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RESEARCH ARTICLE

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# Acellular nerve graft enriched with mesenchymal stem cells in the transfer of the phrenic nerve to the musculocutaneous nerve in a C5-C6 brachial plexus avulsion in a rat model

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## Abstract

**Introduction:** Phrenic nerve transfer has been shown to achieve good nerve regeneration in brachial plexus avulsion. Acellular nerve allografts (ANAs) showed inferior results to autografts, which is why its use with mesenchymal stem cells (MSCs) is currently being studied. The aim is to study the effect of BM-MSCs associated with ANAs in a rat model of phrenic nerve transfer to the musculocutaneous nerve in a C5-C6 avulsion.

**Material and methods:** 42 Wistar-Lewis rats underwent a C5-C6 lesion in the right forelimb by excising a 3 mm segment from both roots, followed by a phrenic nerve transfer to the musculocutaneous nerve associated with the interposition of a three types of nerve graft (randomly distributed): control (autograft) group (n = 12), ANAs group (n = 12), and ANAs + BM-MSCs group (n = 18)

After 12 weeks, amplitude and latency of the NAP and the compound motor action potential (CMAP) were measured. Biceps muscles were studied by histological analysis and nerve grafts by electron microscopy and fluorescence analysis.

**Results:** Statistically significant reductions were found in latency of the CMAP between groups control (2.48 ± 0.47 ms) and experimental (ANAs: 4.38 ± 0.78 ms, ANAs + BM-MSCs: 4.08 ± 0.85 ms) and increases in the amplitude of the CMAP between groups control (0.04388 ± 0.02 V) and ANAs + BM-MSCs (0.02275 ± 0.02 V), as well as in the thickness of the myelin sheath between groups control (0.81 ± 0.07 μm) and experimental (ANAs: 0.72 ± 0.08 μm, ANAs + BM-MSCs: 0.72 ± 0.07 μm) and in the area of the myelin sheath between groups control (13.09 ± 2.67 $\mu m^2$) and ANAs (10.01 ± 2.97 $\mu m^2$) ($p < .05$). No statistically significant differences have been found between groups ANAs and ANAs + BM-MSCs.

**Conclusions:** This study presents a model for the study of lesions of the upper trunk and validates the autologous graft as the gold standard.

## 1 | INTRODUCTION

Brachial plexus injury is a devastating chronic pathology for patients and represents a surgical challenge due to complex anatomy and lesion patterns. Selective lesions of C5 and C6 roots are extremely limiting because they affect shoulder control due to the loss of suprascapular and axillary nerves and elbow flexion, due to the loss of the musculocutaneous nerve (Limthongthang et al., 2013). These three nerves are critically important for proper upper limb function.

In cases of circumscribed nerve tissue loss, reconstruction with interposition of nerve grafts is one of the most widely used therapeutic options. However, increasing the length of required nerve grafts will determine the viability of this technique. In those cases, the prognosis for reinnervation by means of nerve grafts is very poor (Kolar & Kingham, 2014), which makes it necessary to consider distal nerve transfers (Tung & Mackinnon, 2010). Nerve transfers are the only remaining technique, especially for preganglionic injuries (Vasudevan et al., 2013).

There are several options for performing neurotization, such as the use of contralateral C7 or the spinal accessory nerve, but phrenic nerve transfer has been shown to achieve good nerve regeneration when used as a donor nerve in brachial plexus avulsion injuries, and the procedure is associated with low morbidity (Zhang et al., 2004).

It is necessary to highlight the limiting factors posed by autologous nervous tissue used as a donor for nerve grafts, due to the limited number of donor nerves available and the morbidity associated with the donor area. For this reason, there is a need to seek therapeutic alternatives to repair the nerve defect, such as the use of acellular nerve allografts (ANAs) or synthetic nerve conduits. ANAs offer several advantages over synthetic nerve conduits because they maintain their extracellular matrix and three-dimensional architecture (Karabekmez et al., 2009) and do not possess the inconvenience of immunogenicity presented by allografts. Despite this, its results are inferior to autografts, which is why their use when enriched with mesenchymal stem cells (MSCs), is being studied, in order to achieve comparable results (Zhao et al., 2014).

Interest in the use of stem cells from mesenchymal tissue has increased exponentially in recent years, with lines of research oriented in this direction becoming more and more frequent. Studies in animals, especially in the murine model, are increasingly common. Promising results have been obtained in their use, but the mechanism by which this benefit is obtained is not entirely clear (Fan et al., 2014a).

The goal of this study is to develop an experimental model of preganglionic lesions of the upper trunk and reconstruction based on a transfer from the phrenic nerve to the musculocutaneous nerve. Three different types of nerve grafts will be examined.

