IRAP II (ABPS): Vertical vs. Horizontal Incubation, Serum Color Differences

Arthrex Research and Development

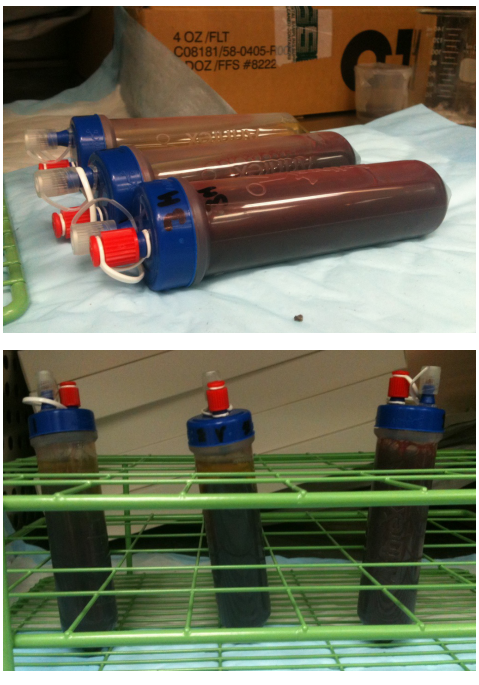
Introduction

The IRAP II system was designed to maximize the production of autologous Interleukin-1 Receptor Antagonist Protein (IL-1ra). The cytokine IL-1β is believed to be the main catabolic protein related to cartilage and bone resorption[1]. IL-1ra competitively inhibits all known activities of IL-1β, thus countering osteoarthritic effects[2]. To obtain the maximum concentration of the IL-1ra cytokine, the IRAP II device should be incubated with whole blood for up to 24 hours at 37 °C. This study illustrated the effects of incubating the device in a horizontal and vertical position.

Methods and Materials

Human blood was delivered into two IRAP II devices; one was incubated at 37 °C in a horizontal position (Figure 1A) and one was incubated at 37 °C in a vertical position (Figure 1B). After 24 hours, the devices were centrifuged and serum was extracted and filtered. The serum was frozen at -81 °C, and after thawing, growth factor analysis (human IL-1ra) was performed. Frozen serum was also sent to the University of Connecticut (Department of Orthopaedic Surgery, University of Connecticut of Health Center, Farmington, CT, U.S.A.) for cell culture studies (human chondrocyte, osteoblast, and tenocyte proliferation); where serum was administered to the cells at time zero and proliferation was analyzed at day two and day five.

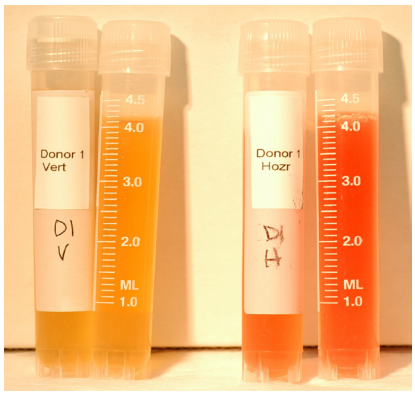
Figure 1: Top (A) horizontal incubation; Bottom (B) vertical incubation

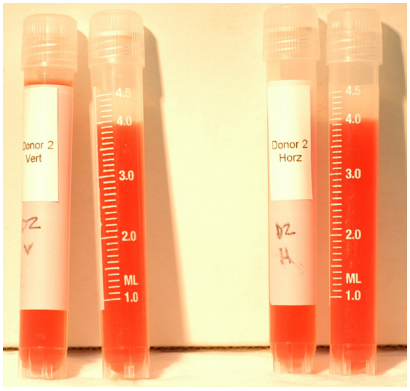


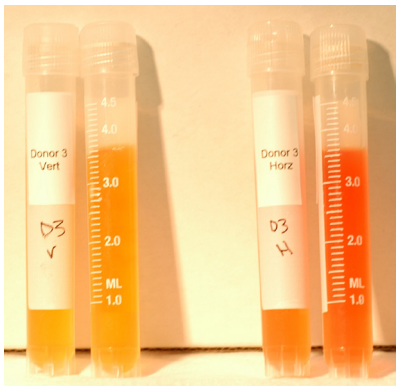
Results

Serum Color and IL-1ra Analysis: Figure 2 illustrates the color differences of the serum produced by each of the donors (donor 1 is the top picture, donor 2 is the middle picture, and the bottom picture is donor 3).

Figure 2: IRAP II (ABPS) serum colors of the three donors (the left two tubes in each picture show vertical incubation and the right two tubes show horizontal incubation)







The average concentration of IL-1ra in the samples was 27,125 ± 3,366 pg/mL and 26,507 ± 3,192 pg/mL (vertical and horizontal, respectively). A t-test (α=0.05) was performed and there was not a statistical difference between these concentrations (p=0.829).

