Potential Use of Live Fluorescence Staining Technique in Surgery

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Abstract

Surgery has evolved considerably in last 30-40 years. With the use of better diagnostic techniques there is a rising need for surgical procedures. There is a lag between the new technology and the delivery of clinical care with it. Currently x-rays based techniques are being used routinely during surgery. This article refreshes the history and use of fluorescence so far. It is also aimed at exploring the use of fluorescence based techniques in surgical practice.

The phenomenon of fluorescence has been described for thousands of years. The term fluorescence was coined in 1852 by George Gabriel Stokes and he described it as ‘wavelength change or refrangibility’. Since then fluorescin based compounds have found its application in various aspects of medical science like fluorescein staining of the eye, fluorescence microscope, fluorescein angiography and immunofluorescence. Fluorescence based techniques are superior in detecting hidden malignant tissue in comparison to conventional surgical treatment.

Fluorescence has astonished us for centuries. Various cancer research organisations are investing heavily in optical imaging. With further development in fluorescence based surgical techniques cancer treatment will be more precise and whole of the surgery will be lot more colorful, astonishing and safe with minimal collateral damage.
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Conflict of interest: None

Introduction

Use of imaging techniques in diagnosis has brought the need for more and more surgery to be done. Surgical treatment requires great detail and precision. In terms of diagnostics main modalities being used are x-rays, ultrasonography, Magnetic resonance imaging, single photon electrospectrometry and positron emission tomography [PET]. Surgical techniques have advanced by using laparoscopy, robotic surgery, and microscopic surgery, but still there is lag between the technology and delivering clinical treatment with surgery. There are various reasons which stop new technology from becoming a clinical practice. These include the following questions before the uptake of technology:
1. Is there a real clinical need?
2. Will technology solve the problem without impeding the current workflow?
3. Is the technology cost effectiveness and user-friendly?

Current Real time imaging doesn’t solve the clinical problem because of low penetration into deep tissues and do not identify the abnormal tissues. Use of fluorescence based techniques is being thought as the way forward to improve surgical techniques.

Fluorescence History

Fluorescent effects have amazed people for thousands of years. Chinese books have been written about fluorescence as far back as 1500BC. Nicolas Monardes [1.Acuña, A]. Spanish physician and botanist in 1565 describe ‘a bluish opalescence of water infusion from the wood of tree named Lignum nephriticum [2.Alexander Poellinger]. In 1574 Charles de L’Ecluse a Flemish botanist did a Latin translation of the Monardes work and described the wood as Lignum nephriticum because of its medicinal virtues for treating kidney ailments. Later many scientists were astonished by this phenomenon through the centuries.

Robert Boyle 1664 investigated Monardes work more closely and found that after many infusions the wood lost its power to give colour to the water and that there are some essential salts responsible for the effect. Sir John Herschel observed fluorescence from quinine sulphate and named it as ‘epipolic dispersion. George Gabriel Stokes (1852) described it as wavelength change or refrangibility. He coined the term ‘fluorescence’ [3.Allweis TM]. Name was derived from ‘calcium difluoride’ also known as fluorite.
Adolph Von Baeyer (1871) a German chemist synthesised a dye named Fluorescein by combining resorcinol and pthalic anhydride. Later more compounds with fluorescence properties like eosin, polycyclic aromatic hydrocarbons and uranin (sodium salt of fluorescein) were found. Paul Ehrlich (1882) first used uranin (sodium salt of fluorescein) in vivo to track aqueous humour secretion in the rabbit eye [4.Bani MR]. Oskar Heimstaedt and Hans Moritz Lehmann (1991-1913) developed the first fluorescence microscope [5.Burke S]. After that fluorescence phenomenon has found its wide application in laboratory and in vivo applications. Scientists have used it in labelling antibodies also known as immunofluorescence, proteins and various fluoremetric methods for measuring chemicals in blood, urine, CSF etc.

