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Melt Extruded PU Hollow Fibers for Nerve Regeneration: *In vivo* Study

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Abstract: Melt-extruded guides for peripheral nerve repair based on PU were realized. Various *in vivo* tests were carried out for the repair of medium (2.0 cm) size defects in the peripheral nerves of Wistar rats. Results from *in vivo* tests were subjected to histological examination after 1-3 months postoperative. Regeneration was found. Electromiographical analysis also evidenced functional recovery after 6 months from implantation. The research showed that PU guides can be used for the successful repair of small and medium size nerve defects with possible improvements by suitable bio-mimetic coatings.

Key words: Melt-extruded guides, defects, implantation, regeneration, coatings, Italy

INTRODUCTION

Peripheral nerve posses the capacity of self regeneration after traumatic injury but functional recovery is often disappointing (Sunderland, 1991). A nerve transection (neurotmesis) requires a surgical realignment (Pfister *et al*., 2007; Ciardelli and Chiono, 2006) and many strategies are developed for the repair of peripheral nerve injuries with substance loss with the common goal to direct the regenerating nerve fibres into the proper distal endoneurial tubes (Ciardelli and Chiono, 2006). The strategies described in literature to manage a nerve lesion with gaps can be roughly classified into two categories: under tension end-to-end suturing of the nerve stumps, commonly used to repair short nerve defect and bridging which includes grafting and tubulization techniques (Millesi *et al*., 1972; Battiston *et al*., 2005). Grafting and tubulization techniques are more effective since, they avoid tension across the repair site.

To date, autologous nerve graft is the method most used to repair nerve with the best results in terms of functional recovery (Ciardelli and Chiono, 2006; Huang and Huang, 2006). Inevitable disadvantage resulting from the use of such technique is the lack of nerves donors and the permanent loss of their functionality (Millesi, 1991; Terzis *et al*., 1997) and the need to perform more than one surgery. Biocompatible guides are an encouraging support for mechanical and chemical stimulation of a regenerating peripheral nerve and can provide the axonal lengthening (Ciardelli and Chiono, 2006). Several materials were tested for the production of artificial devices for nerve regeneration including degradable and not degradable biocompatible materials (Ciardelli and Chiono, 2006). Biodegradable materials offer several advantages such as the possibility of attaching Schwann cells or bioactive molecules on the polymer surface through physicochemical modification (Ciardelli and Chiono, 2006). Delivering of these bioactive molecules during biodegradation is another important characteristic of biodegradable materials. Moreover, numerous studies (Ciardelli and Chiono, 2006; Chalfoun *et al*., 2006; Labrador *et al*., 1998; Ijkema-Paassen *et al*., 2004) showed that an extracellular matrix made up for example, of insoluble laminina, fibronectin or collagen derivatives such as gelatin, promotes the elongation during axonal regeneration especially when it is incorporated in the light of the nervous guidance channel.

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Nerve Guidance Channels (NGCs) should be flexible and sufficiently permeable in order to assure the exchange of fluids between the regeneration environment and the surrounding tissue (Ciardelli and Chiono, 2006).

Materials such as Poly-L-Lactic Acid (PLLA) (Yang *et al*., 2004), the Poly-Glycolic Acid (PGA) (Nakamura *et al*., 2004) or copolymers ε-Caprolactone with dl-lactide or Policaprolattone (PCL) (Verreck *et al*., 2005) or Polyurethane (PU) (Hausner *et al*., 2007; Guelcher, 2008) are reported in the literature as suitable for nerve regeneration. Particularly copolymerization technique allows to obtain materials with specific characteristics in terms of degradation, biomechanical performance, thermal properties and water-soluble allowing to set up nerve guides that are flexible and resistant (Ciardelli and Chiono, 2006).

