# Increased Proteoglycan Syntheses in Primary Human Bovine and Chondrocytes in Biomimetic PEG Hydrogels Containing Type I Collagen and Hyaluronic Acid Signaling Motifs

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**Statement of Purpose:** By 2030, 67 million Americans are projected to suffer from osteoarthritis, a disease characterized by the degeneration of cartilage and underlying bone in a joint[1], with 25 million subjected to severe forms which limit their physical activity[2]. Current surgical interventions (microfracture, autologous chondrocyte transplantation, use of filling material, etc…) typically produce fibrous cartilage, which degrades with constant loading instead of more durable hyaline cartilage found in healthy joints. Tissue engineering offers the potential to generate biomimetic hyaline cartilage with mechanical properties identical to native materials. Current strategies primarily focus on either synthetic or natural systems in isolation. We hypothesize that the inclusion of natural extracellular matrix (ECM) components in synthetic poly(ethylene glycol) dimethacrylate (PEGDM) matrices leverages the respective advantages of both systems while limiting their inherent drawbacks. The combination leads to enhanced cartilage formation through increased ECM production. Primary bovine and human chondrocytes were cultured in 10% PEGDM hydrogels containing varying concentrations of type I collagen (Col I) and hyaluronic acid (HA), the effects on ECM production and tissue formation were examined over 6 weeks of in vitro culture.

**Methods**: Hydrogel preparation: 10% PEGDM (~8000 g/mol) Opti-MEM I reduced-serum medium solution was prepared containing 0.1% Irgacure 2959. For ECM additive testing, 1% Col I and 0.5% or 1% HA was added to the 10% PEGDM Opti-MEM solution. Hydrogels were photopolymerized using ~2.3 mJ/cm$^2$ UVA light for 5 min. 1 million bovine (2-3 week old) or human (knee arthroscopy) chondrocytes, which were expanded in monolayer culture for 1 week, were encapsulated in a 100 µL of 10% PEGDM Opti-MEM solution with or without ECM additives to create samples (~1 cm diameter, 0.3 cm thick) were cultured up to 6 weeks in Opti-MEM containing 50µg/ML ascorbate and 100ug/mL primocin. Media was changed 3 times a week.

Histology: Proteoglycan staining was conducted on 10% formalin fixed whole samples with 0.01% thionin. For biochemical testing, samples were desiccated overnight in a vacuum centrifuge.

Biochemistry: Sulfated gylcosaminoglycans (sGAGs) were quantified with dimethylmethlene blue and collagen content was quantified using dimethylaminobenzaldehyde to observe chloramines T-oxidized hydroxyproline. All biochemistry was normalized against sample without cells cultured in media for the same duration.

**Results:** Glycosaminoglycan staining indicates the inclusion of Col I and HA in the matrices promotes bovine ECM production for both bovine and human chondrocytes. Figure 1 illustrates human chondrocyte ECM production after 6 weeks of culture in the matrices. PEGDM matrices seeded with bovine chondrocytes containing 0.5% HA and 1% Col I and PEGDM matrices containing 1% Col I were found to contain roughly 2.6 times and 1.9 times more chondrotin sulfate respectively than matrices containing only PEGDM and 2.4 times and 1.8 times more hydroxyproline respectively than matrices containing only PEGDM after 6 weeks of cultures. Human chondrocytes showed similar biochemical results.

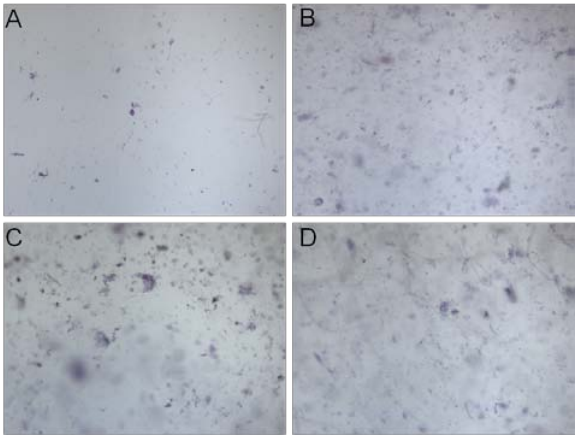


**Figure 1**: Glycosaminoglycans (thionin) staining of matrix produced by primary human chondrocytes after 6 weeks of culture in 10% PEGDM (A) , 10% PEGDM + 1% type-I collagen (B), 10% PEGDM + 1% type-I collagen+0.5% hyaluronic acid (C), 10% PEGDM + 1% type-I collagen+1% hyaluronic acid (D) , Magnification= 4x.

**Conclusions:** The inclusion of low concentrations of Col I and HA promotes ECM production by both bovine and human chondrocytes. Future work will examine the effect of these ECM on gene expression and examine the pathways activations triggering this response.

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