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Biomaterials 27 (2006) 3515–3518
Biomaterials
www.elsevier.com/locate/biomaterials

Leading Opinion

Peripheral nerve regeneration: An opinion on channels, scaffolds and anisotropy ☆

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Received 24 January 2006; accepted 20 February 2006
Available online 14 March 2006

Abstract

Peripheral nerve regeneration presents a significant clinical challenge and the current state of the art using autografts to repair long peripheral nerve gaps is unsatisfactory. In this manuscript, the analytical framework that determines the fate of grafts (autografts or biomaterial-based grafts) is discussed. Also outlined are parameters and variables that might be manipulated to enhance the efficacy of scaffolds designed for peripheral nerve regeneration. The importance of using appropriate animal models and outcome measures in evaluating biomaterials-based scaffolds or other engineered constructs suitability for bridging peripheral nerve gaps is highlighted.

Keywords: Neutral tissue engineering; Hydrogels; Nanofibers

1. The problem

Peripheral nerve regeneration is a serious clinical problem. In 1995, there were in excess of 50,000 peripheral nerve repair procedures performed in the United States [1]. The data, however, probably underestimates the number of nerve injuries, as not all surgical or traumatic lesions can be repaired. Coaptation of the two nerve ends is commonly used to repair short nerve defects. When larger nerve gaps exist (20 mm or longer in humans), the current clinical gold standard for repairing larger nerve deficits involves using sensory nerve autografts. An analysis of clinical outcomes with Autograft use suggests that a critical need for engineered alternatives exists. Autografts are plagued by issues such as a shortage of donor nerves, a mismatch of donor nerve size with the recipient site, and occurrences of neuroma formation; and even in the best-case scenarios, complete recovery of function is rare. Increasing evidence suggests that the modality of the donor nerve might matter, with mixed nerves having better outcomes than commonly used sensory nerves such as sural nerves [2]. In addition, peripheral nerves might also express inhibitory Proteoglycans such as Chondroitin sulfate Proteoglycans [3]. Therefore, the need for synthetic alternatives to autografts is compelling and would be of great surgical benefit if they could match or exceed autograft performance.

2. Nerve guidance channels (NGCs)

To date, much of the research effort has focused on nerve guidance channels to enhance regeneration across nerve gaps. While they improve regeneration when compared to no intervention, guidance channels rarely approach or match the performance of autografts when the gaps are 10 mm or longer (in rats). This includes numerous studies with varying permeability of the guidance channels [4,5], involving electrically active channels [6,7], as well as degradable guidance channels [8,9]. While a few groups still pursue research trying to modify the NGC characteristics, there is emerging consensus that bridging long peripheral

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doi:10.1016/j.biomaterials.2006.02.030

nerve gaps will involve filling NGCs with scaffolds/constructs that promote regeneration.

3. Nerve guidance channels may need to carry other scaffolds

The rationale for filling NGCs with 'engineered' constructs is the following. When nerve gaps are short and inherent regeneration is possible, a fibrin cable forms across the nerve gap [10,11] allowing for Schwann cell infiltration and the formation of the Bands of Bungner, which are oriented columns of laminin-1 and aligned Schwann cells. Regenerating fibers then enter the gap and follow these Bands of Bungner, reach the distal end of the severed nerve, enter it and go on to re-innervate the original target. Conventional wisdom and experimental data both point to the fact that the modality of the regenerating fibers is mixed in this process, but that the brain re-learns to control the target tissue/organ to a great degree [12,13]. When the nerve gaps are large, the formation of the fibrin cable as well as the Bands of Bungner is compromised, necessitating exogenous support to enable the regenerating fibers to cross the large nerve gap (>15 mm in rats).

4. Rational design of scaffolds for peripheral nerve repair

Pursuing this logic, several groups have implanted natural and synthetic biomaterials, cells, microfibers, nanofibers, chondroitinase ABC digested autografts, and Schwann cells seeded in Matrigel to enhance regeneration across peripheral nerve gaps. An analysis of these various approaches reveals that 4 essential components of grafts are typically manipulated to enhance regeneration across peripheral nerve gaps. These components are the growth permissive substrates (hydrogels or nano/micro fibers), neurostimulatory extracellular matrix (ECM) proteins or peptides (typically LN-1 or LN-1 fragments), trophic factors (bFGF, NGF or BDNF), and glial cells or other support (Schwann cells or stem cells). (see Fig. 1 for schematic). The distribution of these factors in the NGCs determines if these approaches provide isotropic cues for growth promotion or anisotropic cues as described below.

