Engineering Tissue-to-Tissue Interfaces and the Formation of Complex Tissues

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INTRODUCTION TO TISSUE-TO-TISSUE INTERFACES

A significant challenge in the field of tissue engineering is the simultaneous formation of multiple types of tissues and the functional assembly of these tissues into complex organ systems. This grand challenge holds special significance for orthopaedic regenerative medicine, as musculoskeletal motion is facilitated by synchronized interactions between many types of tissue and the seamless integration of bone with soft tissues such as tendons, ligaments or cartilage (Figure 1). These tissue-to-tissue interfaces are ubiquitous in the body and exhibit a gradient of structural and mechanical properties that serve a number of functions, from mediating load transfer between two distinct types of tissue to sustaining the heterotypic cellular communications required for interface function and homeostasis (Benjamin et al. 1986, Woo et al. 1988, Lu and Jiang 2006). These critical junctions are prone to injury and unfortunately, not re-established following standard surgical repair. Failure to regenerate the intricate tissue-to-tissue interface compromises graft stability and long term clinical outcome (Friedman et al. 1985, Robertson et al. 1986); Consequently, there exists an unmet demand for grafting systems for *biological fixation* or *integrative* repair of soft tissues .

Utilizing a combination of cells, growth factors and/or biomaterials, the principles of tissue engineering (Skalak 1988, Langer and Vacanti 1993) have been readily applied to the formation of a variety of connective tissues such as bone, cartilage, ligament or tendon *in vitro* and *in vivo*. More recently, the emphasis in the field of has shifted from tissue formation to tissue function (Butler et al. 2000), with a concentration on imparting biomimetic functionality to orthopaedic grafts and enabling their translation to the clinic. Presently, a significant barrier to clinical translation is how to achieve *biological fixation* or functional integration of the tissue engineered orthopaedic grafts, be it bone, ligaments or cartilage, either with each other and/or with the host environment. The nature of this interface tissue engineering challenge is rooted in the complexity of the musculoskeletal system and

the structural intricacy of both hard and soft tissues. These tissues, each with a distinct cellular population, must operate in unison to facilitate physiologic function and maintain tissue homeostasis. It is thus not surprising that the transition between various tissue types is characterized by a high level of heterogeneous structural organization that is crucial for joint function. For example, as shown in Figure 1, ligaments and tendons with direct insertions into bone exhibit a multi-tissue transition consisting of three distinct, yet continuous, regions of ligament, fibrocartilage, and bone (Cooper and Misol 1970, Benjamin et al. 1986, Wang et al. 2006). The fibrocartilage interface is further divided into non-calcified and calcified regions. In light of this complexity, functional tissue engineering must incorporate *strategic biomimicry* or a more selective design approach in order to regenerate the intricate tissue-to-tissue interface and ultimately, enable seamless graft integration and functional repair.

