Chromophores in operative surgery: Current practice and rationalized development

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Abstract.

Background.

Chromophore-containing molecules feature extensively in surgical practice, with synthetic dyes gaining popularity over endogenous optical adjuncts. New applications for chromophores in diagnostics and operative treatment exploit unique chemical structures suited for illuminating target tissues beyond the visual spectrum, ranging from ultraviolet (UV) to near-infrared (NIR). This review outlines the rationale for surgical chromophore application, the weaknesses and risks in each class of these compounds, and areas of foreseeable potential for employment of specialized contrast agents.

Method.

An English-language literature search applied the following Boolean Search string: “dye OR Lake OR Stain OR chromophore” AND “tox$ OR terato* OR carcino$ OR Allerg$ OR surg$ OR clinic” using EMBASE, PUBMED, PUBMED central and OVIDSp, with back-referencing through Web of Knowledge^{TM}.

Results.

Based on the primary literature, this study proposes a surgically relevant classification system of chromophores in current use, which facilitates risk/benefit consideration for the surgeon who employs them, and which facilitates clinically oriented development.

Conclusions.

The next stage of development for optically active surgical adjuncts must address practical constraints while minimizing risks of adverse effects. Exploiting the technology’s full potential also requires improvements in the usefulness of imagery equipment.

1. Background

Optically active surgical adjuncts are widely used in conventional, current surgical practice across several specialties, with applications ranging from skin marking ink to sentinel lymph node biopsy (SLNB) [1]. The operating surgeon increasingly relies on real-time sensory input to guide precise, accurate identification of tissues. Enhanced visual differentiation of anatomical structures and diseased tissues becomes increasingly important, especially when considering complex, remote or robotized surgery [2]. In the conventional operating suite, the surgeon achieves this distinction via reflection of overhead white light, which is visible to the naked eye (390-700 nm) [3]. Whilst this principle contributes the mainstay of conventional surgery, it suffers from several drawbacks including multiple light shadowing, dependence on the operating surgeon, and experience [4]. Many bind tightly to circulating amylase, thereby being delivered to the intended site (Table 1). Recent literature reports considerable effort aimed at enhancing this sensory input through the use of optically active surgical adjuncts . However, their current use is based on clinical historical precedent, often off-label, and may incur the risk of significant adverse effects. Moreover, recent development of surgical techniques using emitted light in the UV and NIR spectra merit specific consideration. This review reports a snapshot of chromophores in surgical practice, proposes a simple, surgically relevant classification, and evaluates current-best evidence regarding their indications, adverse effects, contra-indications and salient controversies surrounding their use.

2. Review Methodology

Literature was retrieved using the following Boolean Search String: “dye OR Lake OR Stain OR chromophore” AND “tox$ OR terato* OR carcino$ OR Allerg$ OR surg$ OR clinic”. EMBASE, PUBMED, PUBMED central, and OVIDSp data bases were searched for articles in English, published or in press until 31 August 2015. Literature was back-referenced using Web of Knowledge^{TM} software.

3. Literature review

3.1 Current clinical deployment

All visually active entities depend on the presence of an atom or group of atoms in the molecule that gives it colour. This moiety is termed the chromophore [5], regardless of the classical distinction between dyes, stains mordants and lakes.

A synopsis of the common chromophore-containing molecules used in current surgical practice, and the related specialties in which they continue to be currently useful is provided in Table 1.

3.1.1 Endogenous versus exogenous chromophore-containing molecules

Endogenous optical adjuncts are typically naturally occurring chromophores, which depend on absorption or reflection of light that is then detected unaided by the operator. Pathogenic accumulation typically imparts specific optical characteristics to tissues and forms the basis of visual differentiation in conventional surgery. This

Table 1. Chromophores in current surgical practice, and typical specialties in which their use is exploited

