

## Free Floating Microcultures of Human Fetal Ventral Mesencephalon

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In the reported clinical trials of human fetal ventral mesencephalic transplants to the brain of Parkinson patients the tissue was transplanted either as small solid pieces or as cell suspensions. In all cases the tissues were implanted immediately after explantation. Delayed surgery and the *in vitro* maintenance of the tissue prior to transplantation would present several advantages. For example, this would allow one to estimate the approximate number of neurons transplanted and to measure dopamine content and tyrosine-hydroxylase (TH) activity *in vitro* expressing the activity of the cultures. Furthermore tissues *in vitro* could be treated with various growth factors.

Here we present a protocol to maintain human fetal ventral mesencephalic tissue in free floating microcultures. Ventral mesencephalon of human fetuses was obtained from material of induced suction abortions. Out of 38 abortions the mesencephalon could be identified in 16 cases (50% in fetuses age 8-10 weeks post mens. and 31% in fetuses age 11-13 weeks post mens.) based on macroscopic criteria. The landmarks which allowed identification were first the isthmus rhombencephali and second the IV ventricle (caudal to the mesencephalon) and the recessus ventriculi III (rostral to the mesencephalon) as well as the mesencephalic flexure. However the tissue was most often fractured at the isthmus.

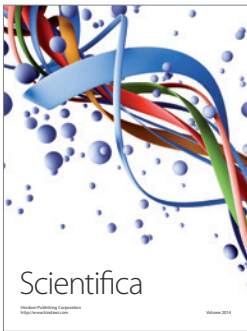
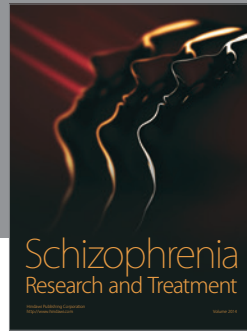
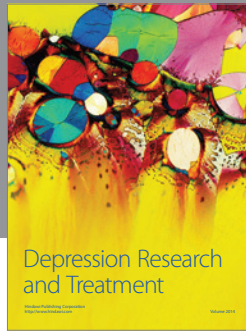
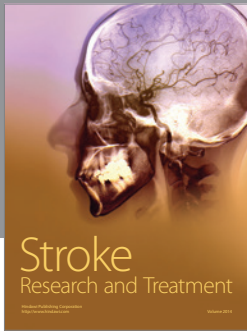
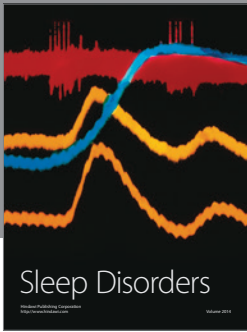
The ventral mesencephalon was dissected out of the identified brain tissue and divided into four longitudinal pieces. These pieces were transversely chopped in 250  $\mu\text{m}$  steps by means of a tissue chopper (McIllwain). These tissue pieces (one piece per tube) were transferred into plastic tubes (Falcon No. 2095) (one culture per tube) containing 1 ml of medium. One hun-

dred ml of medium consisted of 55 ml Dulbecco MEM (Gibco No. 041-02320), 32.5 ml Hanks BSS (Gibco No. 041-04020), 1.5 ml glucose 20%, 10 ml FCS (Gibco No. 013-06290), and 1 ml of 23.8% HEPES. A triple-antibiotic (Gibco No. 043-05240D) was used during the first 4 days *in vitro*. Ten ng/ml NGF, 10 ng/ml BDNF or 10 ng/ml bFGF was added to the medium. The tubes were placed in a revolving drum (60 revolutions per hour) in an incubator with 5%  $\text{CO}_2$ . The medium was changed at 3 and 4 day intervals. After 5-14 days *in vitro*, these cultures were pooled in groups of 5-10 cultures and processed for electron microscopy, histology in frozen sections or enzyme and transmitter assays.

The cultures survived and developed further *in vitro*. After 5 days *in vitro*, the tissue pieces had turned into small round balls of 0.5 to 1 mm in diameter. Histological examinations of mesencephalic cultures showed good survival of the tissue. TH positive cells were demonstrated by immunohistochemistry and dopamine and tyrosine hydroxylase was detected in biochemical assays. When free floating microcultures were explanted onto coverslips after 14 days *in vitro* and grown by the roller tube method for one week, newly formed processes extended from the explant.

These experiments show the feasibility of maintaining human fetal ventral mesencephalon *in vitro* by means of free floating microcultures. It is suggested that the transplantation of these cultures be tested in rats as a next step.

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