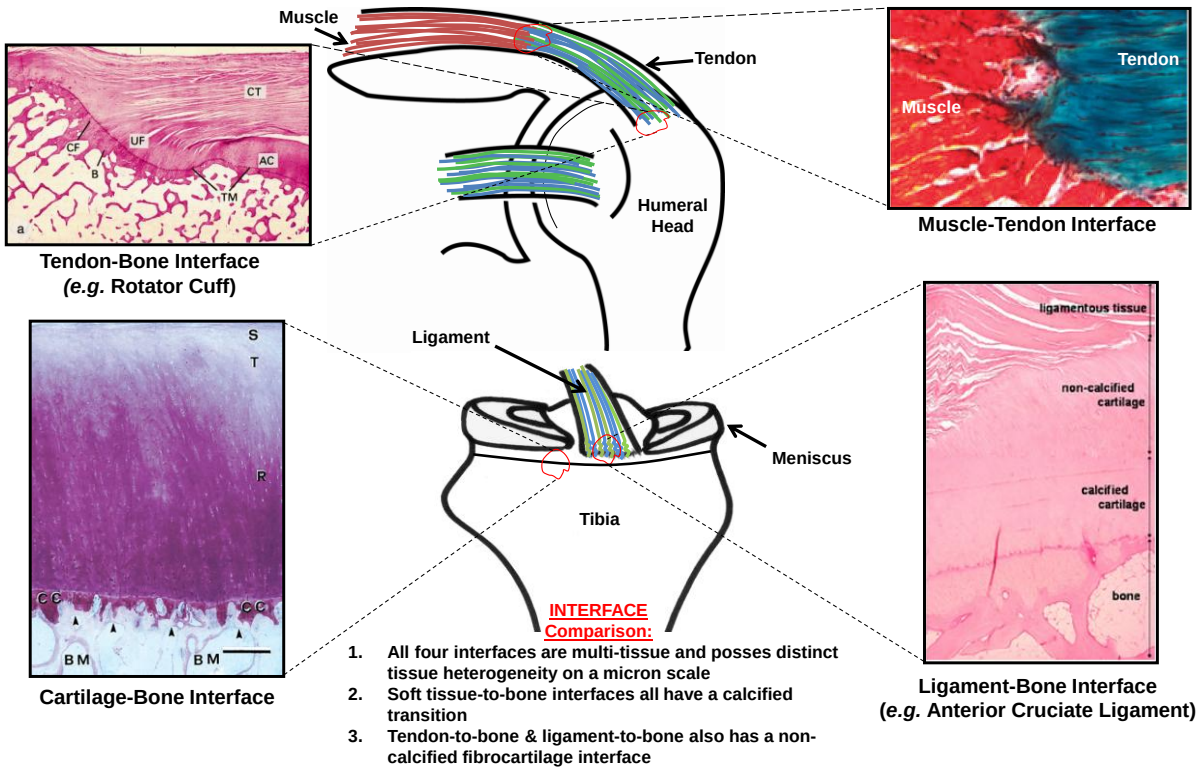

Figure 1. Common Tissue-to-Tissue Interfaces. *Significant structural and compositional homology exist in orthopaedic tissue-to-tissue interfaces, namely the Tendon-Bone (Benjamin and Ralphs 1998), Muscle-Tendon(50),Cartilage-Bone (Hunziker et al. 2002), Ligament-Bone insertion site (Iwahashi et al. 2010). Regeneration of these complex junctions is essential for integrative soft tissue repair and treatment of massive, multi-tissue injuries prevalent in battlefield wounds.*

MECHANISM OF INTERFACE REGENERATION

A fundamental question in interface tissue engineering is how distinct boundaries between different types of connective tissues are re-established post-injury. When tendon is re-sutured to its original attachment site, cellular organization resembling that of the native insertion have been observed *in vivo* (Fujioka et al. 1998). It was also reported that while healing following ligament reconstruction does not lead to the re-establishment of the native insertion, a layer of interface-like tissue is formed within the bone tunnel (Rodeo et al. 1993, Grana et al. 1994, Blickenstaff et al. 1997). These observations collectively suggest that when trauma or surgical intervention results in non-physiologic exposure of normally segregated tissue types (*e.g.*, bone or ligament), interactions between the resident cell populations in these tissues (e.g. osteoblasts in bone, fibroblasts in tendon, stem cells/progenitor cells in both tissues) are critical for initiating and directing the repair response that lead to the re-establishment of a fibrocartilage interface between soft tissue and bone. In 2006, Lu and Jiang (Lu and Jiang 2006) proposed a working hypothesis for interface regeneration, suggesting that osteoblast-fibroblast interactions mediate interface regeneration through heterotypic cellular interactions that can lead to phenotypic changes or trans-differentiation of osteoblasts and/or fibroblasts. These interactions may also induce the differentiation of stem cells or progenitor cells into fibrochondrocytes and thereby, promoting the regeneration of the fibrocartilage interface. This hypothesis has been validated using co-culture and tri-culture models of interface-relevant cell populations (Jiang et al. 2005, Wang et al. 2007). It was found that cellular interactions play a regulatory role in the induction of interface-specific markers in progenitor or stem cells, demonstrating the effects of heterotypic cellular interactions in regulating both the repair and maintenance of soft tissue-to-bone junctions.

