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Animal models of vascularized nerve grafts: a systematic review

Francesca Toia*, Daniele Matta, Federico De Michele, Roberto Pirrello, Adriana Cordova

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Abstract

The aim of this review is to present and compare the various animal models of vascularized nerve grafts described in the literature as well as to summarize preclinical evidence for superior functional results compared to non-vascularized free nerve grafts. We also will present the state of the art on prefabricated vascularized nerve grafts. A systematic literature review on vascularized nerve graft models was conducted via the retrieval with the PubMed database on March 30, 2019. Data on the animal, nerve, and vascularization model, the recipient bed, the evaluation time points and methods, and the results of the study results were extracted and analyzed from selected articles. The rat sciatic nerve was the most popular model for vascularized nerve grafts, followed by the rabbit; however, rabbit models allow for longer nerve grafts, which are suitable for translational evaluation, and produced more cautious results on the superiority of vascularized nerve grafts. Compared to free nerve grafts, vascularized nerve grafts have better early but similar long-term results, especially in an avascular bed. There are few studies on avascular receiving beds and prefabricated nerve grafts. The clinical translation potential of available animal models is limited, and current experimental knowledge cannot fully support that the differences between vascularized nerve grafts and free nerve grafts yield a clinical advantage that justifies the complexity of the procedure.

Key Words: nerve animal models; nerve grafts; nerve regeneration; peripheral nerves; prefabricated vascularized nerve grafts; vascularized nerve grafts

Introduction

Vascularized nerve grafts (VNGs) were first described as pedicled grafts in 1945 and free grafts in 1976, as a tool to improve functional results of nerve regeneration (Restrepo et al., 1985; Kanaya et al., 1992). Compared to non-vascularized nerve grafts, they seem to lead to superior regeneration (Terzis et al., 1995). Both experimental and clinical studies suggest that VNGs have a greater regenerative potential than free nerve grafts (FNGs). In particular, VNGs are generally indicated in cases of very long or large caliber nerve defects (Restrepo et al., 1985; Shibata et al., 1988; Kanaya et al., 1992) and in the case of a traumatized and/or poorly vascularized (Taylor and Ham, 1976; Rose and Kowalski, 1985; Rose et al., 1989) receiving bed, although their role is still controversial.

Do VNGs improve functional outcomes? Is the improvement in results clinically significant? Clinically, vascularized nerve grafts are postulated to enhance nerve regeneration and provide better functional outcomes compared to free nerve grafts, especially for long defects and scarred beds. However, there is no conclusive evidence due to a lack of randomized studies comparing similar groups of patients (with similar injuries, similar defect length, similar bed, same nerve, etc.) who received either a vascularized nerve graft or a free nerve graft. To overcome this limit in the design of clinical studies, experimental studies could prove useful in providing a reproducible injury to compare the potential of vascularized and free nerve grafts. Also, VNGs show several clinical drawbacks, such as low availability of donor nerves, variability of their vascular supply, need for the sacrifice of a major vascular axis, technical difficulty, and length of the procedure. Prefabrication of VNGs and tissue engineering of vascularized nerve conduits could overcome some of these limits (Saffari et al., 2020).

Most of the experimental studies on VNGs were done during the 90s, but recently there has been a new wave of interest due to their upcoming potential applications.

The aim of this review paper is to present and compare the different animal models of VNGs described in the literature, discussing their respective values and individual drawbacks in comparing the two types of grafts' potential in a "reference" injury, which is not easy in clinical research. Also, we will present the state of the art on prefabricated VNGs, to provide a reference for future preclinical research.

Methods

A systematic literature review on VNGs models was performed. The search was performed in the PubMed database on March 30th, 2019, with "vascularized/vascularized nerve graft/grafts" as the search term (title and/or abstract field).

Papers were selected based on the abstract or full-text review (**Figure 1**). Inclusion criteria were articles in English, Italian, Spanish or French language, introducing an original or a modification of an existing animal model of vascularized nerve graft, or presenting an animal study on VNGs. Exclusion criteria included language other than English, Italian, Spanish, or French, studies not concerning experimental VNGs, and anatomical or clinical studies. Further search for relevant articles included references to selected articles (**Additional Table 1**).

Data on the animal, nerve (donor, recipient, defect length), and vascularization model, on the recipient bed (vascular/avascular), on the evaluation time points and methods, and results of the study were extracted from selected articles and analyzed.

The initial search gave back 111 papers proposing a model of vascularized nerve graft and/or comparing vascularized and non-VNGs. After abstract/full-text review, 31 papers were selected.

Extracted data are presented in **Additional Table 1**.

Results

Animals and nerves

The rat was the most commonly employed animal model in 17 studies, mainly Sprague-Dawley or Wistar, followed by rabbits in 11 studies; dogs in 2 studies, and pigs in 1 study.

In the rat model, the sciatic nerve was most commonly used, in 10 studies, followed by the femoral nerve in 5 studies. The mean nerve graft length was 1.6 cm (range: 0.7–3 cm).

In the rabbit model, the sciatic or the median nerves were most commonly used (4 studies each). The mean nerve graft size was 2.7 cm (range: 1–5 cm).

In the dog model (Townsend and Taylor, 1984; Lux et al., 1988), the saphenous nerve was used with longer nerve grafts (5 and 10 cm), while in the pig model (Taylor and Ham, 1976), the femoral nerve was used.