5. A case for anisotropy in scaffold design

Anisotropic distribution of the four components influencing peripheral nerve regeneration may enable faster or better regeneration, by exploiting the differential response of growth cones to changes in structural (oriented scaffolds vs. non-oriented scaffolds) or biochemical features (gradients of trophic or ECM proteins). Prof. Letourneu's pioneering work suggests that growth cone extension across gradients (even if it is down an LN-1 gradient) is superior to growth across uniformly distributed LN-1 [14]. In our own laboratory we have recently demonstrated that DRG neurite extension in gradients of immobilized LN-1 was superior to that in uniformly distributed LN-1 gels, even when the LN-1 concentration was below the saturation point of LN-1 dose–response curve for the cells in question (E9 chick DRGs) [15]. This suggests that gradients exploit an innate response of growth cones that is not possible with uniform, isotropic distribution of LN-1 or trophic factors such as NGF or BDNF. Prof. Shoichet's

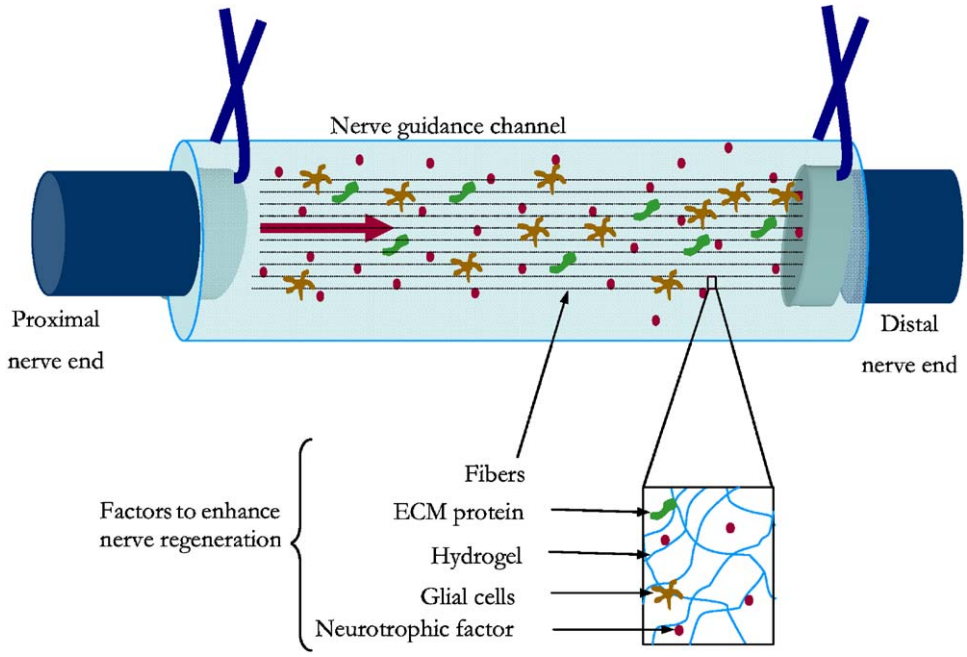

Fig. 1. A schematic illustration of the components of 'grafts' that influence peripheral nerve regeneration. The components include Scaffolds (hydrogel or fibers), ECM proteins, glial or other cells and neurotrophic factors. The spatial distribution of one or more of these components determines the degree of anisotropy of the graft.

group has elegantly shown that NGF gradients in 3D can steer growth cones and influence the extent of neurite extension [16,17]. It is possible that gradients of LN-1 and/or BDNF/NGF might exert synergy, and enable the neurostimulatory cues to be more effective than when they are distributed isotropically.

6. The third dimension: a closer look

Another important consideration in designing strategies for enhancing peripheral nerve regeneration are the kinds of approaches one takes to fill the NGCs with bioactive features (physical/structural as well as biochemical/biological). There has been a vigorous debate on the need for the development of 3D substrates/gels/scaffolds because they are more 'biomimetic'. However, in general, neurite extension on 2D surfaces, including tissue culture plates, is better than when cells are embedded in 3D substrates (although neurites that are several millimeters long can be cultured in 3D substrates). From several years of active research in the area of developing 3D constructs, this author has come to believe that the ideal constructs may involve distributing 2D-like substrates/surfaces in 3D space, suggestive of a '*Z*' direction anisotropy across the cross-section of 3D scaffolds. Gomez and Letourneau demonstrated several years ago that given a choice, growth cones will follow the preferred of 2 substrates [18,19]. This is consistent with (unpublished) observations that when culturing peripheral and central primary neurons (DRGs, retinal ganglia) as well as cell lines (PC 12 cells), in 3D scaffolds such as collagen, agarose, agarose derivatized with Laminin-1, agarose derivatized with LN-1 peptides, Matrigel, and HEMA, the cells that sink to the bottom of the well always extend the longest processes!

