Research Article

International Journal of Alzheimer's Disease Research

Applications of Tissue Nano-Transfection Technology (TNT): A Systematic Review

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Submitted: 18 Jan 2023; **Accepted:** 30 Jan 2023; **Published:** 07 Mar 2023

Citation: *Hagar, E., Hagar, A. H., Hagar, D. H., Hagar, W. H. (2023). Applications of Tissue Nano-Transfection Technology (TNT): A Systematic Review. Int J Alzheimers Dis Res, 1(1), 14-20.*

Abstract

Aim: *The purpose of this systematic review was to provide an overview of recent advances in applications of Tissue Nano-transfection and to assess its applicability in the treatment of various diseases.*

Materials and Methods: *A systematic literature search was conducted in 2 electronic databases (PubMed and Science direct). The research findings were incorporated from inception. Initial screening was performed to select articles for review based on title and abstract. The full texts of selected articles were then evaluated, and relevant articles were chosen to be included in this review.*

Results: *29 articles were identified during the literature search. There are 16 From Science Direct and 13 in PubMed. This review included 8 research articles.*

Conclusion: *TNT advancement has made a positive impact on the future of treatment of certain diseases by providing one-touch and in-site treatments. There is a need to scale up TNT experiments on animals and humans, as well as testing this approach on a broader range of diseases. Although tissue Nano-transfection technology is still in its early stages, it has already proven to be a highly promising approach in the field of regenerative medicine.*

Keywords: Tissue Nano-Transfection, Nano-Chip, Reprogramming Factors, Regenerative Medicine

Introduction

Background

TNT is a non-invasive method that utilizes a Nanochip loaded with biological cargo, which contains reprogramming factors to convert one cell into another [1].

Reprogramming factors are loaded into the channels of a Nano-chip, which is then applied to the skin. An electric current will drive programming factors into skin cells [2]. This technology reprograms mature cells by transforming them into pluripotent stem cells. These cells can generate all types of body cells as well as replace damaged ones [3].

In contrast to other cellular therapies that rely on the implantation of cells reprogramed in vitro and then implanted in vivo, which may stimulate immune response and eventually lead to cell rejection, TNT allows for the safe reprogramming of cells in the living body while being monitored by the local immune system. Therefore, there is no need for immunosuppression [4]. It is a non-invasive method that takes only a few seconds. It does not necessitate a lengthy multi-step laboratory procedure. It is risk-free and does not pose risks such as insertional mutagenesis or cancer. It can be used as a point of care strategy [1].

TNT could be used to heal skin wounds, repair ischemic tissue, convert skin fibroblasts into electrophysiological active induced neuronal cells, genetically labeling exosomes with fluorescent proteins, gene delivery to treat lymphedema, decrease limb ischemia, non-viral gene delivery to nerve tissue, drive reprogramming factors intracranial to treat ischemic stroke, rescuing necrotizing tissues.

Main text

Materials and Methods

Study Identification and Selection

The data were extracted using the Preferred Reporting Items for Systematic Reviews and Meta-Analysis. PubMed and Science Direct were used to conduct a systematic literature search. The electronic search was carried out from Inception to August 31, 2022. The keywords used in the electronic database searches

were Tissue Nano-Transfection. A first-level screening was performed to select articles for review based on Title and Abstract. The full texts of selected articles were then examined, and relevant articles were chosen for inclusion in this review.

Inclusion and Exclusion Criteria

The full transcript of all eligible studies was procured for evaluation against the following inclusion criteria:

1. Research articles published in English language.

The following were the study exclusion criteria:

1. Lack of focus on a well-defined TNT general approach involving the use of a Nano-chip to deliver cargo and a cargo to reprogram cells.
2. Article review.
3. Conference abstract.
4. Books.
5. Full text is not available.

Data Extraction

2 authors reviewed all included articles for Bias risk and extracted data using a data extraction form.

Risk of Bias Assessment

Articles chosen based on inclusion and exclusion criteria were critically evaluated to determine their quality according to the method described in SYRCLE's tool for assessing the risk of Bias in animal studies [5]. The following domains were used to evaluate the risk of Bias in individual studies: selection Bias (random sequence generation, Baseline characteristics, and allocation concealment), performance Bias (Random housing and Blinding), detection Bias (Random outcome assessment and Blinding.), attrition Bias (incomplete outcome data), and reporting Bias (selective outcome reporting).

