INTRODUCTION:
Focal cartilage defects are a common occurrence in sports injuries and early osteoarthritis. Most clinical resurfacing techniques involve accessing the mesenchymal stem cells (MSCs) from under the subchondral bone by either drilling or performing a microfracture procedure. Most animal models of cartilage repair use drilled osteochondral defects while debridement alone and microfracture procedures are more seldom evaluated. In this study, we evaluated the repair of osteochondral and microfracture defects in the rabbit and the non-human primate (NHP) in order to determine which model is superior as a model of human cartilage defect repair.

METHODS:
Animal studies received IACUC approval and involved 12 skeletally mature New Zealand rabbits and 12 skeletally mature cynomolgus macaques. Osteochondral defects were made with a diamond-impregnated dental drill to a depth of 3 mm. Microfracture procedures were performed by debriding a 3.5 mm diameter area through the calcified cartilage, and creation of multiple penetrating microfracture holes using a 0.028 kirshner wire. 3.5 mm debridement alone defects (without microfracture) were also created. Rabbits had unrestricted cage activity and were euthanized at 4 weeks post-operatively. The NHP knee was cast at 70° flexion for 2 weeks post-operatively. At 2 weeks post-operatively, 15 minutes of range of motion exercises were instituted under anesthesia three times a week. Surgery on the opposite knee was performed 6 weeks after the first surgery and animals were sacrificed 6 weeks after the second surgery to allow a direct comparison of healing at the 6 and 12 week timepoints within the same animal.

RESULTS:
Rabbit Defects (Figure 1A and B)
At 4 weeks post-operatively, rabbit osteochondral defects were almost completely filled with Safranin-O rich cartilage. Active subchondral bone remodelling and reformation was apparent. Rabbit microfracture defects contained minimal repair tissue and were markedly inferior to osteochondral defects.

NHP Defects (Figure 1C and D)
At 12 weeks post-operative, NHP osteochondral defects had incomplete subchondral bone reformation and were filled with fibrocartilaginous tissue containing minimal chondrogenic cells. The tissue stained poorly with Safranin-O indicating minimal extracellular proteoglycan. At 6 weeks, the microfracture defects on the condyles were partially filled with fibroblastic tissue with few chondrogenic cells. The response within the subchondral bone to the microfracture holes was primarily osteoclastic resorption. At 12 weeks post-operatively, the defects were no longer grossly apparent, and histologic analysis showed complete filling with chondrogenic tissue and active repair by the subchondral bone. Interestingly, the microfracture defects on the trochlear grooves of the NHPs never filled with tissue and the response of the subchondral bone was aggressive resorption which was not followed by new bone reformation, even out to 12 weeks post-operatively. This contrasted markedly to the excellent repair response on the condyles of the same animals. Debridement without microfracture at 12 weeks on the condyles looked histologically identical to the microfracture defects. However, these defects always showed histologic penetration through the calcified cartilage, due at least partially to osteoclastic resorption, and so had access to MSCs from the bone marrow.

DISCUSSION:
Rabbit osteochondral defects are the most widely used model of cartilage repair and demonstrated a rapid fibrocartilaginous repair. Microfracture in the rabbit showed dramatically inferior repair at the same timepoint. In contrast, NHP osteochondral defects had a much slower repair with incomplete bone reformation through 12 weeks. The microfracture defects in the NHP had a dramatic improvement in repair on the femoral condyles from the 6 to the 12 week timepoint. At 6 weeks, the microfractures through the subchondral plate underwent remodelling and osteoclastic resorption so that a net further loss of bone and calcified cartilage was apparent. The 12 week defects were filled with a more differentiated fibrocartilage. The immature repair of the 6 week NHP microfracture defects lends support for extending the clinical post-operative rehabilitation period beyond this timepoint. The NHP debridement alone defects at 12 weeks had comparably excellent repair to microfracture defects on the condyles. The debridement alone group always had access to the marrow MSCs from either the surgery procedure itself, or from the osteoclastic resorption of the calcified cartilage. Therefore, repair should not be attributed to MSCs from other sources when using debridement models. The 12 week NHP microfracture defects on the trochlear groove had dramatically inferior repair that we attribute to altered biomechanical loading that allowed for a dramatic bone loss and little indication of repair. This study gave an appreciation for the efficacy of the microfracture procedure which improved dramatically from 6 to 12 weeks on the femoral condyles. Different results were apparent when different sites or species were evaluated. We recommend the use of the NHP microfracture model on the femoral condyles to approximate healing of the human as this is the most closely related species, most cartilage defects are on the condyles, and the microfracture procedure is widely performed in the clinic.

REFERENCES:

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MICROFRACTURE IN NON-HUMAN PRIMATES IS THE PREFERRED MODEL FOR REPAIR OF HUMAN CARTILAGE DEFECTS
+*Glasson, S (E-Genetics Institute/Wyeth); *Powers, J (E-Genetics Institute/Wyeth); *Blanchet, T (E-Genetics Institute/Wyeth); *Peluso, D (E-Genetics Institute/Wyeth); **Gill, T (E-Genetics Institute/Wyeth); *Carito, B (E-Genetics Institute/Wyeth); *Morris, E (E-Genetics Institute/ Wyeth)
+*Genetics Institute/Wyeth, Cambridge, MA. 617-665-5344, Fax: 617-665-5390, sglasson@genetics.com

Figure 1. Repair of rabbit osteochondral (A) and microfracture (B) defects at 4 weeks. Repair of NHP osteochondral (C) and microfracture (D) defects at 12 weeks.

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**MGH, Boston, MA.