Culture Technique of Human Nervous Cells Collected from Traumatized Brains

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Abstract

Introduction: The human brain is composed of a complex network of cells that interact with each other through direct and indirect pathways. Studies about culture of human brain cells have been limited to postmortem experiments and only more recently have been obtained from living patients. These harvests of human nerve tissue samples occurred only during neurosurgeries for control of refractory epilepsy and resection of brain
tumors. **Methods:** We describe in this work a technique for culture of nerve cells removed during urgency neurosurgeries in injured brains (patients who suffered traumatic brain injury and needed surgery), aiming the culture of injured cells for later study of nerve regeneration. These samples of nerve tissue were collected from carefully selected patients: in cases of depressed skull fracture with laceration of the dura-mater and cerebral cortex, with spontaneous exit (caused by trauma) of brain tissue, which obviously required neurosurgery; and in patients who presented traumatic intracranial hematomas with subcortical component and expansive effect, requiring surgical removal through a small opening in the cerebral cortex, known as corticectomy, for drainage of the clot. After obtaining, this material was immediately stored and sent to treatment and plating, being maintained in culture medium in a CO2 incubator. **Results:** In the medium, the presence of neurons and glial cells can be verified few days later. **Conclusions:** It is concluded that is possible to culture injured nerve cells through the technique employed, thus opening a field of research for the study of substances with presumed neuroprotective function.

**Introduction**

The human brain is composed of a complex network of cells that interact with each other through direct and indirect pathways. Neurological diseases and drugs act specifically on different cell types, therefore, studies delineated with a single cell type are the best way to evaluate this variability and specific effects in each cell (Spaethling et al., 2017).

However, most of these neuronal cell culture studies were performed in rodents (Dueck et al., 2015; Miyashiro; Dichter; Eberwine, 1994; Tasic et al., 2016; Zeisel et al., 2015). Studies in humans have been limited to postmortem experiments (Hawrylycz et al., 2015; Lake et al., 2016), cancer cell lines and only more recently, cell culture were obtained from live patients (Darmanis et al., 2015; Zhang et al., 2015).
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al., 2016). These collections of human nerve tissue samples occurred during neurosurgeries of refractory epilepsy and brain tumors (Darmanis et al., 2015; Spaethling et al., 2017; Zhang et al., 2016). The investigation of diseases that affect neurons, as well as the effect of drugs, is therefore hampered by the paucity of studies and experiments on human nerve cells of living patients.

The development and validation of a neuron culture technique that allows investigating its function and its response to several stimuli is therefore important and has already been used by other authors to distinguish and classify different cellular subtypes (Darmanis et al., 2015; Spaethling et al., 2017). We describe a technique for the collection and culture of nerve tissue samples from patients who suffered traumatic brain injury (TBI), requiring neurosurgery in some specific situations: in cases of skull sags with laceration of the dura mater and cerebral cortex, with spontaneous exit (caused by the trauma) of the cerebral tissue, that obviously needed neurosurgery; and in patients who presented traumatic intracranial hematomas with subcortical component and expansive effect, requiring surgical removal through a small opening in the cerebral cortex, known as corticectomy, for drainage of the clot.

Our objective is to describe and validate a technique for the culture of human nerve cells from collections of biological specimens from patients who suffered traumatic brain injury and required emergency neurosurgery.

The culture of these cells can be used later for the study of the nervous regeneration with the addition of several chemical substances, with supposed neuroprotective action. Drugs and medications can also be tested.

Methods

The biological samples were obtained from the brain tissue of patients traumatized with TBI, attended at the Regional Hospital Tarcisio de Vasconcelos Maia (HRTM), in Mossoró - RN, who required emergency neurosurgery, in some very specific si-
tutions, detailed in item 2.2.

**Legal Requirements**

The collection of the material was only carried out with the authorization of the legal representative of the patient through the signing of the Term of Free and Clarified Assent (TALE). The collection was carried out in accordance with the specific legislation (Resolution 441/2011 and ordinance of the Ministry of Health 2201/11). This research project was approved by the local Research Ethics Committee (CAAE 65640517.0.0000.5294) on April 26, 2017.

