EXPERIMENTAL DEVELOPMENT OF THE METHOD OF SEAMLESS CONNECTION OF THE DURA MATER USING HIGH-FREQUENCY BIPOLAR ELECTRIC WELDING AND SUBSTANTIATION OF ITS GREATER TIGHTNESS, COMPARED TO THE TRADITIONAL METHOD OF CONNECTION USING SUTURE MATERIAL

Dmytro Zabolotnyi
Prof., Doctor of Medical Science, Major Researcher, Director
SI “O.S.Kolomiychenko Institute of Otolaryngology, NAMSU”, Kyiv, Ukraine

Olena Kvasha
Aspirant
SI “O.S.Kolomiychenko Institute of Otolaryngology, NAMSU”, Kyiv, Ukraine.

Summary. One of the main tasks of surgical treatment of patients with frontal sinus neoplasms with intracranial spread is to seal the subdural space, which is achieved by hermetically connected dura mater. The purpose of this study was to investigate the structure of the tissues of the dura mater and dura mater-fascia junction using bipolar electric welding. The obtained data shows, that electric welding provided a stable advantage in the tensile strength and tightness of the electric welded connection, compared to the connection using suture material. The value of the tensile strength for the connection of the DM with the DM was established - 32.795±0.1 (increase in strength compared to the suture connection by 1.35 times) mm Hg, for the connection of the DM with the aponeurosis of the skull 30, 71±0.37 (1.26 times increase in strength compared to the suture connection), for the connection of DM with the fascia lata was 29.67±0.33 mmHg. (increase in strength compared to a seam connection by 1.22 times). Also the methods of light microscopy and infrared spectroscopy were used. When evaluating the results, no β-structured aggregates were observed in the tissue samples under study. There was minimal traumatic damage to the adjacent dura mater tissue and minimal thermal damage from vaporisation. Pacchioni granulations, venous pial, and cortical vessels of the dura mater were preserved. The study observed complete identity of the wave values of amide A, B, amide I, II spectra; preservation of amide A, B peaks. Preservation and curvature of the N-H arm and O=C-N band were changed within acceptable limits. The findings indicate the absence of β-structured protein aggregates in the junction area, which excludes the possibility of fibrous structures and, as a result, does not contribute to the formation of a meningeal scar. The structure of the collagen protein in the junction area is normal, altered but intact, with preservation of its functions. This indicates the feasibility of using the method of high-frequency bipolar electric welding for surgical closure of defects of the dura mater, as an alternative to closure with suture material.

Keywords: electrosurgery; infrared spectroscopy; dura mater defects; cerebrospinal fluid leak, fascia lata.
The state of art. One of the most frequent complications of radical removal of frontal sinus tumours with intracranial growth is a disruption of the closed physiological circulation of cerebrospinal fluid (CSF), i.e., the emergence of external and occult CSF liquorhea. This is caused by insufficient tightness of the closure of defects in the dura mater (DM) at the final stage of surgery. These complications can be prevented by using a special method of joining dura mater defects using bipolar high-frequency electric welding (HFEW) developed jointly with the E.O. Paton Institute of Electric Welding of the National Academy of Sciences of Ukraine (NAS) [1]. Protein-associated electothermal tissue adhesion ensures hermetic binding of the tissues of the DM without the use of foreign materials and the formation of a coagulation scab. To evaluate the new method of DM connection, special attention should be paid to the structure of the zone of direct tissue connection, its similarity or difference from the surrounding tissues. The answer to this question can be obtained using histological and biophysical methods (confocal infrared spectroscopy).

O.I. Palamar et al. [2] found that a violation of the integrity of the dura mater during elective neurosurgical and otorhinolaryngological operations causes a risk of developing CSF liquorhea in the postoperative period with a frequency of 15.9-27.5%. Insufficient sealing of the DM, according to V.V. Kishchuk et al. [3], contributes to the leakage of CSF outside the subdural space, resulting in the emergence of external CSF liquorhea through the surgical wound and other natural openings (otoliquorrhea, rhinorrhea), as well as latent CSF in the form of subaponeurotic accumulation of cerebrospinal fluid, which may stay undiagnosed for some time. One of the main tasks in the surgical treatment of patients with this pathology, according to multiple studies by B. Marlier et al. [4], is the sealing of the subdural space, which is achieved by a hermetically sealed dura mater. However, if the defect in the dura mater is not linear and, when stretched, leads to overstretching of the dura mater and compression of the brain tissue, dura mater plastics material is required. According to comparative studies by B. Coucke et al. [5], autografts are considered to be the ideal material for DM plastics: periosteum, broad fascia of the thigh, temporalis muscle aponeurosis, and only then – synthetic artificial materials.

