Schematic Diagram of Human and Mouse Skin. The human (A) and mouse (B) skin showed visible differences in thickness and structure.

Moreover, limitations in certain areas in which TNT can be placed must be further researched. However, before more specifics are discussed, the voltage required for the genetic cargo to effectively reach the desired target area must be optimized depending on the type of tissue, area, and depth of injury. As areas like the heart are vulnerable to electrical currents of 50 mA, which can cause cardiac arrest, the area of treatment is also a source of concern [20]. In addition, electric fields have been found to be capable of inducing a mismatch or mutation, which indicates the importance and priority of discovering a voltage at which TNT will not induce the aforementioned effects on specific areas [4]. Not to mention that electroporation can possibly also lead to cell death [3]. Although previously discussed, time is also a source of concern, especially whether it is significant in determining effectiveness. If the TNT is topically applied longer, will it have a greater effect on the patient, or will it simply not produce significant differences? In one experiment, a one-time treatment of the dorsal skin of mice lasting only for a brief moment led to "increased angiogenesis" [6].

Roughness and flatness of the surface of the site of treatment are a source of concern since there might be gaps between it and the TNT chip, which will "lower efficiency of drug delivery and reduce the uniformity of drug distribution" [11]. Even with some limitations, official descriptions of TNT claim that "in less than a second, the nanochip can deliver treatment at the injury site and convert skills to vasculogenic cells" [1]. Despite this promising statement, the specifics of whether or not the time it takes to reach the desired area effectively will be affected by the depth of the injury is also not discussed in depth. Some recommend using a silicon hollow-needle array with sharp tips, but, as will be discussed later, silicon needles are not as tough nor strong as metal needles, indicating that needle deformities could result from the topical application [11]. This would result in undistributed drug application to the treatment site, rendering TNT less useful. In essence, there are numerous problems in correlating the benefits of TNT on mice to humans due to the difference in the constitution, especially regarding the skin tissue where the TNT is topically applied.

Clinical Aspects

Currently, the clinical side of TNT has yet to be fully addressed. TNT has not been tested on humans as of yet, which raises the pressing issue for TNT of the need for more clinical research on people. Current research mainly focuses on murine and does not indicate any conditions that may either be worsened or created if TNT is applied to humans due

to biological limitations [8, 6, 18]. Furthermore, not every patient that has vascular injuries is the same. They each have different medical histories, constitutions, and areas that are injured that must be considered when conducting TNT treatment [9]. From the lack of research, it is evident that the clinical applicability of TNT must be researched further to determine the effects that it has on people with certain diseases, conditions, or ages. Something important to note, however, is that, currently, there have not been any significant mishaps with TNT topical application as in harmful mutations.

In the medical field, there are two steps in treating a patient: diagnosis and treatment. Diagnosis is crucial in successfully treating a patient because, without a correct diagnosis, correct treatment cannot be applied. This, of course, applies to TNT. Diagnosis often results from an aggregation of both a patient documenting their injured areas and identification from a medical device similar to ultrasound and CT scans in tandem with the doctor's own judgment. In the case of TNT, what machines will be used to identify where to place the TNT chip? Without knowing what processes and machines TNT requires, discerning the potential application of TNT is convoluted when various variables are present. This need is further emphasized by the factor of golden hour, which is the concept that injured patients should receive care within an hour from the time they were injured [15]. This is imperative to TNT because the golden hour harbors the question of whether or not TNT should be employed in emergency settings. If TNT requires patients to undergo certain processes to ascertain the area to treat similar to CT scans or angiography, which varies in length accordingly, then there would be some situations where TNT may not be the recommended form of treatment due to the process taking an extensive quantity of time to treat a patient [15]. Current research points to TNT as a form of therapy or perhaps chronic conditions rather than a critical wound that requires immediate treatment. Arriving at this conclusion, however, would require more knowledge regarding the situations in which TNT would be the most ideal to use. In order to research this, however, the necessity of clinical trials for humans is evident.

