INTRODUCTION

Severe trauma to the extremities resulting in simultaneous injury to multiple tissue types – such as bone, muscle, blood vessel, and nerve – presents many clinical challenges. The complex nature of the injuries often results in multi-stage treatment to salvage the limb and diminished long-term function even if aesthetic reconstruction is successful. Current models of musculoskeletal trauma typically consist of critically-sized single tissue defects and are therefore not useful for investigating strategies for multi-tissue repair. Our goal in this experiment was to develop and characterize a novel model of composite lower limb bone and nerve trauma to facilitate future investigation of new technologies designed to promote functional regeneration.

METHODS

All procedures were reviewed and approved by the Georgia Tech IACUC. Thirteen-week-old female Lewis rats received unilateral surgeries encompassing four groups (n=8-9 each): 8mm mid-femoral bone defect (B), 14mm sciatic nerve gap (N), composite 8mm bone defect in combination with 14mm nerve gap (C), and 14mm sciatic nerve autograft (A).

Each bone defect was encompassed by an implanted PCL nanofiber mesh tube and treated with 2μg of BMP-2 delivered in pre-gelled 2% RGDFunctionalized alginate. Treatment of each nerve gap consisted of implanting a polysulfone nerve guidance channel containing a single PAN-MA thin-film with aligned fiber topography. Treatment in the nerve autograft group consisted of excising the 14mm segment, rotating 180° and suturing back in place. Sixteen weeks were allowed for regeneration post-surgery. Bone regeneration was assessed longitudinally via in vivo radiographs and microcomputed tomography (µCT) scans. Post-mortem, femur explants were again scanned prior to mechanical testing in torsion. Age-matched intact femurs served as controls. Evaluation of nerve regeneration included determination of compound nerve action potential conductance velocity in a terminal electrophysiology procedure. Histology was performed on nerve tissue explants to visualize and quantify regenerating axons in cross-sections from the middle region of the defect. Age-matched unoperated nerves served as controls. Assessment of gait was performed longitudinally using Noldus CatWalk equipment and software. Data were analyzed using t-test, ANOVA (Tukey’s post-hoc), or Kruskal-Wallis (Mann-Whitney post-hoc), as appropriate (significance at p<0.05).

RESULTS

Blinded evaluation of in vivo radiographs revealed similar bony bridging rates for Groups B and C, with all but one sample from Group B having bridged by 8 weeks post-surgery. No differences were observed between groups for new mineral volume or density parameters at any time point (Fig. 2). In addition, no difference was observed in failure strength between Groups B and C. Both groups did fail at significantly lower loads than Group N and intact femurs. Group N femurs also saw a reduction in strength compared to intact (Fig. 3A).

Electrophysiology measures showed no difference in conductance velocity between Groups N and C, but both were less than Group A and intact nerves. While showing improved performance, Group A conductance was still significantly slower than intact nerves. Nerves from Group B had no difference compared to intact (Fig. 3B). Although quantitative measures of axon regeneration are pending, preliminary qualitative results indicated no significant difference between Groups N and C. Also, axon diameter appeared noticeably slower than intact nerves.

Gait parameters indicated Group C experienced a significant functional deficit compared to Groups B and N. No group with nerve injury experienced a restoration of gait to pre-injury levels. The dynamic gait parameter Duty Cycle indicated that Group B did not experience a significant decrease from pre-injury baseline (Fig. 5).

DISCUSSION

In this experiment we have successfully established and characterized a novel small animal model of composite lower limb bone and nerve trauma. Interestingly, local bone and nerve regeneration did not appear affected in the composite injury group compared to single injury counterparts. Despite comparable tissue regeneration, the composite injury group experienced a marked functional deficit compared to single injury groups. Taken together, these results indicate the importance of identifying treatment strategies focused not only on specific tissue regeneration outcomes, but also restoration of function.

ACKNOWLEDGEMENTS

This work was supported by the Armed Forces Institute for Regenerative Medicine (AFIRM).