Safety and efficacy evaluation of a human acellular nerve graft as a digital nerve scaffold: a prospective, multicentre controlled clinical trial

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Abstract

This study developed a human acellular nerve graft (hANG) as an alternative to autogenous nerve and reports on its safety and efficacy. There were two groups comprised of 72 patients that received digital nerve repair with hANG (test) and 81 that received conventional direct tension-free suture repair of the nerve defect (control). The efficacy of the treatment was evaluated by static 2-point discrimination (s2PD) and Semmes-Weinstein monofilament testing. Safety was evaluated by local wound response and laboratory testing. Mean age of patients in the test group was 33.0 ± 11.1 years (range 18-61 years) and in the control group 36.9 ± 13.4 years (range 15-77 years) (p = 0.0470). Mean time from injury to repair in the test group was 23.7 ± 52 days (range 0-200 days) and in the control group 1.5 ± 10.4 days (range 0-91 days) (p = 0.0005). Mean length of nerve graft was 1.80 ± 0.82 cm (range 1-5 cm). All surgeries were performed successfully and without complications. The excellent and good rate of s2PD in the test group was 65.28% and 95% CI was 51.98-78.93%. s2PD in the test group improved over time and average distance was 12.81 ± 5.99 mm at 6 months postoperatively. No serious adverse or product-related events were reported. These results indicate that hANG is a safe and effective for the repair of nerve defects of 1-5 cm in size. © 2015 The Authors. Journal of Tissue Engineering and Regenerative Medicine published by John Wiley & Sons, Ltd.

Keywords allogenic nerve; digital nerve; nerve transplantation; tissue engineering; extracellular matrix; nerve regeneration

1. Introduction

Damage to peripheral nerves can lead to significant morbidity in the ability to manipulate and sense the environment. With neurotmesis (complete nerve division), there is no spontaneous return of nerve function. Thus, surgical repair is used to approximate nerve ends and restore motor and sensory function. End-to-end neurorrhaphy is the treatment of choice for the reconstruction of severed nerves when the segmental defect is amenable to direct repair. However, in some cases of radical tumour resection and traumatic soft tissue defects, direct nerve repair is impossible because of the presence of a large segmental defect. The gold standard for the reconstruction of segmental nerve defects is an autogenous nerve graft because it can provide tension-free nerve continuity without adverse effects at the graft site. However, although autogenous nerve grafts have been widely used, donor site morbidity remains inevitable (Millesi et al., 1976; Rappaport et al., 1993; Staniforth and Fisher, 1978; Terzis et al.,

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1997). Moreover, if the nerve being considered for reconstruction is a genuine sensory nerve, it is doubtful whether sacrificing a donor sensory nerve is a reasonable option. These considerations have led investigators to find a suitable nerve graft substitute.

Recent studies have shown that an acellular nerve prepared by chemical extraction is cell-free; thus, immunogenicity is greatly reduced while the scaffold structure of the extracellular matrix is retained. When used to bridge nerve defects, it can guide, support and promote nerve regeneration (Hudson et al., 2004). Animal experiments have shown excellent outcomes of peripheral nerve defect repair using autologous acellular nerve graft (Zhang et al., 2010; Zhong et al., 2007). Our research has been focused on nerve graft materials for more than 20 years. We developed a human acellular nerve graft (hANG) using human peripheral nerve as raw material and it was demonstrated to be safe and reliable in the repair of peripheral nerve defects in monkeys (Hu et al., 2007, Wang et al., 2008; Wang et al., 2010). However, clinical experience is still limited. As a result, the purpose of the current study was to examine the clinical safety and efficacy of using hANG to repair digital nerve defects.

2. Methods

This clinical trial was conducted in four centres after being approved by the ethics committees of each centre. After discussion with senior experts in the field, we designed this study as a prospective, multicenter, parallel and non-inferiority clinical trial, and used direct suture repair of the injured nerve for the control group. When the nerve being considered for reconstruction was a genuine sensory nerve, it became doubtful whether sacrificing the donor sensory nerve was a reasonable option. The standard approach is to use autologous nerve grafting; however, donor site complications such as neurological dysfunction, neuroma formation and scar formation can occur. Thus, in clinical practice, a digital nerve defect in most patients is sutured directly by full isolation of both ends of the damaged nerve and by extreme flexion of the interphalangeal joint. However, this method often results in greater tension on the sutured nerve, which can adversely affect outcomes. Thus, in the current clinical trial, digital nerve repair by harvesting autologous sensory nerve with the purpose of setting up a control was not only unacceptable for patients but was also a violation of ethical requirements for clinical trials.

