

# Athymic rat model for studying human acellular allograft

Tim C. Keating, BS<sup>1</sup>, Jonathan Isaacs, MD<sup>2</sup>, Satya Mallu, MD<sup>2</sup>

<sup>1</sup>VCU School of Medicine, <sup>2</sup>Division of Hand Surgery, Department of Orthopedic Surgery, VCUHS  
Richmond, VA, USA

## Introduction

Although human acellular nerve allograft is a promising nerve repair tool, optimizing graft application and understanding effective graft dimensions has been hampered by lack of an appropriate animal model. Rodent nerve acellular allograft can be tested in the utilitarian rodent nerve repair model, but testing different size options is limited by the size of the rodent donor animal. Human acellular nerve allograft offers the variety of sizes desired for more complete study but poses a high risk of rejection as xenograft tissue in the rodent model. Athymic nude rats are less prone to reject xenograft tissue due to their immunocompromised state and may offer an animal model for testing human acellular allograft.

## Materials and methods

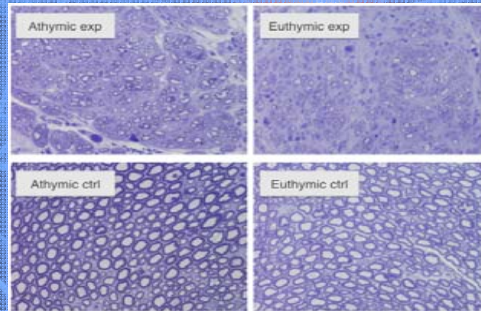
Fifteen athymic nude and 15 Sprague-Dawley rats underwent unilateral excision and repair of a 10mm tibial nerve segment using 10mm of human acellular nerve graft. At 3 months, the rats underwent testing. The gastrocnemius and distal tendon were dissected away from surrounding tissues and attached to a clamp while preserving the proximal neurovascular pedicle. Tetanic contractions were stimulated using a bi-polar electrode applied to the proximal tibial nerve (5V, 1.5sec, 70Hz) and implanted nerve graft and contralateral control nerves were excised and histologic specimens prepared from the middle of the grafts. The specimens were stained with toluidine blue to allow for axon counting, measurements, and myelin quantification. Bilateral gastrocnemius muscles were harvested and weighed, and the rats were euthanized.

## Acknowledgments

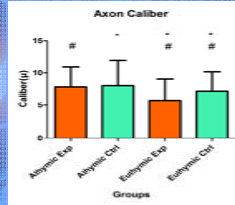
We would like to thank the VCU Department of Animal Care, the faculty of the Microscopy facility in the Department of Anatomy and Neurobiology, the VCUHS Department of Orthopedics and AxoGen Inc. for their assistance in this project.

## Results

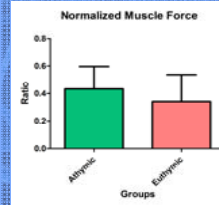
**General observations:** The euthymic rats that underwent nerve repair with human acellular allograft performed poorly in physical measures and histomorphometric analysis compared to other groups.



Following repair with human acellular allograft, nerves from athymic rats demonstrated regeneration of mature, well-formed axons. Nerves of euthymic rats generally demonstrated patchy regeneration of small, poorly myelinated axons. Nerves from contralateral control limbs of both athymic and euthymic rats demonstrated similar histology on light microscopy.



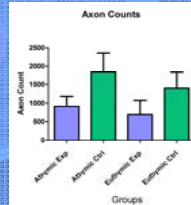
**Normalized axon caliber** was 0.9761 ± 0.3904 in athymics and 0.7995 ± 0.4617 in euthymics. Normalized axon caliber was significantly ( $p < 0.0001$ ) greater in athymic rats compared to control rats.



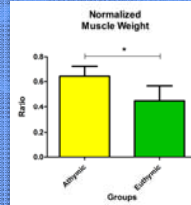
**Normalized developed force** for the athymic group was 0.4335 ± 0.163 versus 0.3408 ± 0.196 for euthymic rats, which was not significantly different ( $p > 0.05$ ).



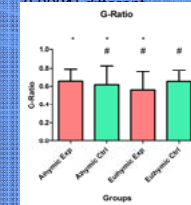
Gross specimens from athymic nerve sections showed nerve fascicles that were well-developed with mature epineurium. Euthymic experimental limbs uniformly demonstrated smaller nerve fascicles, many of which were malformed and hemorrhagic.



**Axon count** of athymics was 1593 ± 741 and 687 ± 384 for euthymics. Control limbs of athymics had a mean axon count per nerve section of 1829 ± 526 and 1396 ± 426 for controls. All groups were significantly ( $p < 0.0001$ ).



**Normalized muscle weight** was 0.6414 ± 0.0829 for athymics and 0.4505 ± 0.1161 for controls. Both experimental groups were significantly different ( $p < 0.0001$ ) than contralateral controls.



**The G ratio** of athymic experimental limbs was 0.6557 ± 0.1366 and 0.5610 ± 0.2008 in the experimental euthymics. Athymic control limbs had a mean G ratio of 0.6169 ± 0.2091 and euthymic limbs had a mean G ratio of 0.6536 ± 0.1235.

## Conclusions

The immunocompromised state of the athymic rat seemed to support more successful nerve regeneration through processed acellular human nerve tissue implanted as nerve xenograft when compared to similar tissue implanted into euthymic or immunocompetent rats. Gross inspection of the human nerve following implantation into euthymic rats demonstrated central necrosis consistent with immune-rejection not seen either grossly or histologically with the athymic rats. Additionally, axon counts, axon caliber, and reinnervated muscle weights all indicated superior axon regeneration in the athymic rats.

Our study confirms the previous notion that xenograft rejection is a hindrance to using human xenograft in a rodent model. The capability to directly test commercially available human acellular nerve grafts in a relatively easy-to-use rodent model would advance our understanding of these nerve grafts and help optimize their use in humans. The use of an athymic nude rat model appears to be a promising tool that warrants further investigation.

## Corresponding author

Jonathan E. Isaacs, M.D.  
Jonathan.isaacs@vcuhealth.org

## Literature cited

Chiu MC, Wang BC, Wang PC, Chang JG, Ngai JC, Shue H, Shue CH, Fann WE (2007) Allograft nerve repair in the upper extremity using processed human allograft. *J Hand Surg Am*. 2007;31:2366-74.

Quinn C, Williams DF, Keating T, Frazier PR, Blalock JC (2012) Use of human acellular nerve grafts in a rodent model of peripheral nerve injury: histological and behavioral outcomes. *J Bone Joint Surg Am*. 2012;34:451.

Johnson PJ, Neuman P, Hunter DM, Mackinnon SE. Nerve axonal microstructure facilitates and biases distribution of regenerated fibers: a pilot study. *Journal of Neurosci*. 2011;31:3330.