The newest types of sutures, staplers, shape memory compression devices, diathermocoagulation, biological adhesives, artificial dura mater are used to connect dura mater defects, but P. Nicolai et al. [6] and L. Häni et al. [7] found that they cause several complications: persistent infection, focal intense cellular inflammatory reaction, dense encapsulation of foreign material, cerebrospinal fluid, haematomas, rejection reactions, and Creutzfeldt-Jakob disease. A. Kinaci & T.P.C. Van Doormaal [8] found that the negative effects of using sutures to connect the dura mater are as follows: leaving foreign material in the tissues, squeezing the tissues that are sutured, which substantially affects the healing process of the wound surface and often causes chronic inflammation in the tissues, allergic reactions to tissues of biological sutures, resulting in wound suppuration, repeated needle trauma, and potentiation of the wound surface by sutures.

Based on the research data of M.M. El-Sayed & E. Saridogan [9], thermal denaturation of proteins – coagulation – occurs when they are heated above 450°C. Coagulation is an inertial process that absorbs energy. The coagulation rate depends on the temperature. T.L. Smith, & J.M. Smith [10] found that living soft tissue should
be heated to a temperature at which protein coagulation should end in a few seconds, and the tissue should be clamped with a certain defined pressure. S. S. Podpriatov et al. [11] found that with an increase in voltage, the quality of the connection increases, but at the same time, the temperature of the surface layer of living soft tissue between the electrodes increases, which leads to unacceptable thermal damage. In another study, the authors concluded that the reason for this phenomenon is that protein coagulation in cells occurs mainly before the destruction of lipid membranes, i.e., before the formation of a single protein space for the protein to be joined [12]. Thus, an effective technical solution was proposed, which is defined by the fact that to destroy cell membranes before the start of protein coagulation, it is necessary to modulate high-frequency stress with rectangular pulses with a frequency of several thousand hertz.

The purpose of this study was to compare the collagen capacity in the area of the dura mater and broad fascia of the thigh after high-frequency bipolar electric welding with the normal collagen structure of the intact dura mater and to assess its ability to regenerate in the weld zone.

MATERIALS AND METHODS

The study was conducted in 2018-2022 in the experimental research laboratories of the State Institution “Academic A. Romodanov Institute of Neurosurgery of the National Academy of Medical Sciences of Ukraine”. The experiment was performed on 18 porcine cadaveric dura mater and 8 porcine cadaveric broad fasciae of the thigh. Immediately after collection, the porcine tissue samples were placed in sterile dishes and thermal bags to preserve the physiological properties of the tissue and delivered to the State Institution (SI) “E.O. Paton Institute of Electric Welding”. Then the material was subjected to selected modes of high-frequency coagulation for biological tissue welding using a hardware welding complex in automatic and semi-automatic modes. The study using infrared spectroscopy (IR spectroscopy) was performed jointly with the SI “Institute of Physics of the NAS of Ukraine”, Department of Physics of Biological Systems, Kyiv. Histological studies were conducted according to the generally accepted review method of staining histological sections with haematoxylin and eosin.

Four rectangular pieces of 4 x 3 cm were prepared from each porcine dura mater. From each porcine broad fascia of the thigh, 6 pieces of rectangular shape 6*4 cm were harvested. The animal material was divided into 3 groups (2 experimental groups and 1 control group): the first group was a simulation of high-frequency bipolar electric welding with porcine dura mater (n=25). The second group was modelling of HFEW of porcine dura mater with porcine broad femoral fascia (n=25). The third group consisted of samples of intact porcine cadaveric dura mater (control) (n=30). In Groups 1 and 2, the samples were joined using high-frequency bipolar electric welding in automated modes of the hardware welding complex PATONMED EC 300 M1 (Ukraine) [11].