Dye used	Reference	Uses in current clinical practice studies
Indigo Carmine	[6] [7] [8]	Indigo Carmine: Intravenous indigo carmine injection. Localize ureteral orifices, severed ureters and fistulous communications in radical retropubic prostatectomy and ureteral stenosis during robot-assisted ureteroureterostomy.
Indocyanine Green	[9] [5]	Indocyanine Green: localization of ureteral stenosis during robot-assisted ureteroureterostomy.
Methylene Blue		Methylene Blue: Retrograde vaginal methylene blue testing for localization of urinary fistulae openings. Intravesical methylene blue identification of the diverticular neck during robot-assisted approach for bladder diverticulectomy.
Bonney's Blue	[10]	Bonney's blue: instillation of diluted into the bladder during gynecological operation.
Isosulfan blue	[11]	Outlining lymphatic channels during surgery thoracic duct ligation.
Indocyanine green		Indocyanine green: Visualization of lens capsule in cataract surgery. Internal limiting membrane (ILM) of the retina green during pars plana vitrectomy.
Brilliant Blue Green	[12]	Brilliant Blue Green: Macular hole surgery to stain the ILM.
Trypan blue		Trypan Blue: Measuring cell viability in-vitro. Visualization of lens capsule in cataract surgery. Macular hole surgery to stain the ILM.
Fluorescein	[13]	Visualization of lens capsule in cataract surgery.
Methylene Blue	[5]	Methylene Blue: stains the parathyroid glands for surgical visualization during parathyroidectomy.
Indocyanine green	[14]	Indocyanine Green: Intraoperative blood flow assessment of neurovascular system during angiography.
Indocyanine green	[5]	Indocyanine green: ICG angiography is used to objectively quantify changes in microcirculation pre- and postoperative skin flap.
Fluorescein		Fluorescein: Prediction of breast flaps viability following reconstructive surgeries such as radial mastectomy or subcutaneous mastectomy.

distinction may also be refined by anatomical site (homotopic versus heterotopic), rate, and pattern of accumulation. Clinical application of such chromophore-containing molecules, in diagnosis and to guide operative treatment, still forms the core of established medical practice. However, it is also fraught with difficulty, including limited sensitivity, specificity and operator dependence. Cutaneous primary malignant melanoma is a pertinent example: the number of pigmented lesions that need to be excised to diagnose one cutaneous malignant melanoma (numbers needed to treat, NNT) is in excess of 8:1, which is operator and experience dependent . Even the well-established guidelines for conventional surgical lateral margins of excision of primary melanoma are far from satisfactory, having only been proven to reduce local recurrence rates (at the cost of significant morbidity) without affecting survival rates . The advent of photonic devices such as LASER has enabled less collateral damage when compared to conventional surgery [15]. However, such techniques still rely on human detection of melanin, and therefore at best could be considered as non-inferior treatment modalities.

3.1.2 Visible versus Ultraviolet and Infrared Applications

Exogenous optical adjuncts include chromophores not normally present in the human body. Whilst most human endogenous chromophore-containing molecules are situated within the visible part of the spectrum, synthetic chromophores do not suffer from this limitation, and clinically salient examples extend from UV through visible to the Infra-Red (IR) spectrum. The use of exogenous chromophore-containing molecules emitting visible light broadly falls into three categories: delineation of anatomical margins; indicators of oncopathogical spread, and as miscellaneous therapeutic agents.

Methylene blue, the first fully synthetic drug introduced in humans, is injected to delineate surgical tracks, including fissures, fistulae, sinuses and abscesses [16] and as a substitute to Patent Blue V (PBV) for SLNB. Similarly, Bonney's blue is a mixture of gentian (crystal) violet and brilliant green, as an equal parts mixture dissolved in ethanol [17]. It is ubiquitously found within operating theatres and is used for a plethora of applications, including as a marker for planning surgical incision or resection. Indigo carmine has been used to detect amniotic fluid leakage or urine. In the latter application, it is rapidly filtered into urine which it colors blue, facilitating detection of urinary leaks [18]. It is important to note that such materials in current surgical practice have been in continuous use since that time, and have just been accepted on the bases of tradition. Such materials have been in clinical use well-before current FDA requirements.