Recipient bed

In 21 studies, VNGs were transferred to a recipient vascular bed, namely a tissue with a good vascular supply, while in 10 studies they were transferred to an avascular recipient bed, which means a tissue previously irradiated, scarred, or burned, which are not able to provide good vascular support to

Plastic and Reconstructive Surgery. Department of Surgical, Oncological and Oral Sciences. University of Palermo, Palermo, Italy
***Correspondence to:** Francesca Toia, MD, PhD, francesca.toia@unipa.it.
https://orcid.org/0000-0003-4903-815X (Francesca Toia)

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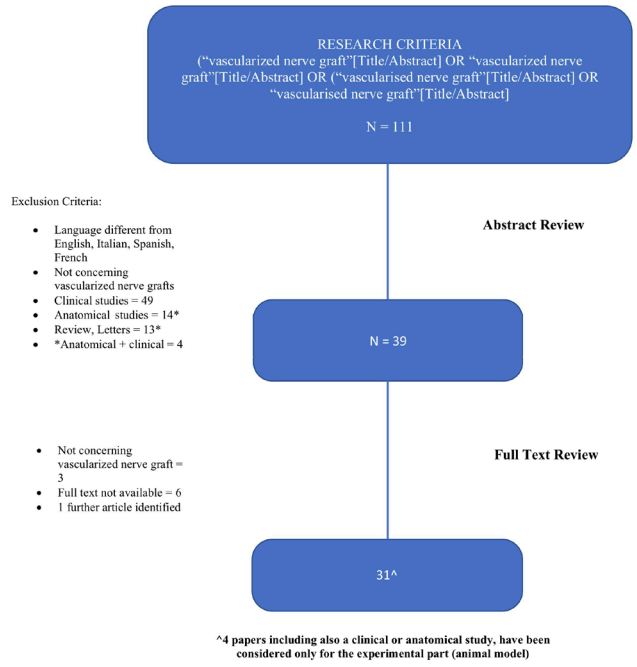

Figure 1 | **Strategy and research criteria for systematic review of the literature.**

the graft. In all but one study, the avascular bed was realized by enveloping the nerve in silicon or custom-made sheet or tube.

Prefabricated vascularized nerve grafts

Five studies reported on prefabricated VNGs or conduits; in one study vascularization was brought from an Artero-Venous loop based on femoral vessels to the sciatic nerve in its anatomical site, while in four studies a nerve graft or an amnion tube were first harvested and then implanted near the femoral vessels to receive neo-vascularization (**Figure 2**). Better results compared to non-VNGs were generally reported, but the implant of a harvested nerve graft near the femoral vessels failed to build a functional VNG in one study.

Evaluation methods

Outcomes of VNGs were generally superior to those of FNGs in rats than in rabbits; better results were reported in 10/17 or 58% of studies in rats and only in 4/11 or 36% of those in rabbits.

In most studies, nerve regeneration was evaluated by histomorphology and/or electrodiagnostic studies, while only 4/31 or 12.9% (Seckel et al., 1986; Best et al., 1993; Bertelli et al., 1996; Donzelli et al., 2016) of studies included functional tests (**Figure 3**).

The main evaluation method used was histomorphology, which was used in all but one study with no evaluation at all (model description only). Hematoxylin and eosin were by far the most popular staining in 48% of studies; with this method, nerve regeneration was deemed superior in VNGs to FNGs in 47% of the studies in the rat model but equivalent to FNGs in studies in the rabbit model. Other evaluation methods included toluidine blue in 35% of studies, Masson's trichrome in 9.6%, Streptavidin biotin (Hatoko et al., 2003), Bodian's silver impregnation (Kärcher and Kleinert, 1986), uranyl acetate (Pho et al., 1985; Matsumine et al., 2014), fluorescein dye (Ozcan et al., 1991), luxol fast blue (Pho et al., 1985; Serel et al., 2010), India Ink (Townsend et al., 1984) as alternative or in adjunct to hematoxylin and eosin staining. The number and diameter of myelinated fibers, number of large myelinated axons, and axon diameter were evaluated in most studies (Restrepo et al., 1985; Ozcan et al., 1991, 1992; Koshima and Harii, 1994; Matsumine et al., 2014) Intraneural scarring was also evaluated by Seckel et al. (1986) who reported no significant differences among VNGs and FNGs in this characteristic. Alfa-Beta-Gamma Catenins levels were evaluated in 1/31 studies (Hatoko et al., 2003).

Electrodiagnostic studies were reported in 13/31 (42 %) of studies. Functional tests instead were reported only in four studies (Kanaya et al., 1992; Best et al., 1993; Bertelli et al., 1996; Donzelli et al., 2016): walking track analysis was used in two studies (Best et al., 1993; Donzelli et al., 2016), grasping test (Bertelli et al., 1996) and gait analysis (Kanaya et al., 1992) in one study each.

Results of the histomorphologic and functional evaluation were usually concordant. In Bertelli et al. (1996) study for example showed a quicker recovery for VNGs but similar long-term results compared to FNGs.