**Criteria for Inclusion**

Age between 18 and 70 years; signature of TALE by the legal guardian; patients who require emergency neurosurgery due to TBI in very specific situations: in cases of patients with cranial dandruff with laceration of the dura-mater and cerebral cortex, with spontaneous (caused by trauma) brain tissue, which obviously need neurosurgery; and in patients with traumatic intracranial hematomas with subcortical component and expansive effect, requiring surgical removal through a small opening in the cerebral cortex, known as corticectomy, for drainage of the clot.

**Patient Characteristics**

**Patient 1**

A 29-year-old male, who suffered a motorcycle accident with TBI, having ingested alcoholic beverages, being operated on for fracture-skull sinking, acute subdural subdural hematoma and lacerations of the dura mater and cerebral cortex in the right temporal lobe non-eloquent), with spontaneous exit (caused by the trauma) of the nervous tissue.

**Patient 2**

A 45-year-old male, suffered a traffic accident (motorcycle collision in a car), with a fractured skull, dural and cortical lacerations in the left temporal lobe, and a lower temporal gyrus topography.
**Patient 3**

Young male, TBI due to traffic accident, with right temporal lobe explosion (voluminous right temporal contusion with large subcortical component). This patient was submitted to temporal lobectomy, and it was possible to collect a larger quantity of material, mainly from the cerebral cortex, which was used to prepare explants (fragments of nervous tissue).

**Pre-Operative Preparation**

After the indication of the need for surgical intervention, the patients were referred to the operating room of the surgical center in the usual manner, being routinely received by the nursing and anesthesiology team for anesthesia and subsequent surgery, as is the protocol for all patients who are operated. There was no change in the routine of the pre-surgery visits. Meanwhile, in another room, a member of the research team presented TALE with the legal guardian for the patient to read and authorize or not to collect the material.

**Surgical Procedure**

Surgical interventions followed the internationally agreed steps for the treatment required for the patients in question, according to the following sequence: surgical table positioning, asepsis and antisepsis, placement of sterile surgical fields, surgical incision in the skin and subcutaneous tissue, rotation of the cutaneous or myocutaneous flap, craniotomy (opening of the skull) and opening of the dura mater. In this stage of the surgical procedure, after drainage of acute subdural hematoma, a small area of cortical laceration of the right temporal lobe was visualized in patient 1, and the biological sample of brain tissue was collected and the surgery followed its normal steps with the hemostasis, closure of the wraps (dura mater, skull, muscle, subcutaneous tissue, skin) and dressing. I reiterate that the neurosurgical technique was in no way modified (except at the time of material collec-
tion), in order to guarantee the best available treatment for the patient and to minimize the risks involved.

**Signing the Sample for Culture**

After collection, this material was immediately stored in Leibovitz-15 preservative medium (L-15: GIBCO Invitrogen Corporation) and shipped for treatment and plating on the addition of D-10 culture medium, being maintained in CO2 greenhouse. The technique of human nerve cell culture was adapted from the work of Lucena et al. (Lucena et al., 2014) - performed with rodents - with some modifications and described in detail in item 2.7 below.

**Culture Technique**

Inside the laminar flow, the brain tissue was processed and submitted to the technique of cleaning and obtaining tissue explants for posterior plating.

New 15 ml Falcon conical tubes with 4 ml of Dulbecco’s modified Eagle medium medium supplemented with 10% fetal bovine serum and ceftriaxone, a medium designated D-10, all obtained from Cultilab®, were prepared and received the nerve tissue to perform the preparation. 35 mm culture plates (P35) for plating were prepared with 1 ml of fetal bovine serum, which was removed and discarded after 30 minutes, and then 3.0 ml of D-10 was added to the P35, where, The plating of the explants was performed, and the plates were kept in a humid oven at 37 °C with 5% CO2 and 95% air. In contrast light microscopy with phase contrast was used for the observation of the explants and the cell migration at the bottom of the plates. When the explant cells reached 70-90% confluence at the bottom of the plate, the basic medium was removed and 2 ml of trypsin / EDTA (0.25% trypsin containing 1 mM EDTA-Cutilab / Brazil® ). The cell suspension was then placed in Falcon-type conical tube with the same volume of D-10 medium for 10 minutes, in order to inactivate trypsin. The suspension was centrifuged
at 1500 rpm for ten minutes; after this procedure, the supernatant was discarded and the cells resuspended in 1 ml of medium.

