

## Mitochondrial DNA Mutation in Microglia Can Be Treated by SCNT Cloning and Not by Reprogramming of Olfactory Ensheathing Cells in the Multiple Sclerosis Treatment

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### Abstract

**Background:** Multiple sclerosis (MS) is a demyelinating chronic inflammatory neurological disease of the central nervous system (CNS), characterized by activation of microglia. Mitochondrial mutations (mtDNA.mutat) and dysfunctions in microglial cells are thought to centrally contribute to the damaging effects of neuroinflammation seen in MS.

**Objective:** The somatic nuclear transfer (SCNT) technology offers a more practical mode of therapy in MS, This method would attempt to dilute and/or progressively replace the (mtDNA.mutat) and activated microglia with cloned olfactory ensheathing cells (OEC) with remyelinating and scavenging properties which would attempt to limit the progression of MS.

**Proposed Methods:** Applying SCNT-derived embryonic stem (ES) cells based therapy by cloning olfactory ensheathing cells (OEC), engineered with an autologous nuclear component of the recipient OEC with a healthy donor oocyte. The inner cell mass of the subsequently developed blastocyst would be the source to generate the microglia. The novel proposed transcribrial route device offers a painless mode of cell transplantation to the brain.

**Expected Results:** This mode of generating SCNT cloned glia and its transplantation to the brain is expected to replace the (mtDNA.mutat) and activated microglia of the patients with MS and use the regenerative and remyelinating and properties of the OEC's, as has been seen in recent clinical trials in paraplegic patients with spinal cord injuries.

**Conclusion:** The use of SCNT in contrast to the reprogramming adult somatic cell is expected to develop isogenic ES cell-based therapies for the prevention and treatment of MS associated with mtDNA mutations that may open a new avenue of designer's targeted cell therapy unique for the patients with MS. The proposed "transcribrial device" to access the brain can be an advantageous route of delivery of cloned cells to the brain.

**Keywords** Multiple Sclerosis; OEC's; Neurodegenerative diseases; Stem cell therapy; Regenerative medicine; SCNT

### Introduction

Multiple sclerosis (MS) is a demyelinating chronic inflammatory neurological disease of the central nervous system (CNS); characterized by neurodegeneration and astroglial proliferation [1] Despite major advances in the current understanding of the pathogenesis of MS, the inflammatory cascade involved in MS remains unknown; although there is considerable evidence implicating the involvement of mitochondria in axonal and glial injury mechanisms [2]. Mitochondrial dysfunction in microglial cells has been observed in several animal models of neurodegeneration and aging, in this regard the mitochondrial DNA damage in particular is most pronounced in microglial cells, especially compared to neurons [3]. There appears to be a role of complex genetic factors exerting a profound influence in MS [4].

Neuronal injuries and neurodegenerative (ND) diseases of the central nervous system (CNS), like MS are exclusive in that they eventually get repaired by gliosis (fibrosis), leading to damage to the neurological tissue and resultant neurological deficits in the affected individual. Widespread microglial activation is a histopathological hallmark of progressive MS and inhibition of this aspect of innate immunity could potentially have beneficial effects on both progressive and relapsing forms of MS [5]. There is currently no treatment for the loss of neuronal function after damage to the nervous system, multiple attempts at nerve re-growth across the peripheral nervous system (PNS) and (CNS) transition have not been successful [6].

Majority of ND, including (MS) is characterized by activation of microglia and mitochondrial dysfunction in microglial cells is thought to contribute to the harmful effects neuroinflammation seen in these diseases [3]. One of the most distressing aspects of MS treatment is the failure of development of disease-modifying therapies that slow disability in progressive forms of MS. At present, none of the drugs do attempt to reduce the inflammatory process and target either the primary progressive or secondary progressive MS [5]. Very trivial

studies in animal models have come up in the field of cell therapy as a treatment modality for this demyelinating disease.

### Current scenarios in the drug management of Multiple Sclerosis

As a result of the past 20 years of research done for treatment of MS, various forms of auto injectable interferon beta (IFN  $\beta$ ) and Glatiramer acetate (GA) got into the market with GA 40 mg given subcutaneously reduced the relapse rate at 1 year [7] and became widely used as first line disease treatments. Natalizumab, and to a much lesser extent Mitoxantrone, are second-line intravenously infused treatments. With teriflunomide in patients with relapsing MS study shows teriflunomide's clinical efficacy in reducing the relapse rate and neurological deficits in MS over a period of 2 years [8]. Several phase III studies in relapsing MS are ongoing for second-generation sphingosine-1-phosphate receptor 1 immunomodulators which shows promise in reducing some of the side effects associated with such an agent like bradycardia, abnormal hepatic enzyme elevations, and lung fibrosis [5] Vitamin D deficiency has been recently identified as a risk factor and its potential in possible disease modifying role and awaits clinical trials in relapsing MS [9].

### Ongoing trends in stem cell therapy of Multiple Sclerosis

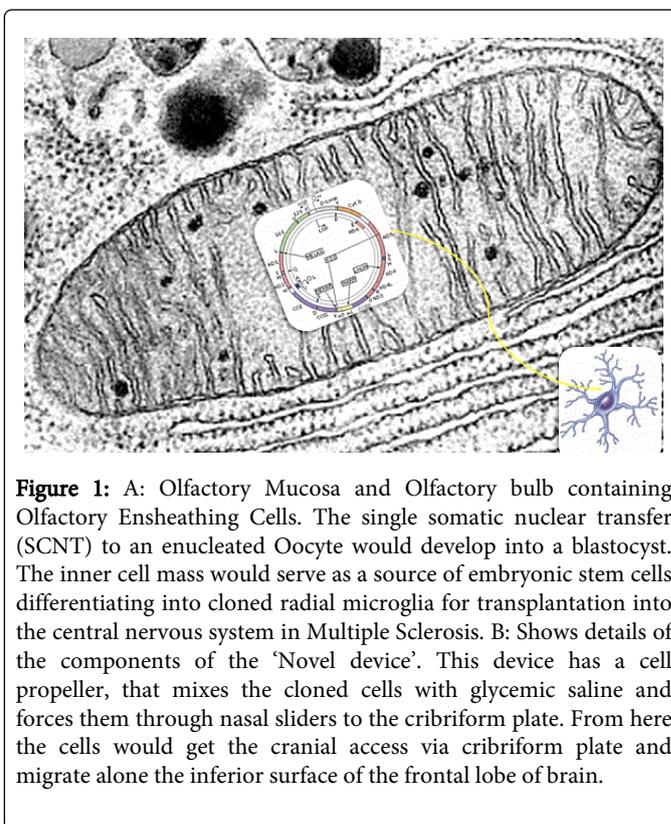
In humans, the neural tissue development largely ceases during adulthood, but in two areas of the brain, the hippocampus and olfactory bulb, there is strong evidence of regeneration of substantial numbers of new neurons (10). Studies done in detail on the use of (ESs) in MS have revealed that the actual limitation is the preparation of sufficient human oligodendrocytes precursor cells in getting tissue-specific progenitor cells from human ES's and the dangers that there are always risk of tumorigenesis in neural cells derived from ESs, limiting the potentialities of science and therapy in such studies (11). Another shortcoming of the autologous Mesenchymal Stem Cell (MSC) and induced pluripotent stem cells (iPS), transplantation in the treatment of MS is that of carrying the inherited (mt DNA) mutation to the CNS. These autologous MHC would tend the disease to recur. For MSC there is lingering doubt whether the MSC derived neurons are functional, (12) and as similar fate, of disease recurrences could be expected if it ends up differentiating into a glia.

### Recommendation for a novel cell therapy in Multiple Sclerosis

From extensive reviews on the cell therapy methodologies it can be understood that partial successes or disappointments encountered in treating MS with autologous embryonic stem cell transplants have occurred due to immune rejection and/or persistence of inherited or acquired mutated mitochondrial DNA in the glial cells in the case of autologous iPS transplants. Although transplantation of Mesenchymal Stem Cell (MSC), or induced pluripotent stem (iPS) cells generated from autologous somatic OECs and reprogrammed into ES-like cells may provide an additional source of autologous stem cells for treating MS, both would be of no potential benefit as would continue to harbor the same mitochondrial mutations (mtDNA) as possessed by glia in CNS [13]. The field of mitochondrial-targeted therapeutics is one that is growing fast and is of great importance and an increasing evidence points towards mutation leading to altered mitochondrial trafficking and fusion-fission dynamics in several ND diseases including MS [14]. MS can be targeted for slowing down the progression, prevention of

onset and possible treatment by, perhaps, the proposed genetically modified glial cells cloned by somatic nucleus transfer (NT) of Olfactory ensheathing cells (OEC's) to a healthy donor oocyte devoid of mt DNA mutations. OECs normally perform regenerative, scavenging and innate immune functions in the olfactory region and have been investigated thoroughly in related to spinal cord injuries, amyotrophic lateral sclerosis and other ND diseases where research suggests that these cells possess a unique ability to remyelinate injured neurons [15].

Here we propose glia cloned with an autologous nuclear component of the recipient's OEC's, union by combination of whole somatic OECs with a healthy oocyte cytosol [13]. (Figure-1), offers an alternate speculative likelihood of producing NT-derived ES cells in which mutated mtDNA would be substantially diluted/destroyed by the mt DNA of oocyte, happens with the mitochondrial DNA of the spermatozoa, shortly after fertilization. The resulting inner cell mass from the blastocyst stage of development would become a source of induced pluripotent stem cells that would differentiate into a radial glia like OEC's with the inherent properties of axonal and myelin generation and scavenging functions. The transplantation followed by the self-renewal of this iPS at sites of demyelination would attempt to repair the gliosis and dilute the activated microglia populations that are the leading cause of defects seen in MS. After being reported that transplantation of heterologous fetal OECs into the frontal lobes it has been seen to slow down the rate of clinical progression in ALS patients, subsequent neuropathologic analysis did not support a therapeutic efficacy for ALS patients even after OEC transplantation via the ventricles, although transplantation of OECs engineered to produce neurotrophins might be helpful to improve the condition in ALS [15].



**Figure 1:** A: Olfactory Mucosa and Olfactory bulb containing Olfactory Ensheathing Cells. The single somatic nuclear transfer (SCNT) to an enucleated Oocyte would develop into a blastocyst. The inner cell mass would serve as a source of embryonic stem cells differentiating into cloned radial microglia for transplantation into the central nervous system in Multiple Sclerosis. B: Shows details of the components of the 'Novel device'. This device has a cell propeller, that mixes the cloned cells with glycemic saline and forces them through nasal sliders to the cribriform plate. From here the cells would get the cranial access via cribriform plate and migrate along the inferior surface of the frontal lobe of brain.

Culturing and transplanting this cloned glia that expresses identical HLA antigens would not evoke an autoimmune rejection while the mitochondria's of oocyte of a healthy donor would possibly dilute the activated microglia slow down the ongoing neuronal degeneration [4,13,14]. The additional rewarding benefit of such transplantation would be that the regenerative and scavenging potentials of OECs in contrast to the over reactive microglia present in the CNS of the MS patients, would help recover the damage already caused by the degenerative process, a feature missing in mesenchymal stem cells (MSC) and iPS. The use of the NT to develop isogenic ES cell-based therapies for the prevention and treatment of MS associated with mtDNA mutations may open a new avenue of designer's targeted cell therapy unique for the patients with MS.

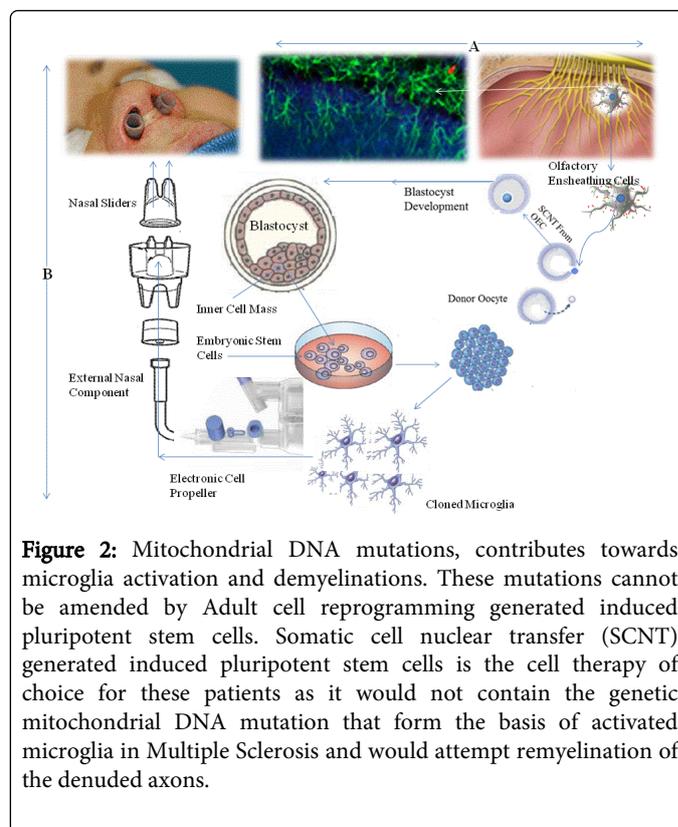
### Weighing the benefits ahead of Transplantations

The nuclear profiling of the cloned glia by microarray for differential expression of mtDNA and nuclear DNA of these engineered cells prior to transplant would clearly highlight the benefits of the planned transplantation. A similar gene profiling of the patient's glial cells would help understand the differences in their protein expression and the gains of the procedure in the ongoing MS in the patients. Once evaluated and approved for transplant, these cloned cells like their parent OEC's can be labelled and tracked by a magnetic resonance imaging (MRI) device when being dispersed in the central nervous system. A recent study has made use of a novel type of micron-sized particles of iron oxide (MPIO) to label and track these transport-mediated cells via MRI [16].

### Novel delivery via Transcribrial route device

The advantage of transplantation techniques done in past via Intrathecal, Intraventricular, intra-parenchyma and several other routes can be considered for the aforementioned cloned glial cells. Methods of cells being delivered to CNS through an intranasal route has been reported in the past [17]. The nasal route has therapeutic effects that appear to be equivalent to those induced by intravenous route adult neural stem cells (NSCs) and lead to protection from CNS inflammation and the promotion of remyelination and neural repair. Such an effective alternative route of administration could significantly improve the efficacy of NSC-based therapy in MS (18). There is potential of this intranasal route principally for the therapy of neurodegenerative disorders, this delivery method provides the option of chronic treatment which may enhance the number of delivered cells in order to achieve therapeutic benefit [19] Figure 2.

We have recently proposed a "transcribrial route device" that is composed of three connected components [20] to access the brain across the cribriform plate of the ethmoid bone. This can be of advantage because of being painless and the trimmed ball-valve terminal could prevent any regurgitation back into the delivery system. A local anaesthetic application prior to the cell therapy could minimize the reflex sneezing that is expected on insertion of the nasal components. Unlike other routes this route would clearly avoid the risks of escape of the cells into the lungs and various neurological complications of other cell therapy delivery systems.



**Figure 2:** Mitochondrial DNA mutations, contributes towards microglia activation and demyelinations. These mutations cannot be amended by Adult cell reprogramming generated induced pluripotent stem cells. Somatic cell nuclear transfer (SCNT) generated induced pluripotent stem cells is the cell therapy of choice for these patients as it would not contain the genetic mitochondrial DNA mutation that form the basis of activated microglia in Multiple Sclerosis and would attempt remyelination of the denuded axons.

### Statement of Conflict of interest

The author declares that he has no conflict of interest with any institution and/or financial organization regarding the material discussed in the manuscript.

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