Polyurethanes represent a main class of synthetic elastomers applied for long-term medical implants (Pinchuk, 1994; Lamba *et al*., 1998; Gunatillake *et al*., 2001). They present tuneable chemical properties, excellent mechanical properties, good blood compatibility and can be designed to degrade in biological environments (Zdrahala and Zdrahala, 1999). The main problem in their use as biodegradable materials in biomedical field is due to the possible release of toxic diamines when conventional isocyanates are used in their synthesis (Lamba *et al*., 1998). To overcome this problem, aliphatic diisocyanates such as an L-lysine Derived diisocyanate (LDI) (Storey *et al*., 2004) and 1,4-diisocyanatobutane (Guan *et al*., 2005) were applied in the synthesis of polyurethanes for tissue engineering applications. Bruin *et al*. (1988) realized PU networks using star-shaped polyester prepolymers reacted with LDI. However, the high cross-linking degree inhibited the use of the process standard techniques. Hirt *et al*. (1996) studied (Saad *et al*., 1996) the synthesis, characterization and degradation of linear polyurethanes prepared from LDI and a series of polyester macrodiols (having poly-3-(R)-hydroxybutyrate as the hard segment and glycolide and e-caprolactone copolymers as the soft segments). *In vitro* studies showed that these materials were biocompatible did not activate macrophages and gave good level of cell adhesion when processed in the form of porous scaffolds. These results were confirmed *in vivo* (Saad *et al*., 1997). Subsequent research of Zhang *et al*. (2002) involved the preparation of cross-linked polyurethanes based on LDI and glycerol in the form of sponges as scaffolds for tissue engineering. *In vivo* preliminary studies showed that structures supported cell growth. Polyurethane based microporous scaffolds were also designed for meniscus regeneration by Spaans *et al*. (2000). More recently (Skarja and Woodhouse, 1998, 2001) proposed a series of L-phenylalanine based diamine (amino-phenyl acetic acid 4-(2-amino-2-phenyl-acetoxymethyl)-cyclohexylmethyl ester, Phe diester) containing polyurethane- ureas with tuneable degradation properties for soft tissue repair applications. In a recent research by Ciardelli *et al*. (2004) was proposed the synthesis, characterization and degradation of novel linear polyurethanes. The polymers were obtained using LDI as diisocyanate, a cyclic diol (1,4-cyclohexane dimethanol, CDM) and Phe diester as chain extender and poly (e-caprolactone) diol (PCL diol) or tri-block copolymers based on Polyethylenglycol (PEG) and Poly (e-caprolactone) (PCL) as macrodiols. Introduction of such tri-block PCL-PEG-PCL copolymers, prepared according to a procedure involving a bulk.

About 10 mm long NGC of polyurethane was implanted in rats with a 8 mm gap of transected sciatic nerve and nerve regeneration, degradation of the polymer and inflammatory response of surrounding tissues were investigated (Borkenhagen *et al*., 1998).

Moreover nerve regeneration in rats using biodegradable polyurethane tubular scaffolds was evaluated by Hausner *et al*. (2007). In this study the authors used scaffolds coated with diluted fibrin sealant.

The aim of this study was to carry out the possible employment of a biodegradable polyurethane scaffold filled with gelatin and poly-L-lysine and used as nerve guidance channel in peripheral nerve regeneration. The researchers used as experimental model rats in which an 18 mm defect in the sciatic nerve was created. Recovery function of the treated legs in context with electrophysiological and histological data are discussed.

MATERIALS AND METHODS

The researchers used a synthesised Poly (Ester-Urethane) (PU) having a commercial PCL diol (M_n = 1250; Aldrich) as a soft segment, hexamethylene diisocyanate (HDI, Aldrich) as a hard segment and 1, 4-cyclohexane dimethanol (CDM, Aldrich) as a chain extender (Rechichi *et al*., 2008).

Extrusion: Melt-extruded conduits were produced using a single-screw extruder (VSF-MAC.GI s.r.l., Italy) with 20/1 barrel length/diameter ratio and a ring-shaped die with 1.5 and 1.0 mm external and internal diameter, respectively (Fig. 1). The operating parameters were the rotation speed of the screw and the six temperature values of the different zones along the extruder length: The three temperatures along the extruder barrel (T_1, T_2 and T_3 starting from the feed zone) and those of the jaws at the end of the extruder barrel (T_4) of the extruder head (T_5)

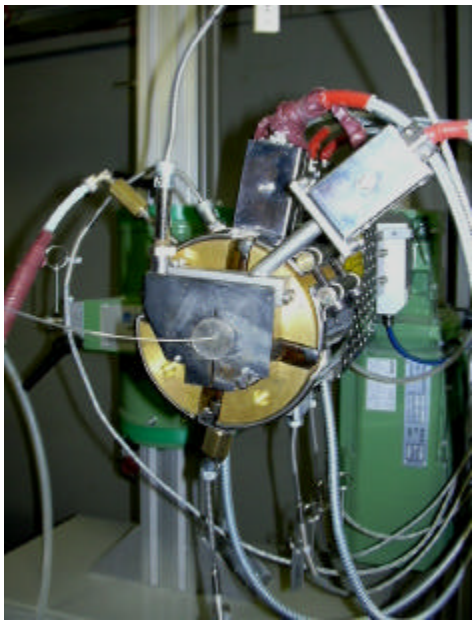

Fig. 1: Extrusion apparatus for melt-spinning hollow conduits

and of the spinneret (T_6). Pellets were accurately dried under vacuum overnight before extrusion. The rotation speed of the screw was set at 5-10 rpm whereas the T_1-T_6 temperature profile was selected on the basis of the thermal properties of the extruded material: 110, 115, 120, 125, 125, 130°C for PCL and 95, 95, 100, 100, 100, 100°C for PU.

