International Journal Dental and Medical Sciences Research
Volume 5, Issue 1, Jan-Feb 2023 pp 126-132 www.ijdmsrjournal.com ISSN: 2582-6018

Photoacoustic Imaging (PAI) – Modified ultrasound to assess tissue perfusion and vasculature using gold nanoparticlesin head and neck cancers

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Submitted: 09-01-2023 Accepted: 19-01-2023

ABSTRACT
Photoacoustic imaging (PAI), as the name suggests, is the fusion of two imaging modalities- optical imaging and ultrasound (USG).
It amalgamates the high contrast and specificity of optical imaging with the strong temporal resolution and increased penetration depth of ultrasound to provide structural, functional as well as molecular information of tissues based on the absorption of incident laser light in the near infra-red (NIR) spectrum by endogenous tissue components such as hemoglobin (Hb), oxyhemoglobin (HbO2), lipids, and water. This hybrid imaging modality has been effectively used in the diagnosis of several cancers such as breast, skin, thyroid, and prostate cancers apart from the detection of adverse health conditions such as atherosclerosis in humans. The ability to effectively image vasculature and lymph nodes and assess tissue perfusion based on the measurement of oxygen saturation (SaO2) has enabled its use in diagnostic as well as therapeutic oncology. This paper aims to present a review of photoacoustic imaging and highlight its role in head and neck cancers.
Keywords: Photoacoustic imaging, non-invasive, vasculature imaging, oxygen saturation, perfusion, lymph node metastasis

Abbreviations: Photoacoustic imaging (PAI), Ultrasound (USG), Near infra-red(NIR), Hemoglobin (Hb), oxyhemoglobin (HbO2), oxygen saturation (SaO2)**,** Positron Emission Tomography (PET), dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI), blood oxygen level-dependent magnetic resonance imaging (BOLD-MRI), nanoparticles (NPs), sentinel lymph node (SLN), gastrointestinal tract (GIT), photodynamic therapy (PDT), reactive oxygen species (ROS), radiotherapy (RT), indocyanine green (ICG), Multispectral Optoacoustic Tomography (MSOT), enhanced permeability and retention (EPR), surface plasmon resonance effect (SPR). polyethylene glycol (PEG), reticuloendothelial system (RES), sentinel lymph node biopsy. (SLNB), glycol-chitosan gold nanoparticles (GC-AuNPs). epidermal growth factor receptor (EFGR)

I. INTRODUCTION

Photoacoustic imaging (PAI) or optoacoustic imaging is an emergent, non-invasive, non-ionizing, a real-time imaging modality that providesa measurement of the absolute blood SaO2 which inversely correlates with hypoxia in tissues. PAI is based on the identification of unique optical absorption signatures of endogenous tissue components such as Hb, HbO2, lipids, and water without the need fortracers. [1,2,3,4]

Although Positron Emission Tomography (PET) imaging is the current gold standard of diagnosis, poor oxygen perfusion in hypoxic regions restricts access to the hypoxia-targeted imaging probes to map the spatial heterogeneity in these regions. This is also true for other tracer-based hypoxia imaging modalities such as phosphorescence quenching, electron paramagnetic resonance, dynamic contrast enhanced-magnetic resonance imaging (DCE-MRI), etc. While blood oxygen level-dependent MRI (BOLD-MRI) can infer the hypoxia distribution in tumor tissue by imaging the transition of deoxyhemoglobinto HbO2 in blood, BOLD MRI cannot estimate absolute blood sO2. [5,6]

Principle[7,8,9,10]

PAI is based on a 'light-in-sound-out' technique instead of the 'sound-in-sound-out' principle of traditional ultrasound (USG). Hence, the name 'photo-acoustic', integrates incident light photons of optical imaging and acoustic waves of USG. This technique is based on the photoacoustic effect first described by Alexander Graham Bell in 1880.

Although USG is an economically viable, readily available, and effective technique for high-resolutionsoft tissue imaging at reasonable penetration depths (a few centimeters), it has low sensitivity. Additionally, USG provides only structural information and thus may not be

International Journal Dental and Medical Sciences Research
Volume 5, Issue 1, Jan-Feb 2023 pp 126-132 www.ijdmsrjournal.com ISSN: 2582-6018

completely suited for medico-oncological diagnosis.