Typically, there are 4 phases in the clinical development of a method of treatment. The 1st phase gauges the safety of the treatment; the second phase determines the efficacy and the side effects; the third phase confirms the findings within a large patient population and monitors adverse reactions; and the fourth step tests diverse patient populations for long-term safety (Figure 4). To begin, many of the patients and people must volunteer for the clinical trial. The concern regarding the clinical trials is how they will experiment on humans. Touching upon a previous point, the genetic cargo that will be used is an issue. If the genetic cargo is not standardized for each possible use, then clinical trials are not necessary at that point in time. To measure the efficacy as well as the safety of the TNT for patients, the control of the experiment must be the genetic cargo so that results can be accurately discerned. The other perspective and the most pressing concern is from the person administering the treatment. Does TNT require training people to deliver the treatment? Despite claiming that TNT is "simple to use," there is still vagueness that must be clarified: who will train them, how long will the training take, and how will people be tested on their proficiency [1]? Clinical needs must be met in order for TNT to aid and improve the lives of many patients truly.

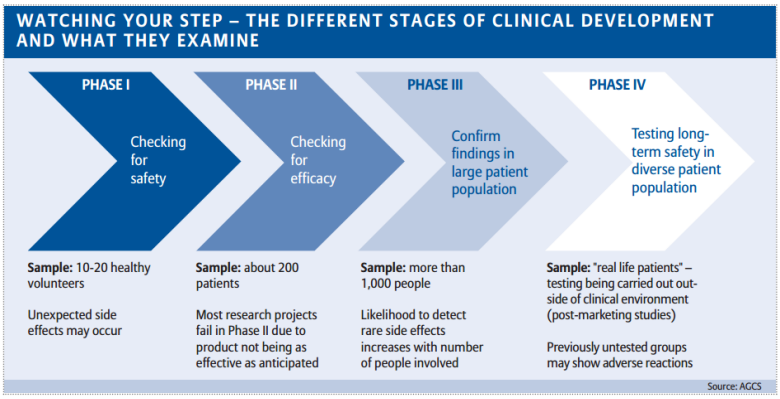

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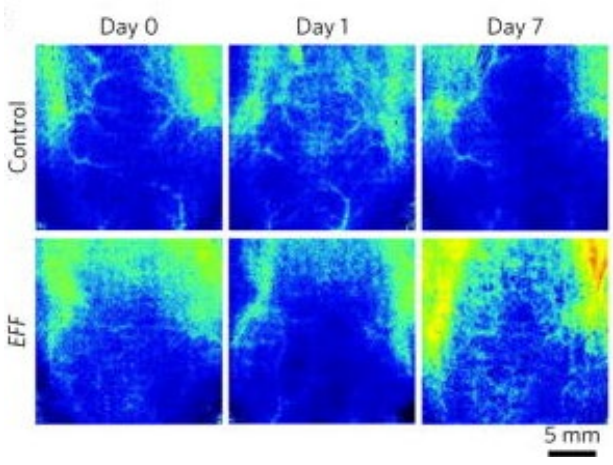
Figure 4. Clinical Development Phases. Schematic and description of the clinical development stages.

Commercial Aspects

Despite its promising potential as a medical treatment, commercial needs must be addressed in order for TNT to be practical and help others. Perhaps the most pressing issue regarding commercial needs is the production of equipment. After all, no substantial gain would be brought forth from TNT if the technology was limited by its capabilities to be produced. Prior research regarding TNT utilized 200 μm double-side-polished silicon wafers with nanoscale opening patterns using a GCA 6100C stepper with extremely precise measurements and conditions to make TNT, illustrating the necessity of machines that can work on a nanoscale [6]. This raises the question of whether large-scale production of TNT can even begin, especially since the TNT chip fabrication process takes 5-6 days [7]. to 5-6 days to fabricate a single chip implies the complicated and arduous process by which it is created. This means that it will not necessarily be readily mass-produced in a factory. TNT often uses silicon hollow-needle arrays, which are less challenging than metal hollow-needle arrays in regards to manufacturing capabilities and price, but silicon is less tough and strong, suggesting that needles could be disfigured upon light penetration of skin [21].

