Radioprotection of Tendon Allografts and Bioburden Inactivation using Ebeam Radiation

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Statement of Purpose: Musculoskeletal allografts provide an unmatched alternative to autografts for use in injuries requiring reconstructive procedures. Although with an annual rise in allograft implantation, the risk of disease transmission is a major concern and primary deterrent to patient acceptance. Ionizing radiation has been effective at neutralizing bioburden through direct transfer of high energy and free radical manipulation, both causing disruption of nucleic bonds. At sufficiently high doses, these processes also affect matrix proteins causing the loss of mechanical integrity. In order to use ionizing radiation as a sterilization method successfully, allografts would have to be protected against radiation damage. Our research seeks to optimize a radioprotective treatment involving a combination of crosslinking and free radical scavenging aimed at counteracting radiation effects. In this study, we specifically want to determine: 1) if the treatment could maintain allograft mechanical properties after high dose radiation, 2) if sterilization is still effective at inactivating bioburden in grafts after radioprotective treatment.

Methods: Soleus tendons were harvested from male New Zealand White rabbits purchased frozen from Bioreclamation Inc. (Liverpool, NY). Tendons were first crosslinked with an EDC (1-ethyl-3-(3-dimethyl aminopropyl) carbodiimide) solution followed by soaking in an ascorbate/riboflavin free radical scavenger cocktail solution. Another group was soaked in saline to investigate radiation effects. Tendons were then shipped in solution to E-beam services Inc. (Cranbury, NJ) for sterilization at 25 and 50 kGy. These sterilized groups were compared to native tendon. Tendons were pulled in tension to failure using an instron testing machine to obtain mechanical properties.

Treatment effects on bioburden were determined before and after sterilization. This study was performed in conjunction with Gibraltar Labs (Fairfield, NJ). Tendons were harvested aseptically and inoculated with 1e5 cfu of *Bacillus pumilis*, then randomly allocated to three groups. The first was a control group remained untreated, and used to confirm bioburden load as well as account for changes in bacterial growth during the treatment process. Our protective treatment was applied to the remaining two groups: non-irradiated and irradiated. Bioburden analysis of non-irradiated tendons after treatment was used to estimate a minimally lethal dose. Any bioburden recorded after radiation exposure would suggest that sterilization efficiency was compromised.

Results: Radiation effects on untreated tendon showed decreases in mechanical properties, and continued the trend with higher radiation dose. Comparatively, our treatment combination of crosslinking and free radical scavenging showed successful radioprotection of tendon in the presence of e-beam radiation. Radioprotected tendons had significantly greater strength, elastic modulus and toughness compared to PBS soaked tendons. Mechanical properties were comparable or higher than native tendons. (Fig. 1A)

Bioburden analysis showed no significant changes after performing radioprotective treatment. Accordingly, 19.9 kGy was calculated to be the target radiation dose. Results show that this dose was successful in neutralizing nearly all the bioburden present with all samples reported to possess less than 1 cfu. (Fig. 1B). At this radiation dose, there was no difference in bioburden levels between radioprotected and untreated tendons.

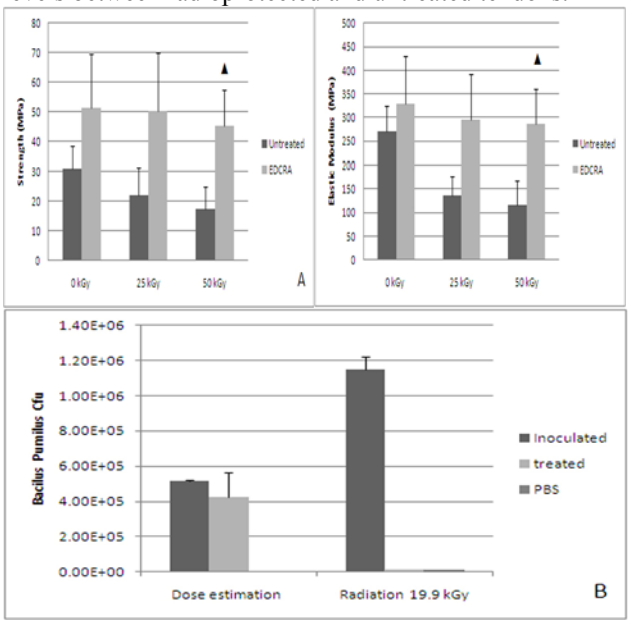

Figure 1 (A) Radioprotection of allograft strength and modulii after exposure to ebeam radiation (B) Treatment influence on bioburden sterilization (▲ $p<.05$)

Conclusions: Our results suggest that mechanical properties of tendon allografts can be protected at high doses. Radioprotection can be attributed to EDC and scavenger components of our treatment, which drive the formation of crosslinks in tissue collagen to compensate for reduction caused by radiation, and sequester free radicals to minimize interaction with matrix collagen, respectively. Although, the true extent of radioprotection and quality of graft tissue cannot be determined without *in vivo* studies, which are currently being performed.

Bioburden analysis conducted in this study was motivated by the potential for free radical scavengers to protect bacteria, therefore requiring higher doses to achieve sterility. Our data suggests that there is no protective effect on bioburden by radioprotective treatments during sterilization. This study must be repeated with other forms of bioburden including viruses to ensure effective sterilization of a range of pathogens.

Terminal sterilization of allografts would allow for safe and regular allograft distribution. Ionizing radiation could be used to achieve this if complimented with radioprotective treatments developed in this research.