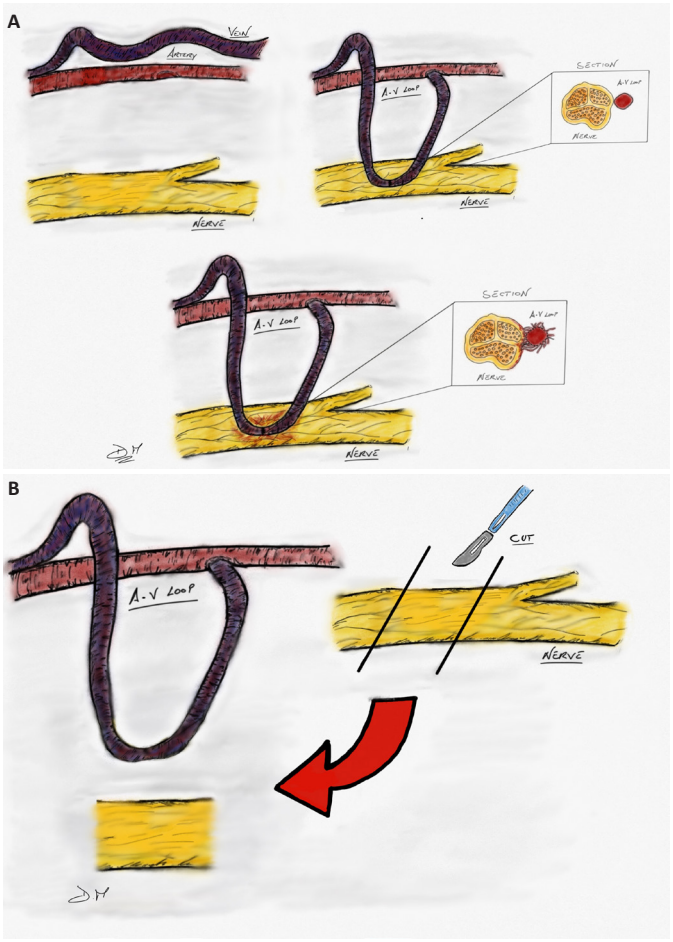

Figure 2 | **Different vascularization methods for prefabricated vascularized nerve grafts.**
(A) a vascular loop is created near a nerve in its anatomical site. (B) a nerve graft is first harvested and then implanted near the vessels to receive neo-vascularization. AV: Artero-venous. EMG: electromyography; ENG: electroneurography.

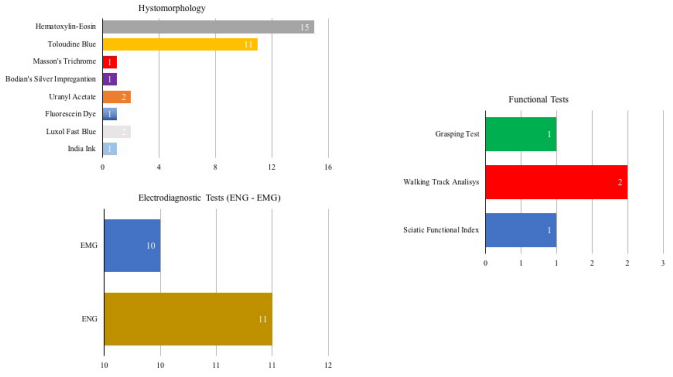

Figure 3 | **An overview of the evaluation methods used in the selected studies.**

Evaluation timing

In most studies, results were evaluated at an earlier time point (4–8 weeks) and/or a later time point (12–36 weeks). Over half of the studies (54.8%) concluded that VNGs perform better than non-VNGs, but 35.3% showed only earlier better results and comparable long-term results to non-VNGs.

Discussion

The review of the literature showed that the rat sciatic nerve is the most popular model for VNGs, followed by the rabbit; however, rabbit models allow for longer nerve grafts – more suitable for translational evaluation – and showed more cautious results on superiority of VNGs. Better early but similar long-term results are generally reported for VNGs compared to FNGs, especially in an avascular bed. Few studies in avascular receiving beds and on prefabricated nerve grafts are available.

The most popular experimental model was the rat sciatic nerve. The popularity of the rat model relays on several factors: it is easy to handle and has very low maintenance costs; nerve regeneration is similar to humans, although faster, 2 mm/day *vs.* 1 mm/day (Varejão et al., 2004). However, in rats spontaneous nerve regeneration process takes places in defect less then 4 mm (Ceballos et al., 1999). The rabbit model is slightly less popular, but allows to study long nerve defects (range 1–5 cm/average 3.1 cm), which makes them more suitable for the study of VNGs, clinically indicated for long nerve defects. Nerve grafts longer than 5 cm were reported only in the pig and in the dog model (Townsend and Taylor, 1984; Lux et al., 1988), for which ethical issues can occur.

Despite clinical indications for VNGs being limited to long nerve defects and/or avascular receiving beds, not only most studies were performed in rats with short nerve defects, but in 2/3 of the studies, a vascular bed was used, with only 10 studies investigating nerve grafts in an avascular bed (Koshima et al., 1985; Ozcan et al., 1991, 1992, 1993; Mani et al., 1992; Cavadas and Vera-Sempere, 1994; Saray et al., 2002; Vargel et al., 2009; Serel et al., 2010; Matsumine et al., 2014).

The histomorphologic evaluation was always reported, often together with electrodiagnostic studies, while functional tests were rarely performed. Despite histological and electrophysiological findings not necessarily having a clear correlation with functional recovery as reported by Kreischer et al. (1993), functional tests were performed in 3 studies (Best et al., 1993; Bertelli et al., 1996; Donzelli et al., 2016).

Generally, the results of the different evaluation methods were comparable and show slightly superior results for VNGs over FNGs in the early postoperative period, but the functional evaluation was more suggestive of comparable long-term outcomes. Bertelli et al. (1996) showed that early recovery after VNGs graft was 20% faster than conventional graft but long-term results were comparable.