PAI adds to the benefits of USG by integrating the advantages of optical excitation with ultrasonic detection. This hybrid modality uses optical properties of a pulsed nanosecond long laser lightsuch as the Nd: YAG laser of wavelength 680-970 nm in the near NIR spectrum as the input source for tissue excitation and USG for detection and image formation.

Upon absorption of incident lightby the components of the tissue of interest, diffuse intracellular photon propagation occurs. The absorption of these photons leads to a slight local rise in temperature. As a result of this localized tissue heating, the tissues thermoelastically expand transiently. The expansion stimulates the generation of wideband USG pressure waves. The acoustic waves can be detected using broadband USG transducers and are converted into sonic images. The intensity (or amplitude) of acoustic signals is proportional to the absorption coefficient and the optical fluence of the tissue.

PAI takes advantage of the fact that the body tissues contain a variety of endogenous chromophores with different absorption spectra, such as melanin, water, lipids, Hb, and HbO2.The total light absorbance in these tissue components is lowest in the wavelength range of 600 -1,000 nm or NIR spectrum or"optical window."Since the absorbance is lowest in the NIR range, the light of these wavelengths is used to obtain sufficient tissue-penetration depth.Information on tissue composition, that is, relative concentrations of (de)oxyhemoglobin, water, lipid, or an injected imaging agent, can thus be calculated by combining photoacoustic data acquired at multiple wavelengths. Numerous relevant clinical parameters can be assessed this way, for example, hypoxia, perfusion, and neoangiogenesis.Many diseases cause changes in tissue composition, such as neovasculature in cancer development and vascular fat deposition in atherosclerotic plaques. PAI has the potential to visualize and quantify these changes in comparison with the normal surrounding tissue.

PAI is thus, a fusion of optical imaging and USG to yield functional and structural information, respectively. With the incorporation of gold nanoparticles (NPs), molecular information of the tissues can be obtained additionally.

Applications[11,12,13,14,15]

PAI has a vast number of applications in oncology and is a part of diagnostic procedures to image several organs of the human body. Concerning oncology, PAI has been used to assess tumor growth by neoangiogenesis, determine hypoxic regions and predict tumor response to radiotherapy (RT), determine tumor-free margins in real-time and enable efficient surgical resection of tumors, evaluation of lymph node metastasis, evaluation of tumor response to anti-angiogenic agents,monitoring of drug delivery with the help of nanocarriersand co-loaded contrast agents,monitoring results of therapeutic interventions by imaging changes in specific characteristics of disease such as tissue hemodynamics and reactive oxygen species (ROS) generation, etc. PAI has been used in previous studies to image tumors of the head and neck, breast, skin, thyroid, prostate, etc.

Body tissue imaging involves the use of PAI in endoscopy of the gastrointestinal tract, laparoscopy, brain cortex imaging, detection of atherosclerotic plaques in blood vessels.non-invasive in-vivo temperature mapping, detection of pH aberrations in conditions such as ischemia, inflammation, cancer, chronic obstructive pulmonary disease, and kidney failure, monitoring and imaging enzyme activity such as alkaline phosphatase using micellar probes, and measurement of periodontal pocket depth.It has also been used to monitor the efficacy of photodynamic therapy (PDT).

Advancements in PAI

PAI has been integrated with tomography as Multispectral Optoacoustic Tomography (MSOT) for imaging fluorescence in proteins. It can be used for high-speed tracking of the biodistribution of fluorescent agents such as indocyanine green (ICG), carboxylate dyes, etc through circulation and uptake in the organs such as the liver, gallbladder, and kidneys. PAI has also integrated with other imaging modalities such as Computed tomography as Photoacoustic Computed Tomography (PACT), Magnetic Resonance Imaging (MRI), and Raman spectroscopy.