## 2 | MATERIAL AND METHODS

### 2.1 | Mesenchymal stem cells preparation

Cells were obtained from the long bones of the hind limbs (femur and tibia) of 12 14-day-old female Wistar-Lewis rats. The process was carried out under sterile conditions in laminar flow cabinets. The extraction of the bone marrow was carried out by means of flushing techniques with Roswell Park Memorial Institute (RPMI) medium with 10% fetal bovine serum and 1% penicillin and streptomycin antibiotic solution. Once the sample was obtained, it was cultured in the same medium on cell culture plates according to the usual protocols, using 0.25% trypsin to carry out cell passages and phosphate saline solution (PBS). The incubation was carried out in ovens at 37°C.

When the MSCs reached 80% confluence in P2, they were labeled with indocarbocyanine fluorescent lipophilic cationic dye (DiI). A stock solution of DiI in dimethylsulfoxide (DMSO) at a concentration of 2 mg/ml was used to obtain a dilution of DiI at 0.1 μM in Hank's balanced salt solution (HBSS) from Gibco™ (Thermofisher, Madrid, SP). The optimal concentration used was based on the results obtained from the cytometric analysis of different cell samples previously labeled with different concentrations of solution, where a labeling of at least 90% of the cells analyzed was considered adequate. Briefly, the labeling of the cells was carried out by adding the DiI solution to the culture plate, after having removed the RPMI medium. Subsequently, the cells were incubated for 5 min at 37°C followed by 14 min at 4°C, after which the working solution was washed and the cells were prepared according to the conventional technique.

### 2.2 | Acellular nerve tissue preparation

The peripheral nerve tissue was obtained by means of a surgical procedure in eight female animals between 8 and 10 weeks old. We extracted 1.5 cm segments of mixed nerves from terminal branches of the brachial plexus. The axillary, musculocutaneous, ulnar, median, and long thoracic nerves were resected. For the decellularization process, the protocol published by Sondell et al. was used (Sondell et al., 1998). Eighteen of the decellularized nerves were cultured with BM-MSCs. The process of inserting the stem cells into the nerve was carried out inside a laminar flow cabinet, prior to the implantation of the nervous tissue within the experimental animal. $3 \times 10^5$ cells per graft were inserted sub-epineurally through small epineural perforations. This procedure required the use of a micropipette (Stripper® Tip, 135 μm, Origio, Denmark).

### 2.3 | Model establishment and experimental design

The experimental design was carried out with 62 rats (Wistar-Lewis [LEW] haplotype RT1l LEW/HanHsd line). The femurs of 12 14-day-old female rats were used to obtain the stem cells and eight 10-week-old female rats were used as nervous tissue donors The protocol was evaluated and approved by the ethics committee for animal experimentation (CEEA: 2015/R02, 07/01/2015).

The surgical procedure was carried out with 42 male rats with a mean weight of 335.13 ± 35.71 grams and 80 ± 10 days old that were divided randomly into three groups; 12 rats in the control group,

12 rats in the ANAs group and 18 rats in the ANAs + BM-MSCs group. All the animals underwent the same surgical procedure in order to produce a lesion of the C5 and C6 roots in the right forelimb, followed by an ipsilateral phrenic nerve transfer to the musculocutaneous nerve, completed with the interposition of a nerve graft.

Inhalation anesthetic induction was performed in an induction chamber with 5% sevoflurane and 1.5 L/min oxygen and was maintained with 2% sevoflurane and 1.5 L/min oxygen. Preoperative analgesia with 0.05 mg/kg buprenorphine was administered subcutaneously.

An L-shaped incision was made at the level of the cervical midline up to the sternal fork, and prolonged following the clavicular ridge toward the right forelimb. To achieve exposure of the roots, a dissection of the anterior scalene muscle, the sternohyoid muscle, and the subclavian musculature was performed. The lateral brachial plexus dissection was carried out in order to identify the origin of the musculocutaneous nerve, which is located between the coracobrachialis muscles and the short portion of the biceps brachii, since the latter will be used as a receptor for nerve transfer (Figure 1). Subsequently, it was necessary to dissect the ipsilateral phrenic nerve down to the point where it enters the thoracic cavity, where it was sectioned. Injury to the C5 and C6 nerve roots was performed at the level of the intervertebral foramen by sectioning and excising a 3 mm nerve segment from both roots.

The repair technique consisted of the transfer of axons from the phrenic nerve to the distal portion of the musculocutaneous nerve via a 0.5 cm graft by end-to-end technique. In the control group we used an autologous nerve graft harvested from a segment of axillary nerve, distal to the lesion, in ANAs group we interposed a heterologous acellular graft and in ANAs + BM-MSCs group a heterologous acellular graft enriched with BM-MSCs, labeled with the vital dye DiI. The graft was sutured in a retrograde position using two simple epineural 10/0 nylon stitches at each end, viewed through an Optomic OP-C12 surgical microscope with ×16 lens and 200, 250, 300, and 400 mm magnification, with a 150w cold light illumination system.