In addition, because of its extremely delicate properties, TNT must be handled carefully, considering that gene cargo transfection occurs through nanoscale openings. If some of them happen to be bent, scratched, or disfigured, it is uncertain what effects they could cause, whether they are inaccurate transfections, or general malfunctions. Furthermore, it is not clear who will be manufactured, although there is a protocol that describes how to fabricate the TNT chip and use it for *in vivo* TNT [21]. Companies must be involved in the commercialization of TNT. Without companies to manufacture, fund, and organize TNT chips and genetic cargo, TNT will not be able to impact the world significantly. Even current researchers acknowledge that "TNT fabrication processes are not compatible with standard semiconductor processes and therefore would limit scalability" [21]. Nevertheless, it is evident that the TNT chip is still immature in development, as it is a relatively new technology.

A critical aspect that also needs to be addressed is the distribution and manufacturing of the genetic cargo. Specific genetic cargoes and reprogramming factors are required to meet specific needs. In an experiment to directly reprogram fibroblasts into induced neurons (iNs), *Ascl1*/*Brn2*/*Myt1l* (ABM) was used, while reprogramming skin cells to induce endothelial cells (iECs) required *Etv2*, *Foxc2*, and *Fli1* (EFF) [6]. Something to note from this experiment is that through the TNT application of EFF, there was visible improvement in the perfusion of the treated area (Figure 5). From this information, it is reasonable to deduce that the genetic cargo is not one-size-fits-all and must be geared either specifically for the patient or subject to reprogramming. Hence, the plausibility of large-scale manufacturing of genetic cargo for patients throughout the world is low, but could be addressed in future research.



[6]

Figure 5. TNT research paper results. Laser speckle imaging revealed enhanced perfusion in the EFF-treated area over time.

TNT's target market remains a source of concern. Currently, there are no established or specific targets for TNT to be issued. Either hospitals or trauma centers should be the primary target markets for TNT for reasons such as the fact that they are where treatments for vascular injuries are generally issued. Furthermore, as TNT is mainly being developed in the United States, it raises the question of the possibility of international commercialization of TNT. TNT will be especially impactful in third-world countries where vascular injuries are a prevalent problem in many third world countries. They are a major contributor to limb loss as a result of the unavailability of vascular facilities [11]. TNT, with its non-invasiveness properties and nonobligatory laboratory-based procedures, could become a primary standard of care for vascular trauma in third-world countries, not to mention the world. However, for TNT to be accessible across the world, a key element remains unaddressed: price. How much would the TNT chip cost? Or the specific genetic cargo? With a single TNT chip fabrication alone taking 5-6 days, it is difficult to estimate the cost of the chip solely based on its physical components. There are costs in producing them, maintaining them, testing them, and even shipping them, which signify how expensive TNT could be. This limits the ability to expand to poverty-stricken countries. In essence, these concerns must be addressed to illustrate the viability of TNT.

Conclusion

All things considered, TNT is a promising, novel medical biotechnology that needs several aspects to consider in order to be implemented in the real world where it can positively impact people. Potential applications of the TNT range vastly. It includes the repair of damaged or diseased tissues and organs, the creation of new organs and tissues for transplantation, and new therapies for different medical conditions. This technology has been tested in animal models and the results have been promising, showing success in treating tumors, diabetic conditions, muscle therapy, and vascular injuries and traumas. In regards to ameliorating vascular injuries and traumas, topical application of TNT shows increased vascularization, perfusion, and angiogenesis, as illustrated by numerous studies.

At the current stage of the research process, however, it is impossible to gauge its full potential without taking many research and logistical steps. Its target market is unspecified and, on a more technical facet, its genetic cargo is not generalized; therefore, unless a specific genetic cargo is catered to different types of injuries, it is incapable of being mass-produced which will bring about significant change. This calls into question the scalability factor of TNT and whether or not it can reach people in need. Furthermore, more testing and clinical trials must be done in order to validate the positive effects of TNT on people with different constitutions, diseases, and medical histories. Until now, TNT has shown promise in both animal models and in vitro studies. However, the results of the current testing of mice cannot be clearly correlated with the benefits that TNT can bring to mice due to the difference in the characteristics of their skin and the medium of TNT. Research that correlates murine vascular studies to human vasculature will be a step to advance TNT into becoming available and viable for clinical trials. In essence, although TNT is still in the early stages of development, it has the potential to transform the domain of regenerative medicine and ameliorate the conditions of millions of people across the world; however, more research is still required to fully understand the safety and efficacy of TNT.

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