



Photo-cross-linked methacrylated polysaccharide solution blends with high chondrocyte viability, minimal swelling, and moduli similar to load bearing soft tissues



James W.S. Hayami^a, Stephen D. Waldman^b, Brian G. Amsden^{a,*}

^a Department of Chemical Engineering, Queen's University, Canada

^b Department of Mechanical and Materials Engineering, Queen's University, Canada

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ABSTRACT

Photo-cross-linked hydrogels produced from solution blends of methacrylated glycol chitosan (MGC), hyaluronic acid (MHA) and chondroitin sulfate (MCS) were examined for their potential use as load bearing soft tissue (LBST) repair constructs. The effect of the degree of prepolymer methacrylation (X, Y or Z; based on a repeating dimer unit) on the final hydrogel properties was investigated using solutions of 6% w/v for MGC (6%MGC-X, where X = 12%, 26% or 58%) and MHA (6%MHA-Y, where Y = 14%, 42% or 96%) and 20% w/v for MCS (20%MCS-Z, where Z = 16%, 26% or 45%). The goal was to adjust the cross-link density to produce hydrogels with moduli greater than 100 kPa to be suitable for load bearing applications while also minimizing swelling after cross-linking ($\pm 10\%$ change in volume), and maintaining high viability and high metabolic activity of the encapsulated chondrocytes. These objectives were achieved by cross-linking solution blends of either the 6%MGC-12 or 6%MHA-42 with the 20%MCS-45 prepolymer at 50% mass fraction (5050 MGC12MCS45 and 5050 MHA42MCS45). Chondrocyte viability within blended hydrogels were greater than 70% after 24 h of culture, but metabolic activity was highest in the MHA based hydrogels (single component and blended) over a 35 day culture period. Overall, the high initial modulus, low swelling and enhanced metabolic activity of the encapsulated chondrocytes within the 5050 MHA42MCS45 hydrogels suggests they may be suitable for use in a reparative load bearing soft tissue construct.

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1. Introduction

Load bearing soft tissues (LBST), such as articular cartilage and the central tissue of the intervertebral disc, can be affected by progressive degeneration either slowly through the natural aging process or more rapidly from disease and injury [1–3]. Current treatments only address the symptoms until damage to the LBST has progressed to the point where invasive surgeries are required to replace the entire

joint with permanent hardware [4]. However, these non-regenerative options alter the natural joint biomechanics, which can lead to additional issues in the surrounding tissues and possible implant failure under normal operating conditions [5,6]. Therefore, LBST in need of repair would benefit greatly from a cellular replacement scaffold that can be used as an earlier treatment option to maintain as much of the original joint architecture as possible, restore immediate load bearing abilities to the joint and regenerate functional tissue with time.

Hydrogels are an ideal medium for cell encapsulation through injectable delivery and *in situ* cross-linking.

* Corresponding author at: Department of Chemical Engineering, 19 Division St, Kingston, Ontario K7L 3N6, Canada. Tel.: +1 613 533 3093.

E-mail address: amsden@queensu.ca (B.G. Amsden).

Provided sufficient mechanical properties can be obtained, a cell-laden hydrogel would be suitable for use as a load bearing soft tissue scaffold; the hydrogel would be able to provide the initial load bearing support required in the damaged joint and the encapsulated cells would be able to regenerate *de novo* tissue over time. An approach that has shown promise is the *in situ* encapsulation of cells within gels formed from cross-linking aqueous solutions of polymers modified with (meth)acrylate groups through photoinitiated free radical polymerization [7–10]. This approach can be achieved in a minimally invasive manner, gel formation is rapid, a wide range of gel physical properties, such as modulus, swelling and degradation, can be generated, and a variety of cell types have been photo-encapsulated with high viability under the appropriate conditions [7–10].