2.1. Patient recruitment

Patients were included if they met the following criteria: 1) had digital nerve injury; 2) were 14-80 years of age; 3) required direct suturing or had a nerve defect that was 1-5 cm in length and required nerve transplantation; 4) provided informed consent; and 5), duration of the injury was < 6 months. Exclusion criteria were: 1) acute infection, severe wound contamination, unstable vital signs and inability to conduct a functional assessment of nerve repair due to damage to the skin; 2) neurological and other diseases such as diabetes that could potentially affect the nervous system; 3) chronic diseases including gout and collagen vascular diseases, alcoholism, liver impairment and renal dysfunction; 4) inability to comply with treatment, post-operative rehabilitation and follow-up; and 5) a defect > 5 and < 10 mm. Withdrawal criteria were: 1) patients diagnosed with neurological or autoimmune disease during the trial and 2) patients who did not meet the inclusion criteria that were included by error. All patients provided informed signed consent to participate in the trial.

2.2. Sample size calculation

If the satisfaction rate of direct sutureting was 90% according to the Semmes-Weinstein (SW) monofilament test and the non-inferiority clinical standard was ± 15% compared with the control group using as a statistically significant level of 5% (both sides) and a degree of certainty (power) of 80%, then a minimum of 63 cases was needed in each group. Considering a 5% dropout rate, we expected to enroll 70 patients in each group.

2.3. Scaffold preparation

Fresh peripheral nerves were harvested from traumatically amputated upper limbs. Median, ulnar, superficial radial, common digital and proper digital nerves were collected from a location above the elbow. The median and ulnar nerves were isolated longitudinally in accordance with the anatomy of the nerve bundles and bundles with diameter similar to the proper digital nerve were harvested. Informed consent was obtained from each donor and the protocol was approved by the Ethics Committee of the First Affiliated Hospital, Sun Yat-Sen University. All nerve donors were screened for the following: hepatitis B by HBsAg, HBeAg, HBeAb and HBCAb; hepatitis C by HCV-IgG and IgM; syphilis by treponema pallidum particle assay (TPPA), rapid plasma reagin test (RPR) and toluidine red unheated serum test (TRUST); AIDS by anti-HIV antibody testing; and type I HIV by fluorescence RNA qualitative analysis by nested fluorescence PCR. If any of the above showed positive, donor nerves were not used. In addition, the extracted decellular nerves were subjected to cobalt-60 irradiation (25 kGy γ irradiation). After inactivation, nerves were sent to the HIV/AIDS National Confirmation Laboratory for HIV detection and to the Bioactive Substances and Virus Safety Testing Laboratory at the National Center of Biomedical Analysis for the detection of pseudorabies virus (PRV), bovine viral diarrhoea virus (BVDV) and porcine parvovirus (PPV). All test results were negative.

A modified protocol based on the Sondell method (Sondell et al., 1998) was adopted for scaffold preparation. Briefly, donor nerves were shaken in deionized distilled water for 6 h and then the water was replaced by 46 mM Triton X-100 in distilled water. After agitation for 24 h, the
scaffolds were rinsed 3 times with 10 mM phosphate-buffered saline (PBS) solution (10 min per rinse). The nerves were then shaken for another 24 h in 96 mM sodium deoxycholate dissolved in deionized distilled water. These steps were repeated again before a final wash with 10 mM PBS solution. The ratio of tissue to solution was 1:100 (v/v). All steps were performed at room temperature and scaffolds were subsequently stored in 10 mM PBS at 4 °C.