Scanning Electron Microscopy (SEM): Sections of melt-extruded guides were obtained by sample fracturing in liquid nitrogen and then, evaluated for surface morphology by scanning electron microscopy after coating with gold (SEM, Jeol JSM 5600 LV).

Surgical procedure: Male and Female adult Wistar rats, weighing 350 g at the start of the experiment were divided randomly into three groups of 6 animals each. The end-point for these experiments was set up at 1 month (group 1, n = 6), 2 month (group 2, n = 6) and 6 months (group 3, n = 6) postoperative.

All procedures were performed with the approval of the Local Ethical Committee of Pisa University (D.Lgs.vo 116/92). Surgery was carried out under deep anaesthesia with the aid of an operating optical microscope OPMI 7 (Zeiss, Jena, Gemrany). The sciatic nerve of one side (indistinctly right or left) was exposed at the lateral side of the thigh and carefully freed from surrounding tissues. The sciatic trunk was transacted and the nerve stumps fixed in a PU guide (2 mm i.d., 2 cm long) coated with gelatin and poly-L-lysine with two epineurial stitches of 9-0 monofilament nylon applied between the tube wall and the two stumps. A gap of 20 mm was left between the stamps. After nerve surgery, the soft tissues overlaying the nerve were reapproximated with 5-0 polyglecaprone 25 (Monocryl®, Ethicon) and the skin was closed with 3-0 polyglecaprone 25 (Monocryl®, Ethicon). The opposite limb of each animal served as an uninjured control. The animals of the three groups were deeply re-anesthetized and eutanized at 1 (group 1, n = 6), 2 (group 2, n = 6) and 6 months (group 3, n = 6), a nerve segment containing the guide and 5 mm of the nerve segment proximal to the proximal suture and at least 5 mm distal to the distal suture was then excised. The morphology of the guide and surrounding tissues were carefully noted. The samples were fixed with a 4 wt./vol.-% formaldehyde solution in PBS.

EMG analysis: EMG signals were recorded during general anaesthesia by a Biopac® system using stainless steel electrodes with a sampling rate of 500 Hz. The signal was filtered with an 50 Hz analog notch filter and digital filtered in the Matlab® environment (4 Hz high-pass) and then rectified. Signal envelope was obtained using a running average filter over 100 samples. For standardizing the evaluation of signals, the beginning and end of muscle activity was defined as a detectable increase of the EMG amplitude over the background signal. To obtain a synthetic and homogenous assessment max amplitude of enveloped signals (I_{EMG}) and qualitative parameters (presence of twitch in absence of stimulation and the absence of twitch after a stimulation). To avoid dispersion of max amplitude of enveloped signals data we performed a quantization procedure with the algorithm:

$$I_{EMG}^{max} = \begin{cases} 0.01 & \text{if } \max(I_{EMG}) \le 0.01 \\ 0.1 & \text{if } 0.01 < \max(I_{EMG}) < 0.5 \\ 0.5 & \text{if } \max(I_{EMG}) \ge 0.5 \end{cases}$$

Morphological evaluation: Nerves with synthetic polymers were fixed in phosphate-buffered 4% formalin for >24 h. After fixation, guides were longitudinally cut to expose the internal nerve. Specimens were trimmed and cut into 2 mm long fragments and labeled as proximal (proximal stump joined to the tube wall), medial (fragments of regenerated nerve inside the tube) and distal (distal stump joined to the tube wall). Specimens were post-fixed in 1% osmium tetroxide (Electron Microscopy Sciences, PA, USA) at 4°C for 2 h and then dehydrated in ethanol and infiltrated and embedded in epoxy resin (Electron Microscopy Sciences, PA, USA). About 1 μm thick

transverse sections were stained with toluidine blue (Fluka, Switzerland). Sections were observed with optical microscope (Leica DM 2500M, Italy).

