INTRODUCTION: Insulin-Transferrin-Selenium (ITS) medium supplement is available as a means of reducing serum concentration while maintaining normal cell functions. Previously, medium containing 1% ITS + 0.2% FBS was evaluated vs 10% FBS for total GAG accumulation in a chondrocyte-seeded self-assembling peptide gel, a material scaffold for cartilage tissue engineering [1]. After 3 weeks the ITS + FBS culture showed similar GAG accumulation as the FBS culture. This result indicates ITS may be suitable for complete or partial replacement of serum in culture medium for tissue engineering. In this study, cell proliferation and type II collagen production are assessed at early times in seeded peptide gels cultured in ITS medium +/- 0.2% FBS relative to 10% FBS, with the objective of developing optimal conditions for both cell proliferation and matrix synthesis and accumulation. In the process, we developed a method for quantifying viable cell number in the peptide gel. For reference, cell biosynthesis in the well-defined agarose culture system in ITS media was evaluated relative to 10% FBS culture over a 5 week period.

METHODS: Hydrogel Scaffold Seeding and Culture: Bovine chondrocytes from 1-2 week old calves were isolated by sequential pronase and collagenase digestion. Cells were seeded into 0.5% self-assembling peptide scaffolds (sequence - KLDLKLDDLKLDDL-) or 2% agarose in a flat slab geometry as previously described [1]. Seeded gels were cultured in DMEM supplemented with 1% ITS (ITS), 1% ITS + 0.2% FBS (ITS/FBS), or 10% FBS (FBS). DNA/Cell Content in Peptide: DNA quantification via Hoechst dye analysis was unsuccessful using the method of Kim et al. [2], due to interference associated with the peptide material. As an alternative, an MTS viable cell kit (Promega) was used to determine viable cell density in seeded peptide scaffolds. In order to interpret MTS values, a calibration curve for 3-D cultures was first established using agarose cultures. Five agarose slabs were seeded at cell densities ranging between 10-30 x 10^6 cells/ml. Samples were punched and analyzed on day 2, 3, and 5. Each group of 11 plugs, 6 were incubated in MTS medium for 2 ½ hours on a shaker table. SDS was added (final concentration = 2%) to stop the MTS reaction. After 30 minutes, medium samples were tested for optical density. The remaining 5 plugs were digested and analyzed for DNA content using the Hoechst dye assay. Mean MTS output was plotted vs mean DNA content to establish the calibration curve (Fig. 1). MTS data were then obtained for seeded peptide plugs using the established method. Viable cell counts were determined from MTS output, and cell densities are reported as viable cell populations in plug volume. To further verify MTS results, samples were incubated with BrdU for 20 hours and fixed on days 3, 7, and 13. Biosynthesis: Immunostaining for BrdU incorporation and collagen II was performed on day 13 in all media conditions. BrdU incorporation verified MTS analysis, showing proliferating populations in FBS gels on day 3 and 7 (data not shown). DNA values for the FBS and ITS/FBS samples showed a marginal increase in strength, while ITS samples were an order of magnitude increase in strength, while ITS samples were as delicate as acellular gel. Delay or suppression of matrix accumulation may limit the effectiveness of both ITS media for in vitro culture of tissue engineered implants. Agarose - ITS and ITS/FBS medium supplements are acceptable alternatives to 10% FBS for culture of chondrocytes in agarose gels. MDMEM + 1% ITS is a fully-defined medium that allows for biosynthesis similar to 10% FBS. Addition of 0.2% FBS to ITS increased biosynthesis over both single-component media. As observed in peptide cultures, surface de-differentiation is limited with both ITS media, reducing potential artifact for normalization of biosynthesis to plug DNA. The agarose results indicated that ITS medium is potentially equivalent or superior to 10% FBS for biosynthesis. However, reduced collagen II deposition in peptide gels illustrates the sensitivity of chondrocyte/medium interactions to scaffold properties.