Introduction: Clinically, there is an interest in minimally invasive solutions that lessen pain symptoms and restore joint mobility in the early stages of cartilage degeneration and/or damage. However, little is known about wear resistant materials, which articulate against cartilage without compromising the tissue during wear. Since wear testing of articular cartilage began in the 1970s[1], researchers have struggled to maintain a viable, alive tissue state, as well as to continuously report wear rates over time. To keep chondrocytes alive and metabolically active, a close replication of in vivo conditions regarding temperature, CO₂, humidity, and load is necessary. Also, the tribological system characteristics should be maintained to provide accurate and insightful results. Here, we attempt to validate a novel in vitro cartilage wear testing protocol which may be helpful to screen new cartilage friendly materials. We hypothesized that polyethylene (PE) generates more cartilage wear and tissue damage than cobalt chromium (CoCr) alloy.

Materials and Methods: Six CoCr and six PE balls (diameter: 32±0.2mm; roughness: 0.013um (CoCr), 1.08um (PE)) provided by an implant manufacturer were used in this study. These balls were mounted on a specially equipped tribological testing apparatus, which was housed in an incubator[2]. They articulated against cartilage disks under compression using complex motion trajectories with a migrating contact area as input. Cartilage Procurement: The cartilage explants were obtained from six calf hind legs from a local abattoir. Stifle joints of the 24-week old animals were opened under sterile conditions within 24 hours of slaughter. A 14 mm punch was used to procure 5 disks from every trochlea, which were trimmed to approximately 2-3mm thickness leaving the superficial zone intact.

Testing: After a 5 day preculture, 2 disks from each joint were exposed to wear testing (one against CoCr; one against PE); the remaining disks served as controls. A 50N compressive load was applied for wear testing. The ball oscillated with ±30 deg at 1 Hz; the disk rotated with ±15 deg at 0.1 Hz around an offset axis. All tests were performed for 3 hours per day for 3 days. Wear/Damage Analysis: After each three hour test, medium (DMEM:F12 (1:1) supplemented with ‘miniTTS’) was individually collected and analyzed for proteoglycan (PG)/GAG release (DMMB assay), PG synthesis (S35- incorporation), and media weight for detection of eventual wear debris. After dialysis, samples were frozen and lyophilized under high vacuum for 48 hours. The weights of the tubes were compared with the weights of empty tubes. After 3 days of wear testing, the cartilage explants were collected and examined for cell viability (live/dead assay) and matrix morphology (histology with Safranin O stain).

Results: The weight gain was significantly higher for tubes filled with media from PE compared to that from CoCr and control (p<0.001, Fig.1a). Similarly, the PG/GAG release was significantly higher for PE when compared with CoCr and control (p<0.001), but the difference between CoCr and control was not significant (p=0.098). The daily averaged values (mean ± s.d.) were 299±11, 89±23 and 39±4 ug/ml for PE, CoCr and control respectively. DMMB data for control and CoCr positively correlated with above weight gain measurements (R2=0.29 and 0.23; p<0.05), while, no such correlation was found for PE. When normalized to wet weight, no difference in S35-incorporation was found between the three groups, although on average they were highest for PE (883 CPM) followed by control (830 CPM) and CoCr (708 CPM). Overall, cell viability was maintained throughout wear testing and there was no difference between groups when the entire explant thickness was evaluated (p=0.53). However, cell viability in the top 15% of the CoCr tissue samples was greatly reduced when compared with control (Fig.1b, p<0.001). Although, PE showed no reduction in cell viability of the remaining 15% top-layer after testing, examining the histological sections suggests that the superficial zone was worn off. Morphologically, the cartilage surfaces articulating against CoCr stayed intact, contrasting the explants tested against PE.

Discussion: In this short-term in vitro test, tissue degradation was considerably higher for the cartilage articulated against PE compared with CoCr. This is in agreement with clinical observation[3] and animal models[4], where it was found that PE is not suitable for movable weight-bearing prostheses articulating against cartilage. Histology and the comparison of lyophilization and DMMB data suggest that the collagen matrix is not only disrupted but more likely abraded from its surface, which will be the focus of further studies. Interestingly, the cells below the abraded surface stayed alive. The increased S35-uptake could suggest hypermetabolic activity of the cells in an attempt to repair the disrupted surface. In contrast, damage for CoCr was mild which is in agreement with clinical observation where CoCr is the material of choice for hemiarthroplasty. However, its clinical success is variable which might be related to the observed cell death. In summary, an in vitro wear test for artificial materials articulating against cartilage has been established and appears to be a promising tool for material screening.


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In Vitro Wear Testing of Living Cartilage Tissue

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