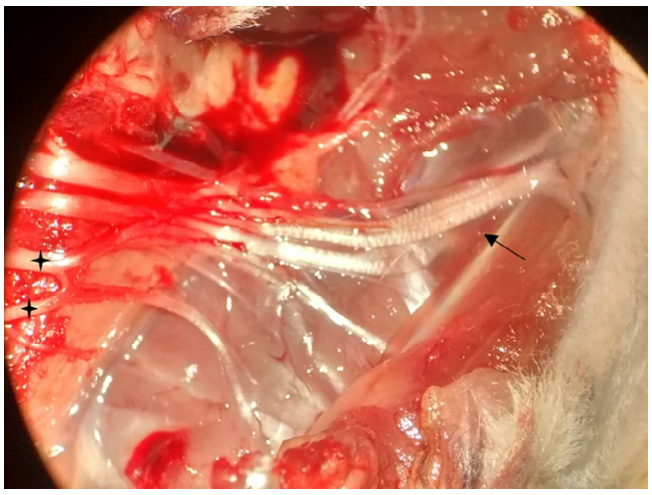

**FIGURE 1** Intraoperative image taken through the surgical microscope. Note the orientation of the animal, which has the most cephalic portion close to the surgeon. C6-C5 roots are indicated with asterisks and musculocutaneous nerve with a star

The follow-up time was 12 weeks, during which a daily clinical evaluation was carried out to assess the appearance of muscle contraction in the biceps. After that time, an electrophysiological record was performed to measure the amplitude and latency of the nerve action potential (NAP) and the compound motor action potential (CMAP). The distal portion of the nerve graft was processed for study by electron microscopy, the biceps muscle by histological analysis and proximal half of the nerve graft (ANAs + BM-MSCs group) by fluorescence analysis.

## 2.4 | Electrophysiological examination

The electrophysiological record was carried out at 12 weeks of follow-up under general anesthesia after exposing the phrenic nerves, the nerve graft segment and the musculocutaneous nerve, as well as the biceps muscle. The stimulation electrode was placed on the phrenic nerve and a monopolar electrode was used for recording, located in contact with the musculocutaneous nerve, maintaining a constant distance of 1 cm from the stimulation electrode with a stereotaxis apparatus. With the animal in the same position, the electromyographic recording was carried out, transferring the recording electrode to the biceps muscle, placing it at 1.5 cm.

The aim was to measure the amplitude and latency of the NAP and the CMAP, for which an amplifier (AM Systems, Jaen, Spain) associated with a Power 1401 interface (Cambridge Electronic Design Limited, Cambridge, United Kingdom) was used. The data were processed with the Spike 2 program (Center d'Estudis Demogràfics, Barcelona, Spain).

## 2.5 | Histological analysis

The biceps muscle of all the animals were fixed in 10% formaldehyde for 24 h, followed by subsequent dehydration in gradient alcohol and inclusion in paraffin. Four-micrometer microtome cuts were made, after which deparaffinization was carried out at 60°C with xylene and its rehydration by immersion in 100° alcohol, 96° alcohol and water. Harris hematoxylin staining was performed for 5 min, followed by a water rinse and subsequent application of eosin for 1 min. It was completed with dehydration in 96° alcohol, 100° alcohol, and immersion in xylene, allowing its study under light microscopy (Olympus).

The proximal half of the nerve graft from the animals belonging to ANAs + BM-MSCs group was processed using a freezing technique. Plates with O.C.T fixative were introduced after placing the nerve segment in a vertical position in a 100% isopentane (C5H12) solution previously cooled in liquid nitrogen, to achieve rapid freezing. The samples were transported on dry ice to a freezer for storage at −80°C until processing. For the immunofluorescence analysis, the sample was permeabilized with 0.1% Triton in PBS for 10 min at room

