Plastination Biopsy Samples from the Central Nervous System as a Solution for Time Domain Optical Coherence Tomography Noninvasive Investigation Method

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A biopsy sample that was taken from neurosurgery then introduced into the solution of formaldehyde was examined with Time Domain optical coherence tomography. The results are inconclusive and so that preserving was accomplished by plastination the tissue sample in which the degree of rigidity of the sample is controlled without destroying the tissue.

Keywords: Plastination, Optical Coherence Tomography, nervous system

Once in the tissue biopsy sampling, equally of normal or pathological origin, the autolysis is started . To preserve the integrity of tissue so it can then be examined in various conventional and new methods it is needed to be preserved. In these methods of conservation and analyzing the tissue are included plastination and analyzing of the specimen with optical coherence tomography method which allows examination of tissue after plastination without further preparation.

Jin U. Kang has spent years working with lasers and optical fiber, studying what happens when light strikes matter and recently on a new challenge: brain surgery. Kang builded a tool to help brain surgeons locate and get a clear look at cancerous tissue. In some cases, Kang says, this device could eliminate the need to cut into the brain for a traditional biopsy, a procedure that can pose risks to the patient [1]. The idea is to provide instant high-resolution pictures of a small segment of the brain without actually touching the tissue [2, 3]. These pictures could let the doctor conduct a 'virtual biopsy' to see where the tumor is and whether it is benign or malignant. And when it's time to cut out the cancer, these images could help a surgeon see and avoid healthy tissue [4, 5]. To give doctors this detailed view of brain tissue, Kang's device employs ultra-thin optical fiber, the material used in long-distance communication systems, to direct harmless low-powered laser light onto the area the surgeon wants to examine [6, 7]. When the light strikes the tissue, most of it bounces away in a scattered, incoherent manner. But using a technique called optical coherence tomography, the small portion of light that is scattered back can be collected and used to construct a high-resolution three-dimensional picture of the tissue, down to the cellular level. These images are significantly sharper than those produced by MRI or ultrasound equipment, and should give surgeons a better look at the boundaries of a tumor and the presence of blood vessels and healthy tissue that must be preserved [8].

Experimental part

After opening the skull using the craniotomy procedure an incision of different shaped is made in the dura mater exposing the brain. Most tumors of the brain grow inside the cerebral parenchyma. An incision is made into the cortex (corticectomy) after visual inspection will determine the surface extension of the tumor. In those cases where the tumor is too deep, the intraoperative ultrasound probe is used in order too verify the location, find the shortest way and avoid the eloquent areas of the brain. Usually is preferred a trans-sulcal route. Depending on the tumors vascularization, size, consistency an en bloc resection of the tumor could be possible. In order to protect the brains normal structure, in most cases an piecemeal fashion resection of the tumor is used, debulking and shrinking the tumor by enucleating the center and gradually decreasing the tumor volume. Finally, the goal of the surgery is to achieve a gross-total resection if it is possible and subjecting fragments of the tumor to an anatomical pathological examination. It is important that outer fragments of the tumor as well as from its center and from the cerebral tissue surrounding to be subject to the anatomical pathological examination because some tumors contain a mixture of different grade cells. In our case, an incision in the middle of the tumor was made, fragmenting the tumor using the tumor forceps and a bipolar coagulation under irrigation of sterile water. We achieved a gross-total resection of the tumor and fragments from the center and the outer area of the tumor were sent to the anatomical pathological examination.

Parts of these fragments were taken to be evaluated by a non invasive method, optical coherence tomography, working in Time Domain mode. The wavelength used was 1300 nm and the procedure consists in obtaining C scans from the samples.

C-scans are made from many T-scans along either of X, Y, repeated for different values of the other transverse coordinate, Y, X respectively in the transverse plane. The repetition of T-scans along the other transverse coordinate is performed at a slower rate than that of the T-scans, which determines the frame rate. In this way, a complete raster is generated. Different transversal slices are collected for different depths Z, either by advancing the optical path difference in the OCT in steps after each complete transverse (XY) scan, or continuously at a much slower

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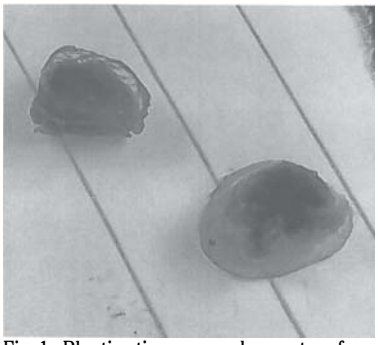

Fig 1. Plastination procedure: step four representing the hardening of the sample.

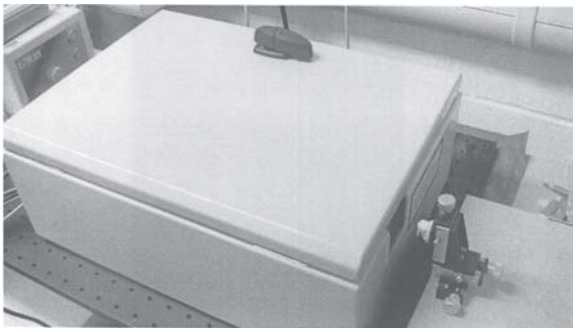

Fig. 2. The aspect of the Time Domain Optical Coherence Tomography System used for the imagistic evaluation in this study