2.4. Surgical procedure

Three sizes of hANGs (ZDMED-1520, -1540 and -1560) were used. They were 1.5 ± 0.5 mm in diameter and 20 ± 1.0, 40 ± 1.0 and 60 ± 1.0 mm in length, respectively (Figure 1). The damaged nerve was exposed by conventional methods. Nerve stump debridement was performed until the normal nerve papilla was exposed. Nerve epineurium and bundles were trimmed to facilitate suturing. When the nerve stump retracted following isolation, measurement of diameter and length of defect was performed after normal tension was restored. After identification of the lesion, direct suture was performed (when defect was 5 mm) or a suitable graft (when defect was > 10 mm) was unpacked and rinsed in saline for 20 min. Once the graft was cut to the required length, it was then used to bridge the defect area with tension-free end-to-end anastomoses with a 9-0 interrupted suture under a 10x microscope. Finally, the implanted graft was placed on a tissue bed with good blood supply and covered with healthy soft tissue. The concomitant fracture and injured tendons and vessels, when present, were repaired simultaneously. For nerve defects of 5-10 mm, direct suturing was performed by full isolation of both ends of the damaged nerve and by extreme flexion of interphalangeal joint. This type of repair placed greater tension on the anastomosis and thus, these patients were not included in the control group.

2.5. Follow-up

Patients were hospitalized for at least two weeks until sutures were removed. Patients were followed-up at 1, 3 and 6 months after discharge. Dropout was defined as a patient not completing the 6-month follow-up period. None of the patients received immunosuppressive therapy. The evaluation of safety included local and systemic reactions. Evaluation of local reactions included duration and extent of wound swelling and pain, volume and colour of exudates and duration of exudation. Evaluation of the systemic reaction included body temperature, routine blood tests, liver and renal function tests, immune function testing (CD4/CD8 levels), erythrocyte sedimentation rate, C-reactive protein, HIV antibody test, hepatitis testing and syphilis test. Related adverse events were recorded as well.

Efficacy evaluation included static 2-point discrimination (s2PD) measured by a Touch-Test two-point discrimination and Semmes-Weinstein (SW) monofilament examination by Touch-Test™ Sensory Evaluators (specifications: 6.65, 4.56, 4.31, 3.61 and 2.83). SW and s2PD testing was conducted by a third party; the contralateral digital nerve was blocked during examinations. Physicians administering the tests were blind to whether patients were in the control or experimental groups. Interpretations of the classification of sensory recovery are shown in Tables 1 and 2. When performing monofilament testing of pressure threshold, we defined 2.83, 4.31 and 3.61 as satisfactory and 4.56 and 6.65 as unsatisfactory results (Table 3).

2.6. Statistical methods

Data were analyzed statistically by an independent data management service (Division of Biometrics, National Center for Cardiovascular Diseases, Peking, China).

Figure 1. Human acellular nerve graft (hANG)

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Table 1. Classification of sensory recovery

| Grade | Recovery of sensation | s2PD (mm) | m2PD (mm) |
|-------|-----------------------|-----------|-----------|
| S0    | No recovery of sensation in the autonomous zone of the nerve |           |           |
| S1    | Recovery of deep cutaneous pain sensation within the autonomous zone of the nerve |           |           |
| S1+   | Recovery of superficial pain sensation |           |           |
| S2    | Recovery of superficial pain and some touch sensation |           |           |
| S2+   | As in S2, but with over-response |           |           |
| S3    | Recovery of pain and touch sensation with disappearance of over-response |           |           |
| S3+   | As S3, but localization of the stimulus is good and there is imperfect recovery of 2PD (7-12 mm) |           |           |
| S4    | Complete recovery |           |           |

Table 2. Modification of the Mackinnon-Dellon scale for stratification of 2PD results*

| Classification | Mackinnon-Dellon scale | s2PD (mm) | m2PD (mm) |
|----------------|------------------------|-----------|-----------|
| Excellent      | S4                     | ≤ 6       | ≤ 3       |
| Good           | S3+                    | 7-15      | 4-7       |
| Poor           | S3 and below           | ≥ 16      | ≥ 8       |

*Weber RA, Breidenbach WC, Brown RE, et al. 2000; A randomized prospective study of polyglycolic acid conduits for digital nerve re-construction in humans. Plast Reconstr Surg 106:1036-45.

2.6.1. Analysis sets

Full analysis set (FAS). The intention-to-treat (ITT) principle was used to establish the full set of subjects. For subjects not included in the efficacy evaluation, last observation carried forward (LOCF) and worst observation carried forward (WOCF) principles were used to transfer the missing data.

Per-protocol set (PPS). The sub-group remaining after exclusion for breach of protocol according to exclusion criteria. Safety set (SS). Subjects receiving the product therapy, and at least one safety evaluation of the subject set. Efficacy analysis was based on PPS, demographic analysis was based on FAS, and safety analysis was based on SS.

