Biostability, Biocompatibility and Mechanical Properties of a Hyaluronan-Polyethylene Copolymer

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Statement of Purpose: A biomaterial was designed for the permanent repair/replacement of articular cartilage, which must demonstrate mechanical properties that support, or match, those of the articulating surface and exhibit osseocompatibility for anchorage of the implant into the subchondral bone. A series of hyaluronan (HA) and maleated high density polyethylene (MA-*g*-HDPE) copolymers (HA-*co*-HDPE) were evaluated for use as an osteochondral plug in the repair and/or replacement of articular cartilage. It was hypothesized that the addition of HDPE would improve the mechanical properties of HA while retaining its bioactive properties.

Methods: HA-*co*-HDPE was prepared with 10 and 50% (w/w) HA [1] and compression molded under vacuum at 110°C and 150 MPa. Weight loss was used to characterize the enzymatic degradation of HA-*co*-HDPE and MA-*g*-HDPE. Samples were placed in a hyaluronidase (Sigma) phosphate buffered solution (PBS) solution; PBS soak controls were placed in PBS only (without enzymes). Samples were incubated at 37°C; the enzyme solution and PBS were replaced every other day. At each time point, samples were rinsed with distilled water and vacuum dried at room temperature. The human osteoblast cell line hFOB1.19 (ATCC, Manassas, VA) was propagated in an appropriate complete growth medium. HA-*co*-HDPE samples were disinfected and equilibrated in PBS prior to cell seeding. Tissue culture polystyrene (TCPS) was used as a control. Cells were seeded at a density of approximately 9000 cells/cm^2 and incubated at 39.5°C. Cell viability was evaluated after 24 hr and 7 d (Live/Dead® viability/cytotoxicity kit, Molecular Probes) *via* fluorescence microscopy. Relative alkaline phosphatase (ALP) expression (BioAssay Systems QuantiChrom alkaline phosphatase assay kit) was measured 7 d post-seeding. HA-*co*-HDPE and MA-*g*-HDPE were tested in unconfined dynamic compressive mode (Perkin Elmer Diamond Dynamic Mechanical Analyzer, DMA) at 37°C in PBS from 0.01 to 10 Hz, giving the storage modulus (E') and tan δ.

Results: HA-*co*-HDPE and MA-*g*-HDPE samples subjected to hyaluronidase for 56 days showed nearly the same weight loss as samples incubated in PBS for 56 days. The LIVE/DEAD® stained images of HA-*co*-HDPE samples at 24 hr and 7 d post-seeding showed viable (green) cells growing on all of the samples at each time point. HA-*co*-HDPE exhibited a higher ALP activity compared to the TCPS control. E' values of all the HA-*co*-HDPE samples increased with increasing frequency (Fig 2). E' of all the HA-*co*-HDPE samples was lower when compared to the MA-*g*-HDPE control samples. The tan δ of all the HA-*co*-HDPE samples (0.22-0.26) was higher compared to MA-*g*-HDPE (0.18) at 1 Hz; the tan δ of the HA-*co*-HDPE samples was close to that of articular cartilage (~0.24).[2]

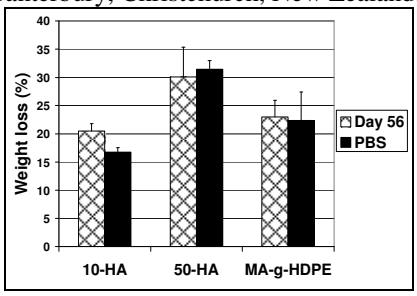

Figure 1. Weight loss for HA-*co*-HDPE and MA-*g*-HDPE after 56 days in hyaluronidase solution verse PBS.

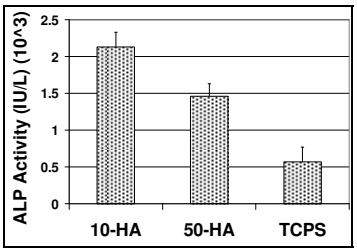

Figure 2. ALP activity 7 d post-seeding for HA-*co*-HDPE.

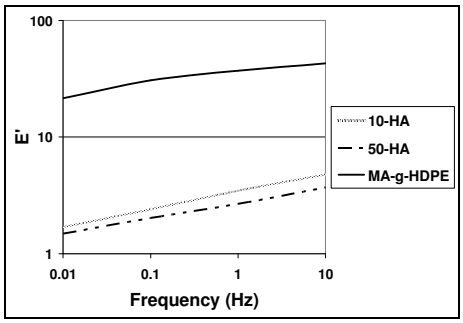

Figure 3. E' of HA-*co*-HDPE and MA-*g*-HDPE under unconfined compression.

Conclusions: The weight loss of HA-*co*-HDPE was largely due to the release of low molecular weight species as opposed to enzymatic attack via hyaluronidase. HA-*co*-HDPE is thus suitable as a permanent implant. HA-*co*-HDPE was found to be both non-cytotoxic and osseocompatible toward hFOB1.19 cells. The cells present on HA-*co*-HDPE surfaces exhibited higher ALP levels compared to TCPS. HA-*co*-HDPE is a compliant material with mechanical properties similar to articular cartilage; the unconfined storage modulus for the HA-*co*-HDPE samples were close to the unconfined dynamic stiffness of bovine articular cartilage: 4.10 MPa at 0.1 Hz in PBS at 37°C.[3] It has been shown by adjusting the amount of HA in the final composite the mechanical properties can be optimized for a variety of applications.

References: 1. Oldinski, R *et al.* 54th ORS 2008. 2. Schwartz, CJ and Bahadur, S. Wear, 2007;262:1315-1320. 3. Woodfield TBF *et al.* Biomaterials, 2004;25:4149-4161.

Acknowledgements: Schwartz Biomedical, LLC, Indiana 21st Century Research and Technology Fund.