Decellularized Tendon-Bone Composite Grafts are Less Immunogenic and Stronger than Untreated Grafts – an In Vivo Experimental Study in Rat

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Introduction:
-tendon-bone insertion site (TBI) injuries are challenging
-healing is typically through scar formation at the insertion site
-tissue engineered tendon-bone composite graft may be an alternative to direct tendon-to-bone suture

Aim:
-to show that decellularized grafts (TBI) are stronger and less immunogenic than untreated grafts in vivo

Hypothesis:
-replacing immune-competent Sprague Dawley (SD) rat TBIs with decellularized composite tendon-bone allografts from Wistar rats would lead to less inflammation than reconstruction with untreated Wistar allografts
-as a result of reduced inflammatory destruction, decellularized grafts would display greater strength properties than untreated grafts after 2 and 4 weeks in vivo.

Materials and Methods:
Composite Achilles-calcaneus TBI grafts were harvested from Wistar rats and divided into 2 groups. Grafts in Group 1 were physicochemically decellularized detergents and ultrasonication. Grafts in Group 2 were untreated controls. SD rats underwent Wistar TBI allograft reconstruction of bilateral hindlimb Achilles tendon-calcaneus bone tendon-bone insertion sites using a pair-matched design. SD rats were killed at 2 or 4 weeks and the reconstructed hindlimbs were harvested. The extent of B-cells and macrophage infiltration was determined using immunohistochemistry (IHC). The explants were subjected to mechanical testing to determine the ultimate failure load. Statistical analysis was performed using a paired Student’s T-test.

Results:
At 2 weeks, there was increased B-cell and macrophage infiltration in the untreated compared with the decellularized group, both in the capsule surrounding the TBI and the tendon substance. There was improved ultimate failure load (33.6±7.5N vs 24.0±9.8N, respectively, p=0.044) in the decellularized group.
At 4 weeks, there was persistent B-cell and macrophage infiltration in the untreated group. The decellularized group also demonstrated persistently greater ultimate failure load (46.5±17.7N vs 22.7±7.3N, respectively, p=0.042) compared with the untreated group.

Conclusions:
-decellularization of tendon-bone composite grafts removes cell surface antigens leading to a decreased immune response when used for allograft reconstruction.
-these grafts showed better biomechanical properties at 2 and 4 weeks when compared with untreated tendons.
-decellularization is an important step in the processing of tissue engineered tendon-bone composite grafts for upper extremity TBI reconstruction.