The obtained samples were immediately fixed in a solution of neutral formalin (sequentially 5-7-10%, 24 hours), dehydrated in ethanol, toluene, and sealed in paraffin (Thermo Scientific Richard-Allan Scientific, Paraffin type 6 REE 8336, Kalamazoo, USA). Sections of 7 µm thickness were made on a HM430 microtome (Microm, Germany), stained with haematoxylin and eosin, picrosirius red (Direct Red 80, Magnacol Ltd, UK) and embedded in balm (Thermo Scientific Richard-Allan
Scientific Cytoseal 60, REF 8310-16, Kalamazoo, USA). Microsections were examined using an Axiophot microscope (Carl Zeiss, Germany). The morphometric study of the thickness of the dura mater was performed using Carl Zeiss software (AxioVision SE64 Rel.4.9.1, Germany). Tissue samples of 3 groups with a volume of up to 0.5 mm³ were examined by infrared spectroscopy at 5 points from each compound sample. To analyse the infrared absorption spectra, thin sections of the post-manipulation tissue were made and dried at room temperature between two CaF₂ plates (fluorite glass, transparent in the infrared region). Next, the films on the substrates were placed in the cuvette chamber of the device to record the absorption spectra. The spectra were recorded on a Bruker INVENIO-R spectrometer (Germany) in a wide spectral range (from 3,800 to 800 cm⁻¹) at room temperature and humidity of about 60% in a cuvette chamber. The sample under study was placed in one of the interferometer arms or at its outlet in mixed beams.

RESULTS

The development of the technology of high-frequency bipolar electric welding was carried out in four stages. At the first stage, the development of specialized bipolar instruments was carried out, which are suitable for DM welding, aponeurosis of the skull, fascia lata. At the second stage, the development and selection of automated and semi-automated welding modes and connection techniques were carried out.

At the third stage, a study of the tightness of the DM connected using HFEW was carried out, the comparison of these data with the value of the tightness of the DM, connected according to the traditional method, and the determination of its indicators of tensile strength and indicators of change in elasticity, comparison of the data of the results with intact pork cadaver DM, intact porcine aponeurosis, intact porcine broad fascia of the thigh.

At the fourth stage, the histological properties of the tissues of the joint area were studied using the method of confocal infrared spectroscopy and morphological studies.

With the participation of engineers from the E. O. Paton Institute of Electric Welding of the National Academy of Sciences of Ukraine, Ph.D. H. S. Marinskyi and co-authors developed specialized bipolar tools for welding DM, aponeurosis, broad fascia of the thigh. (Utility model patent No. 135155 dated 06/25/2019) (Fig.1)

![Fig. 1. Variants of electrowelding bipolar instruments in variants of flat and tooth-shaped surfaces of electrodes for applying a multi-point electrowelded joint have been developed.](source: photographed by the authors)
At the second stage of the research, the main task was the development and selection of modes of connection of biosimulators of DM, broad fascia of the thigh.

In order to study the effect of HFEW on the tissue of the DM and the fascia lata, a morphological evaluation of the results of joining tissues using the EK 300 welded complex was carried out in the above compared groups 1 and 2. During the study, the impact of automatic and semi-automatic modes was evaluated of the welding complex on the fabric structure, on the presence of signs of charring and vaporization, on the formation of perforations in the fabric and, as a result, loss of tightness.

With the participation of engineers of the E. O. Paton Institute of Electric Welding of the National Academy of Sciences of Ukraine, Ph.D. H.S. Marinskyi and co-authors, we formulated the medical and technical requirements for a specially developed mode of electric welding of the TMO and the broad fascia of the thigh, under which a seamless connection is stably ensured. The necessary medical and technical requirements were established: presentation of a series of electrical rectangular high-frequency current pulses, generated by the algorithm taking into account the proven modes (2,3,5), at an alternating current frequency with an indicator of 66 kHz and a temperature on the electrodes within the range of 41–450°C.

The obtained data statistically confirmed the effectiveness of the use of certain modes of VBZV, in which satisfactory results were obtained, and it was established that the most effective modes of using VBZV for TMO tissue, aponeurosis, and broad fascia of the wall are:

- mode 2 (Automatic mode, load 10 Ohms, output power 120W, current frequency 350±44kHz) — for welding TMO with TMO;
- mode 5 (Semi-automatic mode, load 20 Ohms, output power 180 W, current frequency 400±44kHz) - for welding TMO with a wide hip face;
- mode 3 (Semi-automatic mode, load 15 Ohms, output power 140 W, current frequency 370±44 kHz) — for welding TMO with TMO/aponeurosis.

