The Effects of Different Sources of Fetal Bovine Serum on Chondrocyte Growth

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Introduction: Fetal bovine serum (FBS) is obtained from the blood of a bovine fetus and is utilized as a serum supplement for in vitro cell cultures to simulate physiological environments. Compositional differences in each sample can pose an issue of variability that may affect the integrity of experiments. Various biochemical techniques like mass or fluorescence spectrometry have shown composition analysis of FBS media, but these methods have been mainly limited by cost. Literature has shown compositional variations among different FBS lots via proteomic measurements of FBS media prior to and after usage in cell cultures [1]. This study examines the effects on chondrocyte culture of these six FBS samples obtained from different biotechnological companies. The growth factors TGF-β, IL-1β, bFGF, and IGF were chosen due to their regulation in chondrocyte proliferation for quantitative measurements. Effects on growth were examined by growing chondrocytes in monolayer in the different FBS samples over 21 days.

Methods: Seven FBS samples were obtained from different well-known companies (Table 1).

ELISA ASSAYS. Sandwich ELISAs were performed on each sample of FBS using Abcam ELISA kits that tested for each of the growth factors TGF-β, IL-1β, bFGF, and IGF. Each sample was loaded in triplicates. Insulin-Transferrin-Selenium (ITS) media served as the negative control. For quantitative analysis, a mitochondrial-based fluorescence assay was used to measure absorbance to determine the amount of each growth factor in each FBS sample.

CHONDROCYTE MONOLAYER GROWTH. Frozen bovine articular chondrocytes were thawed and cultured until confluence, with the media changed every three or four days. Upon reaching confluence, the chondrocytes were re-plated onto nine 96-well plates at 5000 cells/well using 100µl of the specific FBS media, with triplicates for each FBS sample. The media on the plates was changed every two or three days. FBS samples 2 and 7 were not utilized due to insufficient stock serum upon the completion of the ELISA assays. To examine the trend in chondrocyte growth, each of the nine 96-well plates represented one time point in our study. Each time point was separated by two or three days. 48 hours prior to assaying the plate to determine cell density, triplicates of the standard curve values were seeded onto the plate of interest. Cell density was measured using PrestoBlue, a fluorescence-based viability assay that determines the reducing ability of metabolically active cells. Subsequently, the plates were washed with 1x PBS and added with Live-Dead stain solution for qualitative analysis of the cell population. This fluorescence-based stain uses ethidium bromide and calcein AM to determine dead cells and live cells, respectively. After incubation at 37°C, the cells were imaged under the Apotome fluorescence microscope with the Axiol software to confirm the cell density determined by our PrestoBlue assay. An analysis of variance (ANOVA) was used to compare the different culture conditions.

Results: ELISA ASSAYS. All of the FBS samples, except bFGF FBS #6, yielded negative results for the levels of each growth factor. Results obtained from the IL-1β were inconclusive and therefore not included.

CHONDROCYTE MONOLAYER GROWTH. At each time point, chondrocyte population numbers were determined with PrestoBlue (Figure 2) and confirmed from qualitative analysis using Live-Dead stain. Cell density among the different types of media did not vary until day 9. At day 16, the peak of population growth was reached. On day 16, the cell densities for each of the FBS types were significantly different from the negative control ITS media: FBS 3 (p <0.001), FBS 4 (p<0.05), FBS 5 (p<0.001), and FBS 6 (p<0.001). Chondrocytes grown in ITS media showed consistent decreases over time, and indicated negative population numbers starting from day 9.

Discussion: The ELISA assays yielded mostly negative results. This suggests that there are no significant detectable concentrations of the examined growth factors in the different FBS samples. However, chondrocyte monolayer population trends indicate that culture in the different FBS types do lead to a detectable difference in population growth, whether due to growth factor variations or to other compositional differences. Only after nine days did we observe significant differences in the cell density for the various FBS media and the ITS media, suggesting that the supplemental effect of FBS media may not be apparent during the early stages of chondrocyte monolayer growth. The general decrease in population growth after day 16 may be attributed to the overcrowding of chondrocytes in the provided well space, leading to a reduction in population numbers. Divergence of population growth rate starting from day nine may be explained by two factors: 1) differences in FBS sample composition and 2) phenotype changes on the chondrocytes themselves. First, although the growth factors examined did not yield positive concentrations in the ELISA assays, other unexamined growth factors may influence chondrocyte growth. It may also be possible that specific growth factor activation is required for positive detection. Second, literature has suggested that monolayer chondrocytes grown in plastic surfaces may dedifferentiate over a long period of time due to the lack of cartilage matrix [2]. Therefore, the significant growth between day nine and day sixteen may be due to an increase in dedifferentiated...
When performing cell culture with FBS, lab experiments should take care to consider these demonstrable differences in chondrocyte growth. Further, caution should be taken when culturing cells in ITS media for long periods of time, as this may affect their long-term viability and phenotypical morphology. To determine the phenotype developments, future goals aim to utilize various molecular techniques to track the chondrocyte phenotypes over the same timeline using specific markers, such as col I and col II. Additionally, it may be beneficial to examine whether these diverging trends may be observed in chondrocytes grown in pellets due to its support for more wholesome cellular proliferation [3].

**Significance:** Though specific growth factors important to chondrocyte growth were not detected in significant amounts in the different fetal bovine sera (FBS) tested, chondrocyte monolayer growth in these different types of FBS media have shown significant differences compared to the ITS negative control. Lab experiments should be cognizant of these potential differences when performing cell culture with FBS media.

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**References:**

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<tr>
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<th>Fetal Bovine Serum Samples</th>
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<tbody>
<tr>
<td>1</td>
<td>ITS (insulin-transferrin-selenium)</td>
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<tr>
<td>2</td>
<td>Axenia Biologix VitroPure</td>
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<td>3</td>
<td>Axenia Biologix CultraPure</td>
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<td>Corning Cellgro Mediatech</td>
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**Table 1.** Seven different FBS samples were obtained, with an emphasis on the variety of the source to include differences in the company and the country of origin.
Figure 1. Relatively concentrations of various growth factors (IGF-1, bFGF, and TGF-β) compared to known standard curve. All calculated R² values were above 0.95. In (C), TGF-β samples were performed with an activated and an inactivated set.
**Figure 2.** Chondrocyte population monolayer growth in different ITS or FBS media over three weeks.

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