PBV is a triphenylmethane derivative that is highly visible at small concentrations [5]. It is extensively used in lymphatic visualization and SNLB through injection at a primary tumor site [5]. However, its sensitivity and specificity are contended. Most surgeons use it in combination with a radiolabeled colloid, but its preparation, including filtration to limit colloid size, varies widely [5]. A recently launched

radiopharmaceutical specifically designed for lymphoscintigraphic intraoperative SLNB detection (Tilmanocept) contains multiple diethylene-triamine-penta-acetic acid moieties which tightly bind to ^{99m}Tc, while its multiple mannose moieties act as ligands for multivalent binding to CD 206 mannose receptors on the surfaces of reticuloendothelial cells resident in lymph nodes [19]. Whilst PBV is currently still considered to be the gold standard for SNLB [20], in two rigorously conducted phase III trials, Tilmanocept® correctly identified an additional 20% of melanoma-positive sentinel nodes that were not detected by blue dye [21]. PBV is rapidly quenched upon exposure to light. Moreover, its color is pH dependent (becoming yellow-orange in acidic conditions) and reduction-oxidation sensitive (being oxidized to a red form in solution). These changes in absorption may potentially explain the decreased sensitivity when compared to Tilmanocept.

Individual chromophore-containing molecules have also found particular niches in the treatment of particular conditions. For nearly 100 years, application of methylene blue combined with light has been known to have viricidal properties, and has been consequently applied in the treatment of *Staphylococcus aureus* and Hepatitis C . High-dose methylene blue has been described as an antidote to cyanide poisoning [5].

UV light ranges in wavelength from 10 to 400 nm, but does not penetrate as deeply into human tissues as NIR. Fluorescein angiography (UV) was used for intraoperative assessment of microvascular perfusion, but its use was limited by long half-life (up to 18 hours for clearance, preventing re-evaluation) and rapid leakage into the interstitium, enhanced by local ischemia, leading to false positive results . Fluorescein also suffers from a well-documented risk of anaphylaxis-type reactions [22]. In contrast, indocyanine green (ICG) is a cyanine class chromophore which is visible, but also fluorescent in the NIR range (see Figure 1). IR frequencies penetrate deeper than UV, so their use in angiography facilitates imaging deeper patterns of circulation than fluorescein angiography. ICG has consequently experienced a wide range of novel applications, including measurement of cardiac output, hepatic function, and blood flow; as well as ophthalmic angiography . ICG angiography has been recently transposed to microvascular surgery by Gurtner and colleagues, its short half-life allowing multiple reassessment, as opposed to fluorescein [23]. The cyanine also been applied to SLNB .

However, NIR chromophores themselves are not risk-free. ICG, a well-researched chromophore useful in the NIR range, has well-documented and frequently overlooked potential adverse effects. In aqueous solution, ICG starts to oligomerize when concentrations >3.9 mg/L are reached. This phenomenon leads to non-applicability of Beer's Law (the linear relationship between absorbance and concentration of an absorbing species), making interpretation difficult [24]. Above 103 mg/L, ICG forms large aggregates resulting in self quenching, and consequently reduced fluorescence, apart from the potential for thrombogenesis [25].

Degeneration in aqueous solution is due to solvent radicals and ions, leading to structural loss [26].

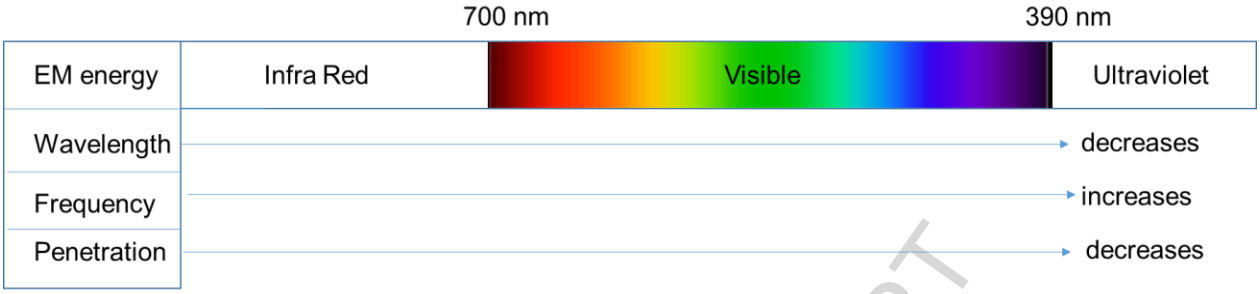

Figure 1: The electromagnetic spectrum and its relation to visible, ultraviolet and infra red light, wavelength, frequency and tissue penetration. EM electromagnetic; nm nanometer.

