Dextran Hydrogel Used as a Phospholipid Delivery System for Viscosupplementation

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Introduction: The use of viscosupplementation agents based on hyaluronic acid (HA) has been promoted to enhance the fluid film lubrication of degenerated joints. Though earlier models consider HA as the predominant articular boundary lubricant[1], later work[2] indicates that HA affects the viscosity of the synovial fluid with insignificant cartilage boundary lubrication. Lubricin was observed to impart the lubricating properties of synovial fluid, one of the constituents being phospholipidic in nature[3]. This study was aimed at developing a Dextran based hydrogel (DPL) containing a physiologically relevant concentration of dipalmitoyl phosphatidylcholine (PC) as a viscosupplementation and boundary lubricant delivery agent. [4,5]

Materials and Methods: Hydrogels were fabricated by coupling Dextran (Sigma D-1662 [Sigma Chemical Co., St. Louis, MO]) of 40,000 MW with glycidyl methacrylate. Gelation occurred using an initiator system of ammonium peroxydisulfate and N,N,N',N'-tetramethyl-ethylene diamine. Two types of hydrogels were synthesized: with and without PC (two concentrations: 0.36 mg/ml (normal phospholipid concentration in synovial fluid) and 0.72 mg/ml (higher concentration to compensate for dextran-DPPC binding); α-DPPC (Sigma P-0763)) with a degree of cross-linking of 14 mole ratio percent. The rheological properties (cone-on-plate, Paar Physica) of these hydrogels were tested and compared to normal ankle bovine synovial fluid. Gels were exposed to confluent lapine synoviocytes for 1, 4 and 7 days using a contact method. Assays performed included BCA for total protein, MTS for cell metabolism and proliferation, and live/dead assay (n=4/group). Zymograms were used to examine enzyme activity with variables of standards, activated gelatinase, cells, cells + DPL, cells+PC-DPL, cells+PC, and PC-DPL (n=4). An in vivo prophylactic study was conducted for 70 days using the normal lapine knee as a model where 0.25cc of the hydrogel with or without PC (0.36 mg/ml and 0.72 mg/ml) was injected in the joint space using the contralateral joint as sham (physiological saline) (n=5/group). Femoral condyles, patellae, tibial plateau, and synovial capsule/membrane of both knees were fixed in 10% buffered formalin. Decalcification was performed for bone tissues using a Decal solution. Following paraffin embedding, sections were stained with hematoxylin-eosin, safranin-O/ light green, and Masson's trichrome

The release of PC from the hydrogel was modeled by mixing the gels with 7-nitro-2-1,3-benzoxadiazol-4-yl (NBD) 16:1 tagged fluorescent polar lipid (Avanti Polar Lipids, Inc.) (1:99 Mol% ratio) and examined at 0, 0.25, 0.5, 0.75, 1, 2, 4, 8, 20, and 24h. Samples (n=4) were vortexed 2 min, then centrifuged for 5min at 1000 rpm prior to aliquot removal and assayed fluoremetrically (EX:460 and EM:534 on the Gemini Spectromax EM).

Results and Discussion: Chemical analysis using Fourier Transform Infrared (FTIR) spectroscopy confirmed the synthesis of dextran-based hydrogels. Addition of either concentration of PC to DPL provided a rheological behavior closely representing that of normal synovial fluid (μ=0.04-0.08) at shear rates up to 1000 sec-1. The rheological properties of dextran-based hydrogels indicated a non-Newtonian behavior: viscosity decreased with increasing shear rates. The addition of DPPC decreased the viscosity of these hydrogels, rendering the gel more easily injectable and possibly more easily mixed in a fluid-containing cavity such as a synovial joint. No change in viscosity was observed as a function of temperature (25, 37, and 45°C) for the DPL alone (p=0.942). However a significant decrease in viscosity of the DPL at either DPPC concentration at the two defined temperatures was observed (t-test; α=0.05**;** p=0.014).

MTS 4d results indicated no significant difference between samples. BCA total protein (μg/mL) results at 1d and 4d were significantly different between DPL to cells alone (p=0.027), PC 0.72mg +DPL (p=0.005), and DPL to PC 0.72mg+DPL (p=0.010), and the other samples were not significantly different. Zymogram results indicated that all variables containing synoviocytes excreted gelatinase. The addition of PC–DPL may aid in controlling enzymatic release from the synoviocyte cell cultures. Elution of PC-NBD 16:1 from DPL over 24h was 19.03% of the total with 11.53% released during the first hour. No histological adverse effects on synovium and articular cartilage were observed in vivo for all groups (Figure 1).

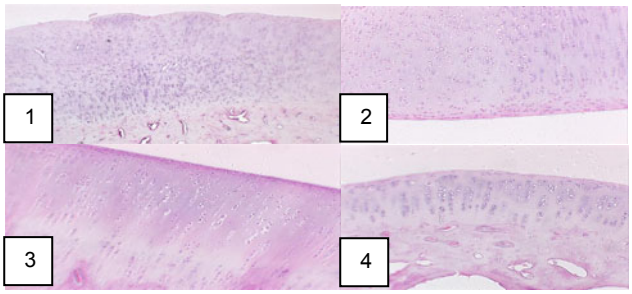

Figure 1. Articular cartilage (H&E; 40X) 1:DPL; 2:DPL+PC(0.36mg/1.5mL); 3: DPL+PC (0.72mg/mL) ; 4. Contralateral joint

Conclusions: From this study, it can be concluded that lipid-containing polysaccharide hydrogels can be successfully made from dextran and phospholipids. PC release from the gel remains to be investigated for longer period of time in tribological conditions observed in vivo.

References: 1. J Phys 119: 244- 252, 1953. 2. Br J of Rheum 37: 137- 142, 1998. 3. J Biochem 161: 473- 485, 1977. 4. J. Orthop. Res. 19, 671-676, 2001. 5. US Patent 6,800,298.

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