Studies were categorized into three categories

1. High quality: All domains have a Low risk.
2. Fair quality: 1 criterion not met or 2 Unclear but unlikely to affect the validity of the findings
3. Poor quality: 1 criterion not met or 2 criteria Unclear and likely to influence results of the study. If 2 or more criteria were not met, the study is identified as having a high risk of Bias. All studies of high and fair quality were considered for the qualitative synthesis.

Results

Study Identification and Selection

The initial electronic search yielded 29 citations. No additional records were identified using other search sources. 2 duplicates were identified and removed. 7 articles were excluded during the initial screening based on their title and abstract. Finally, 20 papers were selected, but only 8 were included in the study as full texts.2 review articles, 3 conference abstracts, 3 books,2article with no full text available, and 2 articles examining approaches other than TNT were excluded (chart1).

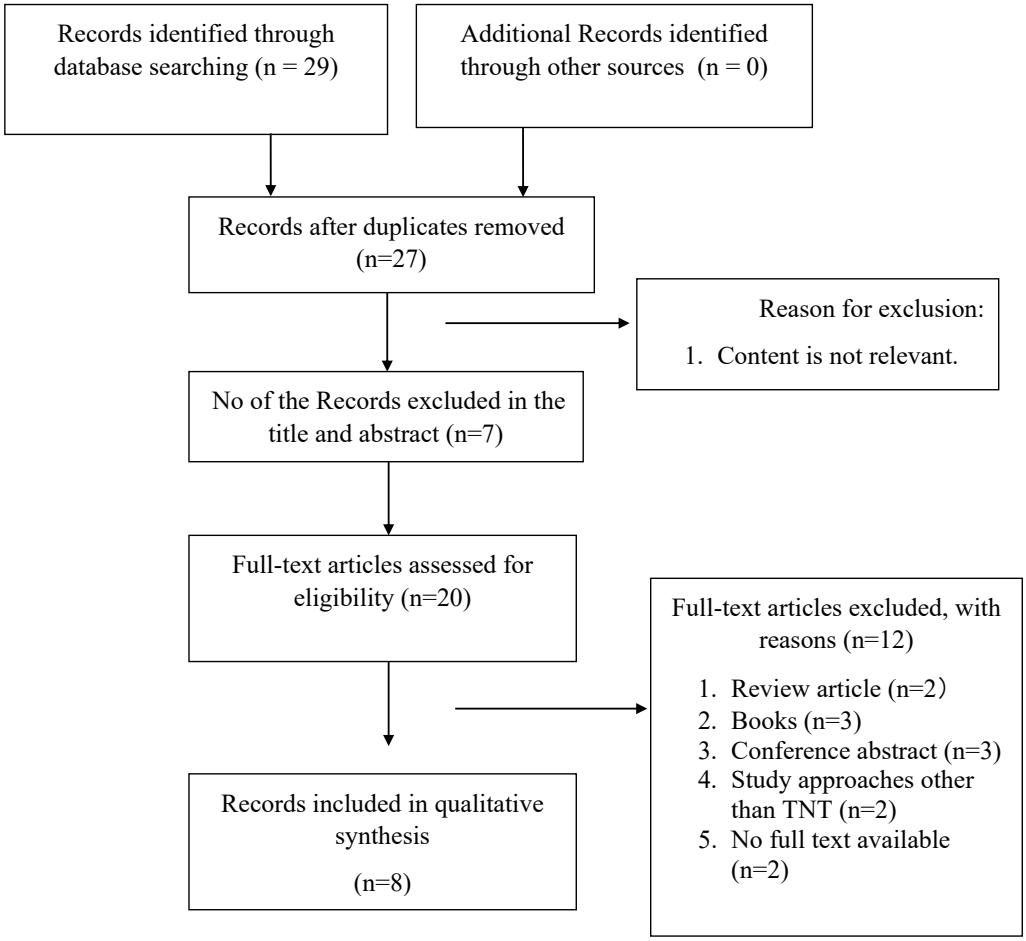

Chart 1: Flow chart for the study selection process.

