

Poster: free communications

## Cell-growth promoting fractions originated from bovine blood clot in combination with the porcine ocular fluid

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Sera are generally obtained from drawn and collected whole blood from adult, calf or fetal animals. Following the natural clotting process, which may take several hours at 4°C, the blood consists of serum and blood clot containing 95% of red blood cells, 5% platelets, less than 1% and numerous amounts of fibrin strands. In comparison to a PRP (platelet rich plasma), blood clot containing 4% of red blood cells, 95% platelets and 1% of white cells. The specific cell-growth promoting components are the platelet derived growth factor (PDGF) and the transforming growth factor Beta (TGF Beta). Both of them are contained in the Beta granules of the platelets. Fibronectin and vitronectin are also the components of the PRP. They are the cell adhesion molecules found in plasma and fibrin itself. The experiments presented herein were aimed to isolate, characterise and to test in vitro on different cell cultures the growth promoting material from the bovine blood clot. The bovine blood was collected and allowed to form the clot. Afterward the whole content was centrifuged at 2500 RPM for 20 minutes and the supernatant (serum) was aspirated off. The sediment ("clot") was quickly washed with the sterile buffered saline pH=6.9 for 20 minutes, and centrifuged for 25 minutes at 2500 RPM for 25 minutes. The supernatant (Fraction I) was collected and frozen. To the remaining "clot" the PBS pH=7.2 was added and left for 1 hour at +4°C. After the centrifugation of the suspension at 2500 RPM for 25 minutes, the supernatant (Fraction II) was collected and frozen. To the sediment ("clot") the PBS pH=7.4 was then added for 18 hours (Fraction III). All the fractions were sterilised by 0.2 membrane filtration. The content was analysed by PAG-SDS electrophoresis. The cell growth promotion/inhibition activity in the comparison to the SR-2.055P (Serum replacement based on porcine ocular fluid) and FCS (Fetal calf serum) was tested on the Chicken embryonal fibroblasts, WISH, HAC-3/T2 (Human amniotic cell lines), PLA-2 (Adult pig kidney cell line), Bovine intestinal epithelial cell line, WiREF (Wistar rat embrional fibroblastoid cell line) and CaCo-2 (Colon cancer carcinoma cell line). The results of the experiments shows: (1) The attachment and growth of primary cultures (Chicken embryonal fibroblasts) is not affected after the use of bovine blood clot fractions. (2) Fractions (I-IV) shows the growth promotion, but different according to the group of cells used in the test. (3) The strongest growth enhancement was found when transformed cells (WiREF, CaCo-2) were tested. (4) The optimal content was 8-10% in Eagle's medium. In this range up to 95% value of the SR-2.55P could be obtained. (5) When different fractions (I, II, III) were combined with the porcine ocular fluid the slight enhancement can be found only when the ratio 1:2 was used.

*Keywords: cell growth promoting fractions, bovine blood clot, porcine ocular fluid*