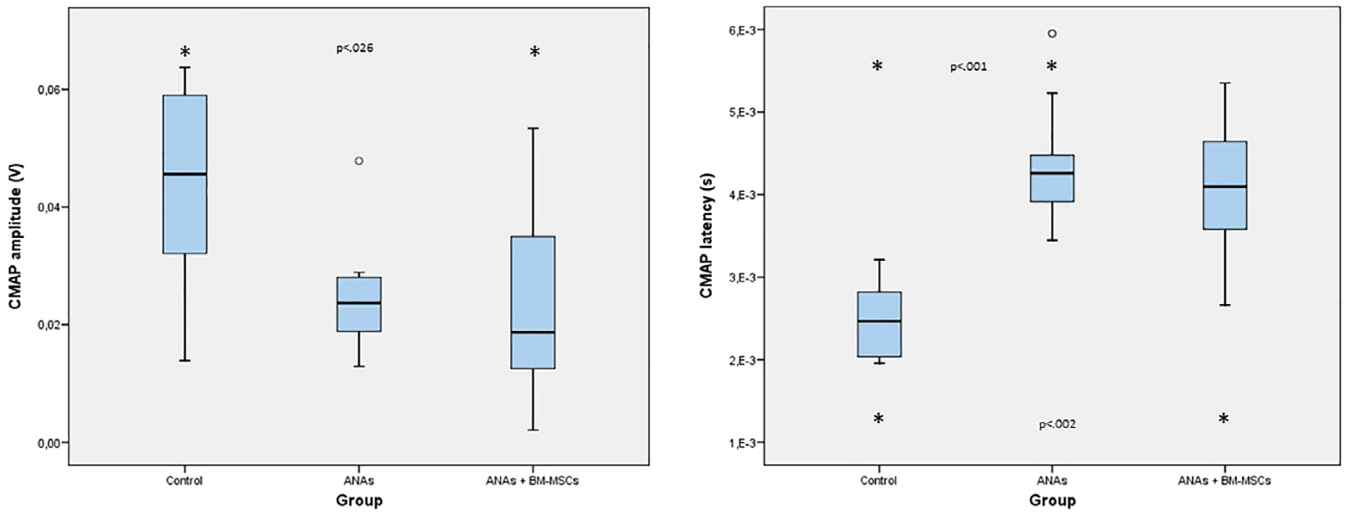


**FIGURE 2** Differences in the amplitude and latency of CMAP. Despite not finding statistically significant differences between both experimental groups, amplitude is significantly greater in the control group and latency time is less in the control group. Outliers are represented as circles. *$p < .05$. CMAP, compound motor action potential

**TABLE 1** Statistical analysis of the electrophysiological findings. No statistically significant differences have been found between groups ANAs and ANAs + BM-MSCs in the amplitude and the latency of CMAPS. *$p < .05$ statistically significant

| | Amplitude (V) mean ± SD | Comparison with control | Latency (ms) mean ± SD | Comparison with control |
|---|---|---|---|---|
| Control | 0.04388 ± 0.02 | | 2.48 ± 0.47 | |
| ANAs | 0.02525 ± 0.01 | $p = .093$ | 4.38 ± 0.78 | *$p = .001$ |
| ANAs + BM-MSCs | 0.02275 ± 0.02 | *$p = .026$ | 4.08 ± 0.85 | *$p = .002$ |

Abbreviations: ANAs, acellular nerve allografts; MSCs, mesenchymal stem cells.

temperature, after which a PBS wash was performed for 5 min. Finally, Vectashield mounting medium with DAPI (4′,6-diamidino-2-phenylindole) was covered with 100 µl for analysis with a conventional fluorescent microscope (Olympus).

## 2.6 | Electron microscopy

The distal portion of the nerve graft was processed for study by electron microscopy. The samples were fixed with a PBS solution of 1.5% glutaraldehyde, 1.5% paraformaldehyde, and 0.1% thamic acid for 12 h at 4°C, followed by a post fixation in 1% osmium tetroxide, dehydration in acetone in gradient and finally inclusion in a low-viscosity epoxy resin SPURR. Sixty-nanometer sections were made with an ultramicrotome, which were stained with toluidine blue.

The parameters analyzed were the area and thickness of the myelin layer, the area and diameter of the nerve fibers and the percentage of myelin fibers, for which an analysis software (Soft Imaging System GmbH, Olympus, Shinjuku, Japan) was used. They were quantified in two random sections of each sample, with a magnification ×2000, using a JEM 1010 transmission electron microscope (Jeol, Akishima, Japan). Images were taken with a Mega View III digital camera.

## 2.7 | Statistical analysis

SPSS 24.0 software (IBM Inc, Armonk, New York) was used for the statistical analysis. A descriptive analysis was carried out of all the variables included in the study, which were expressed in terms of mean ± SD. The acquired data were not normally distributed, therefore we have used the nonparametric Kruskal-Wallis test for independent samples, using the correction for multiple comparisons. We have considered $p < .05$ statistically significant.

# 3 | RESULTS

For the surgical protocol, 42 male rats were used. All animals showed clinical reinnervation consisting of involuntary flexion of the elbow synchronously with respiration between the third and fourth postoperative week, this being in the control group at 20.69 ± 2.14 days of average, in the ANAs group at 21.08 ± 2.61 days and in the ANAs + BM-MSCs group at 21.28 ± 2.49 days. No differences were observed in the time of appearance of the first contraction data between the groups. The grooming test could not be used to monitor clinical motor recovery because none of the animals achieved voluntary control for elbow flexion due to the use of the phrenic nerve as a donor.