As some tissue components have similar absorption spectra within the NIR range, poor contrast is obtained on imaging these endogenous chromophores. To overcome this, exogenous contrast agents have been used to enhance the PAI contrast. Moreover, exogenous imaging agents can potentially facilitate deeper tissue imaging. Non-

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targeted high-molecular-weight imaging agents have been used that extravasate from the bloodstream and accumulate in tissues that offer high vascular permeability such as inflamed and malignant tissues. This property of exogenous agents is based on the enhanced permeability and retention (EPR)effect. Targeted imaging agents such as Evans blue and methylene blue,directed at specific receptors, proteins, or enzymes of certain molecular pathways have also been used for PAI to provide information on molecular processesand cellular biology in the tissues. [16,17]

Incorporation of nanoparticles (NPs) into PAI:[18,19]

NPs are small-sized molecules, of size ranging from a few to several hundred nanometers. These particles are broadly classified into two types- plasmonic and non-plasmonic and available in several forms such as nanospheres, nanoshells, nanorods, nanoclusters, nanocage, and nanostars. NPs have been incorporated into PAI to yield valuable molecular information.

The plasmonic nanoparticles are made of a noble metal (gold or silver) and their use is based on the surface plasmon resonance effect (SPR). This effect takes place when the electromagnetic field of incoming light interacts with the conduction electrons on the NP surface, resulting in the mutual oscillation of these electrons at a resonance frequency relative to the lattice ofpositiveions.At this resonance frequency, the incoming light is absorbed by the NP, with an optical absorption five times greater than the absorption for dyes.The most commonly used plasmonic nanoparticles in PAI are nanoshells, nanorods, and nanocages, usually made of gold.

The advantage of these plasmonic nanoparticles is that their optical properties(absorption and scattering) are highly tunable over the NIR spectrum by changing their size or shape, which makes them very attractive for biomedical applications. An additional important advantage is that the surface characteristics of these NPs are easy to chemically modify, which is important for in vivo use (e.g., to decrease cytotoxicity or to increase circulation time and stability) and for molecular targeting. Functional groups such as polyethylene glycol (PEG) or integrins can be straightforwardly attached to the surface.

However, they can deform after extended exposure to laser irradiation and their long-term safety is a concern, especially with the larger particles that do not fully clear the body as they are taken up by the reticuloendothelial system(RES). NP deformation affects the photoacoustic signal and results in inconsistent imaging results over time. A thin layer of silica coating can increase the stability of the NPs and allow them to maintain their original shape for a longer time. An extra advantage of the silica coating is that these hybrid NPs can act as photoacoustic nanoamplifiers due to more efficient heat dissipation to the tissue. Nanoparticles can also be loaded with drugs and used as delivery vehicles to allow for simultaneous delivery of therapeutic and imaging agents in vivo, or their intrinsic properties can be used for photothermal ablation therapy.This provides great opportunities for image-guided therapy/theranostics (combining therapy and diagnostics).

Role of PAI in head and neck cancers

Although PAI has been used previously for imaging cancers of the breast,ovary,thyroid,skin, and prostate, this paper aims to highlight its role in head and neck cancer. PAI imaging benefits in head and neck cancers can be broadly classified under three categories i.e. imaging tumor hemodynamics, lymph node metastasis detection, and invivo molecular imaging.[20,21,22,23,24,25,26]

A. Imaging tumor hemodynamics:

The SaO2O2 levels are indicative of tissue hypoxia. RT, a commonly used treatment modality for head and neck cancers has low efficacy in hypoxic tissues and regions. PAI can be used to predict the response of tumors to RT by measurement of the SaO2.Thus, PAI has prognostic value in tumors. [27,28,29]

A tumor grows by neo-angiogenesis or the development of new blood vessels in the tumor tissues. Since PAI can effectively image vasculature based on the absorption of the incident light by endogenous tissue components such as hemoglobin and oxyhemoglobin, this hybrid imaging modality can be used to assess tumor growth and spread byneo-angiogenesis. It has also been used to monitor the efficacy of anti-angiogenic drugs.[30,31,32,33,34,35,36]

B. Detection of lymph node metastasis:[37,38,39]

Tumors lead to significant morbidity and mortality by metastasizing to different tissues and organ systems of the human body. PAI can be used for rapid, real-time assessment of sentinel lymph nodes (SLN) for metastatic alterations. This facilitates accurate tumor staging and complete

eradication of the tumor thereby preventing recurrence, in a non-invasive, non-ionizing manner and at a faster pace,compared to sentinel lymph node biopsy. (SLNB)

The tumor cells are injected with glycol-chitosan gold nanoparticles (GC-AuNPs). These NPs bind to host immune cells and these NP tagged cells accumulate in the SLNs.