3.2 Applied chemical structure

In order to understand the risk/benefit imparted by the use of chromophore containing molecules, a functional understanding of their common chemical structures is important. Each chromophore consists of an arrangement of conjugated double bonds, termed a delocalization electron system, and includes a chain of methine groups (-CH=) [27]. Electron accepting or donating atoms, such as oxygen and nitrogen, are also present at the ends of most colored compounds [25].

Endogenous chromophores broadly fall into two types: porphyrin-type and melanin structures (see Figure 2). The porphyrin ring is central to many naturally occurring chromophores including haemoglobin, bilirubin, biliverdin, and cytochromes [5]. It also occurs in natural, exogenous chromophores such as the chlorophylls [28]. Melanin, in contrast, is produced by the oxidation of the amino acid tyrosine, followed by polymerization [5]. This natural chromophore exists in three forms: eumelanin, pheomelanin, and neuromelanin [29]. Eumelanin exists in two forms, brown eumelanin and black eumelanin. Pheomelanin is a cysteine-containing red polymer of benzothiazine units, present in red hair. Finally, neuromelanin is found in the central nervous system including the medulla locus coeruleus, the substantia nigra, and the zona reticularis of the adrenal gland [5]. Melanin is a frequently employed target in conventional LASER therapy [30].

Literature presents several complex and overlapping classification systems for exogenous chromophore containing molecules [5]. Whilst these systems may be exhaustive, they are of limited value to the operative surgeon, as they are based on industrial process rather than in vivo use and metabolic fate (see Table 2). In contrast, this section briefly discusses the applied significance of common chemical structures of chromophores in current surgical use, as part of a simplified, functional classification (see Figures 3 & 4).

3.2.1 Triarylmethane derivatives

Triarylmethane is the basic skeleton of this class. Gentian (methyl) violet and brilliant green are two examples. Bonney's blue is very commonly used as a surgical ink, and is composed of brilliant green and gentian violet [31].

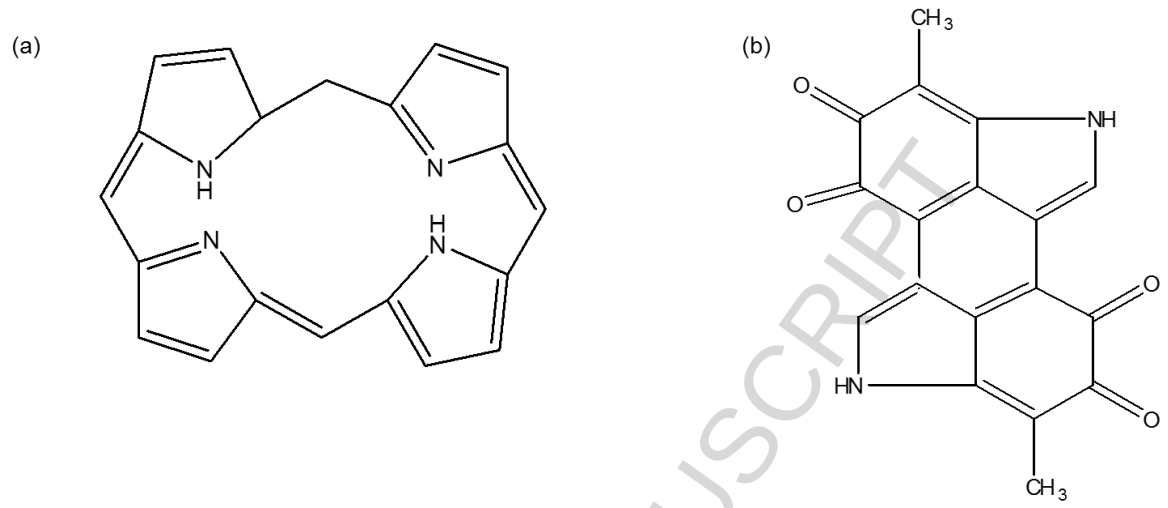

Figure 2: (a) Chemical structure of the porphyrin ring illustrating the classical electron delocalisation system of single and double bonds