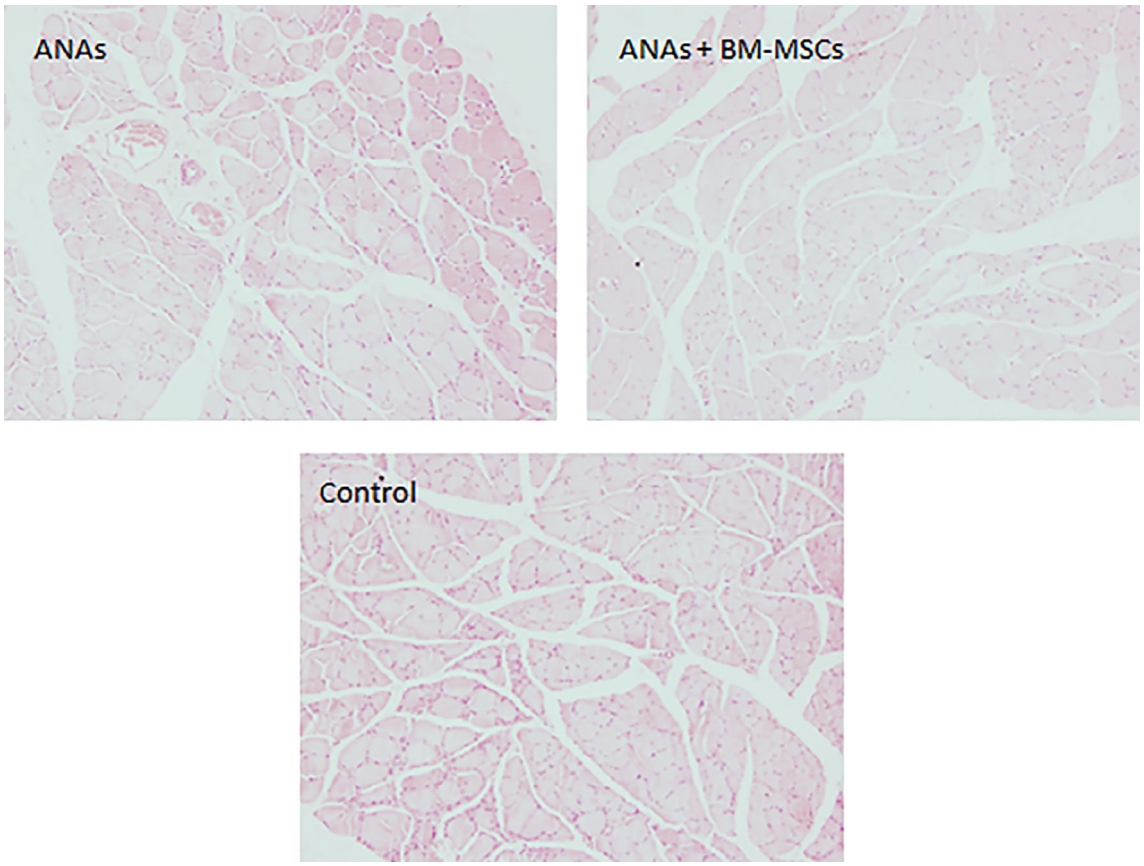


**FIGURE 3** Hematoxylin–eosin stain from sections of the biceps muscle of the ANAs group, ANAs + BM-MSCs group and control group. The similarities in the histomorphology in the three groups can be observed. (magnification ×10). ANAs, acellular nerve allografts; MSCs, mesenchymal stem cells

## 3.1 | Electrophysiological findings

Statistically significant differences was observed in the electromyography, both in the amplitude of the CMAPs, which was significantly higher in control group (0.04388 ± 0.02 volts) compared with ANAs + BM-MSCs group (0.02275 ± 0.02 volts, $p = .026$) and in the latency of the CMAPs, which was significantly lower in the control group (2.48 ± 0.47 ms) compared with ANAs group (4.38 ± 0.78 milliseconds, $p = .001$) and ANAs + BM-MSCs group (4.08 ± 0.85 ms, $p = .002$) (Figure 2), but not between the two experimental groups ($p > .05$) (Table 1).

The differences found in the electroneurography between the groups, when analyzing the NAPs, were not statistically significant, but the drift of the data suggests action potentials with greater amplitude and lower latency in the control group with respect to the experimental groups.

## 3.2 | Histological findings

In the hematoxylin eosin preparations from the biceps muscle, no differences were observed in the histomorphology between the groups,

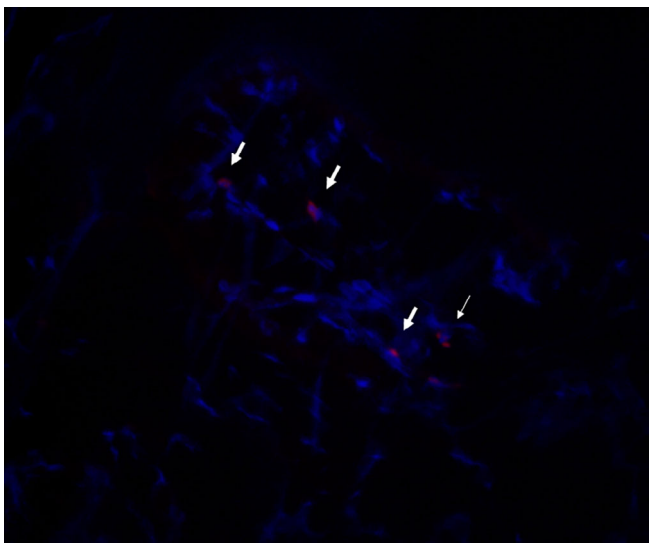

**FIGURE 4** Immunofluorescence of nerve graft sections from ANAs + BM-MSCs group. Cell nuclei marked with DAPI (blue) and MSCs marked with DiI (red). White arrows point to MSCs. (magnification ×20). ANAs, acellular nerve allografts; MSCs, mesenchymal stem cells