Equilibrium modulus values for human articular cartilage have been reported to be between 500 to 1000 kPa, depending on the age and health of the tissue [3,11]. In addition, reported aggregate moduli for confined compression of the degenerated and healthy human nucleus pulposus tissue are between 440 ± 190 to 1010 ± 430 kPa, respectively [12]. To generate hydrogels with these mechanical properties requires high cross-link densities, which are typically achieved by using a high prepolymer solution concentration and/or a high degree of (meth)acrylation of the prepolymer. The encapsulation of cells in photo-cross-linked hydrogels having stiffnesses approaching those of load bearing soft tissues has been hampered by poor cell viability, which has been attributed to the higher concentration of the reactive (meth)acrylate groups required to yield the necessary cross-link densities [13,14]. Therefore, it has been common to use a low prepolymer concentration along with a low degree of (meth)acrylation of the prepolymer to reduce cytotoxic effects on the cells. Generally, these hydrogels have low moduli of around 10 kPa and a high degree of swelling, with swelling ratios close to 40 [13,15]. A variety of load bearing soft tissue cell types and stem cells encapsulated in these low modulus hydrogels have been able to increase the hydrogel stiffness up to approximately 100 kPa, either through cell proliferation and/or the accumulation of extracellular matrix, but only after 4–12 weeks in culture [16–20]. As well, the significant increases in the initial hydrogel dimensions would reduce the targeted mechanical properties and initial cell densities of the hydrogels [21,22]. In an *in situ* gelation situation, hydrogels would not have the opportunity to reach equilibrium before implantation. Therefore, in the field of LBST repair, there is a need for hydrogels with initial moduli approximating that of native LBST that are also dimensionally stable after cross-linking, while supporting chondrocyte-like cell behavior and the generation of *de novo* cartilage tissue over time.

In these earlier studies cell viability was measured utilizing fibroblasts and auricular chondrocytes [13,17]. Cell viability following photo-encapsulation varies with cell phenotype, and in general, cells that proliferate faster exhibit greater cell death [23]. As articular chondrocytes and nucleus pulposus cells, which are the cells of interest in the regeneration of LBST, grow more slowly than do

fibroblasts and auricular chondrocytes [13], it was hypothesized that chondrocytes isolated from articular cartilage could be photo-encapsulated with high viability within hydrogels possessing moduli approach that of LBST.

The use of prepolymers synthesized from the major polysaccharide constituents of the native tissue under study is common practice in the field of tissue engineering. Typical natural polysaccharides used to form hydrogels for LBST studies are hyaluronic acid and chondroitin sulfate [24,25]. Hyaluronic acid (HA) is a high molecular weight ($>1 \times 10^3$ kg/mol), anionic, non-sulfated polysaccharide common in load bearing connective soft tissues [26,27]. Chondroitin sulfate (CS) is a lower molecular weight (<50 kg/mol) sulfated polysaccharide that forms aggregates with the protein aggrecan. In LBST, aggrecan is responsible for creating hydrostatic pressure, which in turn structurally supports the compressive loads on the tissue. These polysaccharides are readily methacrylated under relatively straightforward reaction conditions [13,28,29]. Chitosan is a cationic polysaccharide isolated from crustacean shells, which can be modified with glycol moieties to provide solubility at neutral pH. Methacrylated glycol chitosan (MGC) has been used to produce photo-cross-linked hydrogels suitable for cell encapsulation and LBST studies [10,30].

In this study, hydrogels were prepared from the individual prepolymers of methacrylated glycol chitosan, hyaluronic acid and chondroitin sulfate using a range of solution concentrations and degrees of prepolymer methacrylation with the objective of obtaining hydrogels with moduli approaching that of native LBST and exhibiting minimal swelling following cross-linking. Initially, prepolymer concentrations with low degrees of methacrylation were adjusted to obtain hydrogels with moduli greater than 100 kPa. The lowest prepolymer concentration that could meet the modulus requirements was selected to maintain a reasonable prepolymer solution viscosity that could be injected. Control of the hydrogel swelling through the cross-link density was also utilized to stabilize the original hydrogel dimensions during equilibrium swelling and to further improve the initial modulus of the hydrogels. Controlling hydrogel swelling from the original cross-linked dimensions was deemed important for maintaining a stable implant at a defect repair site. Hydrogel modulus and swelling extent were further manipulated by mixing and co-cross-linking MGC and MHA with MCS prepolymers at different mass ratios. Chondrocytes isolated from articular cartilage were then photo-encapsulated within these hydrogels and their viability and metabolic activity measured over a 35 day period.

2. Methods and materials

2.1. Materials

Sodium hyaluronate (number averaged molecular weight, $M_n = 116$ kg/mol, dispersity index, $\bar{D} = 1.3$, 99.8% purity) was obtained from Lifecore Biomedical (St. Paul, MN, USA) and used as received. Bovine chondroitin sulfate ($M_n \approx 50$ kg/mol, $\geq 90\%$ purity) was purchased from LKT

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