INTERFACE STRUCTURE-FUNCTION RELATIONSHIP AND DESIGN INSPIRATION

From a structure-function perspective, the complex multi-tissue organization across the soft tissue-to-bone junction is inherently related to the nature and distribution of the mechanical stress experienced at the ligament-bone junction. It has been reported that matrix organization at soft tissue-to-bone transitions is optimized to sustain both tensile and compressive stresses.

Characterization studies (Bullough and Jagannath 1983, Benjamin et al. 1986, Woo et al. 1988, Oegema, Jr. and Thompson, Jr. 1992, Matyas et al. 1995, Ralphs et al. 1998, Thomopoulos et al. 2003, Spalazzi et al. 2004, Moffat et al. 2008) of the soft tissue-to-bone insertion have revealed remarkable organizational similarities between many tissue-to-tissue interfaces (Figure 1). They often consist of a multi-tissue, multi-cell transition, as well as exhibiting a controlled distribution of mineral content that, along with other structural parameters such as collagen fiber organization, result in a gradient of mechanical properties progressing from soft tissue to bone.

These observations have provided invaluable clues for the design of biomimetic scaffolds for engineering tissue-to-tissue interface. Specifically, a stratified or multi-phased scaffold will be essential for recapturing the multi-tissue organization observed at the soft tissue-to-bone interface. In order to minimize the formation of stress concentrations, the scaffold should exhibit phase-specific structural and mechanical properties, with a gradual increase in mechanical properties across the scaffold phases. To this end, introducing spatial control over mineral distribution on a stratified scaffold can impart controlled mechanical heterogeneity similar to that of the native interface. Compared to a homogenous structure, a scaffold with pre-designed, tissue-specific matrix inhomogeneity can better sustain and transmit the distribution of complex loads inherent at the multi-tissue interface. It is emphasized that while the scaffold is stratified, a key criteria is that these phases must be interconnected and pre-integrated with each other, thereby supporting the formation of distinct yet continuous multi-tissue regions. Furthermore, interactions between interface-relevant cells serve important functions in the formation, maintenance, and repair of interfacial tissue. Therefore, precise control over the spatial distribution of these cell populations is also critical for multi-tissue formation and interface regeneration. Consideration of these biomimetic parameters will collectively improve the design of stratified scaffolds optimized for promoting the formation and maintenance of controlled matrix heterogeneity and interface regeneration.

BIOINSPIRED SCAFFOLD DESIGN FOR INTERFACE TISSUE ENGINEERING

The multi-tissue transition from ligament to bone at the ACL-to-bone interface represents a significant challenge for functional interface tissue engineering. Inspired by the native interface, Spalazzi *et al.* pioneered the design of a tri-phasic scaffold (Figure 2) for the regeneration of the ACL-

to-bone interface (Spalazzi et al. 2006a, Spalazzi et al. 2006b). The scaffold consists of three continuous phases, each engineered for a specific tissue region of the interface: Phase A is a polymer fiber mesh for fibroblast culture and soft tissue formation**,** Phase B consists of polymer microspheres and is the interface region designed for fibrochondrocyte culture, and Phase C is comprised of sintered polymer-ceramic composite microspheres for bone formation (Lu et al. 2003). The scaffold is innovative in that it is in essence, a "single" scaffold system with three distinct yet continuous phases, designed to support the formation of the multi-tissue regions observed across the ligament-bone junction. To form the ligament, interface and bone regions, fibroblasts, chondrocytes and osteoblasts were seeded onto Phases A, B and C, respectively. Interactions between these cell types on the stratified scaffold have been evaluated *in vitro* (Spalazzi et al. 2006b) and *in vivo* (Spalazzi et al. 2006a). Extensive tissue infiltration and abundant matrix deposition with tissue continuity maintained established across scaffold phases. Interestingly, matrix production compensated for the decrease in mechanical properties accompanying scaffold degradation, and three continuous regions of ligament, interface and bone-like matrix was formed *in vivo* (Figure 2).