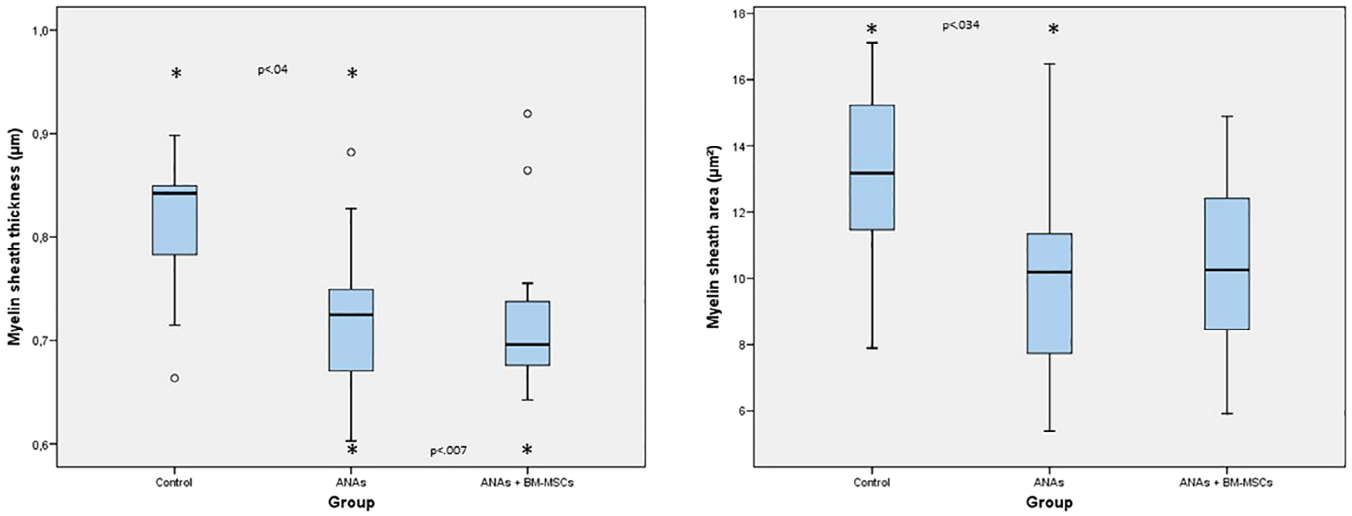


**FIGURE 5** Differences in the thickness and the area of the myelin sheath between the groups. No statistically significant differences were found between the experimental groups, but there were differences in the thickness between both and the control group. The control group presents a larger area than the experimental groups, finding statistical significance in this difference with ANAs group. Outliers are represented as circles. *$p < .05$. ANAs, acellular nerve allografts

**TABLE 2** Statistical analysis of the electronic microscopy findings. No statistically significant differences have been found between groups ANAs and ANAs + BM-MSCs in the thickness and the area of the myelin sheath. *$p < .05$ statistically significant

| | Amplitude (V) mean ± SD | Comparison with control | Latency (ms) mean ± SD | Comparison with control |
|---|---|---|---|---|
| Control | 0.81 ± 0.07 | | 13.09 ± 2.67 | |
| ANAs | 0.72 ± 0.08 | *$p = .04$ | 10.01 ± 2.97 | *$p = .034$ |
| ANAs + BM-MSCs | 0.72 ± 0.07 | *$p = .007$ | 10.44 ± 2.61 | $p = .068$ |

Abbreviations: ANAs, acellular nerve allografts; MSCs, mesenchymal stem cells.

neither in the size of the cells nor the architecture of the tissue (Figure 3).

Fluorescence was observed in ANAs + BM-MSCs group in relation to the presence of MSCs preoperatively marked with DiI dye, which indicates their survival in the implanted tissue (Figure 4).

### 3.3 | Electron microscopy findings

The data analysis revealed a statistically significant difference between the groups when analyzing the thickness of the myelin sheath of the fibers, which was significantly greater in the control group (0.81 ± 0.07 µm) compared with ANAs group (0.72 ± 0.08 µm, $p = .04$) and ANAs + BM-MSCs group (0.72 ± 0.07 µm, $p = .007$), and the area of the myelin sheath, which was significantly greater in control group (13.09 ± 2.67 µm$^2$) compared with ANAs group (10.01 ± 2.97 µm$^2$, $p = .034$) (Figure 5), but not between the two experimental groups (Table 2).