At the third stage, a study of the tightness of the DM connected using HFEW was carried out, the comparison of these data with the value of the tightness of the DM connected according to the traditional method and the determination of its tensile strength index and the index of elasticity change were carried out. A study of these indicators was conducted for the connection of the DM biosimulator with the DM, the DM with the aponeurosis, the DM with the broad fascia of the thigh. These results were compared with intact pig carcass DM.

On 5 animal (porcine) DMs, under the experimental conditions, 5 mesh-shaped twists of the DM were formed with a connection using a suture material — with the help of a continuous wrapping suture with a step of 1.5–3 mm using a monofilament polypropylene atraumatic suture material with a diameter threads 4/0-5/0 according to Donnadi.

Segments of DM sac-like junction using suture material were about 3 cm long, with a junction created in the middle and bottom, which were transferred to the laboratory table for further examination.

A piston-type syringe was attached to the clamp and lumen of a bag-connected HFEW biosimulator, which was filled with 0.9% NaCl solution. An electronic manometer DPG8000 M4026/1203 from Omega, USA was used. For the sake of standardization, the sphygmomanometer readings were monitored after every 3 attempts to administer the solution.
After closing the lumen with a piston syringe, a 0.9% NaCl solution stained with methylene blue was administered at a temperature of 40°C with a pressure of up to 15 mmHg/min. The sphygmomanometer reading was recorded when fluid leakage occurred in the area of the tissue connection. Thus, it was determined the pressure at which the joint breaks with the help of suture material, i.e., the tensile strength indicator for the suture material. In study 1 — 23.2±0.8 mmHg, in study 2 24.9±0.8 mmHg, in study 3 — 25.3±0.8 mmHg. Art., in the 4th study 22.9±0.8 mmHg, in the 5th study 24.1±0.8 mmHg (p<0.05).

The average arithmetic value was set at 24.08±0.8 mmHg. (±0.8 mmHg is the standard deviation for this sphygmomanometer). It was determined that the different tensile strength indicators for DM connected with the help of suture material are within the standard deviation, which allows us to use this average value of tensile strength relative to the hydraulic tension pressure. The average increase in the diameter of the connected biosimulator with the help of suture material at the time of liquid leakage (loss of tightness) was 12% when it was overstretched by pressure (p<0.05).

Given the homogeneity of the obtained data, further research was stopped at this stage, and the obtained data (tensile strength for the DM biosimulator connected with suture material — 24.08±0.8 mmHg) were established as the main value for conducting comparative studies with the connection using HFEW.

Separately, the natural resistance to rupture of the intact porcine DM was determined, which was 222.8±134.8 (p<0.05). The rupture resistance of an intact DM has an important, but not exclusive, value in maintaining a hermetic connection, which is confirmed by world literature data.

It was established that when the tightness of the joint of the DM, the edges of the defect of which were connected with the help of suture material, was breached, this primarily occurred in the places where the needle was punctured during the application of the seam, as a result of their cutting, which indicated the inhomogeneity of the physical properties of the joint and the lack of elasticity (stretching) in this area, which is caused by overcompression of the suture tissue and the presence of a significant number of DM needle puncture sites.

In this way, the value of the tensile strength of the joint for the connection of DM with DM was determined — 32.795±0.1 (increase in strength compared to the seam connection by 1.35 times) mm Hg, for the connection of DM with the aponeurosis of the skull 30.71±0.37 (increase in strength compared to the suture connection by 1.26 times), for the connection of DM with the broad fascia of the thigh 29.67±0.33 mmHg. (increase in strength compared to a seam connection by 1.22 times). These indicators had a significant advantage over the reference indicator of strength when connecting DM with suture material of 24.2±3.2 mmHg. (p <0.5).

It was established that the HFEW connection is highly elastic: the diameter of the connection during the tensile strength test before the moment of failure increases by 19.3% from the initial one when connecting DM with DM, by 17.6% when connecting DM with aponeurosis, by 14.7% at the connection of the DM with the broad fascia of the thigh, against 12% at the suture (p = 0.04).