RESULTS AND DISCUSSION

Scanning Electron Microscopy (SEM) results: Figure 2 shows scanning electron microscopy (SEM, JEOL JSM 5600 LV, USA) images of the fractured sections of melt-extruded PU tube. Inner diameter of tube was generally 400-800 μm for PU whereas the wall thickness was about 300-500 μm. Tube walls are thick due to the higher swelling behaviour of PU melt at the exit of the spinneret. Moreover, PU inner and outer surfaces were smooth.

EMG analysis results: Figure 3 and 4 show, respectively enveloped EMG signals and their maximum values of both limbs (intact and with nerve guide) of a single rat during the entire experimental period. Signals taken from all rats have the same behaviour as it is shown in Fig. 5 where I_{EMG} max values, mediated on all rats, after the quantization procedure are plotted. This graph demonstrates that after 45-60 days from operation, limbs with nerve guides have the same behaviour of intact limbs (full recovery) and that the recovery process starts to be evident from 15°C day. The decrease of I_{EMG} max value of intact limb between 15 and 30 days can be explained as a common post-operative effect.

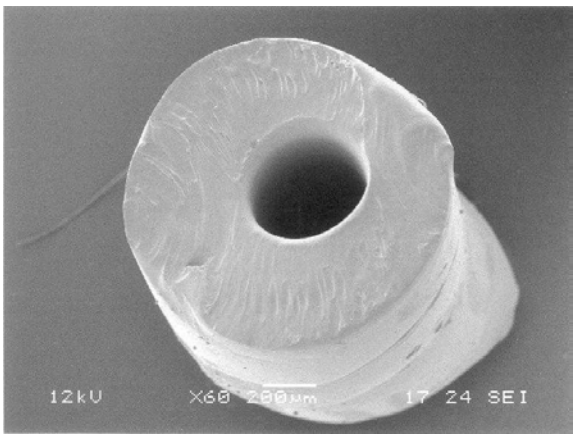

Fig. 2: SEM images of fractured sections of melt-extruded PU guide

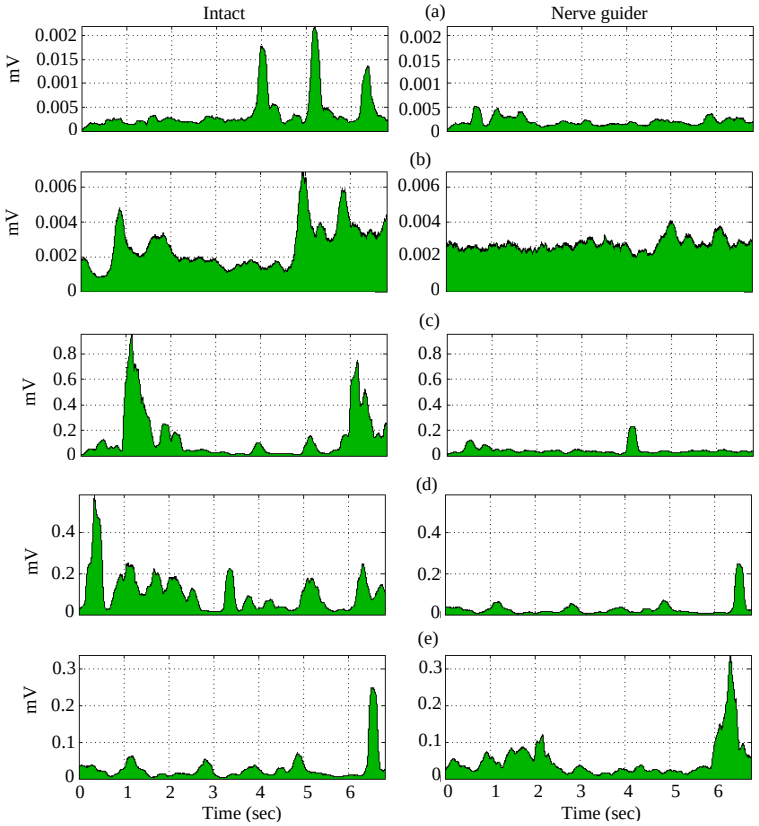

Fig. 3: Enveloped EMG signal (IEMG) of both limbs (intact and with nerve guide) of a single rat. Signals were taken at 7 (a), 30 (b), 45 (c), 60 (d) and 90 (e) days from operation