No significant differences were observed between groups in fiber diameter and fiber area. No significant differences were found either in the percentage of myelinated and non-myelinated fibers. However, there was a significant difference between groups in the total number of fibers, this being statistically greater in control group ($p < .01$) (Figure 6).

## 4 | DISCUSSION

Brachial plexus treatment is far from providing ideal results, requiring in most cases that we assume added morbidity in nerve graft donor areas and sacrifice the function of the transferred nerve. This study aims to compare three types of nerve graft in an experimental model for brachial plexus injuries that is simple, easily reproducible and also offers versatility to generate new study models.

The literature supports the murine model for the pathology of the brachial plexus (Angélica-Almeida et al., 2013). There are small anatomical variations in terms of the topography if we compare it with human. The most significant is the contribution of the C7 roots to the musculocutaneous nerve, as described by Bobkiewicz et al., since in humans it consists of C5 and C6 (Bobkiewicz et al., 2017). This contribution does not interfere with the model, since, in order to carry out the transfer, although the lesion is selective of the superior roots, the complete section is made at the proximal level of the musculocutaneous nerve, which avoids a possible reinnervation through C7.

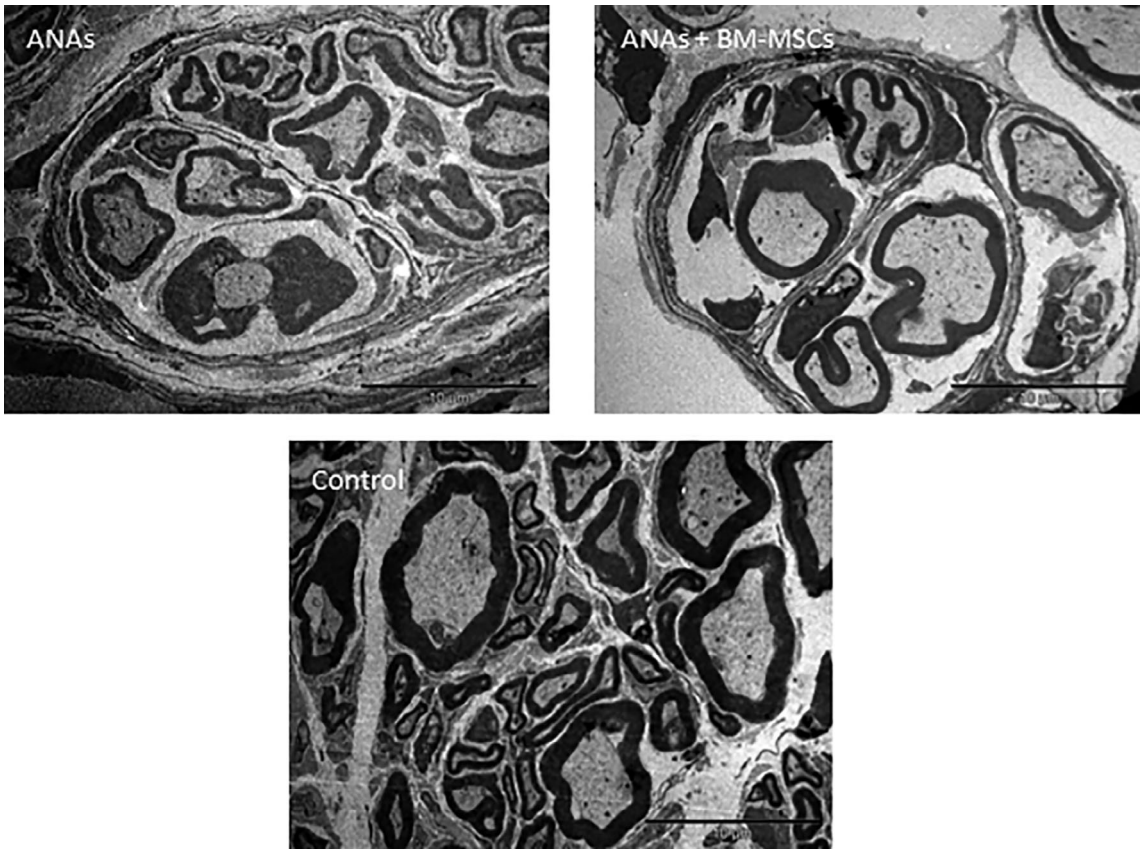


**FIGURE 6** Transmission electron microscopy of nerve grafts from ANAs, ANAs + BM-MSCs and control groups. The preservation of the basement membrane can be seen, as well as the greater diameter of the myelin sheath in the control group. (magnification ×4000). ANAs, acellular nerve allografts; MSCs, mesenchymal stem cells

Bertelli et al., contrary to Greene's proposal, showed that the biceps muscle was only innervated by the musculocutaneous nerve, but they found, 1 month after denervation, that rats which had only the musculocutaneous nerve sectioned scored higher in the grooming test than those which had both the musculocutaneous nerve and the deep branch of the radial nerve sectioned (Bertelli et al., 1995). In our study, the presence of elbow flexion synchronously with respiration by the action of the phrenic nerve was evaluated, and therefore the possible interference of the radial nerve function does not influence our evaluation.