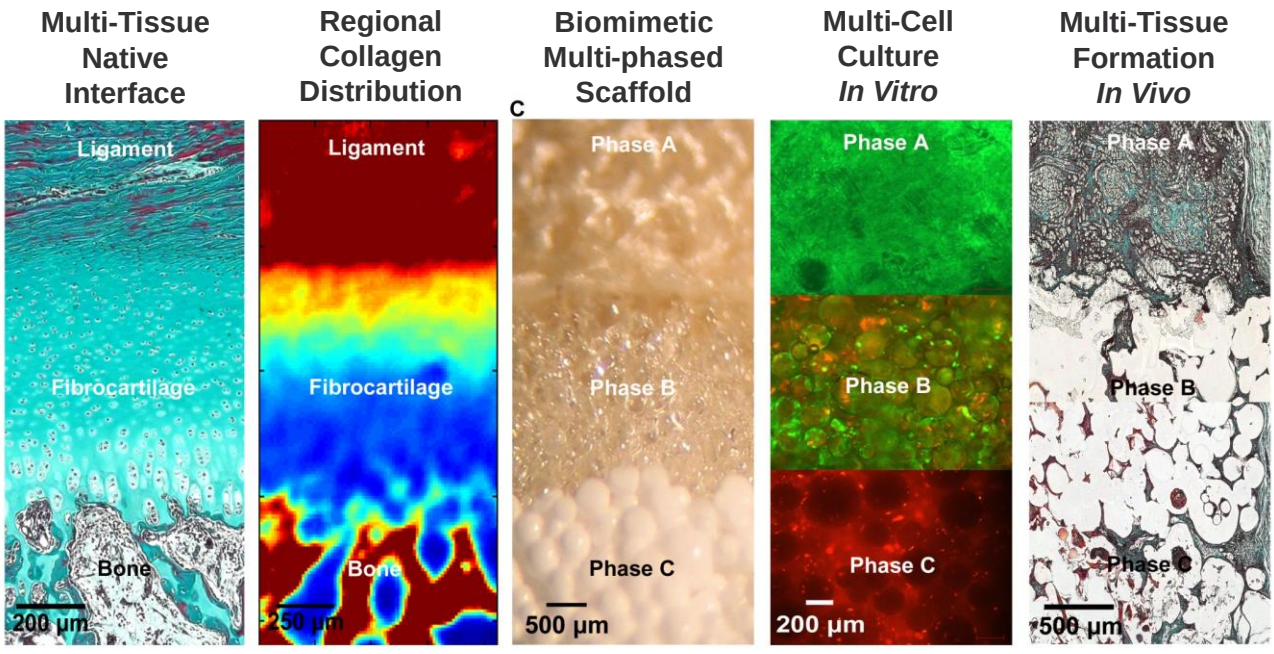

Figure 2. Bioinspired Stratified Scaffold Design for Interface Tissue Engineering and Integrative Soft Tissue Repair

In addition to stratified scaffolds, there is a tremendous interest in designing scaffolds with a gradient of properties (Harris et al. 2006, Singh et al. 2008, Chatterjee et al. 2011, Seidi et al. 2011). These novel scaffolds with either a compositional (Erisken et al. 2008, Singh et al. 2010,

Ramalingam et al. 2012) or chemical factor (Phillips et al. 2008, Li et al. 2009) gradient have the potential to address the need to recapitulate the complex transition of mechanical and chemical properties that are characteristic of tissue-to-tissue junctions, offering direct regional control and allowing for scaffold heterogeneity that mimic the complex native interface. More specifically, these scaffolds consist of a relatively gradual and continuous transition in either composition or structural organization, resulting in a linear gradient in mechanical properties. The primary advantage of the compositional gradient-based scaffolds resides in their ability to more closely mimic the native tissue-to-tissue interface. Current design challenges for engineering biomimetic gradients stems from a question of scale, in other words, how best to recapitulate the micro- to nano-scale gradients that have been reported at the tissue-to-tissue interface. Thus the stratified scaffold approach may represent a simpler approach, whereby a *gradation* of key compositional and functional properties is pre-established by focusing on forming specific tissue region of interest and pre-integrating these tissue regions through stratified design. Regardless, design parameters for interface regeneration must be prioritized and *strategic biomimicry* be adopted in functional interface scaffold design.