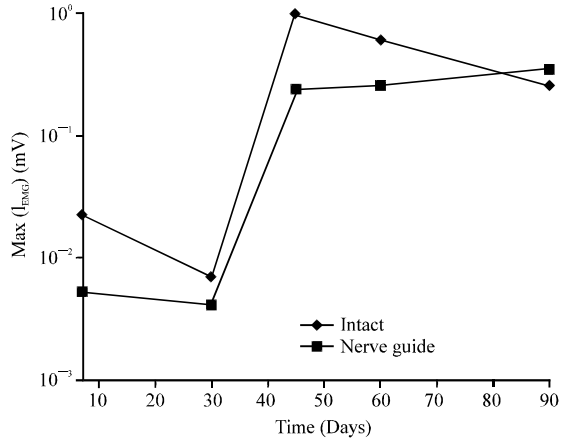

Fig. 4: Semilog-y graph of IEMG maximum value of the same rat analyzed in Fig. X, over the entire experimental period

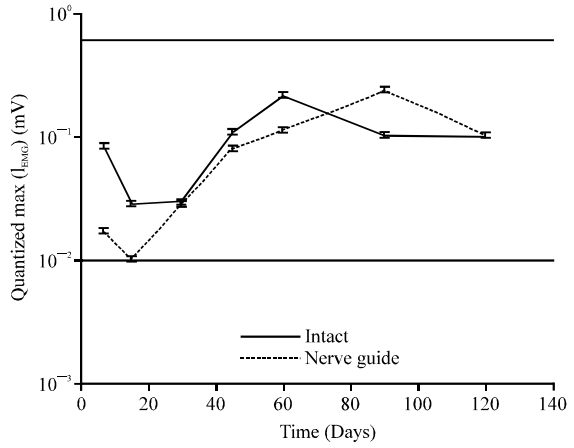

Fig. 5: Semilog-y graph of enveloped EMG maximum values mediated on all animals, after the quantization procedure described in the text. Black straight lines indicate threshold values (0.01 and 0.5 mV). Error bars indicate an error of 5%

Morphological evaluation results: Macroscopically, a regenerated nerve within the polymeric scaffold, firmly connected with proximal and distal stumps (Fig. 6) was detected in 2 rats (2/6; 33.33%) of the first group (30 days) in 3 rats (3/6; 50%) of the second group (60 days) and 4 rats (4/6; 66.67%) of the third group (160 days). A short regenerating nerve was present only in the proximal side of the polymeric scaffold without connection with the distal stump in 2 rats (2/6; 33.33%) of the first group in one rat (1/6; 16.67%) of the second group and in two rats (2/6; 33.33%) of the third group. In 2 rats (2/6; 33.33%) of the first and second group an empty polymeric scaffold was detected with absence of any nerve structures. In one case a necrotic and hemorrhagic tissue was observed whereas in another case the proximal stump have lost the connection with the polymeric scaffold. Microscopically, proximal stumps in all rats were characterized by numerous axonal degenerations involving mainly large fibers and numerous groups of axonal sprouting and regenerating fibers associated sometimes with a moderate endoneurial fibrosis and perineurial thickening (mainly at 60 and 160 days). Regenerated nerve within the polymeric scaffold was a morphologically normal nerve (Fig. 7a, c, e). Indeed, nerve was characterized by a thin perineurium, mildly cellular, rich of small caliber blood vessels and an endoneurium rich of numerous tightly packed regenerating fibers with a thin myelin sheath compared to the axonal caliber. After 30 days, nerve fibers were all small caliber fibers (Fig. 7a) whereas after 60 days medium caliber fibers were detected (Fig. 7c) and in rats after 160 days also scattered large caliber nerve fibers were seen (Fig. 7e). A slight fibrosis was observed at 60 and 160 days. Distal stumps in rats with a regenerated nerve within the polymeric scaffold, showed rare or multifocal regenerating fibers (most numerous at 160 days) associated with a moderate (60 days) or severe (160 days) endoneurial fibrosis (Fig. 7b, d, f).

Numerous axonal degeneration was also detected in distal stumps (Fig. 7f) in all rats associated with proliferation of Schwann cells (Bungner band) (especially in the first group), a less number of fibroblasts and sometimes, many small blood vessels lined by an activated endothelium. Distal stumps in cases with and empty polymeric scaffold or with a short regenerating nerve only in the proximal side, showed moderate/severe endoneural fibrosis, numerous axonal degenerations and infiltration of many macrophages rich of myelin debris. No regenerating fibers were observed.

Muscular denervation atrophy was present in all rats and remained unchanging during all the observation period. Self-mutilation lesions due to denervation/re-innervation were evident in the first 15 dat after surgical operation. The ulcers at tarsus were known mainly by 30°C after surgery and have been improving over time and with the progress of the process of nerve regeneration.