Most studies showed an increase in the axonal number and diameter and the neural to connective tissue ratio. However, results vary based on the animal model, length of the nerve grafts, and vascularization of the receiving bed. For instance, in the rat, Restrepo et al. (1985) showed that VNGs at all study time points (5–15 weeks) performed better in terms of the number of axonal fibers and remyelination; Koshima and Harii (1985) showed that VNGs more effectively promote axonal regeneration and functional recovery, especially in avascular beds. Recently, Zhu et al. (2015) described a new vascularized nerve graft model for facial nerve repair with good results, in which VNGs showed better functional recovery. Conversely, Mani et al. (1992) did not show any significant differences in terms of rate and quality of regeneration of myelinated nerve fibers in long-term results (44 weeks) between VNGs and FNGs (graft length 30–40 mm) in avascular beds in a New Zealand albino rabbit animal model; they concluded that VNGs in rabbits only provide an early blood supply in the graft but do not yield a better functional outcome. However, about 20% of the studies showed superior long-term results; Hems et al. (1992) reported that VNGs performed better than FNGs in the long-term assessment of autologous peroneal nerve graft in a New Zealand Rabbit model but with limited statistical significance; conversely Donzelli et al. (2016) on the same model with autologous axillary nerve graft showed that VNGs are associated to a more rapid regeneration process, to an improvement in the quality of axonal regeneration and a faster functional recovery than FNGs but with no final functional recovery differences in the long term assessment. Functional recovery was significantly better in the VNGs group than in the FNGs group in the early-time assessments with a significant increase in the number of vessels and the regenerated axons; conduction velocity was significantly higher in the VNGs group. These differences in the short-term assessments (30 days) were, however, decreased to only 5% in the long-term assessments (90 days).

Another interesting finding in a study by Hatoko et al. (2003) is related to the activity of beta-catenin in nerve regeneration; they reported that the beta-catenin level increases following nerve grafts and could therefore be used as a recovery evaluation parameter; however, the expression of this catenin is not influenced by vascularization of the nerve graft according to the findings of Hatoko et al. (2003).

Concerning functional evaluation, Bertelli et al. (1996) suggested the use of the grasping test as an evaluation method. Donzelli et al. (2016) used the walking track analysis (indirect evaluation method) and the sciatic functional index; based on their results, Donzelli et al. (2016) supported the idea that axonal count is the best method to evaluate the effect of VNGs on peripheral nerve regeneration.

Prefabricated VNGs could potentially overcome some of the clinical disadvantages of VNGs, namely the need for a sacrifice of a vascular axis and the morbidity due to nerve harvest; however, probably as a consequence of low clinical interest, they have been poorly studied and limited scientific evidence exists; also, the models described are complex and showed variable results: some models have proved unsuccessful (Serel et al., 2010), others have shown promising results (Kanaya et al., 1992; Best et al., 1993). Cavadas et al. (1994) described a model of prefabricated vascularized nerve graft through vessel implantation in the rat; they made an artero-venous fistula in the groin area between superficial inferior epigastric vein and femoral artery and then secure the artero-venous loop to the sciatic nerve. Histologic examinations showed the formation of intraneural vessels and surrounding capillary network but functional results were not investigated. Years later, Saray et al. (2002) reported being able to preserve Schwann cells and the structure of the graft in a rabbit model by using an artero-venous bundle. Later, in 2010, Serel et al. (2010) using Saray et al. (2002) technique but in a different animal model (Wistar albino rat model *vs.* New Zeland white rabbits) showed, differently from Saray et al. (2002) results, vacuolar degeneration and myelin loss; this could be partially explained by the interruption of the axon from the body of the neuron (so deprived of trophic factors); these findings were not observed in Cavadas et al. (1994) study (Wistar albino rat model), where continuity of the axon to the body of the neuron was preserved.

Different techniques and materials have been proposed to produce prefabricated vascularized biogenic nerve conduits (Muangsanit et al., 2018); silicone or PVC tubes were mainly investigated. The synthetic conduit is generally inserted near a vascular bed or a donor nerve for a period ranging between 7 and 21 days; during this time the conduit undergoes a resurfacing with a newly formed pseudo synovial sheet, which can be used as a biogenic nerve conduit (Lundborg and Hansson, 1980) or as a nerve graft for small nerve gaps (Wolford and Stevao, 2003) after synthetic conduit removal. This technique is promising and could significantly reduce the morbidity of the donor site by replacing healthy nerve components with a biogenic nerve conduit. However, issues related to the long delay and the complexity of the procedure required to generate the conduits remain (Merle et al., 1989; Dahlin et al., 2001; Lundborg and Hansson, 2004).

Experimental prefabricated vascular nerve grafts could theoretically provide a reference experimental model for providing a blood supply to other promising and popular tools in nerve reconstruction, such as nerve conduits, allogeneic grafts, or cadaveric nerve allografts, but few studies have already focused on these targets (Merle et al., 1989; Weberet al., 2000; Dahlin et al., 2001; Lundborg and Hansson, 2004; Carlson et al., 2018).

Conclusions

No conclusive evidence can be drawn from the literature on the superiority of VNGs. Although they seem to perform better than FNGs, the advantage reduces in the long-term follow-up. Also, although desirable, no reference model can be identified from the literature in terms of animal and nerve models and evaluation methods. The most encouraging results reported in rats compared to rabbits, and the limited length of grafts that can be obtained in either model, question the appropriateness of these animal models to test the efficacy of the longer and thicker human nerves. Also, good results were often achieved in a well-vascularized bed, which yet represents an ideal situation far from the real one requiring a VNG.