2.6.2. Statistical analysis and evaluation

Enrolment and completion status. Enrolment at each centre was identified and all subjects from each set were analyzed. Dropout and eliminated cases were listed. Efficacy analysis. Units for analyses were the patient and neural levels, separately. For analysis by subject, we chose the nerve whose functional recovery was the worst. Count data between groups was compared by McNemar’s test. Other indicators of efficacy between the two groups were the same as baseline demographic analysis. Groups were compared using the adjusted centre effect, the Cochran-Mantel-Haenszel chi-square test, the 95% confidence interval (CI) estimate of the SW test satisfaction rate and the s2PD excellent and good rates; differences between groups were also calculated. The unit for analysis was the patient. We compared and described laboratory examinations from the trial group before and after treatment to study trends in changes. All statistical analyses were two-tailed and evaluated at a significance level of \( p = 0.05 \). EpiData 3.0 software was used for data entry and SAS® 9.13 software was used for logical inspection and statistical analysis (both free downloads).

3. Results

A total of 159 patients were enrolled in the study. After withdrawals and dropouts, 72 patients remained in the test group and 81 in the control group (N = 152). Mean age of patients in the test group was 33.0 ± 11.1 years (range 18-61) and mean age in the control group was 36.9 ± 13.4 years (range 15-77); the difference was significant \( (p = 0.0470) \). There was no significant difference in gender distribution between the groups (test group, 93.6% male; control group, 90.1% male; \( p = 0.4230 \)).

A summary of injury data is presented in Table 4. Most patients had acute injuries with open wounds, redness and exudation. The most common injured sites were the thumb, index and middle fingers. The radial and ulnar digital nerves had the same frequency of injury \( (p > 0.05) \). The most common reasons for injury were cuts, contusions and compressions. Most patients had skin injuries with accompanying fractures and injured tendons. The mean time from injury to repair in the test group (23.7 ± 52 days; range 0-200 days) was significantly greater than that in the control group (1.5 ± 10.4 days; range, 0 to 91 days) \( (p = 0.0005) \). Of note (Table 4), the \( p \)-value for comparison of combined damage in the two groups was \( p = 0.4767 \) \( (> 0.05) \) whereas the \( p \)-value for the analysis of vessel and tendon damage in the sub-groups was \( p < 0.05 \). These results indicated that heterogeneity of the two groups was the result of whether or not vessel and tendon damage was present. Total number of nerves repaired in test group patients was 100 for the control group, 123 \( (p = 0.0242) \). Mean length of the nerve graft was 1.80 ± 0.82 cm (range 1-5 cm) but most defects were < 3 cm. All surgeries were successful.

Table 3. Interpretation of monofilament testing of pressure threshold

| WEST monofilament (N) | 1   | 2   | 3   | 4   | 5   |
|----------------------|-----|-----|-----|-----|-----|
| SW monofilament number | 2.83 | 3.61 | 4.31 | 4.56 | 6.65 |
| Force (g)            | 0.07 | 0.2  | 2   | 4   | 200 |
| Interpretation of threshold | Normal sensation | Reduced tactile sensation | Reduced protective sensation | Loss of protective sensation | Residual sensation |

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### 3.1. Evaluation of efficacy

Results of s2PD and SW monofilament tests at 6-months post-op are shown in Table 5 and results of neural and patient levels are shown in Table 6. The 95% CI of subtraction for the satisfied rate (trial minus control group) for SW monofilament testing at the neural level was -3.34 to 9.23% and at the patient level -6.07 to 10.87%. Sensitivity analysis and preference scores of SW monofilament testing using LOCF and WOCF principles for missing data transfer also showed that the non-inferiority conclusion was credible (Table 7).

As to s2PD, there were no differences in FAS and PPS between the groups at 6-months post-op (p>0.05; Table 8). Furthermore, s2PD improved over time in the test group (Table 9). The average distance was 12.81 ± 5.99 mm at 6-months post-op.