When evaluating the results of a morphological study performed using light microscopy at 400x magnification and haematoxylin-eosin staining, important structural features of the HFEW joining were revealed. Light microscopy of the joining of the dura mater with the broad fascia of the thigh (Fig. 2) revealed the interweaving
of collagen fibres with the fibrillar structures of the broad fascia of the thigh, which creates a stable weld, and complete preservation of the orientation of collagen fibres, which form the basis of the extracellular matrix skeleton and are the basis of hermetic state. There was no dry coagulation necrosis, which indicates a better prognostic variant of the course of dura mater defect healing.

Fig. 2. The area of the “welded” dura mater suture with the broad fascia of the thigh, haematoxylin-eosin stain, x400
Source: photographed by the authors

The morphological study of the joining of the dura mater with the dura mater (Fig. 3) revealed heterogeneity in the density of collagen fibres, looseness of the collagen structure, areas of tissue oedema, areas of fibroblast accumulation, and complete preservation of the orientation of the dura mater collagen fibres. There is minimal traumatic damage to the adjacent dura mater tissue, indicating minimal thermal injury due to vaporisation. The preservation of Pacchioni granulations, venous pial and cortical vessels in the brain tissue indicates the absence of coagulation necrosis. These structures are actively involved in fluid resorption and maintaining the balance of intracranial pressure.

Fig. 3. Section of the “welded” suture between dura mater and dura mater
Source: photographed by the authors
The technique of confocal infrared spectroscopy has made it possible to control the optical properties of biological substances and objects to increase sensitivity and increase the penetration path of visible and near-infrared light photons into deeper regions, which improves the recognition of the molecular structure of the sample under study.

When using computer analysis to calculate the relative area of the bands of the components depicted in the amide A and B spectrum (Fig. 4), the results presented indicate that the protein of the thigh broad fascia tissue, connected by HFEW, is almost completely identical to the normal thigh broad fascia tissue. The complete identity of the wave values of the amide A and B spectra, the preservation of the amide A and B peak, the preservation and bending of the N-H arm and the O=C-N band are observed.

When using computer analysis to calculate the relative area of the bands of the components depicted in the amide I and II spectrum (Fig. 5), the results presented indicate that the peptides and polypeptides of the broad fascia of the thigh tissue joined by HFEW are almost completely identical to normal broad fascia of the thigh tissue. The complete identity of the wave values of the amide A and B spectra, the preservation of the amide A and B peak, the preservation and bending of the N-H arm and the O=C-N band are observed.

The computer analysis used to calculate the relative area of the bands of the components depicted in the spectrum of amide A and B (Fig. 6) indicates that the tissue in the area of HFEW compared to healthy dura mater is characterized by a bending of the N-H arm and oscillations of the amide A band (by 0.6 ATR (attenuated total reflectance)).
Fig. 5. Characteristic absorption bands of amide III waves protein of intact broad femoral fascia and broad femoral fascia protein connected by bipolar high-frequency electric welding in the infrared range

*Source: compiled by the authors*

total reflectivity, the characteristic presence of the C=O band of oscillations at 3,732 cm⁻¹ with the preservation of the amide A peak, the presence of valence vibrations of the side chains shifted by 0.4-0.1 ATR, the presence of overstretching of the CH₂ band in the valence vibrations of the side chains at wave values of 2,960-2,850 cm⁻¹.

Fig. 6. Characteristic protein absorption bands of amide I and amide II waves of intact dura mater and dura mater protein connected by bipolar high-frequency electric welding in the infrared range

*Source: compiled by the authors*
In the spectrum of amide I and II for the tissue in the area of the HFEW (Fig. 7) in comparison with healthy dura mater, there was a characteristic bend of the NH3+ arm (amino group) in the region of the wave value 1,648 cm⁻¹ (compared to 1,640 cm⁻¹ of normal dura mater tissue), the absence of an arm in the region of 1,640 cm⁻¹, due to which the Amide I band is slightly narrowed and a low-frequency shift of the Amide II band (by 0.4 ATR) is recorded. In the wavenumber region at 1,575 cm⁻¹, a low-frequency shift of the Amide II band is observed with no change in the peak, an N-H bending and vibrations in the C-N band (by 0.4 ATR). In the region of the wave value at 1,450 cm⁻¹, an asymmetric bending of the CH3 arm is observed with preservation of its value, in the region of the wave value at 1,400 cm⁻¹, deformation vibrations of the C=O band and symmetric stretching of the CO₂ band are observed. In the region of the wave value at 1,230 cm⁻¹, a bending of the O=C-N arm (by 0.42 ATR) and a bending of the N-H band with the preservation of the Amide III band peak were detected. In the wavenumber region at 1,020 cm⁻¹ and 1,090 cm⁻¹, a 0.01 (ATR) bending of the N-H band is observed with the preservation of the Amide III band peak.

