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Angiogenesis induced by autologous whole bone marrow stem cells seeded on collagen scaffolds in silicone nerve tubes. An experimental study

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Abstract

Objective: This study aimed at evaluating Vascularity of tissues formed in silicone nerve tubes with collagen scaffolds seeded with Bone marrow stem cells (BMSC) as a sign of tissue regeneration.
Materials and methods: Six adult mongrel dogs were included in this study; a 3 cm gap was made on the buccal branch of right side of facial nerve and also on the left side as a "control side". Next mononuclear cells containing mesenchymal stem cells were separated from whole bone marrow using Gradient separation method with Ficoll. Silicone tubes embedded with collagen type I sponge seeded with stem cells and neurogenic media were sutured in the perineural fascia of both ends of the cut nerve on the experimental side, while in the control side empty silicone tubes alone were sutured to the perineural fascia of the cut nerves. Healing was evaluated at 8 and 10 weeks scarifying 3 dogs each time and performing histological analysis.
Results: After 8 weeks the 6 dogs showed limited movement in the facial area supplied by the buccal nerve on the experimental side and the salivary drooling decreased moderately. After 10 weeks the remaining dogs showed observable muscle movements and decreased salivary drooling in contrast to control side. These results go with the histological examination results showing massive multiple blood vessels formation and nerve like structures with partial absorption of the scaffold at 8 weeks then well organized nerve like tissues with dilated multiple blood vessels at 10 weeks.
Conclusion: Collagen scaffolds seeded with mononuclear layer containing stem cells and nerve growth factors showed efficacy at achieving good neo-vascularization which lead to eventual good healing.

Keywords: Angiogenesis; Nerve regeneration; Bone marrow stem cells; Tissue engineering; Tissue regeneration

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1. Introduction & review of literature

The axon regeneration following peripheral nerve injury often fails to restore a complete functional recovery. The causes have been attributed to regrowth of regenerating fibers to inappropriate peripheral targets and because it is not absolutely dependent on the axons' mechanical alignment. Despite refined micro surgical techniques, misdirection of the regenerating axons is one of the most common limiting factors for a correct restoration of functionality with the results of most common construction and repair techniques remained disappointing [1,2].

Entubation techniques were developed to overcome morbidity and problems of grafting. The aims of entubation techniques are to increase: the number, speed and length of the regenerating axons. After the nerve stumps are inserted and sutured into the ends of the tube, they fill with a protein-rich fluid exudate from the nerve stumps which contains growth-promoting substances. A neomatrix of fibrin is formed within the following days providing support for the migration of specialized cells – the Schwann cells – as well as fibroblasts and macrophages. This provides a scaffold for axonal growth from the proximal end towards the distal stump [3].

However; it is becoming possible to influence the recovery of facial nerve function following injury, in terms of compensation for deficits, rescue of viable neurons and regeneration of damaged axons.

The concept of tissue engineering is based on the simulation of nature itself. To engineer functional tissues, cells from host (and/or donor) must be provided with appropriate spatial and temporal cues to enable growth, differentiation and synthesis of an extra cellular matrix of sufficient volume and functional integrity. Tissue engineering goes beyond regenerative medicine and incorporates the unique qualities of engineering design and use of the engineering method bases for developing the approaches used to control biological systems [4].

Vascularity of the healing tissues is a very essential factor in the equation of regeneration, keeping the process without massive fibrosis or necrosis or failure of maturation of the engineered tissues [5].

Mesenchymal stem cells (MSCs), also referred to as marrow stromal cells, multipotential stromal cells or mesenchymal stromal cells, represent a class of plastic adherent progenitor cells that were initially isolated from the non-hematopoietic compartment of bone marrow. MSCs can even be induced to differentiate into other tissues such as myoblasts, cardiomyocytes, hepatocytes and neurons.

Bone marrow stem cells (BMSC) retain the ability to self-renew and differentiate into cells of all blood lineages throughout adult life. These adult BMSC have recently been shown to have the capacity in adult mice to reconstitute the entire hematopoietic system in myeloablated mice. Recent data derived from a number of laboratories indicate that mouse BMSC also have the potential to generate non hematopoietic cell types including epithelial cells, skeletal muscle cells, hepatocytes, endothelial cells, neurons and glial cells. This newly defined capacity of stem cells from bone marrow to give rise to cells of an unrelated tissue is referred to as plasticity [6].

Bone marrow stromal cells could express phenotypes of neurons, also it was reported that *Salvia miltiorrhiza* could induce BMSC to differentiate into neuron-like cells. If BMSC could be converted into neurons instead of mesenchymal derivatives, they would be an abundant and accessible cellular source to treat a variety of neurological diseases [7].

Developmental limitations of tissue-specific stem cells are regulated by the micro environment. Host cells under specific conditions, such as tissue injury or infection, might provide specific signals that counteract these limitations. In adults, stem cells reside in a physiologically limited and specialized micro environment, or niche, that supports stem cells but varies in nature and location depending on the tissue type [8].

According to a large number of reports, collagen nerve guides have been extensively proven to be effective to functional recovery of nerves. Extra cellular matrix of natural nerve tissue is mainly composed of Collagen-I [9]. Collagen is also a commonly used substance to mimic the natural neuronal ECM.

In this study, autologous bone marrow stem cells were harvested from anterior iliac crest of canines, seeded on collagen type I and provided with nerve growth factors and implanted in silicon guiding tubes for facial nerve regeneration. The effect of these seeded cells on the new vessels formation (angiogenesis) was studied.

2. Materials and methods

Six adult Mongrol dogs were included in this study. After exposure of the Buccal branch of the Facial nerve bilaterally, two gaps, 3 cm in length were made in the nerve by direct cutting using a sharp 15 bard parker blade. On the right side (study side), a silicone tube, with stem cells seeded on collagen type 1 scaffold inside, was sutured to both ends of the cut buccal