INTRODUCTION
Tendon allografts are used in many musculoskeletal reconstructive procedures. The advantages include ready availability, absence of donor site morbidity and reduced operating time. In addition, allografts are especially useful in intrasynovial applications, because intrasynovial autografts are usually not available. However, while allograft remodeling and incorporation into bone and host tendon are similar qualitatively to autografts, they progress much more slowly than in autografts. Enhancement of allograft incorporation could improve clinical outcomes.

In addition, tendon/bone healing is also problematic because of the difficulty of restoring the normal fibrocartilaginous transitional zone of the normal tendon/bone interface[1]. Finally, a surface initially optimized for gliding may not be ideal to facilitate tissue integration. When the tendon surface is modified by trypsin digestion, the tendon surface becomes visibly rougher and friction increases[2]. The roughened surface also provides a greater surface area for cell adhesion. In many studies, the topographical effects of roughness on cell behavior have been examined in various artificial materials, such as metals and polymers. However, little is known about the effect of roughening of native tissue on attached cell topography.

The purpose of this study was to investigate the effect of roughening the tendon surface, either chemically (with trypsin digestion), physically (by mechanical abrasion), or both, on cell attachment to the tendon surface in an ex vivo intrasynovial tendon (IT) model.

METHODS
Ninety canine FDP tendons of 2nd-5th digits from six dogs were used. FDP tendons and bone marrow were harvested from mixed-breed dogs which were euthanized for other IACUC approved studies that did not involve or affect any tendon related research. The tendons were prepared as they would be for allograft use and divided into five treatment groups as follows: (A) untreated IT, (B) IT with mechanical abrasion, (C) IT with trypsin treatment, (D) IT with abrasion and trypsin, (E) untreated extrasynovial tendon.

Bone Marrow Stromal Cells (BMSCs) Harvest and Culture
Immediately prior to euthanization, tibial bone marrow was aspirated from each dog. The aspirates were centrifuged and cultured with 10 mL of minimal essential medium (MEM) with Earle’s salts (GIBCO, Grand Island, NY), 10% fetal calf serum and 1% antibiotics (Antibiotic- Antimycotic, GIBCO, Grand Island, NY). After 3 days of incubation, the medium containing floating cells was removed and new medium was added to the remaining adherent cells. These adherent cells were defined as bone marrow stromal cells (BMSCs).

Tendon Preparation
The 2nd-5th digit FDP tendons were harvested and decellularized by five freeze-thaw cycles and then lyophilized in a custom-made lyophilizer for 24 hours. All tendons were sterilized with gas one week before use and rehydrated in a 0.9% NaCl for 24 hours in a 37 °C incubator immediately prior to use. In groups B and D, before rehydration, the lyophilized tendons were exposed 15 times with 180 grit abrasive cloths (3M, St. Paul, MN). After rehydration, tendons in groups C and E were treated with 0.25% trypsin (Sigma, T-0303) for 2 hours at 37 °C.

Cell Seeding of Decellularized Tendons and Culture
Each tendon was cut into a 1-cm long portion that was just proximal to distal vincula attachment, and then immersed in MEM, 10% fetal calf serum, and 1% antibiotics in coculture with BMSCs at a density of 1×10^6 cells/mL, and incubated for one or two weeks.

Observation of Cell Attachment
At the conclusion of the one or two week culture period, cells on the tendon surface were stained with LIVE/DEAD Viability/Cytotoxicity Kit (Molecular Probes, Eugene, OR). Four surface photos on each tendon (using C-Apochromat 10X/0.45 W objective lens) were captured using a confocal laser microscope (LSM 5 Live, Carl Zeiss, Thornwood, NY). The percentage of the area covered by live cells was determined using image processing software (KS400, Carl Zeiss), averaging the results of 4 photos per tendon. In addition, the morphology of the tendon surface and attaching cells was observed with scanning electron microscopy (SEM) before and after culture.

Statistics
All results were analyzed with one-way ANOVA and post hoc pairwise comparisons using Tukey’s technique. A P-value < 0.05 for any statistical analysis was considered significant.

RESULTS
Group C tendons had greater coverage by BMSCs than any of the other tendons after both one and two weeks of culture (p<0.05).

DISCUSSION
This study has shown that tendon surface treatment or roughening can affect cell attachment and cell behavior. Trypsin digestion can increase the coverage of BMSCs on the tendon surface, as confirmed with both confocal microscopy and SEM. Due to cell overlap, it was not possible to do precise cell counts in many specimens. However, when cells spread beyond a certain threshold, cell proliferation can be promoted[3] and we believe that proliferation was at least a part of the basis for the increase in cell area for the trypsinized tendons in this study. Regardless, the results here suggest that trypsin treatment could be useful for the enhancement of tendon/bone healing.

The limitation of our study is that we did not assess the entire surface of the tendons through confocal microscopy, instead only sampling the surface. In addition, cell counting was not possible with our methods. Based on these results, we plan further studies using an in vivo model.

ACKNOWLEDGEMENT
This study was funded by a grant from NIH (NIAMS AR057745).

REFERENCES