The cells were deposited in P60 and observed in seven time periods: 24, 48, 72, 96, 120, 144, 168 hours and, in this way, it was possible to evaluate the adherence, proliferation and trophism of brain tissue cells at different times. For cellular observation, a CKX41 (Olympus®) phase contrast inverted microscope with a Moticam 3.0 digital camera (Motic®) coupled was used, and photomicrographs of the groups were made at 24, 48, 72, 96, 120, 144, 168 hours, taking the morphological development of the cells.

**Results**

The results presented below are due to the collections performed in three patients. In the samples, neurons and astrocytes were identified in the cell culture, some days after the collection, according to micrographs below.

**Figure 1.** Patient 1: Exposure of the dura mater after craniotomy. The arrow points to the fracture-sinking region with small dural laceration in the right temporobasal region.

**Figure 2.** Patient 1: Dural opening with exposure of acute subdural hematoma (black clot).

**Figure 3.** Patient 1: Exposure of the cerebral cortex after drainage of acute subdural hematoma. Arrow points to the area of the cortical laceration, with contoured brain tissue, macerated, being sampled from this region.
**Figure 4.** Patient 2: Bone fracture with linear fracture in the left lower temporal region.

**Figure 5.** Patient 2: Dural laceration with hematoma outflow and brain tissue.

**Figure 6.** Nervous tissue of patient 3 transferred to a 35 mm diameter plate.

**Figure 7A.** Nerve cells migrating from the explant to the culture medium after 24 hours. 10x magnification. **Figure 7B.** Magnification revealing the migration of nerve cells from the explant after 72 hours. Increase of 20x. White bar in the lower right corner equals 100 μm.

**Figures 8A and 8B.** Neurons (pointed by arrows) and glial cells (arrowheads). Increase of 20x after 96 hours in culture. White bar in the lower right corner equals 100 μm.

**Figure 9.** Micrograph of nerve cell culture after 168 hours. 10x magnification. White bar in the lower right corner equals 100 μm.
Discussion

Research and experiments with cultures of nerve tissue cells have traditionally been performed with rodents. Only recently has work been done on human nerve cells, including studies for the development and validation of the time elapsed until these cells reach the laboratory. In the study by Spaethling et al., this time interval was approximately 10 minutes after surgical excision, the material being kept in oxygenated medium throughout the transport until arriving in the laboratory. Cells were maintained viable in culture up to 84 days, being used for characterization of cell types and subtypes. In the work of Darmanis et al., the nerve tissue samples were sent in less than one hour, from the surgical room.

In our work, the material was quickly sent to the laboratory (about 10 minutes) and cell viability in culture was achieved within 3 months.

Because the thickness of the cerebral cortex is only 2 mm, the most significant samples with a larger neuronal population are those in which it is possible to collect part of the cerebral cortex (hematomas with an important subcortical component), as in the case of the patient 3. Situations in which there is only cortical laceration with extravasation of nervous tissue, the greatest chance is to obtain white matter and therefore glial cells.

Our samples are of traumatized brains, unlike the works carried out by Spaethling, Darmanis and Zhang. Thus, in the systematization of our culture, it is assumed that our tissue already comes with a process of cellular stress as a function of the trauma suffered by the patient, so it is relevant not to subject it to additional stress, as occurs in chemical and mechanical dissociations and in the spin cycles performed by the authors. We then chose to obtain explants of this tissue and allow the natural migration of the cells, as shown in figure 13. By performing these procedures, we observed a higher cell yield in a short period of time, presenting an alternative form in the methodology of the culture technique.
Conclusions

From the results obtained, it is concluded that it is a feasible technique for the culture of nerve cells collected from living patients during neurosurgeries performed due to TBI. These cells can be used later for several other researches, such as the identification of cell types and subtypes and their functions, neuronal regeneration studies (with the addition of chemical substances with supposed neuroprotective function), effects of drugs and drugs and possibly even creation of a bank of neurons and glia cells.

Although it is an early work with human nerve cells, we can glimpse future experiments, such as the transplantation of these cells (after treated, regenerated and/or differentiated) in sequestrated TBI or stroke patients, or even in patients with neurodegenerative diseases such as Parkinson’s or Alzheimer’s, among others. The persistence of lines of research with these cells and potential uses may then open new horizons in the treatment of various neurological diseases and perhaps modify the evolution and prognosis of the patients affected.
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