NP aggregation is immense in healthy tissues and very limited in metastatic tissues. This leads to poor photoacoustic signals in metastatic tumors. Benign lymph nodes imaged using PAI demonstrate the presence of hilum notch and increased contrast in the nodal center compared to the peripheral regions whereas malignant and metastatic lymph nodes exhibit decreased photoacoustic responsein the center as well as peripheral regions along with the heterogeneous accumulation of contrast agents. Antibodies against the epidermal growth factor receptor (EFGR) have also been used for the detection of lymph node metastasis.

C. Invivo Molecular Imaging: CD31 is highly expressed on the surface of vascular endothelial cells. It has been used for monitoring the density of blood vessels in malignant tissues. CD31 antibodies have been established as an effective marker of angiogenesis. Antibodies such as CD31 tagged to extragenous contrast agents such as gold NPs can serve as early tumor biomarkers.

Advantages[40,41]

PAI provides non-invasive, non-ionizing, safe, relatively economic,real-time imaging of tumor vasculature, tissue perfusion, and assessment of tumor-free margins for efficient and accurate tumor resection. It is capable of imaging at multiple scales, resolving single cells, organelles, and capillaries in vivo. The tissue penetration is deeper compared to traditional optical imaging techniques. The use of exogenous contrast agents is also not required. PAI can be used to image intrinsic (hemoglobin, melanin) as well as extrinsic agents (contrast agents targeted to biomarkers or antibodies, heparin, etc). This hybrid imaging modality can be integrated with ease into clinical USG scanners and eliminates speckles seen on USG. The less relevant background information can be reduced in PAI because non-absorbing objects do not generate photoacousticsignals and spectral imaging/signal thresholding can be used to minimize signals from endogenous absorbers. Additionally, both fluorescent and non-fluorescent targets can be imaged.

Limitations[42]

In human tissues, PAI has been able to achieve limited penetration depth (up to 5 cm). However, specific deep-lying organs including the heart (location under the rib cage), lungs (beneath rib cage and gas-tissue interface), stomach (a hollow structure and under the rib cage), and pancreas (depth) are imaged with limited accuracy using PAI. In addition, the cost of PAI is higher compared to traditional diagnostic methods.

Clinical Significance in head and neckoncology

PAI imaging has enhanced diagnostic oncology byfacilitating tumor angiogenic growth assessment and metastatic lymph node detection, as well as therapeutic oncology by accurate determination of surgical margins and aiding prediction of tumor response to treatment measures such as RT. The structural, functional, and molecular information on tumor oncology provided by PAI could improve diagnostics as well as therapeutics to provide patients with a better standard of care and prolong their overall survival.[43,44,45]

II. CONCLUSION

Single modality imaging techniques provide information about a disease process with limited scope. Hybrid imaging, by fusing one or more imaging modalities synergistically provides a completely new vista to the disease process.

PAI holds great promise in biomedical applications. Its key strength lies in the ability to yield real-timefunctional,structural as well as molecular information of tissues by non-invasive, non-ionizing imaging. Since the last decade, this real-time, cost-effective, high-resolution hybrid imaging modality has matured from its research stage in animal studies and has found its place in imaging human tissues such as the brain, eyes, GIT, thyroid, bones, and joints,skin, and cardiovascular tissues.

In addition, the portability and compatibility of PAI with existing imaging modalities could contribute to theranostic oncological imaging and provide valuable information on important aspects such as tumor angiogenesis, lymph node metastasis and aid in prognostic outcomes by the prediction of tumoral responses to radiotherapy. In cancer, the combination of gold nanoparticles and PAI facilitates highly sensitive, specific detection of

tumor metastasis, the primary reason for morbidities in today's world.

However, future studies are required for swift clinical translation of PAI into clinics and other readily accessible healthcare facilities.

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