Clinical Application in Ophthalmology

Fluorescence has found its wider application in diagnostics. Intravenous fluorescein angiography is a technique involves injecting Fluorescein dye in to systemic circulation and an angiogram of the retinal circulation is taken by photographing the fluorescence emitted after illumination of the retina with blue light at wavelength of 490 nm. Fluorescein angiography is helpful in diagnosing retinal artery stenosis, obstruction, diabetic retinopathy, aneurysms, telangiectasia’s, tumour vascularisation, macular degeneration optic disc swelling.

Use in Surgery

The fluorescence is gaining surgical importance in recent years. Visible light can only penetrate few hundred microns, gets scattered and absorbed in to the neighbouring tissues. As a result the surgeon can only see the surface features. It becomes more important in cancer surgery where precise excision is important in treating patients. There are over 40 million surgical procedures performed every year in United States only and a small error will effect large patient populations. In case of breast cancer operations 20-25% patients has incomplete resections leading to re-resections.[6.Ehrlich P][7.Martirosyan NL]. 1.5% of the patients undergoing surgery have persistent pain after 1year of surgery [8.Milestone]

Real time technology is required and one of them is invisible near- infrared (NIR) fluorescent light. The penetration of NIR fluorescent light is relatively high up to several millimetres producing bright tissues on a black background or a high signal to background ratio (SBR). Special fluorophores are required for imaging the display of abnormal tissues within the surgical field.

There are three different fluorophores based on different technologies are available i.e. filtered broadband sources, light emitting diodes (LEDs), and laser diodes. Choice is not
very simple because of their individual advantages and disadvantages. Furthermore field of vision (FOV) and other surgical instruments are also in consideration while making fluorophores. Several complete near infra-red fluorescence systems are available for intraoperative use in clinical trials namely Novadaq SPY™ system, photodynamic eye (PDE), FLARE™ imaging systems. Novadaq SPY™ is already approved for coronary artery bypass grafting (CABG), plastic and reconstructive surgeries and in transplant medicine. These chemical agents have posed risks like allergic reactions; anaphylaxis and death from anaphylaxis of these agents have been reported. Fluorophores can be lipophillic and hydrophilic and selection depends upon the target of choice. Some of these agents are metabolized in liver and are excreted equally through by liver and kidneys. This property can be used to visualized bile duct, bowel, kidneys, ureter and bladder.

**Oral and maxillofacial surgery:**

Oral lesions secondary to the use of tobacco preparations result in oral ulcers, sub mucosal fibrosis and squamous cell carcinoma. Morbidity because of sub mucosal fibrosis is pre or post surgery is quite high. Ponnam et al. concluded in the pilot study using fluorescence spectroscopy that it can be used effectively in diagnosing oral sub mucosal fibrosis (OSMF)[9.Mito JK]. Another in vivo study using auto fluorescence spectroscopy found it as a very good method for diagnosis and treatment effectiveness for oral sub mucosal fibrosis (OSMF)[10.Monardes, N]. They found a high statistical significance (p<0.001) in all three clinical parameters i.e. maximal mouth opening, tongue protrusion and severity of burning sensation in pre and post treatment groups.

**Breast Surgery**

A recent study using fluorescence near infrared imaging with Indocyanine Green (ICG) has shown some positive results in breast cancer screening and diagnosis [11.Murawa D]. The study involves near infrared imaging before and after Indocyanine Green (ICG) injection in 21 breast lesions. Findings suggest that ICG fluorescence imaging is very useful in differentiating benign breast tissue to malignant tissue. Late fluorescence images mainly represent extravasated dye for the immature and friable tumor vessels and quite clearly demarcate malignant to benign breast tissue. The author concluded that late fluorescence images when used as an adjunct to conventional breast assessment with (breast clinical examination, mammogram and ultrasound scan) might increase specificity of diagnostic work out with incremental benefit. The same principle can be utilized while doing wide local excision, lumpectomy or mastectomy for curative breast cancer surgery and sentinel lymph node excision.
Another recent study by Murawa et al [12.Ponnam SR] compared the detection rate of sentinel lymph node involvement by using Indocyanine Green and conventional method with radioisotope and blue dye injection. The results are encouraging for NIR Fluorescence imaging in detection of axillary lymph node diagnosis and dissection (ALND). However more clinical trials involving large number of patients are required.