![Graph showing characteristic absorption bands of amide III waves protein of intact dura mater and dura mater protein connected by bipolar high-frequency electric welding in the infrared range](image)

**Fig. 7.** Characteristic absorption bands of amide III waves protein of intact dura mater and dura mater protein connected by bipolar high-frequency electric welding in the infrared range  
*Source: compiled by the authors*

The results obtained indicate that the regenerative process occurs in the area of joining the dura mater, dura mater with the aponeurosis, and dura mater with the broad fascia of the thigh using HFEW instead of charring or coagulation necrosis. When using HFEW in the joint area, the collagen structure and the fibrous structure of the broad fascia of the thigh were changed, but not damaged, and retained their
Infrared spectroscopy studies showed that the peptides and polypeptides of the dura mater associated with HFEW were almost identical to normal intact dura mater tissue. The complete identity of the spectral values of the amide A and B waves, the preservation of the amide A and B peaks, the preservation and curvature of the N-H arms and the O=C-N bands were observed.

When comparing tissue samples from the junction of the dura mater with the broad fascia of the thigh, joined by HFEW, the identity in the fluctuations of the amide bands A, B is preserved, the maximum spread allowed for the amide peak A, III. Fluctuations in the valence state of the side chains are allowed, and therefore it can be concluded that the structures of proteins (collagen and glycosaminoglycans) in the samples under study are alive, have minor signs of coagulation necrosis, and are capable of regeneration.

**Conclusions**

Based on the results of the research, it was possible to formulate the requirements for the parameters of the current supply and the impedance algorithm for the connection of DM fabrics using electric welding, which provided a stable advantage in the tensile strength and tightness of the electric welded connection, compared to the connection using suture material. The value of the tensile strength for the connection of the DM with the DM was established - 32.795±0.1 (increase in strength compared to the suture connection by 1.35 times) mm Hg, for the connection of the DM with the aponeurosis of the skull 30.71±0.37 (1.26 times increase in strength compared to the suture connection), for the connection of DM with the fascia lata was 29.67±0.33 mmHg. (increase in strength compared to a seam connection by 1.22 times).

Morphological data indicate that regeneration processes occur in the area of joining the dura mater, the dura mater and the broad fascia of the thigh, performed using HFEW, and not charring or the formation of coagulation necrosis. The structure of the collagen and fibrillar structures of the broad fascia of the thigh after the use of HFEW in the joining area is normal, altered but not damaged, with preservation of its functions. Studies using infrared spectroscopy indicate that the peptides and polypeptides of the dura mater connected by HFEW are almost completely identical to normal tissue of the intact dura mater. A complete correspondence of the wave values of the spectra of amide A and B was observed, as well as the preservation of the peak for both amides, and the preservation and bending of the N-H arm and the O=C-N band. When comparing tissue samples of the dura mater and broad fascia of the thigh, joined by HFEW, the identity in the vibrations of the amide bands A, B is preserved, the permissible maximum change in the peaks of amides A, III, there are vibrations of the valence side chains that are permissible. Thus, it can be concluded that the structure of proteins (collagen and glycosaminoglycans) in the samples under study is alive, with virtually no signs of coagulation necrosis, and capable of regeneration.

The ability of high-frequency bipolar electric welding to connect the dura mater tissue and the broad fascia of the thigh, forming a connection that is alive, elastic, tight, and contains collagen that is fully capable of regeneration, was substantiated. Infrared spectroscopy and morphological data indicate that regeneration processes occur in the area of the dura mater joining made using high-frequency bipolar
electric welding, rather than charring or coagulation necrosis. The structure of the collagen protein in the area of this connection is normal, altered but not damaged, with preservation of its functions.

Future research should aim to develop a thermal resistor that will automatically turn off the bipolar high-frequency welding clamp when the temperature rises above 40 degrees Celsius and causes the threat of thermal damage and vaporisation around the brain tissue.

References: