Human Epineural Sheath Conduits Supported with Human Mesenchymal Stem Cells Enhances Nerve Regeneration in the Nude Rat Experimental Model

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INTRODUCTION

Peripheral nerve injuries occur in 2.8% of trauma patients. [1] Approximately 100,000 patients undergo peripheral nerve surgery in the United States and Europe each year. [2]

Nerve autografts are considered the gold standard in peripheral nerve gap regeneration; however, its disadvantages, such as neuroma formation, scarring, limited availability and prolonged surgery durations, present the need for an alternative method of treatment. [3,4]

In recent years, both biological and artificial conduits have become of particular interest as a alternative therapy for peripheral nerve injuries. Nerve allografts offer an unrestricted source of nerve epineurium, which can be utilized as a bridging conduit between the two nerve stumps to support nerve recovery. The epineurium is a layer of connective tissue surrounding the nerve, constituting its anatomical border. Its acellular properties make it non-immunogenic, which is an important trait, especially compared to traditional nerve allograft which require systemic immunosuppression. The neural origin is the potential advantage of this conduit over other biological tubes. High laminin B2 and VEGF expression provides a highly neuropermissive environment for Schwann cell attachment and axonal ingrowth. [4]

Mesenchymal stem cells, due to their anti-inflammatory and neuroregenerative properties are considered a promising approach as a supportive therapy for peripheral nerve injuries. Thus, we propose a novel therapeutic approach for enhancement of nerve regeneration – the human epineural conduit (hEC) consisting of human epineural sheath (hES) supported with human mesenchymal stem cells (hMSC).

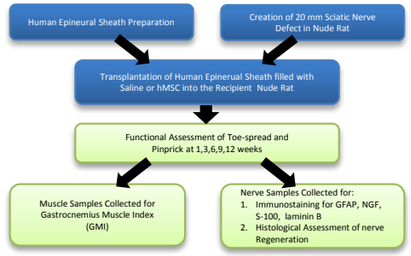
OBJECTIVE

To test the effect of human Epineural Sheath Conduit (hEC), supported with human Mesenchymal Stem Cells (hMSC), on the restoration of 20mm long nerve defect in a nude rat model.

AIMS

1. To assess *in vivo* the regenerative potential of hEC supported with 3x6^10 hMSC in 20mm long nerve gaps in the nude rat model.
2. To assess the role of hMSC in peripheral nerve regeneration.

EXPERIMENTAL DESIGN



METHODS

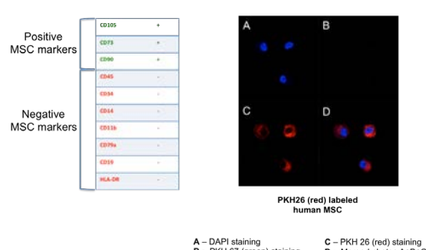
Adult nude rats, weighing 150-250 grams, were divided into 4 experimental groups:

Group	Experimental Groups	F/U (weeks)	# of limbs/group
1	Control - no repair of the defect	12	6
2	Autograft control	12	6
3	hEC repair filled with saline	12	6
4	hEC repair supported with 3x6^10 hMSC	12	6

Human Sciatic Nerve

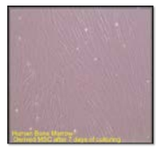
Human sciatic nerves were supplied to our laboratory by the Musculoskeletal Transplant Foundation (MTF). The size ratio between a human and rat sciatic nerve is 10:1. The conduits were prepared by dissecting the branches of 1-3 mm in diameter.

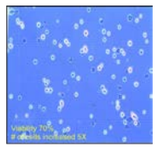
Phenotype characterization and PKH26 labeling of hMSC



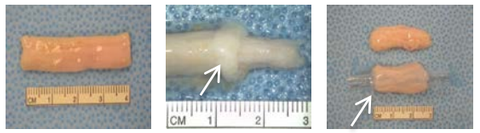
METHODS

Morphology and Proliferation of hMSC at day 7 of cell culturing


hMSC culture


Trypan Blue staining

Surgical Technique – Preparation of the hEC Conduit


1. Human sciatic nerve
2. Epineurium dissected from the fascicles
3. Dissected conduit ready for implantation

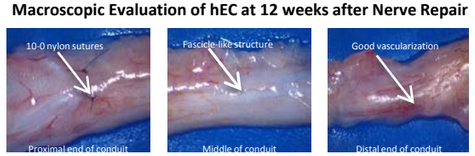
Surgical Technique – Nerve Gap Repair with hEC Conduit

1. Right sciatic nerve of rat
2. Creation of 20mm nerve gap
3. hEC transplanted into the gap

Surgical Technique – hEC Conduit filled with hMSC

1. Filling of the Conduit with hMSC using a syringe
2. Conduit filled with hMSC

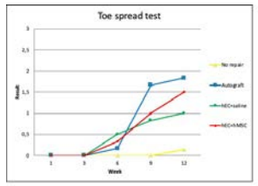
Macroscopic Evaluation of hEC at 12 weeks after Nerve Repair

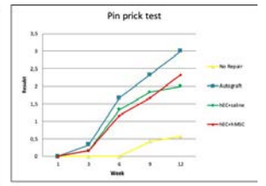


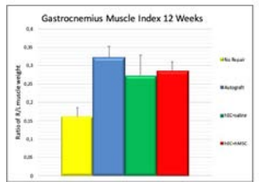
- No adhesions or local signs of inflammation
- Well preserved structure, shape and integrity of the grafts
- Macroscopically fascicle-like structures inside the conduit
- No sings of atrophy of the nerve distally to the conduit
- Good vascularization of the graft

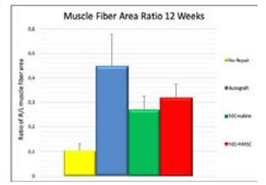
RESULTS

Functional Results









Histological Results

Toluidine blue and immunofluorescent staining results are currently under evaluation.

CONCLUSIONS

- We confirmed the feasibility of hEC creation and its successful application in repair of a 20 mm sciatic nerve gaps.
- Application of hEC + hMSC confirmed better functional results compared to hEC + saline group.
- Our human epineural sheath conduit introduces a novel method for nerve gap repair and may serve as alternative approach to the standard autograft technique.

REFRENCES

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The authors have nothing to disclose.