**Limb Surgery**

Limb surgery for cancers like sarcoma, osteogenic sarcoma can be other surgical challenge where resection margins need to be sufficiently clear of cancer and at the same time preserving the limb length and neurovascular bundle. Margin status is normally investigated by histopathologist after receiving the excised tissue and if positive; it is devastating news for the patient and disheartening to the surgeon. Intra-operative margins assessment and cancer bed assessment is required to ensure complete excision of the tumor. Radiofrequency spectroscopy has been used as tool to assess the resection margins during breast cancer operation [13.Schiller DE]. Initial studies with radiofrequency spectroscopy are encouraging but there are limitations like inability to assess small clusters of malignant cells and inability to assess surgical bed. Recently the use of near infra red imaging using cancer labeled fluorescence agents is being studied in assessing surgical resection margins. Jeffery K et al [14.Stokes, G. G] have used NIR fluorescent probes in genetically engineered mice with conditional mutations in p53 and B-raf genes that developed primary sarcomas.. The author was able to identify tumor cell clusters at the surgical bed and resection margins with 80% more of fluorescence at the tumor bed at the time of surgery. As the technology can examine whole of the surgical bed, it can provide real time analysis of the tumor resection to the surgeon. With further research and development of newer fluorescence techniques can provide high quality surgical resection with preservation of some limb function.

**Cranial Surgery**

Another similar study involving thirty mice for craniectomy and imaging in vivo imaging with laser confocal endomicroscopy to detect the boundary of infiltrative margins [15.Vedeswari CP] Indocyanine green was again used a fluorophore agent. Near Infrared confocal endomicroscopy enhanced the residual tumor detection and provided real time true histological differentiation and significantly improved intraoperative decisions. Cytoreductive surgery in high grade gliomas is vital to establish histological diagnosis and enhances the efficacy of adjuvant therapies.
The Use of fluorescence in diagnosing various kidney problems and bile duct pathologies by virtue of its excretion is hard to get into clinical practice in presence of already established radionuclotide contrast methods. Fluorescence techniques could be useful in doing partial pancreatectomy, thyroidectomy or various other organ surgeries.

Discussion

Fluorescence has long been witnessed from 1500 BC but it is very recent that it has found its clinical use. It has been used successfully amusing us by staining the Chicago River in green or finding connections between them. Fluorescence has found its role in clinical diagnostics quite well in terms of Intravenous Fluorescein angiography (IVFA) and in laboratory based research methodologies like immune-fluorescence and fluorescence spectrophotometry. It is very recent that the need to use fluorescence in clinical surgery has been found. NIR Fluorescence is being compared to previous well established techniques like methylene blue dye and radio nucleotide detection in breast surgery and the early results are encouraging. Neurosurgery has taken up NIR fluorescence technique quite well although long term results are still awaited. NIR fluorescence in limb cancer surgery is still in laboratory animal research phase but with promising results. Various organizations like National cancer institute, the National Institute of Biomedical Imaging and Bioengineering and National Science Foundation has invested heavily in optical imaging. As result optical imaging has matured quite quickly over the last decade. NIR fluorescence imaging is just one of the techniques being practiced and experimented further. With further research there is potential to develop NIR fluorescence and various other fluorescence techniques further to use in day today surgery. No doubt cancer surgery will take the priority in application of fluorescence techniques but later whole of the surgery will be lot more colorful, astonishing and safe with minimal collateral damage.

References


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