Data Extraction

All the included studies were research articles conducted in the United States of America. Data were categorized as following: study author, year of publication, study title, aim of the study, and results. Information is presented in Tables (1, 2, and 3).

Table 1: Summary of Studies

Serial number	Author	Year published	Title of study	Aim of study	Results
1	Ludmila Diaz-Starokozheva et al. (6).	2020	Early Intervention in Ischemic Tissue with Oxygen Nanocarriers Enables Successful Implementation of Restorative Cell Therapies.	To develop tissue repair strategies that could potentially be used as a first line of care. Therefore, researchers combined the use of polymerized hemoglobin (PolyHb)-based oxygen Nanocarriers with Tissue Nano-Transfection (TNT).	• Flaps treated with PolyHbs exhibited a gradual decrease in necrosis as a function of time-to-intervention, with Low oxygen affinity PolyHb showing the best outcomes. • The TNT-based intervention of the flap in combination with PolyHb successfully curtailed advanced necrosis compared to flaps treated with only PolyHb or TNT alone.
2	Kanhaiya Singh et al. (7).	2022	Genome-wide DNA hypermethylation opposes healing in patients with chronic wounds by impairing epithelial-mesenchymal transition.	Determining the importance of keratinocyte-specific editing of TP53 methylation at the wound edge was accomplished by a tissue Nanotransfection-based CRISPR/dCas9 approach.	• Successful demethylation of the TP53 promoter rescued the expression of EMT regulators TP53, ADAM17, and NOTCH1 and promoted ischemic wound closure. • Reversing methylation-dependent keratinocyte gene silencing is a promising therapeutic strategy for improving wound healing

Table 2: Continued Summary of Studies

Serial number	Author	Year published	Title of study	Aim of study	Results
3	Sashwati Roy et al. (4).	2020	Neurogenic tissue Nano-transfection in the management of cutaneous diabetic poly-neuropathy.	To investigate whether the skin's neuro-trophic milieu can be used to rescue pre-existing nerve fibers under chronic diabetic conditions.	• Tissue Nanotransfection (TNT) delivery of Myt1l (TNTABM) resulted in successful neurogenic cell conversion. • Skin neurotrophic enrichment was caused by TNTABM (an interesting finding of this work is that the skin stroma enriches in NGF and Nt3 expression(. • Endogenous NGF and other co-regulated neurotrophic factors such as Nt3 were increased by topical cutaneous TNTABM. • In diabetic mice, TNTABM prevented the loss of cutaneous PGP9.5+ mature nerve fibers. • The first study to show that under in vivo reprogramming conditions, changes in the tissue microenvironment can be utilized for therapeutic purposes such as the rescue of pre-existing nerve fibers from their predictable path of damage under diabetes conditions.
4	Xiaoju Zhou et al. (8).	2020	Exosome-Mediated Crosstalk between Keratinocytes and Macrophages in Cutaneous Wound Healing.	To isolate keratinocyte-derived exosomes (which were genetically labeled with GFP reporter (Exoκ-GFP) using tissue Nanotransfection) from the wound edge and determine their role in the healing process at the wound site.	• Exoκ-GFP has been identified as being critical for functional wound closure and a significant contributor that governs macrophage number, function, and trafficking within the granulation tissue, along with contributing to epithelial barrier properties post-injury.
5	Aladdin H. Hassanein et al. (9).	2021	A Murine Tail Lymphedema Model	To improve the conventional murine tail lymphedema model and deliver genetic cargo to the mouse tail vasculature using TNT.	• The chronic murine tail lymphedema model induces sustained lymphedema over 15 weeks and reliable perfusion to the tail. The modified mouse tail model of lymphedema is a reproducible and clinically translatable animal model of lymphedema. • TNT was used in the mouse tail lymphedema model to allow for focal gene delivery of potential gene-based therapeutics to the lymphatic injury site of the mouse tail, which opens up a promising future for gene-based therapy delivery.

