

## RELEASE OF NEUTROPHIL AND MONOCYTE CHEMOATTRACTANTS INTO MEDIA IN CO CULTURE OF BONE MARROW DERIVED STROMAL CELLS AND EPINEURIUM

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**Background** Epineurium is a preferable biological conduit in repair of peripheral nerve because of its low immunogenicity. In our rat model of sciatic nerve regeneration we have demonstrated that *ex vivo* enrichment of epineurium with bone marrow derived stromal cells (BMSC) improves regeneration process. Our recent study suggested that maintenance of these constructs in culture for up to two weeks prior to transplantation may further benefit a healing process.

**Goal** Since BMSC is a potential source of a variety of cytokines and growth factors it is important to define which specific factors are secreted by BMSC enriched epineurial conduits. In the present study levels of 34 rat cytokines were determined in culture media of BMSC, epineurium, and combined BMSC-epineurium maintained in culture for 7 and 13 days.

**Methods** Stromal cells were prepared from ACI rat bone marrow cells by seeding  $30 \times 10^6$  of bone marrow cells in 25 cm<sup>2</sup> flask using 10 ml alpha-MEM medium complete (containing 10% FBS). After first medium change (72 hrs) 2 cm sciatic nerve epineurium was added to flask with the stromal cells. Cultures containing stromal cells only and epineurium only were maintained in parallel. Medium alone was used as a negative control.

Sample of media have been collected during each change of media (every 72 hours). The level of cytokines in culture media samples was determined using rat cytokine array (RayBio Rat Cytokine Antibody Array G series 2; Norcross, GA) containing antibody to 34 rat cytokines and inflammation related soluble proteins. Signal images of arrays were recorded using Axon 4005 laser scanner. For each spot representing one protein a ratio of signal intensity of sample to signal intensity of negative control was calculated.

**Results** In all three types of cultures (BMSC, epineurium and BMSC/epineurium) and in the both time points (7 and 13 days) the eight following soluble factors were secreted: CINC-1, CINC-2 $\alpha$ , CINC-3, LIX, MCP-1, MIP- $\alpha$ , TIMP-1 and VEGF. These proteins play an important role in neutrophil chemotaxis, monocytes recruitment and angiogenesis. The media obtained from 13 days old cultures contained higher level of these factors than media from 7 days cultures. The media from co cultures of BMSC and epineuria have higher level of these factors than BMSC and epineurium cultured alone. The MMP-8 (collagenase-2), which plays a role in inflammatory cell recruitment, neutrophil apoptosis and collagen metabolism in tissue injuries, was not detected in media of cultured epineurium, but it was secreted into media of BMSC alone and there was an increase of a level of MMP-8 in BMSC-epineurium co culture.

**Conclusion** BMSC enriched conduits used in our rat model for peripheral nerve cellular therapy show expanded secretion of stimulatory factors; therefore these conduits seems to be promising in nerve regeneration *in vivo*.