### Table 4. Summary of injuries

| Test group | Control group | p-value |
|------------|---------------|---------|
| n (%)      | n (%)         |         |
| Side of injury |               |         |
| Left limb  | 60 (60)       | 77 (62) | 0.6915 |
| Right limb | 40 (40)       | 46 (37) |         |
| Finger     | 21 (21)       | 31 (25) | 0.9060 |
| Thumb      | 32 (32)       | 39 (31) |         |
| Index      | 9 (9)         | 12 (9)  |         |
| Ring       | 14 (14)       | 13 (10) |         |
| Little     |               |         |         |
| Direction of figure |       |         |
| Radial     | 51 (51)       | 66 (53) | 0.6926 |
| Ulnar      | 49 (49)       | 57 (46) |         |
| Mechanism of injury |     |         |
| Cut        | 41 (41)       | 46 (37) | 0.0388 |
| Contusion  | 25 (25)       | 50 (40) |         |
| Avulsion   | 11 (11)       | 5 (4)   |         |
| Squeeze    | 22 (22)       | 22 (17) |         |
| Electrical | 1 (1)         | 0 (0)   |         |
| Combined injury |       |         |
| Fracture   | 79 (79)       | 106 (86)| 0.1573 |
| Vessel injury |         |         |
| Muscle or tendon |     |         |

Data are presented as number (percentage). Groups were compared using adjusted centre effect Cochran-Mantel-Haenszel chi-square test.

### Table 5. SW and s2PD results 6 months after surgery (all repaired nerves)

| SW n (%) | Test group n (%) | Control group n (%) |
|----------|------------------|---------------------|
| 2.83     | 16 (16.84)       | 11 (8.94)            |
| 3.61     | 49 (51.58)       | 60 (48.78)           |
| 4.31     | 25 (26.32)       | 42 (34.15)           |
| 4.56     | 3 (3.16)         | 7 (5.69)             |
| 6.65     | 2 (2.11)         | 3 (2.44)             |
| s2PD (mm) |                   |                     |
| 2        | 5 (5.26)         | 1 (0.81)             |
| 3        | 2 (2.11)         | 0 (0.00)             |
| 4        | 7 (7.37)         | 0 (0.00)             |
| 5        | 5 (5.26)         | 1 (0.81)             |
| 6        | 2 (2.11)         | 0 (0.00)             |
| 7        | 3 (3.16)         | 3 (2.44)             |
| 8        | 3 (3.16)         | 0 (0.00)             |
| 9        | 1 (0.55)         | 3 (2.44)             |
| 10       | 0 (0.00)         | 1 (0.81)             |
| 11       | 1 (0.55)         | 9 (7.32)             |
| 12       | 8 (8.42)         | 13 (10.57)           |
| 13       | 7 (7.37)         | 4 (3.25)             |
| 14       | 9 (9.47)         | 22 (17.89)           |
| 15       | 15 (15.79)       | 16 (13.01)           |
| >15      | 27 (28.43)       | 50 (40.65)           |

Data are presented as number (percentage). *No data was available for 5 nerves in the test group.

### Table 6. Results of SW and s2PD testing at neural and patient levels at 6-months post-op (PPS)

| Statistics | p-value | Test group | Control group |
|------------|---------|------------|---------------|
| SW = 3.3951 | 0.1831  |            |               |
| Satisfied  | 90 (95.74) | 113 (91.87) |
| Unsatisfied| 4 (4.26)  | 10 (8.13)  |               |
| Test group minus control group satisfaction rate 95% CI 2.74 (-3.34; 9.23) | | | |
| s2PD = 27.0332 | 0.0 | | |
| Excellent  | 21 (22.34) | 2 (1.63)  |
| Good       | 47 (50.00) | 71 (57.76) |
| Poor       | 26 (27.66) | 50 (40.64) |
| Test group minus control group satisfaction rate 95% CI 11.99 (-0.80; 27.57) | | | |
| Patient levels | | | |
| SW = 1.1197 | 0.5713  |            |               |
| Satisfied  | 68 (94.44) | 75 (92.59) |
| Unsatisfied| 4 (5.56)  | 6 (7.41)   |               |
| Test group minus control group satisfaction rate 95% CI 2.02 (-6.07; 10.87) | | | |
| s2PD = 11.6178 | 0.003 | | |
| Excellent  | 13 (18.06) | 2 (2.47)  |
| Good       | 35 (48.61) | 50 (61.73) |
| Poor       | 24 (33.33) | 29 (35.80) |
| Test group minus control group satisfaction rate 95% CI 2.36 (-11.44; 19.76) | | | |

Data are presented as number (percentage). Groups were compared using adjusted centre effect Cochran-Mantel-Haenszel chi-square test.