The EMG analysis demonstrated that after 45-60 days from operation, limbs with nerve guides have the same behaviour of intact limbs (full recovery) and that the recovery process started to be evident from 15°C day.

Histological examination confirmed the presence of a new regenerated nerve within the polymeric scaffold in 9 rats at 30, 60 and 160 days after surgery. Regenerated nerve was firmly connected with proximal and distal stumps and had similar findings in the three different groups containing numerous tightly packed regenerating fibers characterized by a thin myelin sheath. Fiber caliber and endoneural fibrosis increased progressively with time. The correct regeneration and connection with distal

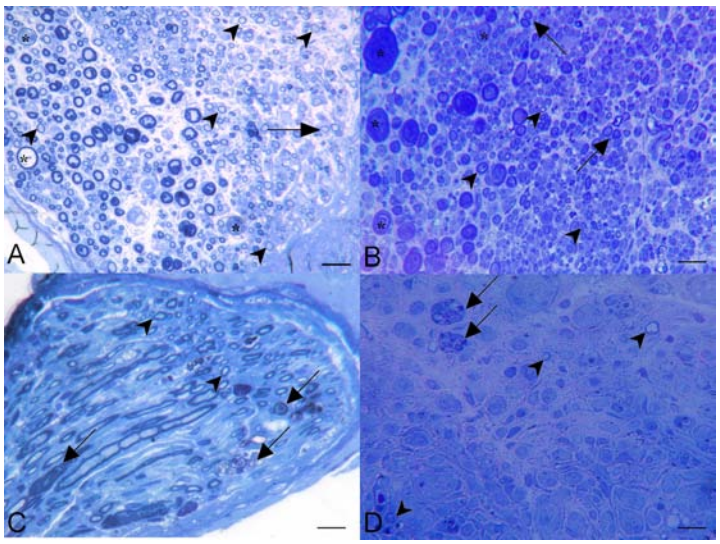

Fig. 6: Sciatic nerve after 160 days (group 3). With removing of the polymeric scaffold, a regenerated nerve is evident that is firmly connected with proximal and distal stumps

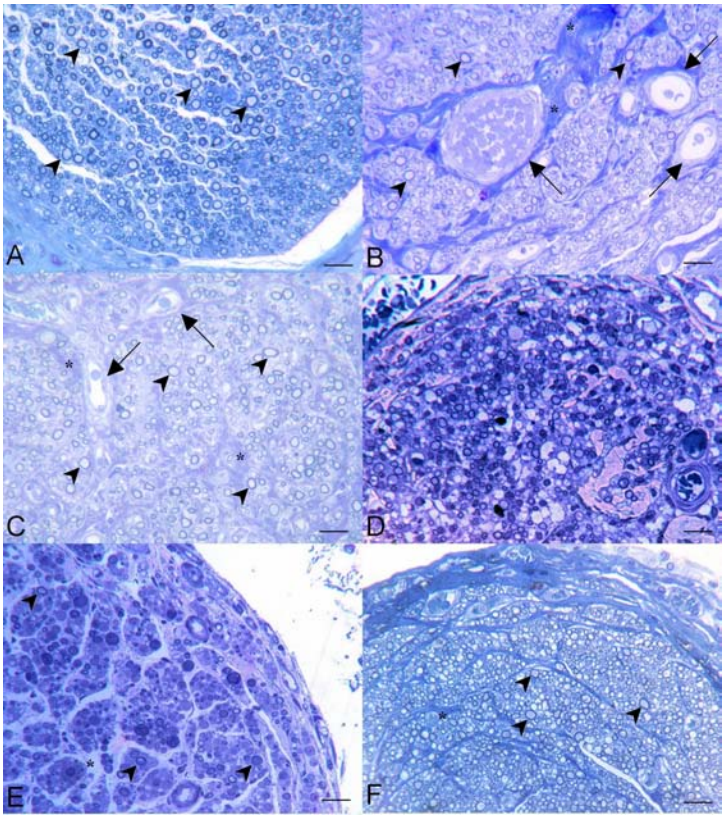

Fig. 7: Claudia regenerated nerves within the polymeric scaffold (A, C, E) and distal stumps (B, D, F) at 30 (A, B), 60 (B, C) and 160 days (D, E) after surgery. Regenerated nerve are composed of numerous small, tightly packed fibers with a thin myelin sheath. Medium fibers are evident at 60 days (C) and scattered large fibers are evident at 160 days (F). Numerous small caliber blood vessels are also evident in a. multifocal regenerating fibers are evident in distal stumps in each groups (arrows). Moderate endoneurial fibrosis is evident, associated with multifocal axonal degenerations (F). Bar = 30 μm (A); 15 μm (C, D, E); 8 μm (B, F)

