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A systematic review of animal models used to study nerve regeneration in tissue-engineered scaffolds

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Abstract

Research on biomaterial nerve scaffolds has been carried out for 50 years. Only three materials (collagen, polycaprolactone and polyglycollic acid) have progressed to clinical use. Pre-clinical animal models are critical for testing nerve scaffolds prior to implementation in clinical practice. We have conducted a systematic review of 416 reports in which animal models were used for evaluation of nerve regeneration into synthetic conduits. A valid animal model of nerve regeneration requires it to reproduce the specific processes that take place in regeneration after human peripheral nerve injury. No distinct animal species meets all the requirements for an ideal animal model. Certain models are well suited for understanding regenerative neurobiology while others are better for pre-clinical evaluation of efficacy. The review identified that more than 70 synthetic materials were tested in eight species using 17 different nerves. Nerve gaps ranged from 1 to 90 mm. More than 20 types of assessment methodology were used with no standardization of methods between any of the publications. The review emphasizes the urgent need for standardization or rationalization of animal models and evaluation methods for studying nerve repair.

Keywords

Peripheral nerve injury; peripheral nerve repair; nerve tube; nerve scaffold; biodegradable

Introduction

The development of an artificial nerve tube as an alternative to autologous nerve grafting is a current focus of interest in peripheral nerve repair. The function of the nerve scaffold is to guide nascent axons from the proximal nerve end to the separated distal nerve stump. It may also provide a conduit for diffusion of neurotrophic and neurotropic factors secreted by the distal stump. The scaffold also provides a physical substrate for growth and prevents

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penetration of fibrous tissue into the regenerating nerve tissue [1]. Since 1880 the development and adaptation of different nerve conduits has been optimized in an attempt to generate the most favorable interaction between the injured nerve and the implanted material [2]. Improving the nervous tissue-biomaterial interface has been approached by altering surface topography, chemistry, energy and charge, as well as filling the scaffolds with different growth-promoting substances or cells [3]. The ideal synthetic nerve scaffold should have a chemical composition that does not induce an adverse body reaction, provide excellent structural features adaptable to the physiologic environment, and possess adequate strength, resistance and elasticity to allow regular motion of the muscles around the conduit without tube collapse [4, 5].

Biocompatibility and chemical-structural safety prior to clinical application is studied using *in vitro* and *in vivo* studies. *In vitro* testing is used principally as a first phase test for acute toxicity and cytocompatibility that minimizes use of animals. *In vitro* testing offers information regarding cytotoxicity, genotoxicity, cell proliferation and differentiation; it is more easily standardized and quantifiable than *in vivo* testing [6, 7]. I*n vitro* assays cannot establish tissue reaction to materials because systemic factors such as foreign body or immune system response, vascularization, oxygen and nutrient supply and waste elimination are completely absent. For these reasons animal models are essential for evaluating biocompatibility, tissue response and mechanical function of any nerve conduit prior to clinical application. *In vivo* studies in diverse animal species permit the evaluation of material over long periods of time and under clinically relevant biological conditions. Although animal models might closely mimic the mechanical and physiological human clinical conditions, one must always consider that these models represent solely an approximation of the human response to pathologic factors [8–10]. Each animal model has distinctive benefits and drawbacks when used in any experimental study of nerve injury.

Data from pre-clinical studies are also used in submissions to regulatory agencies such as the United States Food and Drug Administration (FDA) who decide whether a new device may be used in patients. Regulatory agencies look to investigators to decide whether pre-clinical models are appropriate. The investigator community therefore has a duty to provide advice about appropriate models. It is safe to assume that animal models are only used in situations where investigators believe the scaffold may have potential future human application. To try and understand whether there is any consensus on pre-clinical models for nerve scaffolds, we have reviewed the literature pertaining to animal models used in the evaluation of nerve implants *in vivo*, and provided a comprehensive summary and classification of previous studies on this topic.

Methods and Materials

Inclusion criteria

Inclusion criteria included: (1) an *in vivo* experimental study of peripheral or cranial nerve grafting; (2) an animal species used as experimental model; (3) the use of a synthetic nerve conduit including biodegradable materials, non-biodegradable materials and materials processed from biological sources (e.g. collagen); and (4) the article is written in English. We included experimental studies in which additional agents were also used (e.g., systemic administration of drugs, ultrasound or electrical impulse), as well as studies in which the synthetic implant was filled with a matrix material or a growth factor.

Exclusion criteria include: (1) the absence of a gap between the proximal and distal stump of the injured nerve; (2) the use of an autologous or heterologous tissue (vein, artery, muscle, nerve, perineurium) as a material to synthesize the nerve scaffold (3) human clinical studies.

Because of the paucity of articles available using sheep as an animal model, we included studies that used a scaffold as a sleeve without a gap between the injured stumps.

Study identification

PubMed and Scopus were systematically searched for English language papers (January 1950–December 2010) by entering the following search terms and Boolean operators: “rat”, “rats”; OR “mouse”, “mice”; OR “rabbit”, “rabbits”; OR “dog”, “dogs”; OR “cat”, “cats”; OR “sheep”; OR “pig”, “pigs”; OR “monkey”, “monkeys” as medical subject heading terms and combining them with the text AND “nerve tube”, “nerve tubes”; OR “nerve conduit”, “nerve conduits”; OR “nerve guide”, “nerve guides”; OR “nerve scaffold”, “nerve scaffolds”.

Selection of Articles

Titles or abstracts were evaluated for inclusion. When a title or abstract could not be discarded with certainty, the full text of the article was acquired.

Assessment of study quality and data extraction

Each experimental study was independently analyzed by two of the authors in order to grade the quality of the study design. This selection included the reporting of studies on the following criteria: adequacy of experimental design, quality of outcome measures, and eligibility criteria. For each eligible study, two reviewers extracted all available and relevant data for the experimental groups. These data included demographic and physical information about the animal model used (species, weight and gender); the number of animals included; the injured nerve model; the type of material used; the length of the gap and the nerve scaffold; the characteristics of the experimental groups; and the assessments performed.

Results

Description of included studies

The literature search on animal models yielded 416 studies that met the inclusion criteria (summarized in reverse chronological order in Supplement 1). Comments about articles are included in the supplementary table that may be searched by author, material, nerve or species. Eight different animal species were studied (table 1): rats (n = 308); rabbits (n = 31); mice (n = 31); cats (n = 14); dogs (n = 17), monkeys (n = 10), sheep (n = 4) and guinea pigs (n = 1). Within rodents, inbred, outbred and genetically modified species were used.

Seventeen different cranial and upper or lower limb nerves were studied (table 2). In order of frequency of use they were sciatic (n = 308); peroneal (n = 30); tibial (n = 22); facial (n = 22); median (n = 13); radial (n = 6); ulnar (n = 5); alveolar (n = 5); cavernous (n = 3); saphenous (n = 2) and hypogastric (n=2). The sural, optic, phrenic, recurrent laryngeal lingual and femoral nerves were each chosen in one study. Authors rarely gave a rationale or justification for why a specific nerve was used. Studies that were oriented towards understanding the biology of regeneration tended to use mouse models. Some nerves were used because of potential targeted clinical applications, e.g. facial or alveolar nerves.

Nerve gap length was sometimes determined by size of the animal. The length of the gap grafted by a synthetic scaffold (table 3) was between 1 and 50 mm in the rat model; between 2 and 13 mm in the mouse model; between 2 and 50 mm in the rabbit model; between 10 and 90 mm in the dog model; between 1 and 50 mm in the cat model; and between 1 and 50 mm in the monkey model. In the pig model the gap was 8 mm, and no gap was present between the distal and the proximal stump in the sheep model. Authors rarely discussed the

rationale for a specific gap length when the gap was less than 2 cm. In the larger gaps in rats (more than 2 cm) and in rabbits, dogs, cats and monkeys thee was often a discussion of the concept of a critical gap length. The critical length was usually defined as a gap across which regeneration would not occur without some form of nerve grafting or bridging.

More than 70 synthetic materials were used (table 4). These included completely synthetic degradable and non-degradable materials and materials derived from biological sources such as collagen, chitosan and silk. Within one type of biological material, for example collagen, many different ways for material extraction, processing and scaffold fabrication have been used.

In order to compare the results of studies, it would be important to have some form of standardized or comparable outcome modality. An extensive variety of experimental endpoints have been used (table 5). The most commonly used were some form of qualitative histological analysis (n=334), followed by neuromorphometry (n=209). Number of myelinated fibers, total number of nerve fibers, axon diameter, myelin thickness and g-ratio were most commonly reported from neuromorphometric studies. Here was no consistency in terms of level of nerve to be studied. The importance of tissue processing methods was rarely discussed. Electrophysiological analyses were frequently used (n=165). These included measuring the latency and amplitude of compound muscle action potentials (CMAP) or sensory nerve action potentials (SNAP). Centrally recorded somato-sensory evoked potentials were occasionally used. Comparison of results from electrophysiological studies from different research groups was rarely possible. There was little consistency in stimulation parameters, location of nerve stimulation or of recording of evoked potentials. Some form of functional assessment was used in 67 studies. This included different types of static or dynamic gait analysis, measurement of strength, e.g. voluntary activated grip strength, or specific tasks such as manipulating objects. The only functional assessment using a standardized method by many groups was the sciatic function index [11]. An index of the functional condition of rat sciatic nerve based on measurements made from walking tracks [11] in rat sciatic nerve studies. Other outcome assessments included immunohistochemistry of more than 20 different cellular markers of regeneration, muscle weight, force measurement and morphometry, sensory markers, and gene expression. Direct imaging with ultrasound was only reported in one study.

Discussion

A nerve autograft is the best way to repair a nerve gap in humans. However, limited graft availability and donor site morbidity drive investigation towards a safe, readily available nerve scaffold to repair nerve gaps [12–14]. The studies reviewed here have reached the point of pre-clinical animal testing which is a necessary step before moving to trials in patients. However, after 50 years of published research very few scaffolds with limited clinical application have reached patients [1, 15–21]. The selection of a specific animal model is critical for the design of an experimental study. Study design and conclusions drawn depend on the scientific questions asked in a specific model.

The first criterion of selection is the experimental question. The second factor to consider is the species' unique neurobiology [1] including nerve microstructure and composition, inflammatory response after nerve injury, Wallerian degeneration and capacity for nerve regeneration. Humans [22, 23]. In agreement with Schimandle and Boden [8], animal selection factors should include the cost to acquire and care for animals, availability, acceptability to society, tolerance to captivity and the ease of housing. Comply with national regulatory policies is essential and differs slightly between countries [24]. Other important criteria include tolerance for surgery, resistance to infection, inter-animal uniformity, life

span of the species and biological information and tools available. Maintenance costs and ease of handling are also important factors. Most agree that within any area of research, neither a single animal model would be appropriate for all purposes, nor could a model be rejected as inappropriate for all purposes [8–10].

The rat sciatic model has been the most commonly used (Table 1) for study of synthetic scaffolds. Rats are economical, simple to handle and care for, very resistant to surgical infections, and can be investigated in large groups. It can be used for electrophysiology, functional recovery, muscle and nerve morphology and other assessments of nerve regeneration [9, 25, 26] (table 5). A disadvantage is that relatively short gaps from 1 to 50 mm have been used and the majority have used a gap of 10 mm or less (table 3). This gap small compared with target human nerve lesions and nerve axotomy in rat undergoes complete recovery which does not occur in human nerve injuries. The difference is further complicated by the fact that the rate of peripheral axonal regeneration is slower in humans than in rodents [27, 28]. Chronically denervated distal nerve stump and target tissues become less able to support regeneration with time [29] and axons in the proximal nerve segment become less able to respond to regenerative cues when they are persistently axotomized [23, 30–32]. There are also limited genetic models and immunological probes available for rat compared with mouse. Finally different strains of rats (Lewis, Sprague-Dawley, and others) have been used and there is no information about whether different strains react differently to placement of foreign biomaterials in regenerating tissue.

The major value of the mouse model is the ability to answer mechanistic neurobiological questions. Murine models utilizing transgenic technology over the last fifteen years (Table 1) have allowed more detailed mechanistic studies of the neurobiology of regeneration. Emerging experimental transgenic mice that constitutively express fluorescent chromophores in their axons offer exciting new live imaging possibilities. [33, 34] The major shortcoming of the mouse is the small size which precludes studying axonal regeneration in gaps longer than 13 mm.

The rabbit is one of the more frequently used large animal species for peripheral and cranial nerve research (Table 3). Nerve gaps between 2 and 50 mm have been used in rabbits. Regeneration capacity has usually been assessed by neuromorphometric and electrophysiological analysis in this species. The disadvantage of the rabbit is that hopping and hind limb muscle function are very different from humans. Very few molecular probes, such as antibodies, have been developed for use in rabbits. Finally, rabbits are significantly more expensive to purchase and maintain and more difficult to care for than small rodents.

Dogs and cats have been used for nerve regeneration studies with gaps up to 90 mm (Table 3). The most common method that has been used in both species to determine regeneration capacity has been neuromorphometric analysis. Walking track analysis after orthopedic procedures has been described to measure improvement of motor function in dogs [35–37]. Dogs may be successfully trained for motor and sensory functional analyses. However, there are increasing ethical concerns about the use of dogs and cats in medical research because they are common domestic animals. Both species are expensive to purchase and maintain and there are virtually no molecular probes available for mechanistic studies.

Large mammals such as sheep, pigs and monkeys have been employed to test synthetic nerve scaffolds with gaps up to 60 (Table 3). Studies in these large species are limited by extremely high costs related to animal care, the narrow range of assessments available and the complexity of training for functional testing. Fifteen studies using non-human primates have been reported (Table 3). The ethical debate on use of nonhuman primates in medical research is important to this topic. Because of the presumed similarity between humans and

nonhuman primates, testing safety and efficacy of a synthetic nerve conduit on a small population of nonhuman primates had been considered as an appropriate step before human experimentation. This may no longer be the case given recent policy decisions about primate use [38] which was driven by a report from the Institute of Medicine [39].

Conclusions

The most striking feature of this review is the absence of a standardized, extensively recognized measurements of nerve recovery that make it difficult to compare data across the literature. Although there has been discussion about the best assessments to study nerve regeneration there is no consensus. We believe this is one of the reasons why translation of nerve tissue engineering into new therapies has been slow with few successes in more than fifty years of research. Only four absorbable synthetic nerve conduits involving three materials have obtained the US Food and Drug Administration (FDA) and the Conformity European (CE) approval for clinical use. The large majority of biomaterials used in animal models have not progressed for approval to be tested in clinical trials in spite of the almost uniform benefit described in the experimental papers. It is essential for this area of research to develop established, universally accepted, standardized models of investigation. The process to assess the safety and efficacy of any new synthetic scaffold could involve two steps; the first in an economical, presumably rodent, species and the second, a larger animal such as the sheep. Standardized methods of injury and assessments should be collectively established in the scientific community using well-established procedures available through the ASTM International or the International Organization for Standardization (ISO). The former already has animal model standards and involves a process for standard development that engages the research community. Insistence by funding agencies that standardized models be used as a justification for animal research in nerve injury would make data sharing practical and useful. It would also facilitate regulatory approval.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Animal models: an overview

Species	Rat	Mouse	Rabbit	Dog	Cat	Sheep	Monkey	Pig	Total
Number of articles	308	31	31	17	14	4	10	1	416

Table 2

Nerves used

Nerve	Rat	Mouse	Rabbit	Dog	Cat	Sheep	Monkey	Pig	Total
Sciatic	266	29	6	1	6				308
Peroneal	16		6	8					30
Tibial	15	2	3	2					22
Facial	6		9	1	4	1		1	22
Median	4					3	6		13
Radial			1		2		3		6
Ulnar				1			4		5
Alveolar	1		3		1				5
Cavernous	3								3
Saphenous			2						2
Sural	1								1
Optic			1						1
Phrenic				1					1
Hypogastric				2					2
Laringeal				1					1
Lingual					1				1
Femoral	1								1

Table 3

Gap Lengths Used

Gap (mm)	Rat	Mouse	Rabbit	Dog	Cat	Sheep	Monkey	Pig	Total
1–5	77	30	2		7		7		123
6–10	184	11	21	5	1		1	1	224
11–15	51	1	3	1	1		1		58
16–20	22		3	1			3		29
24–30	5		3	3	2		1		14
40–49			2	1					3
50–60	1		1	1	2		2		7
80				4					4
90				1					1

Table 4

Materials used for Nerve Scaffolds

Material	Rat	Mouse	Rabbit	Dog	Cat	Sheep	Monkey	Pig	Total
Acrylic copolymer	3	2	1						6
Acrylonitrile vinyl chloride copolymer		1							1
Atelocollagen	2								2
Atelocollagen/collagen	1								1
Benzyl ester of hyaluronic acid	1								1
Chitin	1								1
Chitin/chitosan	1								1
Chitosan	11								11
Chitosan/PGA	1			2					3
Chitosan/PLGA	1								1
Collagen	43	1	5	1	4		7		64
Collagen/chitosan	2								2
Collagen/PCL	1								1
Collagen/PLGA	1								1
Corglaes (CRG)						4			4
E-capronolactone-co-trimethylene carbonate	1								1
EDC/NHS crosslinked gelatin	1								1
Ethyl cyanoacrylate	1								1
Ethylene-vinyl acetate copolymer (EVA)	1	1							2
Fibrin	2								2
Fibrocollagen (FFC)			1						1
Fibronectin	1						2		3
Genipin-cross-linked gelatin (GGC)	5								5
Glycolide trimethylene carbonate (GTMC)	2						1		3
Goretex			1						1
GTR	1								1
GTR/collagen	1								1

Material	Rat	Mouse	Rabbit	Dog	Cat	Sheep	Monkey	Pig	Total
Hyaluronic acid(HA)/collagen			1						1
Hydrophilic elastomeric biopolymer	1								1
Keratin	1								1
Lactosorb	1								1
Millipore (cellulose esters)				1	1				2
Nilon					1				1
Photocured gelatine	1								1
Plastic film		2							2
Polyacrylonitrile/vinylchloride (PAN/PVC)	2								2
Polycaprolactone (PCL)	17								17
Polycaprolactone-fumarate (PCLF)	1								1
Polydioxanone	1								1
Poly[(ethylalanato)(imidazol)phosphazene] (PEIP)	1								1
Polyethylene	4	4							8
Polyglactin			2						2
Polyglycolic acid (PGA)	18	1	2	4	2		1		28
PGA/Collagen			1	6	2				9
Polyglicolic lactate	1								1
Poly-3-Hydroxy Butyrate (PHB)	17				2				20
Poly-3-hydroxybutyrate/ Poly-[glycolide-co-(e-caprolactone)](NGC)	1								1
Poly(2-hydroxyethyl methacrylate-co-methyl methacrylate) (PHEMMA-MMA)	4								4
Poly-DL-lactate (PDL)		3							3
Polylactic acid (PLA)	1								1
PLA/PLG	1			1					2
Poly-lactic acid-e-caprolactone (PLAC)			1						1
Poly-lactic-co-6-caprolactone (PLCL)	5								5
Poly-L-Lactide acid (PLLA)	7					2			9
PLLA-chondroitin-sulfate	1								1
Poly (DL-lactide+ɛ-caprolactone P(DLLA-e-CL) (PLC)	7	3							10

Material	Rat	Mouse	Rabbit	Dog	Cat	Sheep	Monkey	Pig	Total
Polylactide-co-glycolide (PLGA)	20								20
PLGA/PCL	1								1
Poly(L-lactide-co-glycolide-co-e-caprolactone) PLGC	2								2
Polymethyl methacrylate (PMMA	1								1
Polyorthoester				1					1
Polyphosphazene	2								2
Polyphosphoester	1								1
Polypropylene	1								1
Polysulfone (PS)	2								2
Polytetrafluoroethylene (PTFE)	2	1	3						6
Polyurethane	1								1
Polyurethane/Poly-lactic acid	1								1
Polyvinyl chloride (PVC)	1								1
Proanthocyanidin-crosslinked gelatine	1								1
Semipermable acrylic XM50 Amicon	3								3
Silastic	7	2	2	1					12
Silastic/bioglass fibers	1								1
Silicone	145	9	5	1	1			1	162
Silicone/cutgut filaments	2								2
Silicone/nitrocellulose (NC)	1								1
Silicone/polyamide filaments	6								6
Silicone/ polydioxanon filaments	3								3
Silicone/ polyglactin filaments	2								2
Silicone/PGA	1								1
Silicone/PLLA filaments	2								2
Silicone/polypyrrole	1								1
Silicone/polyvinylidene fluoride (PVDF)	1								1
Silk fibers	1								1
Sulfopropylacrylamide			1						1

Material	Rat	Mouse	Rabbit	Dog	Cat	Sheep	Monkey	Pig	Total
Teflon		2							2
Tricalcium phosphate/glutaraldehyde crosslinking gelatine	2								2
Trimethylen-carbonate-caprolacton polymer	2								2
Vinylidenefluoride-trifluoroethylen (VT)	1								1

Table 5

Assessment Methodology

Assessment	Rat	Mouse	Rabbit	Dog	Cat	Sheep	Monkey	Pig	Total
Qualitative Histological analysis	244	23	30	12	14	4	6	1	334
Neuromorphometric analysis	146	20	18	8	7	4	6		209
Electrophysiology	107	13	17	9	9	2	7	1	165
Immunohistology	83	2	2	4	1		3		102
Functional motor indices	65	2							67
Retrograde labelling	18	5	1	2	7				33
Functional sensory indices	17	14							31
Muscle weight	24		1			2			27
Functional autonomic indices	6	9		1					16
Muscle contraction test	7	1	1			3			12
RNA expression	8								8
Muscle morphometric analysis	3								3
Fast axonal transport assay	1				1				2
Autotomy evaluation	2								2
Ultrasound evaluation	1								1
Radiological examination				1					1
Gene expression analysis	1								1
Foot pad reinnervation	1								1
Fiberscopic observation				1					1
Calcification analysis	1								1
BrdU staininig	1								1

Supplemental material contains a listing and categorization of all papers reviewed.