In addition to scaffold design, it is expected that cellular contributions will play a pivotal role in mediating the regeneration and homeostasis of the gradation of compositional and mechanical properties inherent at the interface. For example, using a cell-based approach, Ma *et al.* utilized cell self assembly to form bone-ligament-bone constructs by culturing engineered bone segments to ligament monolayers(Ma et al. 2009). Paxton *et al.* utilized a similar methodology with promising results when evaluating the use of a polymer ceramic composite and RGD peptide to engineer functional ligament-to-bone attachments(Paxton et al. 2009). Consequently, controlling cellular response via co-culture, tri-culture or growth factor distribution on the multi-phased scaffolds is a critical strategy to enable the development of local gradients on a physiologically relevant scale.

SUMMARY AND FUTURE DIRECTIONS

In the body, many soft tissues connect to bone through a multi-tissue interface populated by multiple cell types, which serve to minimize the formation of stress concentrations while enabling load transfer between soft and hard tissues. Given its functional significance, re-establishment of tissue-to-tissue interfaces is critical for the formation of multi-tissue systems as well as promoting

integrative tissue repair. The biomimetic interface tissue engineering approach described here is rooted in an in-depth understanding of the structure-function relationship at the tissue-to-tissue interface, and changes at this critical junction during prenatal and post-natal development. Investigations into the mechanism of interface regeneration have revealed the role of heterotypic cellular interactions in directing the formation, repair and maintenance of the tissue-to-tissue interface. Moreover, functional and integrative repair may be achieved by coupling both cell-based and scaffold-based approaches. The vast potential of stratified scaffold systems is evident as they are predesigned to support multi-tissue regeneration by mediating heterotypic cellular interactions, and can be further refined by incorporating well controlled compositional and growth factor gradients, as well as the use of biochemical and biomechanical stimulation to encourage tissue growth and maturation.

It is emphasized that interface tissue engineering will be instrumental for the *ex vivo* development and *in vivo* regeneration of integrated musculoskeletal tissue systems with biomimetic functionality; however there remains a number of challenges in this exciting area. These include the need for a greater understanding of the structure-function relationship existing at the native tissue-to-tissue interface as well as the mechanisms governing interface development and regeneration. Furthermore, the *in vivo* host environment and the precise effects of biological, chemical, and physical stimulation on interface regeneration must be thoroughly evaluated to enable the formation and homeostasis of the neo-interface. Physiologically relevant *in vivo* models are also needed to determine the clinical potential of the designed scaffolds.

In closing, it is anticipated that the successful regeneration of tissue-to-tissue interfaces through a bioinspired approach will promote integrative and functional tissue repair, as well as enable the clinical translation of tissue engineering technologies from bench to bedside. Moreover, by bridging distinct types of tissue, interface tissue engineering will be instrumental for the development of integrated musculoskeletal organ systems with biomimetic complexity and functionality.

REFERENCES

Benjamin, M., E. J. Evans, and L. Copp. 1986. The histology of tendon attachments to bone in man. J Anat. 149:89-100.

Benjamin, M., and J. R. Ralphs. 1998. Fibrocartilage in tendons and ligaments--an adaptation to compressive load. J Anat. 193 (Pt 4):481-494.

Blickenstaff, K. R., W. A. Grana, and D. Egle. 1997. Analysis of a semitendinosus autograft in a rabbit model. Am J Sports Med 25:554-559.

Bullough, P. G., and A. Jagannath. 1983. The morphology of the calcification front in articular cartilage. Its significance in joint function. J.Bone Joint Surg.Br. 65:72-78.

Butler, D. L., S. A. Goldstein, and F. Guilak. 2000. Functional tissue engineering: the role of biomechanics. J.Biomech.Eng 122:570-575.

Cooper, R. R., and S. Misol. 1970. Tendon and ligament insertion. A light and electron microscopic study. J Bone Joint Surg Am. 52:1-20.

Erisken, C., D. M. Kalyon, and H. Wang. 2008. Functionally graded electrospun polycaprolactone and beta-tricalcium phosphate nanocomposites for tissue engineering applications. Biomaterials 29:4065-4073.