Cell Proliferation Studies: Figure 3, 4, and 5 depict the cellular proliferation assays using human chondrocytes, osteoblasts, and tenocytes at two days and five days. Two percent Fetal Bovine Serum (FBS) is used as a control to maintain the cells, but not promote proliferation. One-way ANOVAs (α=0.05) with multiple group comparisons (Tukey Test) were used for statistical analysis.

Figure 3: Chrondrocyte proliferation: horizontal vs. vertical incubation

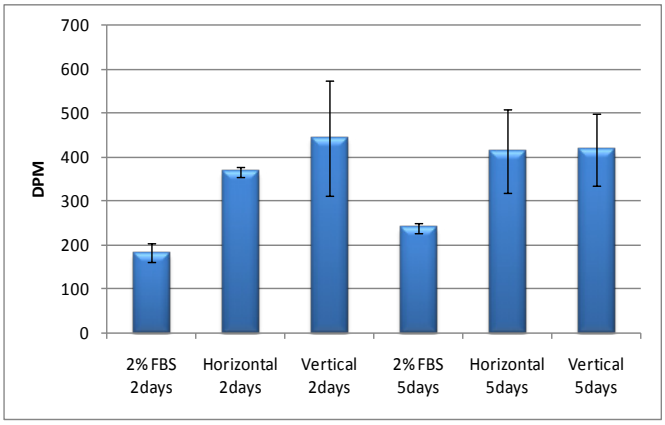


Figure 4: Osteoblast proliferation: horizontal vs. vertical incubation

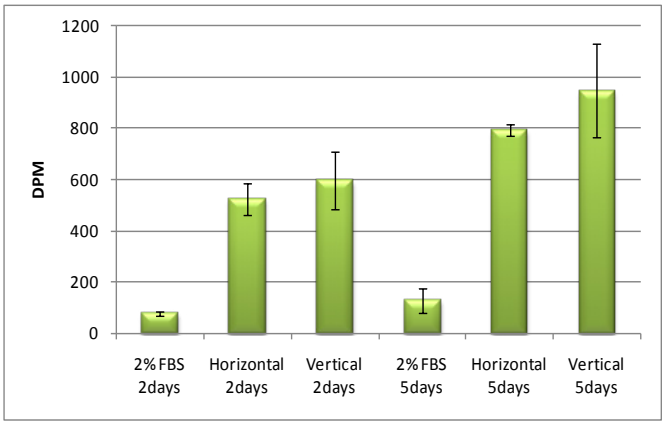
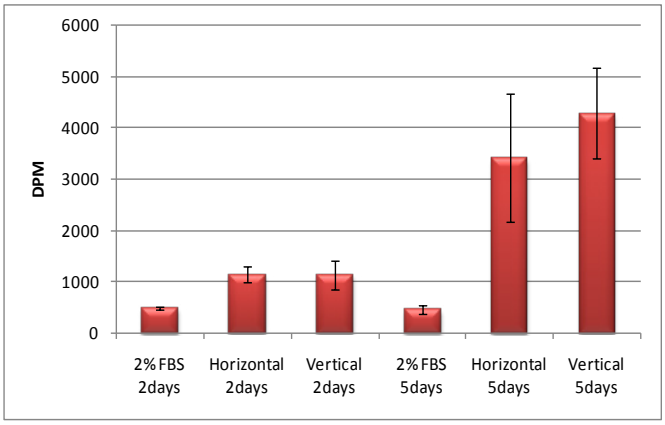


Figure 5: Tenocyte proliferation: horizontal vs. vertical incubation



Chondrocyte Proliferation Statistics

There was not a statistical difference between vertical and horizontal cell proliferation at two and five days (p=0.793, and p=1.000, respectively).

Osteoblast Proliferation Statistics

There was not a statistical difference between vertical and horizontal cell proliferation at two and five days (p=0.928, and p=0.390, respectively).

Tenocyte Proliferation Statistics

There was not a statistical difference between vertical and horizontal cell proliferation at two and five days (p=1.000, and p=0.573, respectively).

Summary

Two of the three donors produced a more yellow colored serum when the vertical incubation technique was used. From a cytokine and cell proliferation perspective, there was not a statistical difference when incubating the IRAP II (ABPS) in a horizontal and vertical position.

References

1. Andersen, LS, et al. Production of interleukin (IL)-1β, IL-1 receptor antagonist and IL-10 by blood mononuclear cells in chronic arthritis. Cytokine. 2000;12(1):62-68.

2. D. Frisbie, C. Kawcak, N. Werpy, R. Park, W. McIlwraith. Clinical, biochemical, and histologic effects of intra-articular administration of autologous conditioned serum in horses with experimentally induced osteoarthritis. Amer Journal Vet Research. 2007;68(3).