stumps was confirmed by the presence of regenerating fibers in the distal stumps. The presence of axonal degenerations in distal stumps especially at 60 and 160 days was referable to aberrant regeneration that haven't found a basal laminal tube or that have tracked along an inappropriate one. At 30 days after surgery, probably, axonal degeneration (Wallerian degeneration) in distal stumps was a consequence of resection of the nerve. The moderate and sometimes fibrosis in the distal stumps could compromise a full recovery of nerve function after regeneration. In cases with evidence of regeneration just in the proximal stumps probably the regeneration was slower than others. However, in that cases, connective tissue within the polymeric scaffold that could compromise regeneration was not detected.

CONCLUSION

This study shows that histological examination confirm that the polymeric scaffold could function as excellent guide for nerve regeneration where proliferation of connective tissue was been inhibited. A correct surgical connection of cut nerve with polymeric scaffold is essential for a correct regeneration.

REFERENCES

Battiston, B., S. Geuna, M. Ferrero and P. Tos, 2005. Nerve repair by means of tubulization: Literature review and personal clinical experience comparing biological and synthetic conduits for sensory nerve repair. Microsurgery, 25: 258-267.

Borkenhagen, M., R.C. Stoll, P. Neuenschwander, U.W. Suter and P. Aebischer, 1998. *In vivo* performance of a new biodegradable polyester urethane system used as a nerve guidance channel. Biomaterials, 18: 2155-2165.

Bruin, P., G.J. Venstra, A.J. Nijenhuis and A.J. Pennings, 1988. Design and synthesis of biodegradable poly(ester-urethane) elastomer networks composed of non-toxic building blocks. Makromol. Chem. Rapid Commun., 9: 589-594.

Chalfoun, C.T., G.A. Wirth and G.R.D. Evans, 2006. Tissue engineered nerve constructs: Where do we stand. J. Cell. Mol. Med., 2: 309-317.

Ciardelli, G, A. Rechichi, P. Cerrai, M. Tricoli, N. Barbani and P. Giusti, 2004. Segmented polyurethanes for medical applications: Synthesis, characterization and *in vitro* enzymatic degradation studies. Macromol. Symp., 218: 261-271.

Ciardelli, G. and V. Chiono, 2006. Materials for peripheral nerve regeneration. Macromol. Biosci., 6: 13-26.

Guan, J., K.L. Fujimoto, M.S. Sacks and W.R. Wagmer, 2005. Preparation and characterization of highly porous, biodegradable polyurethane scaffolds for soft tissue applications. Biomaterials, 26: 3961-3971.

Guelcher, S.A., 2008. Biodegradable polyurethanes: Synthesis and applications in regenerative medicine. Tissue Eng. B: Rev., 1: 3-17.

Gunatillake, P.A., G.F. Meijs and S.J. McCarthy, 2001. Developments in Design and Synthesis of Biostable Polyurethanes. In: Biomedical Applications of Polyurethanes, Vermette, P. (Ed.). Landes Bioscience, Georgetown, ISBN-13: 9781587060236, pp: 160-170.

Hausner, T., R. Schmidhammer, S. Zandieh, R. Hopf and A. Schultz *et al*., 2007. Nerve regeneration using tubular scaffolds from biodegradable polyurethane. Acta Neurochir. Suppl., 100: 69-72.

Hirt, T.D., P. Neuenschwander and U.W. Suter, 1996. Synthesis of degradable, biocompatible and tough block-copolyesterurethanes. Macromol. Chem. Phys., 197: 4253-4268.

Huang, Y.C. and Y.Y. Huang, 2006. Biomaterials and strategies for nerve regeneration. Artif. Organs, 30: 514-522.

Ijkema-Paassen, J., K. Jansen, A. Gramsbergen and M.F. Meek, 2004. Transection of peripheral nerves, bridging strategies and effect evaluation. Biomaterials, 25: 1583-1592.

Labrador, R.O., M. Buti and X. Navarro, 1998. Influence of collagen and laminin gels concentration on nerve regeneration after resection and tube repair. Exp. Neurol., 149: 243-252.

Lamba, N.M.K., K.A. Woodhouse, S.L. Cooper and M.D. Lelah, 1998. Polyurethanes in Biomedical Applications. CRC Press, New York, USA., ISBN-13: 9780849345173, pp: 205-241.