Friedman, M. J., O. H. Sherman, J. M. Fox, W. Del Pizzo, S. J. Snyder, and R. J. Ferkel. 1985. Autogeneic anterior cruciate ligament (ACL) anterior reconstruction of the knee. A review. Clin Orthop 196:9-14.

Fujioka, H., R. Thakur, G. J. Wang, K. Mizuno, G. Balian, and S. R. Hurwitz. 1998. Comparison of surgically attached and non-attached repair of the rat Achilles tendon-bone interface. Cellular organization and type X collagen expression. Connect.Tissue Res. 37:205-218.

Grana, W. A., D. M. Egle, R. Mahnken, and C. W. Goodhart. 1994. An analysis of autograft fixation after anterior cruciate ligament reconstruction in a rabbit model. Am J Sports Med 22:344-351.

Harris BP, Kutty JK, Fritz EW, Webb CK, Burg KJ, Metters AT. 2006. Photopatterned polymer brushes promoting cell adhesion gradients. Langmuir. 22(10):4467-71.

Hunziker, E. B., T. M. Quinn, and H. J. Hauselmann. 2002. Quantitative structural organization of normal adult human articular cartilage. Osteoarthritis.Cartilage. 10:564-572.

Iwahashi, T., K. Shino, K. Nakata, H. Otsubo, T. Suzuki, H. Amano, and N. Nakamura. 2010. Direct anterior cruciate ligament insertion to the femur assessed by histology and 3-dimensional volume-rendered computed tomography. Arthroscopy 26:S13-S20.

Jiang, J., S. B. Nicoll, and H. H. Lu. 2005. Co-culture of osteoblasts and chondrocytes modulates cellular differentiation in vitro. Biochem.Biophys.Res.Commun. 338:762-770.

Langer, R., and J. P. Vacanti. 1993. Tissue Engineering. Science 260:920-926.

Li, X. R., J. W. Xie, J. Lipner, X. Y. Yuan, S. Thomopoulos, and Y. N. Xia. 2009. Nanofiber Scaffolds with Gradations in Mineral Content for Mimicking the Tendon-to-Bone Insertion Site. Nano Letters 9:2763-2768.

Lu, H. H., S. F. El Amin, K. D. Scott, and C. T. Laurencin. 2003. Three-dimensional, bioactive, biodegradable, polymer-bioactive glass composite scaffolds with improved mechanical

properties support collagen synthesis and mineralization of human osteoblast-like cells in vitro. J Biomed.Mater.Res 64A:465-474.

Lu, H. H., and J. Jiang. 2006. Interface tissue engineering and the formulation of multiple-tissue systems. Adv.Biochem.Eng Biotechnol. 102:91-111.

Ma, J., Goble, K., Smietana, M., Kostrominova, T., Larkin, L., and Arruda, E. M. 2009. Morphological and functional characteristics of three-dimensional engineered bone-ligament-bone constructs following implantation. *J Biomech.Eng*. 131:101017-25.

Matyas, J. R., M. G. Anton, N. G. Shrive, and C. B. Frank. 1995. Stress governs tissue phenotype at the femoral insertion of the rabbit MCL. J Biomech. 28:147-157.

Moffat, K. L., W. H. Sun, P. E. Pena, N. O. Chahine, S. B. Doty, G. A. Ateshian, C. T. Hung, and H. H. Lu. 2008. Characterization of the structure-function relationship at the ligament-to-bone interface. Proc.Natl.Acad.Sci.U.S.A 105:7947-7952.

Oegema, T. R., Jr., and R. C. Thompson, Jr. 1992. The zone of calcified cartilage. Its role in osteoarthritis, p. 319-331. *In* K. E. Kuettner, R. Schleyerbach, J. G. Peyron, and V. C. Hascall [eds.], Articular Cartilage and Osteoarthritis. Raven Press, New York, NY.

Paxton, J. Z., Donnelly, K., Keatch, R. P., and Baar, K. 2009. Engineering the bone-ligament interface using polyethylene glycol diacrylate incorporated with hydroxyapatite. *Tissue Eng Part A*. 15:1201-1209.