At present, the clinical translation potential of these animal models is thus limited, and current experimental knowledge cannot fully support that the differences between VNGs and FNGs yield a clinical advantage that justifies the complexity of the procedure.

The results of research on prefabricated VNGs are generally not conclusive and of low interest but could acquire further perspective if addressed towards new tools for nerve regeneration, such as nerve conduits or cadaveric nerve allografts.

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Conflicts of interest: *The authors declare no conflicts of interest.*
Data availability statement: *All data generated or analyzed during this study are included in this published article and its Additional file.*

Additional file:
Additional Table 1: *Animal models and studies on VNGs.*

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Additional Table 1 Animal models and studies on VNGs

References	Animal (sex and number)	Nerve graft implant type	Recipient nerve	Experimental groups and vascularization model	Bed	Nerve defect	Time points	Evaluation method	Conclusions
Arakaki et al., 1993	New Zealand white rabbits (25) 2-2.5 kg	Median nerve (autologous)	1. Orthotopic graft 2. Median nerve	- Vein-vein-vein nerve graft (VVV) - Artery-vein-vein nerve graft (AVV) - Artery-vein-artery nerve graft (AVA) - Vascularized nerve graft based on brachial vessels	Vascular	3 cm	6 h	1. Histomorphology (Evan's blue dye): fluorescent microscope tracing of tagged albumin injected intravenously (to study microcirculation perfusion and permeability of the endoneural capillary)	The VVV graft maintained a normal vascular leakage pattern similar to that of the intact sciatic nerve.
Bertelli et al., 1996	Sprague-Dawley rats (F, 84) 220 g	Ulnar nerve (autologous)	1. Heterotopic graft 2. Median nerve	- Vascularized nerve graft - Non-vascularized nerve graft	Vascular	2 cm	95, 120, 150, 210, 360 d	1. Histomorphology (hematoxylin and eosin, true-blue aqueous solution, ATPasi histochemestry): retrograde labeling studies, Flexor carpi radial studies 2. Grasping test	Recovery with VNG was 20% faster than with conventional graft, but with no advantage in functional recovery in long term assessment.
Best et al., 1992	Lewis (RT1l) ACI (RT1a) rats (18) 260-310 g	Sciatic nerve (autologous, allogenic, syngenic)	1. Orthotopic, autologous 2. Sciatic nerve	Vascularized nerve graft based on the femoral popliteal superior muscular artery (FPSMA) Study 1: autologous graft Study 2: 1. Syngenic graft 2. Allogenic graft 3. Allogenic immunosuppressed graft	Vascular	3 cm	14 wk	Study 1: perfusion assessment: plastic monomer injection and intravascular fluorescence Study 2: 1. Histomorphology: toluidine blue,plastic monomer,evans blue labeled albumin ; morphometry(fiber number and diameter) 2. Electrophysiology: conduction velocity, motor latency, amplitude. 3. Functionality: walking track analysis	The vascularized immunosuppressed allograft showed similar results to the vascularized syngeneic graft. Both were superior to the vascularized allograft without immunosuppression. This model allows comparison of neural function through grafts between animals of known histocompatibility differences
Cavadas et al., 1994	Albino Wistar rat (M, 15) 200-250g	Sciatic nerve (autologous)	1. Orthotopic graft 2. Sciatic nerve	-Prefabricated graft based on a vascular loop (epigastric veins on femoral vessels)	Avascular (silicon sheet)	2 cm	5 wk	1. Histomorphology (hematoxylin and eosin staining) 2. India ink injection (to show nerve blood supply)	This model allows prefabrication of a VNG. At 5 weeks all nerve were neovascularized and remained viable after free transfer.
Donzelli et al., 2016	New Zealand rabbits (M, 20) weights not reported	Axillary nerve (autologous)	1. Heterotopic graft 2. Sciatic nerve	- Vascularized nerve graft based on axillary artery - Non-vascularized nerve graft.	Vascular	-	1, 3 mon	1. Histomorphology (hematoxilin- eosine, toluidine blue): mean axonal count, axon caliber, myelin thickness, number of myelinated fiber, mean axonal diameter 2. EMG and ENG: CMAP, amplitude and latency of signals, NCV 3. Walking track analysis, SFI	VNGs are associated to a more rapid regeneration process and to a faster functional recovery than FNGs. However, the final functional recovery in the long term assessment is not significantly different.
Hatoko et al.. 2003	Wistar rats (M, 50) 250-300 g	Sciatic nerve (autologous)	1. Orthotopic graft 2. Sciatic nerve	- Vascularized nerve graft based on femoral vessels. - Non-vascularized nerve graft	Vascular	1.5 cm	4, 14 wk	1. Histomorphology (hematoxilin-eosine, streptavidin biotin) 2. Western blot analysis: detection of alpha, beta, gamma catenin expression 3. Histochemical: biotiny-lated goat anti-mouse IgG and streptavidin conjugated with horseradish peroxidase	The level of beta catenin increased during nerve regeneration in both the VNGs and FNGs, while the level of alfa and gamma catenins did not increase in both grafts. There was no difference in the levels of the three catenins between the two methods of nerve grafts. This study suggests that beta catenin may play a different role from alpha and gamma for nerve