Table 3: Continued Summary of Studies

Serial number	Author	Year published	Title of study	Aim of study	Results
6	Jordan T Moore et al. (10).	2020	Nanochannel-Based Poration Drives Benign and Effective Nonviral Gene Delivery to Peripheral Nerve Tissue.	To investigate the utilization of TNT as platform nanotechnology for gene and reprogramming-based cell therapy delivery to peripheral nerves.	• TNT-based EFF delivery resulted in increased vascularity, decreased macrophage infiltration, and enhanced electrophysiological healing in crushed nerves especially in contrast to crushed nerves TNT-treated with sham/empty plasmids. • The findings suggest that TNT could be potent platform nanotechnology for in vivo localized non-viral gene delivery and the implementation of reprogramming-based cell therapies for nerve regeneration.
7	Luke R Lemmerman et al. (11).	2021	Nanotransfection-based vasculogenic cell reprogramming drives functional recovery in a mouse model of ischemic stroke.	To use fibroblasts Nanotransfected with Etv2, *Foxc2, and Fli1 (EFF)* to drive reprogramming-based vasculogenesis, intracranially, as a potential therapy for ischemic stroke.	• In stroke-affected mice, intracranial delivery of fibroblasts Nanotransfected with the EFF cocktail results in dose-dependent rises in perfusion, Lowered stroke volume, and significant retrieval of locomotive abilities. • MRI and behavioral tests revealed that mice had 70% infarct resolution and up to 90% motor recovery. • Significant increases in vascularity and neurological cellular activity were confirmed by immunohistological assessment.
8	Daniel Gallego-Perez et al. (12).	2017	Topical tissue Nano-transfection mediates non-viral stroma reprogramming and rescue.	To assess whether TNT-based EFF delivery could result in whole-limb rescue and To implement simple approach to topically reprogram tissues through a Nanochannelled device validated with well-established and newly developed reprogramming models of induced neurons and endothelium.	• The study demonstrated the first time that TNT can be used to deliver reprogramming factors into the skin. • TNT-based EFF promoted vascularization and skin recovery in ischemic conditions. • EFF based on TNT saves entire limbs from necrotizing ischemia. • This simple TNT approach elicited and propagated potently advantageous biological responses via a one-time topical treatment that last for only seconds.

Risk of Bias Assessment

The risk of bias was assessed using the method described in SYRCLE's tool for assessing the risk of bias in animal studies. 5 studies were classified as high-quality with a low risk of bias. 3 studies were classified as fair quality, with an unclear risk of bias that was unlikely to affect the study's outcome. No study was labeled as a poor study. Information is presented in Table 4.

Table 4: Risk of Bias Assessment

Quality Criteria	Studies								
		Sashwati Roy et al.	Xiaoju Zhou et al.	Aladdin H. Hassanein et al.	Jordan T Moore et al.	Luke R Lemmerman et al.	Daniel Gallego-Perez et al.	Ludmila Diaz-Starokozheva et al.	Kanhaiya Singh et al.
Selection Bias	Sequence generation.	Low	Low	Low	Low	Low	Low	Low	Low
	Baseline characteristics.	Low	Low	Low	Low	Low	Low	Low	Low
	Allocation concealment.	Low	Low	Low	Low	Low	Low	Low	Low
Performance Bias.	Random housing.	Low	Low	Low	Low	Unclear	Low	Unclear	Unclear
	Blinding.	Low	Low	Low	Low	Low	Low	Low	Low
Detection Bias.	Random outcome assessment.	Low	Unclear	Unclear	Low	Unclear	Low	Unclear	Unclear
	Blinding.	Low	Low	Low	Low	Low	Low	Low	Low
Attrition Bias.	Incomplete Outcome Data.	Low	Low	Low	Low	Low	Low	Low	Low
Reporting Bias.	Selective outcome reporting.	Low	Low	Low	Low	Low	Low	Low	Low
Total Bias assessment		Low risk of Bias.	Low risk of Bias.	Low risk of Bias.	Low risk of Bias.	Unclear risk of performance Bias.	Low risk of Bias.	Unclear risk of performance Bias.	Unclear risk of performance Bias.