### Table 7. Sensitivity and preference score for SW monofilament testing

| Level of analysis | Analysis set | Missing values filling method | Rate difference (%) | 95% Confidence interval |
|-------------------|--------------|--------------------------------|---------------------|-------------------------|
| Sensitivity analysis of SW monofilament tests | Patient FAS LOCF | -3.1 (-11.9; 6.6) |
|                  | Patient FAS WOCF | -6.0 (-15.0; 4.1) |
|                  | Patient FAS LOCF | 1.3 (-5.0; 8.1) |
|                  | Nerve FAS WOCF | 1.3 (-5.0; 8.1) |
|                  | Nerve PPS NA | 2.7 (-3.3; 9.2) |
| Preference scores of SW monofilament tests (patient level) | Patient FAS LOCF | -0.3 (-4.4; 4.1) |
|                  | Patient FAS WOCF | -3.1 (-7.8; 1.9) |

The following factors were included preference score model analysis: time from injury to repair, mechanism of injury, length of nerve defect, combined injury and age.

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3.2. Evaluation of safety, complications and revisions (FAS, SS)

There were 78 patients included in the safety evaluation. Surgical wounds were divided into 3 types: 1) clean incision, 2) partially contaminated and 3), contaminated. Healing was also divided into 3 types: A) healed well with no complications; B) some inflammation around the incision, edema, hematoma and seroma without purulent drainage; and C), infection with purulent drainage. The condition of the wound preoperatively and after healing was expressed as 1, 2, 3)/A, B, C).

Six patients reported mild wound pain two weeks after the operation and there were three cases of mild redness of the wound but without effusion or exudate. All contaminated wounds had grade II/A healing and clean wound grade I/A healing at one-month post-op. No patients had pain, itching, local erythema, urticaria, rash or other allergic symptoms one month after surgery. Values of routine blood tests, liver and renal function tests, immune function testing (CD4/CD8), erythrocyte sedimentation rate and C-reactive protein were within the normal range preoperatively and at the 6-month follow-up visit in all but two patients. Two patients with multiple traumas had mildly elevated alanine aminotransferase and aspartate aminotransferase, which subsequently decreased to normal levels. There were no material related adverse events. During follow-up, two patients required secondary tenolysis 6-months post-op. During surgery, nerve allografts were visualized and appeared well incorporated into the repair site (Figure 2).

| Analysis sets | Trial group | Control group | p-value |
|---------------|-------------|---------------|---------|
| FAS           | 65.75%      | 64.20%        | 0.8398  |
| PPS           | 66.67%      | 64.20%        | 0.7486  |

Table 8. Comparison of s2PD six-months post-op (patient level)

| Pre-op | 1-month post-op | 3-months post-op | 6-months post-op |
|--------|-----------------|------------------|------------------|
| 20.0±0.0| 18.5±3.8        | 14.4±6.3*        | 12.81±5.99*      |

Table 9. Sensory recovery by s2PD testing in trial group during follow-up

Data presented as mean± standard deviation
*p < 0.05 compared to 1 month post-op

Figure 2. Representative images of hANG repair. A, B) Intraoperative photographs of nerve defects. C) Two weeks after surgery. (D) Three months after surgery. E, F) Intraoperative photograph of tendon and nerve detection.
4. Discussion

Allogeneic nerve is widely available and can be transplanted for the repair of long nerve defects. Nerve allograft acts as a viable biologic conduit through which host motor and sensory axons grow. Although clinical nerve allografting has been attempted as early as the late nineteenth century (Albert, 1885), clinical success had been questionable at best until the era of modern immunosuppression. The advent of microsurgical techniques in the 1970s improved understanding of the neurobiology of peripheral nerve regeneration (Evans et al., 1994), and the implementation of host immunosuppression in the 1980s allowed nerve allotransplantation to become a realistic possibility for otherwise devastating nerve injuries.

In 1988, Mackinnon and Hudson (1992) used an allogeneic nerve graft from a living donor to repair a 23-cm long sciatic nerve defect in an 8-year-old boy. The patient required oral cyclosporin A for two years. Moore et al. (2009) also used allogeneic nerve grafts from living donors to repair nerve defects in 11 patients, and although patients took low-dose tacrolimus (FK506) postoperatively, one developed graft rejection. Bain (2000) used allogeneic nerve transplantation for seven patients and treated the patients post-operatively with CsA and FK506, and one patient developed graft rejection. Since then, many researchers have used this method to develop immune rejection and allergic reactions.

