The Role of Subchondral Bone Microdamage in PTOA Following ACL Rupture

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Introduction: Osteoarthritis (OA) is the most common form of musculoskeletal disease in the United States, affecting approximately 30 million people [1]. Symptoms of OA often only present after years of relatively slow progression. However, OA also occurs subsequent to traumatic joint injury, such as Anterior Cruciate Ligament (ACL) rupture in the knee. This specific circumstance leads to Post Traumatic Osteoarthritis (PTOA), which is a sub-set of the broader condition and makes up 12% of the overall disease burden in the US with associated costs of up to $3 billion annually [2]. The current paradigm for PTOA is that while acute injury eventually causes cartilage degradation, multiple joint tissues are involved from the outset and contribute to overall eventual ‘joint failure’. However, relatively little is known about the concomitant damage of other joint tissues. Since the knee is supported by stiff bone and calcified cartilage, it seems reasonable to propose that those tissues are also damaged by the acute injury, for example by generation of microdamage. Bone remodeling is also known to be involved in the early initiation and progression of OA. Recent intriguing data relates these two phenomena by showing that microdamage induces production of pro-resorptive factors in osteocytes following fatigue loading [3]. The subchondral region has a unique microarchitecture with three distinct regions: Calcified Cartilage (CC), Cortical Plate (CP) and Trabecular Bone (TB). It is not always recognized that these compartments differ not only morphologically but also mechanically and physiologically. Thus, if microdamage does occur following acute knee injury, and if it stimulates remodeling, then characterizing its spatial distribution among the subchondral tissues will be crucial to understanding this system. Here we characterize an in vivo model for ACL rupture, and resultant subchondral microdamage and quantify microdamage according to subchondral mineralized tissue-type as well as increased bone turnover as measured by dynamic histomorphometry.

Methods: We modified the standard in vivo tibial axial loading configuration to include rotational and valgus components (Figure 1), both of which are known to contribute significantly to ACL rupture in humans. Under anesthesia, the knees of female Sprague Dawley rats (n=16, IACUC approved) were fixed at 80-85° flexion, using a novel custom-made fixture, and subjected to a compressive loading ramp (rate 0.1 mm/s) until rupture of the ACL was detected. Outright rupture of the ACL was the test endpoint and was defined by a rapid drop in load which was recorded the system control software. Animals were sacrificed at days 0, 14, 28 and 56 days post injury. Fluorochrome labels were administered at the time of injury and then again 2 days prior to sacrifice. In each animal the contralateral limb served as the control. At necropsy, samples were dissected free of soft tissue, fixed in 10% NBF and either stained en bloc with basic fuchsin, using standard protocols for microdamage analysis, or processed directly for dynamic histomorphometry.

Results: Animal weight was 278.94±18.4[g] and ACL failure load was 65.8±11.4 [N] with coefficients of variation at 6.6 and 17.3[%], respectively. Gross observation confirmed the rupture of ACL in all tested joints, with no evidence of ligament damage in the control group. In the day 0 group, microdamage was found in all three of the subchondral compartments of tested joints and microcrack density was
calculated as 6.69±3.64, 2.65±0.65 and 2.84±0.80 [#/mm²], in CC, CP and TB regions, respectively (Figure 2). These data suggest that microdamage does indeed occur in the subchondral mineralized tissues following acute joint injury, at least in our model system. Furthermore, this suggests that each subchondral tissue is differentially affected - importantly, if the CC and CP regions prove to have osteoclastic activity co-localizing with microdamage this may have important consequences for disease progression in terms of porosity, vascular invasion and bone-cartilage cross talk. Initial data indicate that increased uptake of fluorochrome labels was present in injured joints after 4 and 8 weeks (Figure 3). Furthermore, preliminary data from standard OA scoring on histological sections stained with Safranin O also suggest that cartilage degradation is present after 2, 4 and 8 weeks in these animals.

Discussion: Here we characterize a novel animal model for ACL rupture, which generates subchondral bone microdamage differentially among the subchondral bone structural units as well as subsequent bone resorption. Many of the current animals models of PTOA induce the disease via a surgical procedure or and injection of catabolic factors into the joint capsule. These approaches do not replicate the mechanical environment of injury faithfully since the mechanical overload, which must pass through the joint at the time of injury, is missing. Therefore, by incorporating those aspects, our model allows us to study the contribution of microdamage in the subchondral regions to disease initiation and progression in the overlying cartilage and other surrounding tissues. The magnitude of the failure loads in our study were carefully monitored, while different positioning of the joint during testing would allow lower failure loads, 65N is approximately equivalent to 20X BW in this model. This compares well with the joint reaction forces experienced in the human injury condition. Microdamage appears to be differentially distributed among the three subchondral mineralized tissue regions following this testing protocol. This appears to be related to the remodeling response to injury in this model. Figure 3 shows evidence of increased uptake of fluorochrome labels in the subchondral bone of these animals 4 weeks after injury. We also report that evidence of degradation of the overlying cartilage is also present at this time-point (data not shown). Taken together, these data suggest that there may be a direct link between microdamage in subchondral bone, the subsequent remodeling response and the eventual cartilage break down that characterizes PTOA.

Significance: These data are particularly significant at the translational level. Although the role of subchondral bone in OA has been investigated before, there has never been a clear link between acute microdamage, the subsequent biological responses and eventual cartilage degradation. From a translational perspective, this work may lead to development of novel early intervention bone-targeted treatment strategies, once the underlying cell and molecular mechanisms of this injury-induced disease process are fully characterized and understood.
Increased subchondral fluorochrome uptake