Discussion

The studies included in this review evaluated a variety of different TNT applications, and the data could not be analyzed quantitatively due to heterogeneity. However, the findings of the studies are summarized to provide an overview of the progress and role of TNT. According to the studies included, TNT is effective in the following aspects:

1. Tissue Nano-transfection enabled the delivery of Ascl1, Brn2, and Myt1l (TNTABM), which converted skin fibroblasts into neuronal cells. TNTABM enriched skin stroma, increased NGF and other co-regulated neurotrophic factors such as Nt3, and prevented PGP9.5+ loss in diabetic mice. Thus, using TNT to deliver certain reprogramming factors can protect pre-existing nerve fibers from loss in diabetic patients [4].

2. A TNT-based topical CRISPR/dCas9 strategy was used to develop chimeric versions of dCas9 fused with TET to achieve TP53 demethylation.TP53 demethylation improved wound closure. This proves that TNT can be employed to improve topical wound closure [6].

3. TNT can be used to label exosomes by delivering three plasmids encoding for CD9, CD63, and CD81 with an "in frame" GFP reporter, resulting in GFP expression in exosomes and allowing these exosomes to be easily identified and isolated for further processing[7].

4. As in the mouse tail lymphoma model, TNT can be used to deliver genetic cargo to the site of lymphatic injury. TNT holds great promise for the delivery of gene-based therapies [8].

5. TNT can be used to deliver reprogramming factors genes Etv2, Foxc2, and Fli1 (EFF) to crushed nerves, which results in increased vascularity and better recovery. Tissue Nano transfection, when compared to Bulk Electroporation (BEP), does not hinder toe-spread and pinprick response and does not affect electrophysiological parameters, whereas BEP can cause nerve damage and increase macrophage overexpression [9].

6. TNT is used to treat ischemic stroke in mice by delivering reprogramming factors (Etv2, Foxc2, and Fli1 (EFF)) which are vasculogenic cell therapies. These treatment methods lead

to increased vascularity and revealed infract resolution, indicating that TNT is a particularly promising future approach to treat ischemic stroke [10].

7. TNT was used to deliver reprogramming factors to the skin in 2 injury-induced ischemia mouse models, which resulted in the formation of vascular networks that successfully anastomosed with the systemic circulation, recovered tissue, and managed to rescue the entire limb of mice, implying that TNT could be used to rescue necrotizing tissue [11]. TNT could also be employed to develop tissue early intervention measures to deal with severe tissue ischemia since ischemic tissue can be saved using polymerized hemoglobin (PolyHb)-based oxygen Nanocarriers and Tissue Nano-Transfection (TNT) [6].

Conclusion

Advancements in Tissue Nano-transfection have a great prospect in the prevention and management of various diseases, such as healing skin wounds, repairing ischemic tissue, managing ischemic stroke, treating lymphedema, and labeling exosomes. Tissue Nano-transfection is apparently probable to enhance disease prevention and treatment, but it is still in the development stage, and more clinical studies are necessary to reach an unbiased outcome. There is a need to scale up TNT experiments on animals and humans, as well as testing this approach on a broader range of diseases [12].

Abbreviations

TNT: Tissue Nano-Transfection Technology, PolyHb: Polyhemoglobin, TP53: tumor protein 53, EMT regulators : Epithelial-Mesenchymal Transition regulators ,ADAM17: A disintegrin and metalloprotease 17, NOTCH1: Neurogenic locus notch homolog protein 1, CRISPR/dCas9 : Clustered regularly interspaced short palindromic repeat (CRISPR), based on the fusion of inactive Cas9 (dCas9), MYT1L: myelin transcription factor 1, NGF: Nerve growth factor, Nt3: Neurotrophin 3, PGP9.5 : Protein gene product 9.5, Exoκ-GFP : exosomes labeled with GFP reporter ,FLI1 is a transcription factor named for Friend leukemia virus integration site 1, ETV2 : ETS Variant Transcription Factor , Foxc2: Forkhead transcription factor, Ascl1: Achaete-Scute Family BHLH Transcription Factor 1, BRN2:is POU domain transcription factor , TET: are a family of ten-eleven translocation proteins, CD9: cluster of differentiation9, CD63: cluster of differentiation 63, CD81: cluster of differentiation 81.

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