Millesi, H., 1991. Indication and Techniques of Nerve Grafting. In: Operative Nerve Repair and Reconstruction, Richard, H. and M.D. Gelberman (Eds.). Lippincott Williams and Wilkins, Philadelphia, PA., ISBN-13: 978-0397510740, pp: 525-544.

Millesi, H., G. Meissl and A. Berger, 1972. The interfascicular nerve-grafting of the median and ulnar nerves. J. Bone Joint Surg., 54: 727-750.

Nakamura, T., Y. Inada, S. Fukuda, M. Yoshitani and A. Nakada *et al*., 2004. Experimental study on the regeneration of peripheral nerve gaps through a polyglycolic acid-collagen (PGA-collagen) tube. Brain Res., 1027: 18-29.

Pfister, L.A., M. Papaloizos, H.P. Merkle and B. Gander, 2007. Nerve conduits and growth factor delivery in peripheral nerve repair. J. Peripher. Nerv. Syst., 12: 65-82.

Pinchuk, L., 1994. A review of the biostability and carcinogenicity of polyurethanes in medicine and the new generation of biostable polyurethane. J. Biomater. Sci. Polym. Ed., 6: 225-267.

Rechichi, A., G. Ciardelli, M. Dâ_™Acunto, G. Vozzi and P. Giusti, 2008. Degradable block polyurethanes from nontoxic building blocks as scaffold materials to support cell growth and proliferation. J. Biomed. Mater. Res. A, 84: 847-855.

Saad, B., S. Matter, G. Ciardelli, G.K. Uhlschmid, M. Welti, P. Neuenschwander and U.W. Suter, 1996. Interactions of osteoblasts and macrophages with biodegradable and highly porous polyesterurethane foam and its degradation products. J. Biomed. Mater. Res., 32: 355-366.

Saad, B., T.D. Hirt, M. Welti, G.K. Uhlschmid, P. Neuenschwander and U.W. Suter, 1997. Development of degradable polyesterurethanes for medical applications: *In vitro* and *in vivo* evaluations. J. Biomed. Mater. Res., 36: 65-74.

Skarja, G.A. and K.A. Woodhouse, 1998. Synthesis and characterization of degradable polyurethane elastomers containing and amino acid-based chain extender. J. Biomater. Sci. Polym. Ed., 9: 271-295.

Skarja, G.A. and K.A. Woodhouse, 2001. *In vitro* degradation and erosion of degradable, segmented polyurethanes containing an amino acid-based chain extender. J. Biomater. Sci. Polym. Ed., 12: 851-873.

Spaans, C.J., V.W. Belgraver and O. Rienstra, 2000. Solvent-free fabrication of micro-porus polyurethane amide and polyurethane-urea scaffolds for repair and replacement of the knee-joint meniscus. Biomaterials, 21: 2453-2460.

Storey, R.F., J.S. Wiggins, K.A. Mauritz and A.D. Puckett, 2004. Bioabsorbable composites. II: Nontoxic, L-lysine-based poly(ester-urethane) matrix composites. Polym. Compos., 14: 17-25.

Sunderland, S., 1991. Nerve injuries and their repair: A critical appraisal. Churchill Livingstone, London, ISBN-13: 9780443041617, Pages: 538.

Terzis, J.K., D.D. Sun and P.K. Thanos, 1997. Historical and basic science review: Past, present and future of nerve repair. J. Reconstr. Microsurg., 13: 215-225.

Verreck, G., I. Chun, Y. Li, R. Kataria and Q. Zhang *et al*., 2005. Preparation and physicochemical characterization of biodegradable nerve guides containing the nerve growth agent sabeluzole. Biomaterials, 26: 1307-1315.

Yang, F., R. Murugan, S. Ramakrishna, X. Wang, Y.X. Ma and S. Wang, 2004. Fabrication of nano-structured porous PLLA scaffold intended for nerve tissue engineering. Biomaterials, 25: 1891-1900.

Zdrahala, R.J. and I.J. Zdrahala, 1999. Biomedical applications of polyurethanes: A review of past promises, present realities and a vibrant future. J. Biomater. Appl., 14: 67-90.

Zhang, J.Y., E.J. Beckman, N.P. Piesco and S. Agarwal, 2002. A new peptide-based urethane polymer: Synthesis, biodegradation, and potential to support cell growth *in vitro*. Biomaterials, 21: 1247-1258.