This observation leads this author to suggest that perhaps distributing micron- or nano-sized fibers or films in 3D gels achieves the best of both worlds, provided the fibers are growth permissive. The gels would serve to distribute the fibers in 3D space, and the fibers would provide a 2D surface for regenerating axons. Fig. 2 demonstrates a case where an E9 DRG that is embedded in a permissive agarose gel sends out a process that 'latches on' to a 25 μm nylon fiber embedded within the agarose gel, grows on the fiber, and at the tip of the fiber, the process detaches from the fiber to continue growing in the agarose gel. Therefore, when presented with a choice of nylon fiber surface and agarose gel, processes prefer the nylon surface (2D), and at the tip of the fiber, exit and continue growth within the permissive agarose gel (3D). As the purpose of 3D constructs is to enable the regeneration of a 3D axonal bundle, and to maximize the presentation of trophic/guidance cues in 3D space, distribution of fibers or other 2D surfaces in 3D space (in hydrogels for example) might offer the best of both worlds. However, in embedding fibers or films within hydrogels, it is important to consider maximizing the total cross-sectional area that is physically available to the regenerating nerve without being obstructed by the thickness of the embedded fibers or films. That is, the ideal 3D scaffold would maximize the guidance cues, while minimizing the extent of physically obstructive elements within the 3D scaffolds that are embedded in the nerve guidance channels.

7. Animal models and evaluation of regeneration

Integral to designing and characterizing the ideal engineered constructs for peripheral nerve regeneration are the animal models used, the methods of analysis that determine success, and the criteria used to define success. In rat models, it is imperative that two factors be involved, a gap greater than 15 mm, and controls involving autografts. Secondly, should regeneration in such models be successful, it is important to test the engineered scaffolds in larger animals, with gaps greater than 40 mm, to further validate the intervention strategy.

Most studies of peripheral nerve regeneration use anatomical and histological measures to determine success. It is important to include an evaluation of the quality of the regenerated nerve distal to the lesion site–for instance, the number and quality of neuromuscular junctions need to be evaluated. Equally important is the conduct of rigorous electrophysiological studies that investigate the nature of regenerated nerves, and evaluate the extent to which both sensory and motor nerve fibers regenerate.

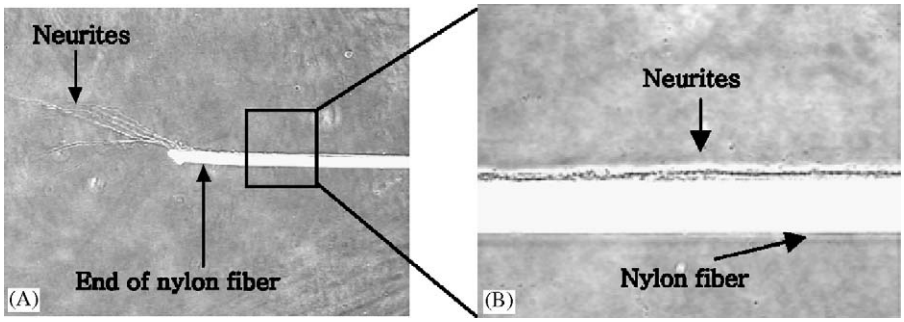

Fig. 2. Light micrograph of DRG neurites growing along laminin coated nylon fibers in 3D in 1% agarose gels. Cell body is to the right (out of the picture). (A) Lower magnification (40 ×). Neurites prefer growing on fibers, however, they continue into the gel when the fibers end. (B) Higher magnification (200 ×) of the square region in (A).

8. Conclusion

In conclusion, although a definitive engineered alternative to autografts has yet to be identified, several promising methods are approaching the performance of autografts. Engineered constructs whose design is inspired by an understanding of the distribution of structural, and biochemical features of autografts are more likely to succeed. Examples of such design include constructs that mimic autografts' anisotropic physical features, including the oriented columns of Schwann cells and laminin-1 as well as biochemical features, such as anisotropically distributed trophic factors and/or extracellular matrix elements.

Acknowledgements

The author acknowledges several former and current post-docs, research engineers and graduate and undergraduate students, including Dr. Xiaojun Yu, Dr. Youngtae Kim, Mr. Michael Tanenbaum and Mr. Mahesh Dodla. Mr. Dodla is also acknowledged for the illustrations. Funding from NIH NS44409 and GTEC, an NSF funded Engineering Research Center at Georgia Institute of Technology/Emory University is acknowledged.

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