The main limitation of the model, apart from the anatomical differences compared to the human plexus, is the possibility of spontaneous reinnervation as described by Mackinnon et al. in a sciatic nerve model, where, after 5 months, they observed spontaneous reinnervation of a nerve section with resection of 1 cm of nerve tissue (Mackinnon et al., 1985). It is true that this effect has not been demonstrated in shorter postoperative times, but it is a factor to be taken into consideration. This is why we resected 3 mm of nerve tissue to minimize the risk of spontaneous reinnervation.

Although there are already studies such as those of Fang et al., which are oriented toward the treatment of preganglionic lesions, addressing them at the avulsed roots level, exposure, especially of the higher roots, is still very complex, and more research is required in this regard (Fang et al., 2018), and therefore the use of nerve transfers continues to be the most viable option. Bergmeister et al. have shown that the use of nerve transfers without an interposition graft can hyper innervate the target muscles, generating neurophysiological changes in them, which allows a functional recovery in many cases close to normal (Bergmeister et al., 2019).

Zhang et al. with the transfer of the phrenic nerve to the musculocutaneous nerve, showed a good result in terms of survival of motor neurons, as well as nerve regeneration and reinnervation of the biceps (Zhang et al., 2004). With the exception of the study by Yang et al., which presents a model using contralateral C7 (Yang et al., 2019), our model is, as far as we know, the first time that an acellular nerve graft associated with BM-MSCs has been presented as a bridge for a transfer from the phrenic nerve to the musculocutaneous nerve.

The choice of the phrenic nerve as a donor has always been very controversial, due to the possibility of generating respiratory disorders. There are studies describing end-to-side transfers to minimize possible sequelae (Jia et al., 2018), but at the moment they have not been shown to achieve a result comparable to the use of end-to-end suture. Since we wished to represent a model as close to reality as possible, we decided to use the phrenic nerve, considering the rest of the options available to restore shoulder stability. It is also true that, due to its anatomical origin, the phrenic nerve can be damaged in

upper trunk lesions, in this case, it would be necessary to consider using other options such as the spinal accessory nerve (Chuang et al., 1995).

As in the study by Zhang et al, we evidenced the first data of biceps muscle contraction synchronously with breathing between the third and fourth week after surgery (Zhang et al., 2004). None of the animals achieved voluntary control for elbow flexion. The position of the animal, with its cephalic portion oriented toward the researcher, was key for the proper approach, which not only gave us easier access to the structures, but also allowed us to perform the surgical technique more easily (Bobkiewicz et al., 2017).

Several studies in animal models show positive results in reinnervation by associating BM-MSCs (Goel et al., 2009; Kurwale et al., 2015). Despite not having found statistically significant differences in our model when comparing the groups with and without stem cells, as evidenced by other studies such as that of Zhao et al. or Wang et al. (Zhao et al., 2014; Wang et al., 2012), we can affirm that decellularized nervous tissue has functioned as a support structure for them, as proposed by Tang et al. (Tang et al., 2013), since in fluorescence studies their survival 12 weeks post-implantation could be observed. The studies by Fan et al. show a favorable effect of stem cells in terms of immunomodulatory capacity, reducing pro-inflammatory factors, in addition to a clear superiority of Schwann-like cells compared to stem cells from bone marrow (Fan et al., 2014a; Fan et al., 2014b). It should be noted that the study by Wang et al. did not find statistically significant differences, as in our work, between acellular grafts with and without stem cells when the thickness of the myelin fibers was analyzed (Wang et al., 2012). It is possible that one of the key factors was to create an optimal microenvironment that facilitated their cellular differentiation into Schwann-like cells, maximizing their plasticity potential (Tohill & Terenghi, 2004).

In our study we once again confirmed that the results obtained with the use of ANAs are far from being those obtained with autografts, and we found that the addition of BM-MSCs failed to provide a sufficient improvement in the parameters of nerve regeneration under study. However we found no differences between the two experimental groups, we consider the model to be relevant, since there are few studies of this type focused on brachial plexus repair, showing the need to encourage the development of new alternatives. We consider that the possible clinical application of ANAs enriched with BM-MSCs should be based on new experimental studies that optimize the creation of a microenvironment that facilitate cell differentiation.

## 5 | CONCLUSIONS

This experimental study presents a new model for the study of selective lesions of the upper trunk and again validates the autologous graft as the gold standard (Sayad-Fathi et al., 2019), finding statistically significant differences in the amplitude and latency of the CMAP, as well as in the thickness of myelin sheath area, when compared with acellular nerve grafts, when used alone or when associated with BM-MSCs. The addition of BM-MSCs to the ANAs fails to provide statistically significant differences in the variables under study.

## ACKNOWLEDGMENTS

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## DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available from the corresponding author upon request.

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