Phillips, J. E., K. L. Burns, J. M. Le Doux, R. E. Guldberg, and A. J. Garcia. 2008. Engineering graded tissue interfaces. Proc.Natl.Acad.Sci.U.S.A 105:12170-12175.

Ralphs, J. R., M. Benjamin, A. D. Waggett, D. C. Russell, K. Messner, and J. Gao. 1998. Regional differences in cell shape and gap junction expression in rat Achilles tendon: relation to fibrocartilage differentiation. J.Anat. 193 (Pt 2):215-222.

Ramalingam M, Young MF, Thomas V, Sun L, Chow LC, Tison CK, Chatterjee K, Miles WC, Simon CG Jr. 2012. Nanofiber scaffold gradients for interfacial tissue engineering. J Biomater Appl. 2012 Jan 27. [Epub ahead of print]

Robertson, D. B., D. M. Daniel, and E. Biden. 1986. Soft tissue fixation to bone. Am.J Sports Med. 14:398-403.

Rodeo, S. A., S. P. Arnoczky, P. A. Torzilli, C. Hidaka, and R. F. Warren. 1993. Tendon-healing in a bone tunnel. A biomechanical and histological study in the dog. J Bone Joint Surg Am. 75:1795-1803.

Singh, M., B. Sandhu, A. Scurto, C. Berkland, and M. S. Detamore. 2010. Microsphere-based scaffolds for cartilage tissue engineering: using subcritical CO(2) as a sintering agent. Acta Biomater. 6:137-143.

Singh M, Berkland C, Detamore MS. 2008. Strategies and applications for incorporating physical and chemical signal gradients in tissue engineering. Tissue Eng Part B Rev. 14(4):341-66.

Seidi A, Ramalingam M, Elloumi-Hannachi I, Ostrovidov S, Khademhosseini A. 2011. Gradient biomaterials for soft-to-hard interface tissue engineering.. Acta Biomater. 7(4):1441-51.

Skalak, R. 1988. Tissue engineering: proceedings of a workshop, held at Granlibakken, Lake Tahoe, California, February 26-29, 1988. Liss, New York, NY.

Spalazzi, J. P., K. D. Costa, S. B. Doty, and H. H. Lu. Characterization of the mechanical properties, structure, and composition of the anterior cruciate ligament-bone insertion site. Transactions of the Orthopaedic Research Society 29, Poster #1271. 2004.

Spalazzi, J. P., E. Dagher, S. B. Doty, X. E. Guo, S. A. Rodeo, and H. H. Lu. 2006a. In Vivo Evaluation of a Tri-Phasic Composite Scaffold for Anterior Cruciate Ligament-to-Bone Integration.525-528.

Spalazzi, J. P., S. B. Doty, K. L. Moffat, W. N. Levine, and H. H. Lu. 2006b. Development of Controlled Matrix Heterogeneity on a Triphasic Scaffold for Orthopedic Interface Tissue Engineering. Tissue Eng 12:3497-3508.

Thomopoulos, S., G. R. Williams, Gimbel J.A., M. Favata, and L. J. Soslowsky. 2003. Variations of biomechanical, structural, and compositional properties along the tendon to bone insertion site. J Orthop Res 21:413-419.

Wang, I. E., S. Mitroo, F. H. Chen, H. H. Lu, and S. B. Doty. 2006. Age-dependent changes in matrix composition and organization at the ligament-to-bone insertion. J.Orthop.Res. 24:1745-1755.

Wang, I. E., J. Shan, R. Choi, S. Oh, C. K. Kepler, F. H. Chen, and H. H. Lu. 2007. Role of osteoblast-fibroblast interactions in the formation of the ligament-to-bone interface. J Orthop Res 25:1609-1620.

Woo, S. L., J. Maynard, D. L. Butler, R. M. Lyon, P. A. Torzilli, W. H. Akeson, R. R. Cooper, and B. Oakes. 1988. Ligament, Tendon, and Joint Capsule Insertions to Bone, p. 133-166. *In* S. L. Woo, and J. A. Bulkwater [eds.], Injury and Repair of the Musculosketal Soft Tissues. American Academy of Orthopaedic Surgeons, Savannah, Georgia.