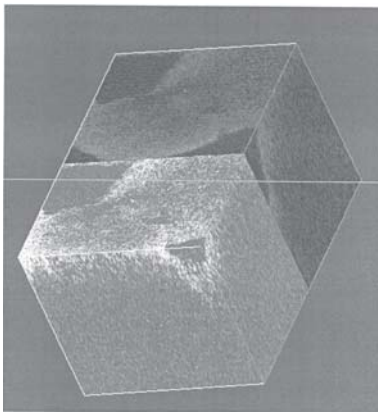

Fig. 3. The 3D reconstruction of the Time Domain OCT investigation of the tumor.

speed than the frame rate. The depth scanning is the slowest in this case. It is more difficult to generate en-face OCT images than longitudinal OCT images as the reference mirror is fixed and no carrier is produced. Therefore, in order to generate T-scans and T-scan based OCT images, a phase modulator is needed in order to create a carrier for the image bandwidth. This complicates the design and introduces dispersion. Research has shown that the X or Y-scanning device itself introduces a path modulation which plays a similar role to the path modulation created by the longitudinal scanner employed to produce A-scans or A-scan based B-scans [9].

Optical coherence tomography examination is performed immediately after extraction and placed in buffered formaldehyde solution and then sent to plastination procedure.

This process of plastination consists of the following methods: sampling tissue, tissue preservation through plastination and examined by optical coherence tomography.

a)Taking tissue: tissue sampling is done as a normal biopsy, our conservation method with a minimum or maximum thickness can be made, allowing you to preserve parts of 1 mm^2.

b) Conservation of tissue to be analyzed is made by formaldehyde and plastination. To stop the autolysis of the sample is introduced immediately after being extracted in a buffered formaldehyde solution, which stops the process of producing and preserving tissue to examine for the next level of plastination. After fixation in buffered formaldehyde solution is introduced into 100% acetone solution for dehydration and degreasing. In the nervous tissue fixation, because of his lipid structure, is used post fixation with osmium tetroxide which protects the lipids that exist within the nervous system, otherwise the acetone attack the lipid structures. The forced impregnation under vacuum is started after dehydration. It is finished when the acetone is replaced by polymers. Forced impregnation is done under vacuum with different polymers, which are chosen according to the things that must be put out, the process is done gradually and not being allowed to be rushed because the forced impregnation preserve excellent the cells. After the forced impregnation with different types of plastic will strengthen their substance is different depending on the type of polymer used in the forced impregnation. Polymers and process for reinforcement are:

- technique S 10: S 10 silicone is mixed with S3 (100:1), at negative temperatures didn't polymerize, only at 40-50 ° C. But S 10 - S 3 mixture will finally polymerized under the action of the gas S6. S 10 is a polydimethylsiloxane with chemical formula $CH_3[Si(CH_3)_2O]_nSi(CH_3)_3$, having the molecular weight of 27,200 g / mol. The viscoelastic properties of polydimethylsiloxane with density of 0.764 g / mL at 20 ° C. S3 is supposed to BE dibutyltindilaurate with oleate. The dibutyltindilaurate has $C_{32}H_{64}O_4S_n$ the chemical formula and 1.066 g / mL at 25 ° C density. S 6 is tetraethyl orthosilicate $Si(OC_2H_5)$ 4 and the density of 0.94 g / mL, whose vapors are irritating the upper respiratory tract. [4]

- . E 12 technique: provides a mixture composed of Biodur E12, 100 pbw, Biodur E1, 30 pbw, and Biodur AE 10, 20 pbw. E 12 is a transparent and less Viscous epoxy resin. E1 have the chemical formula $C_6H_{13}N$ cyclohexylamine and 0.867 g / mL at 25 ° C density. Biodur 10 AE additive is a plasticizer based on dialkylaryldicarboxilic acid ester. Use of this mixture in forced impregnation is subject to the limited up to 32 hours, polymerization reaction is exothermic because the resins harden. Temperature for this technique is room temperature and a dimension of specimens varies between 10 to 200 mm length and 2.1 mm thickness. The addition of larger quantities of plasticizer results in the greater tissue elasticity and transparency. [5]

- P 40 technique: using polyester polymer as forced impregnation, which is very fluid, suitable for impregnation of exceptionally soft tissue.

Results and discussion

After examining the optical coherence tomography method of fixed biopsy the images obtained were blurry, because the existence of fluid and tumor consistency that did not allow proper fit. Also when repeated investigations was needed for the same area and in the same incidence the possibility of scanning was approximately due to the tumor material that allow it to flex very easily. In this way a repeated in vitro evaluation for the same area of the tumor is possible but difficult. This thing will affect also the possibility of comparing the OCT slices with the histological ones done in order to validate the findings. When the plastination of the tumor was completed (fig. 1), the sample was presented as a solid one and in this way the OCT investigations could be done proper for exactly the same area and allow us to validate the findings with the histological method. In this way the uncontrollable mobility of the tissue is resolved and the OCT in Time Domain could be performed in good conditions (fig. 2). From the OCT slices 3D models were generated in order to have a better vision of the entire samples. This tool allows to navigate also inside the reconstruction in order to evaluate different morphological aspects of the investigated tissue (fig. 3).

Conclusions

After examining the optical coherence tomography method of fixed biopsy the images obtained were blurry, because the existence of fluid and tumor consistency that

did not allow proper fit we can examine if the piece fresh are done properly, the plastination offers to tissue the characteristics to be examined by optical coherence tomography.

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