									regeneration, and that the expression of these catenins is not influenced by the vascularization of the nerve graft.
Hems et al., 1992	New Zealand white rabbit (18) 3-3.5 kg	Peroneal nerve (autologous)	1. Orthotopic graft 2. Peroneal nerve	- Free nerve grafts - Vascularized nerve grafts based on a gluteal artery branch - Freeze-thawed muscle autografts	Vascular	5 cm	36 wk (250 d)	1. Histomorphology: total myelinated nerve fiber counts, fiber and axon diameter) 2. EMG and ENG: sensory receptive area, isometric myogram for the extensor digitorum	VNGs performed better than FNGs (limited statistical significance)
Iwai et al., 2001	Fischer strain rats (140), 180-220 g	Sciatic nerve (syngeneic)	1. Orthotopic graft 2. Sciatic nerve	-vascularized nerve graft based femoral artery and vein (end-to-end anastomosis) -free nerve graft	Vascular	1.5 cm	2, 4, 6, 8, 12, 16, 24 wk	1. Histomorphology (hematoxylin and eosin staining,) 2. EMG: evoked potentials gastrocnemius muscle 3. Other: CAT activity, wet weight of the gastrocnemius muscle	VNG showed better early outcomes, but no significant advantage after 6 weeks.
Kanaya et al., 1992	Sprague-Dawley rats (F, 75) 250 g	Sciatic nerve	1. Orthotopic graft 2. Sciatic nerve	- Vascularized nerve graft based on the caudal femoral vessels - Non-vascularized nerve graft - End-to-end repair	Vascular	2.5 cm	4-36 (every 1-2 wk)	1. Histomorphology (tolouidine blue staining) 2. Functional assessment and behaviour: gait analysis, sciatic function index. 3. EMG and ENG: contraction force, NCV, peak amplitude of the action potential, CAPA, axonal count.	This study shows that VNGs is functionally superior to a FNGs in a normal recipient bed.
Kärcher et al., 1986	Sprague-Dawley rats (10) weights not reported	Femoral nerve (autologous)	1. Etherotopic graft 2. Sciatic nerve	- Vascularized nerve graft based on an arteriovenous fistula (femoral artery to vein) - non-vascularized graft	Vascular	1.5 cm	1, 2, 3, 4, 5 mon	Histomorphology (hematoxylin and eosin, trichrome-masson, Bodian's silver impregnation)	VNGs showed more rapid and better regeneration
Koshima et al., 1985	Sprague-Dawley rats (75) 250-350 g	Sciatic nerve (autologous)	1. Orthotopic graft 2. Sciatic nerve	- vascularized nerve grafts based on caudal femoral vessels - free nerve grafts	Vascular	1.5 cm	1-24 wk	1. Histomorphology (toluidine blue staining): diameters of myelinated axons, fiber caliber, number of myelinated fibers, number of large myelinated axons) 2. EMG and ENG: evoked potentials gastrocnemius muscle, MNCVs	VNGs appear to yield better functional outcomes.
Koshima et al., 1985	Wistar strain rats (M, 39) weights not reported	Sciatic nerve	1. Orthotopic graft 2. Sciatic nerve	- Vascularized graft based on the ascending branch of the caudal femoral vessels - Non-vascularized nerve graft Right sciatic nerve with three different blood supplies vs FNG	Avascular (silicon tube)	1.5 cm	1-24 wk	Histomorphology (toluidine blue): diameter of myelinated axons, number of myelinated nerve fibers, number of myelinated axons larger than 5 microm.	VNGs showed more rapid axonal recovery, especially in the earlier postoperative period.
Lux et al., 1988	Mongrel dogs 10-15 kg	Saphenous nerve (autologous)	1. Orthotopic graft 2. Saphenous nerve	- Vascularized saphenous nerve graft based on saphenous vessels - Non-vascularized nerve graft	Vascular	5 cm	1, 3, 7, 14 d (histologic study) 0, 1, 3, 6 d (microsphere study)	1. Histomorphology (hematoxylin and eosin) 2. Blood flow study: radioactive microsphere.	This model allows evaluating blood flow qualitatively and quantitatively. VNGs show an advantage in blood flow during the first post-operative days.
Mani et al., 1992	New Zealand albino rabbit (M/F 76)	Sciatic nerve (autologous)	1. Orthotopic graft 2. Sciatic nerve	- Vascularized nerve grafts based on femoral perforators - Free nerve grafts	Vascular Avascular	3-4 cm	Short term evaluation (2, 5, 9, 14	1. Histomorphology (methylene blue azure II staining): myelinated fiber diameter, frequency distribution, thickness of the	In the long term, the rate, size, and myelination of regenerating nerve fibers through VNGs and FNGs did

	3.5 kg			in a vascular and avascular graft bed	(silicon sheet)		d) Long term evaluation (44 wk)	myelin sheath, axon diameter) 2. EMG and ENG: conduction distances, latency, amplitude of motor action potentials. 3. Angiography: presence or absence of blood vessels, revascularization patterns, rate of longitudinal revascularization	not differ significantly, despite the absence of blood supply to the latter in the initial stages.
Matsumine et al., 2014	Lewis rats (7) weights not reported	Median nerve (autologous)	1. Heterotopic graft 2. Facial nerve	- Vascularized island median nerve based on the median artery and vein - Non-vascularized nerve graft	Avascular(silicone tube)	0.7 cm	30 wk	1. Histomorphology (toluidine blue, uranyl acetate, lead stain solution): number of myelinated fibers, regeneration of axon, myelin thickness, axon diameter. 2. ENG: CMAP	This study developed a rat model of vascularized median nerve transplantation to the buccal branch of the facial nerve. It showed that VNGs more effectively promoted axonal regeneration and functional recovery than the FNGs.
Ozcan et al., 1990	Sprague-Dawley rats (F, 30) 250-300 g	Femoral nerve (autologous)	None	-1-cm vascularized nerve graft model 4 subgroups: 1. A-V fistula 2. no fistula 3. no-blood flow 4. control -2-cm vascularized nerve graft 1. A-V fistula 2. no fistula	Avascular (silicone tube)	1 cm 2 cm	1 wk	1. Histomorphology (fluorescein dye) 2. Microangiography	This model showed that, when distally ligating the femoral vessels without the creation of an A/V fistula, blood flow into the nerve segment remains uncompromised and can be used as VNGs model.
Ozcan et al., 1991	New Zealand rabbits (11) 4-4.5 kg	Median nerve (autologous)	1. Heterotopic graft 2. Facial nerve	Vascularized nerve graft model based on brachial vessels with an distal A-V fistula.	Vascular	1 cm	3 mon	1. Histomorphology (hematoxylin and eosin, tolouidine blue): myelinated axons count; 2. Microangiography: micropaque perfusion	This study introduce an heterotopic VNG model, with a graft diameter similar to that of the reconstructed nerve.
Ozcan et al., 1992	New Zealand white rabbits (F, 15) 4/4.5 kg	Median nerve (autologous)	1. Heterotopic graft 2. Infratemporal facial nerve	- Vascularized nerve graft based on an arteriovenous fistula (brachial vessels) - Non-vascularized nerve graft - No repair group	Avascular (bony bed)	1 cm	3 mon	1. Histomorphology (hematoxylin and eosine, toluidine blue,uranyl acetate): muscle fiber diameter, number of myelinated and unmyelinated nerve fibers, total of myelinated axons, myelin sheath thickness 2.EMG and ENG: insertional and spontaneous activity, peak amplitude and latency	Bone as a recipient bed for a nerve graft is far less than optimal Mean axonal counting, nerve conduction and morphometric muscle study results were better in the VNGs group, but differences were not statistically significant. Morphometric nerve analysis differences between the two groups were found to be significant.
Ozcan et al., 1993	Lewis rats (31*) 250-300g. * in which only 10 nerves repaired with VNGs and 10 nerves repaired with NVNGs	Femoral nerve (autologous)	1. Orthotopic graft 2. Femoral nerve	- Prefabricated vascularized amnion tubes Study 1: amnion tubes implant (subcutaneous, near femoral or epigastric vessels) Study 2: 1. Vascularized amnion conduit based on an inferior epigastric vessel	Avascular(silicone sheet)	1 cm	3 mon	1. Histomorphology (hematoxilin-eosin, toluidine blue): axonal counting, fiber diameter, myelin sheath thickness. 2. Microangiography: micropaque perfusion.	The vascularized amnion conduits showed comparable nerve regeneration to VNGs, and superior nerve regeneration when compared to non-vascularized amnion conduits and FNGs.

				pedicle. 2. Non-vascularized amnion conduit group 3. Vascularized nerve graft group based on femoral vessels 4. Non-vascularized nerve graft 5. Control group					
Pho et al., 1985	White rats (18) 200 g	Femoral nerve (autologous)	1. Ortothopic graft 2. Femoral nerve	- Vascularized nerve grafts based on femoral vessels - Free nerve grafts	Vascular	2 cm	2, 4, 6, 12 wk	Histomorphology(haematoxylin-eosine, phosphotungstate alum haematoxylin, Wilder's, Masson trichrome, Lucol fast blue, Palmgren): vascularization, collapse of reticulum framework, axonal regeneration, remyelination	There was no difference in the degree of vascularization, reticulin framework collaps, rate and extent of axonal regeneration and remyelination between the two groups.
Restrepo et al., 1985	Rabbits (18) weights not reported	Sciatic nerve (autologous)	1. Orthotopic graft 2. Sciatic nerve	- Vascularized nerve graft based on a proximal vascular pedicle. - Non-vascularized nerve graft	Vascular	4.5 cm	5 -15 wk	Histomorphology (Blue II): thickness of myelin sheath, regenerating axons, vascularization, diameter of fiber	VNGs showed earlier myelinizaton and better fiber maturation (superior number and great diameter). No functional study)
Saray et al., 2002	New Zealand white rabbits (20) 2.5-3 kg	Sciatic nerve (autologous)	1. Heterotopic graft (femoral region) 2. Sciatic nerve	- Prefabricated vascularized nerve grafts based on femoral vessels - Free nerve grafts (femoral vessels ligated proximally and distally)	Avascular (custom-made tube)	3.5 cm	3, 7, 14 d	1. Histomorphology(haematoxylin eosin) 2. Microangiography	This model of prefabricated nerve graft does not require microvascular anastomosis for the arteriovenous fistula. The PVNG exhibited neovascularization and preserved viability of the Schwann cells.
Seckel et al., 1985	Sprague-Dawley rats 225-250 g	Sciatic nerve (autologous)	1. Orthotopic graft 2. Sciatic nerve (peroneal fascicle)	- Vascularized nerve graft (nerve transected and sutured through an epineural window) - Non-vascularized nerve graft (epineurium dissected away)	Vascular	1 cm	3, 4, 6 wk	Histomorphology (toluidine blue dye): axonal counts, remyelination of the axons, total fiber area, fibrosis and intraneural scarring.	This study failed to show difference in the number of regenerated axons or in the amount of intraneural scarring between the two groups. (
Serel et al., 2010	Wistar albino rats (10) 200-250 g	Sciatic nerve (autologous)	1. Etherotopic graft (femoral region) 2. Sciatic nerve	Prefabricated vascularized nerve grafts based on femoral vessels	Avascular (silicon sheet)	1.5 cm	4 wk	1. Histomorphology (haematoxyline-eosine, luxol fast blue staining) 2. EMG: evoked compound action potentials	This model does not allow to build a functional VNG.
Shibata et al.,1988	New Zealand white rabbits (43) 2 kg	Median nerve (autologous)	1. Orthotopic graft 2. Median nerve	- Vascularized nerve graft based on brachial artery and vein. - non-vascularized nerve graft	Vascular	3 cm	10, 24 wk	1. Histomorphology (osmic acid): axon numbers, mean axon diameter, muscle weights, 2. EMG and ENG : NCV, muscle contraction force.	No statistical difference was indicated in this comparison of VNGs with FNGs for most measurements. Muscle strength was superior (20%) for VNGs, but may be clinically not significant.
Tada et al., 2001	Wister rats (M, 60) 250-300 g	Sciatic nerve (autologous)	1. Orthotopic graft 2. Sciatic nerve	- vascularized nerve grafts based on caudal femoral vessels - free nerve grafts	Vascular	1.5 cm	20 wk	1. Histomorphology (toluidine blue staining) 2. Western blot analysis and level of E caderine expression 3. Immunofluorescent staining (E caderine)	The level of E- cadherin expression was significantly higher in VNGs than in FNGs, and may affect rapidity of nerve regeneration.
Tark et al.,	New Zealand	Sciatic nerve	1. Orthotopic graft	- vascularized nerve graft based on the	Vascular	4 cm	2, 3, 4 mon	Histolomorphology (toluidine blue):	Morphometric comparisons of

2001	white rabbits (33) weights not reported	(autologous)	2. Sciatic nerve	inferior gluteal vessels - non-vascularized nerve graft				myelinized fiber distribution, vascularization, nerve fiber diameter, Schwann cells	axonal regeneration showed better results in VNGs.
Taylor et al., 1976	White pigs 15-20 kg Patients (data not extracted)	Femoral nerve (autologous)	1. Orthotopic/heterotopic graft 2. Femoral nerve (homo or contralateral side)	pedicled and free vascularized femoral nerve graft based on femoral vessels (sectioned distally or proximally and distally and re-anastomosed for free transfers)	Vascular	6 cm	2 wk	Macroscopic examination: pulsation and bleeding	They describe a model of island/free vascularized femoral nerve graft
Townsend et al., 1984	Human cadavers (13) (data not extracted) Greyhound dogs (15) weights not reported	Saphenous nerve	Orthotopic	- Saphenous artery nerve with/without vein, taken as a unit (vessels anastomosis) - Non-vascularized nerve	Vascular	10 cm	6-12 wk	Histomorphology: india ink, fluorescein	The composite vascularized grafts were associated with more rapid axonal regeneration and remyelination.
Vargel et al., 2009	Wistar albino rats (M,40) 250-3000 g	Femoral nerve (autologous)	1. Orthotopic graft 2. Saphenous nerve	- Prefabricated venous nerve graft (saphenous nerve and femoral vein) - Flow through venous nerve graft (proximally and distally ligated femoral artery, saphenous nerve and femoral vein transected and repaired) - Arterialized venous nerve graft (proximally and distally ligated femoral vein, saphenous nerve and femoral artery transected and anastomosed through the a femoral vein graft) - Free nerve graft (vessels wrapped)	Avascular (silicon sheet)	1.5 cm	10 wk	1. Historphology: (hematoxyline eosine, masson's thricrome staining): density of myelinated fibers, fiber diameter, axon diameter, myelin thickness 2. EMG and ENG: NCV, amplitude of the negative peak, peak-to-peak amplitude, total area of CNAP	Flow through venous nerve graft and arterialized venous nerve graft showed the better results, with all VNGs performing better results than conventional nerve grafts.
Zhu et al., 2015	New Zealand White rabbits (18) 2.5-3 kg	Central Auricular nerve (autologous)	1. Heterotopic graft 2. Facial nerve	- Vascularized nerve graft based on auricular vessels - Non-vascularized nerve graft - Vascularized and non-vascularized nerve graft based on auricular vessels	Vascular	2 cm	4 mon	1. Histomorphology (hematoxylin and eosin): myelinated nerve fiber density, mean diameter of regenerated myelinated nerve. 2. Immunohistochemistry (mouse anti 200kDA neurofilament heavy antibody, mouse anti CD31 antibody, anti-rabbit polyclonal antibody) 3. ENG: NCV, action potential's velocity, amplitude, and latency	This study introduces a new vascularized nerve graft model for facial nerve repair. VNGs showed better functional recovery and more regenerated axons.

AVA: Artery-vein-artery; CMAP: compound muscle action potential; CNAP: compound nerve action potential; EMG: electromyography; ENG: electroneurography; FPSMA: femoral popliteal superior muscular artery; MNCV: motor nerve conduction velocity; NCV: nerve conduction velocity; VVV: vein-vein-vein.