Studies have confirmed that myelin is the main component causing rejection and the Schwann cell (SC) is the most important target of the host immune response. Collagen in the three layers of neural and base membranes is not the major antigenic component and the immune response caused by it very mild. Rovak et al. (2005) found that the immunogenicity of acellular nerve produced by chemical reaction was significantly reduced and that it could be used as an antigen-free graft. Autologous Schwann cells can migrate into the transplanted nerve to wrap the regenerated neural fibres and form a myelin sheath.

An ideal nerve repair technique should: 1) eliminate tension at the repair site; 2) permit immediate reconstruction at time of injury; 3) not require sacrifice of another functioning nerve; 4) not create a scar at a site not already injured; 5) not add appreciable operative time; 6) not place a foreign material into the body permanently; 7) not create the potential for chronic nerve entrapment; and 8) promote neural regeneration (Dellon et al., 2001). In addition, an ideal reconstructive technique should not require the patient to be chronically medicated (immunosuppressive treatment in the case of nerve allografts).

If the immunogenicity of allograft nerve can be effectively removed, it will be an ideal graft. The nerve allograft can be pretreated by degeneration, freezing, periods of storage in a variety of solutions, irradiation (ultraviolet, high-voltage, γ) and lyophilization (freeze-drying). Degeneration, freeze-thaw and low-dose irradiation have been shown to be ineffective, with the recipient still presenting a rejection response. High-dose irradiation and lyophilization destroy the Schwann cells, and this decreases the host’s immune response; however, nerve regeneration across the pretreated allograft is impaired. As early as 1982, Johnson et al. (1982) treated human peripheral nerve with a chemical detergent to obtain extracellular matrix and studied its chemical composition. Since then, many researchers have used this method to clear the cellular components in nerve. This reduces immune rejection after transplantation and the fibrous skeleton of the retained extracellular matrix serves as a channel for nerve regeneration. In prior studies, we treated human tibial nerves with trinitrotoluene and sodium deoxycholate and found that Schwann cells and axons identified by S-100 and neurofilament expression were absent and the scaffolds were cell-free and rich in collagen-I and laminin, with a microarchitecture similar to the fibrous framework of human peripheral nerves (Figure 3) (Yang et al., 2011; Zhu et al., 2004). Furthermore, we evaluated the biocompatibility of the matrix by analyzing its cytotoxicity, haemolysis and skin sensitization, and results showed that the scaffolds had very mild cytotoxicity and haemolysis, and skin sensitization was not observed (Zhu et al., 2004). However, none of the methods described can completely remove the immunogenicity of allograft nerve; thus, the potential for a hANG recipient to develop immune rejection and allergic reactions remains.

Almost all similar studies lack systematic evaluation when verifying the clinical safety of different materials. Karabekmez et al. (2009) used a new type of acellular nerve, AVANCE, to repair digital nerve defects. In that study, local pain, infection, rejection response and need for graft removal were measures chosen for safety evaluation. In the current study, safety was evaluated by local wound response and laboratory testing. Results showed that all patients had grade II/A healing of contaminated wounds and grade I/A healing of clean wounds after one-month follow-up. All patients except two with minor elevations of liver enzymes, likely due to multiple traumas, had normal laboratory test results. No patients experienced pain, itching, local erythema, urticaria, rash or other allergic symptoms one-month post-op and no currently known infectious agents were detected.

The final results of SW testing showed that when the length of the graft was < 5 cm, the hANG group’s efficacy was not inferior to that of the direct suture group, while
s2PD examination failed to prove this. Static two-point discrimination is an important function of digital nerves. In the current study, we defined s2PD’s as: excellent, ≤ 6 mm; good, 7-15 mm; and poor, ≥ 16 mm. Different studies have reported various efficacies using different materials. For example, good neural recovery rates were as follows: 86% using polyglycolic acid (PGA) (average, 1.7 cm; range, 0.5-3.0 cm) (Mackinnon and Dellon, 1990); 76.5% with Neurutube (range, 1.0-4.0 cm) (Battiston et al., 2005); 88% with Neurotube (range, 1.0-2.0 cm) (Bushnell et al., 2008); 75% (average, 3.8 cm; range, 1.2-6.6 mm) (Lohmeyer et al., 2009); and 65.28% in the current study, which is somewhat lower than other relevant reports. However, these reports used a smaller sample volume and the statistical methods used were different from those used in the current study, making direct comparisons difficult. Although the non-inferiority hypothesis was not proven by s2PD examination, we concluded that the good and excellent rate range using hANG to repair nerve damage was 51.98-78.93%. Furthermore, the difference in excellent and good rates was 2.36 and 95% CI was -11.44 to 19.76, which indicated that efficacy was -11.44% less and 19.76% better than direct suturing when using hANG to bridge nerve defects in 95% of cases. 

Currently, most materials for clinical transplantation for nerve defect repair are conduits of synthetic origin such as PGA, polylactic acid (PLA), polyhydroxybutyrate (PHB) and poly(DL-lactide-epsilon-caprolactone) (PLCL). hANG has several advantages over these materials. First, a large body of experimental evidence has shown that extracellular matrix provides a surface for the ingrowth of Schwann cells and axons migrating from nerve stumps. Second, using nerve conduits is time-consuming and meticulous haemostasis is necessary before insertion of the nerve ends into the conduit to prevent bleeding into the lumen, since the formation of a blood clot inside the tube could be detrimental to nerve regeneration. To prevent the potential for blood clots, rinsing with normal saline after each suture and injecting heparinized saline into the lumen is required. 

Karabekmez et al. (2009) used an acellular allogeneic nerve graft (AxoGen® Inc, Alachua, FL, USA) to repair eight digital nerve defects due to mechanical trauma and two pure sensory nerve defects on the back of the hand in seven patients. The mean length of the defects was 2.23 cm (range 0.5-3 cm). The combined good and excellent s2PD rate was 100% after a mean follow-up of 9 months. However, the combined excellent and good s2PD rate in the test group of the current study was 65.28%, lower than that reported by Karabekmez. A multicenter study by Brooks et al. (2012) on processed allografts (AxoGen) for peripheral nerve reconstruction reported a meaningful recovery rate of 87%. A difference between our study and prior studies is that the patients in our study did not receive sensory re-education and maximum follow-up time was 6 months whereas in other

Figure 3. A) Longitudinal sections of fresh nerve (H&E staining 40x). B) Longitudinal sections of hANG (H&E staining 40x). (C) Cross-sections of hANG (scanning electron microscopy 5000x). D) Longitudinal sections of hANG (scanning electron microscopy 5000x)
studies, variable periods of sensory re-education were provided (Mackinnon and Dellon, 1990; Weber et al., 2000). We believe that if we had provided sensory re-education and lengthened follow-up time that our results would have improved.

The current study was not randomized; as a result, there was concern regarding the comparability of the two groups. There were significantly more tendon and vessel injuries in the control group, more patients with contusions in the control group, and time from injury to surgery was significantly longer in the test group; as a result, the baseline between groups was unbalanced. This is a specific major drawback of non-randomized clinical trials. The p-value for the comparison of mechanism of injury in the two groups was 0.0388 (< 0.05) and could have reduced the effect of nerve repair in the control group. In our statistical analysis, we applied the method of propensity score to adjust the baseline, thus making the two groups comparable. The following factors were included in the propensity score model analysis: time from injury to repair, mechanism of injury, and combined injury and age. Furthermore, the groups were compared using the Cochran-Mantel-Haenszel chi-square test to reduce central differences. Even when we excluded the control group and switched our study to a clinical trial without control, we still observed that the combined good and excellent rate for s2PD of hANG for repairing 1-5 cm digital nerve defects was 65.28% (asymptotic state law 95% CI 51.98-78.93%). As to efficacy, we assumed a satisfaction rate of non-inferiority standard of ± 15%, which means that the non-inferiority hypothesis was proven even if the satisfaction rate of the test group was < 15% of that of the control group. Moreover, if the nerve considered for reconstruction was a genuine sensory nerve, it is doubtful whether sacrificing a sensory nerve was a reasonable option.

5. Conclusions

hANGs retain the natural structure of the nerve and only minimally elicit a host immune response since most, if not all, immunogenic substances were removed during preparation of the grafts. We found no adverse events and results showed that its efficacy in restoring digital nerve function was similar to that of other materials on the market, suggesting that it is both safe and effective. The use of hANGs can avoid the pain caused by allogeneic nerve grafting, loss of nerve function and other complications caused by allogeneic nerve grafting. It can also reduce surgical time and damage.

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Conflict of interest

The authors have declared that there is no conflict of interest.

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