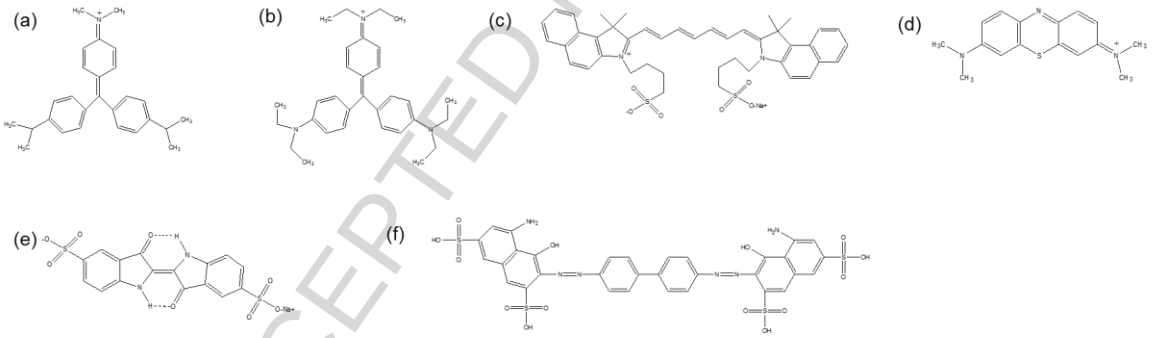

Figure 3: Chemical structure of commonly used exogenous chromophores illustrating their delocalisation system of single and double bonds

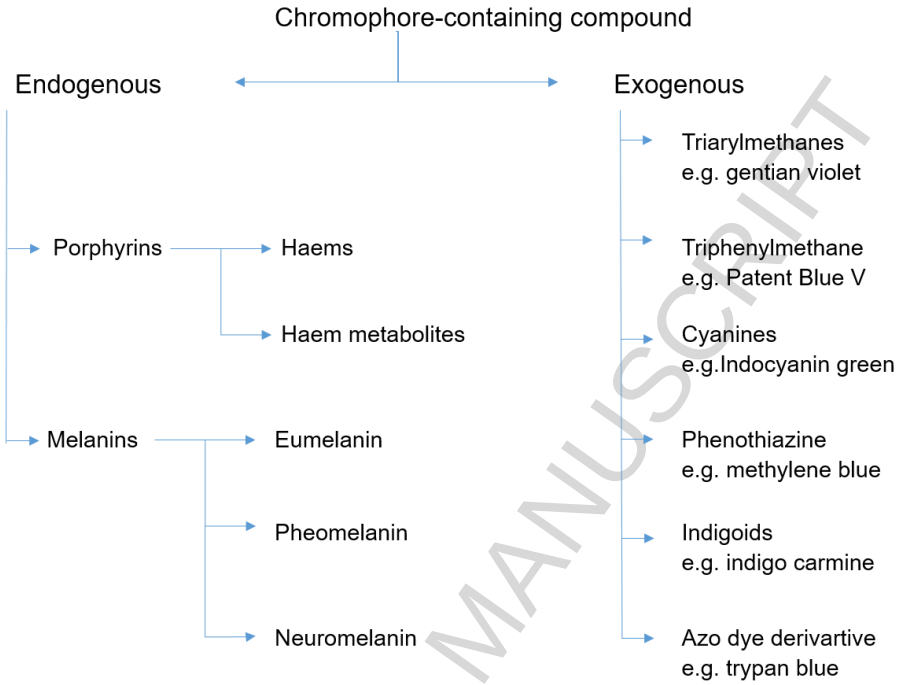

Figure 4: The main types of chemical structure for chromophore-containing molecules in current clinical use, with examples.

3.2.2 Triphenylmethane derivatives

Many members of this class change color with pH, and some even display fluorescence. PBV, and its isomer Isosulfan Blue, are members of this class. Within the European Union, PBV is authorized as a cosmetic and a food colorant.

3.2.3 Azo derivatives

In most azo dyes, the delocalization system includes a benzene or naphthalene aromatic ring [32]. A wide range of possible substituent side groups determine the color, and many contain more than one azo linkage. Azo dyes could be carcinogenic, as a result of their metabolism either in the human body and/or bacterial [33]. Reductive cleavage or degradation into component aromatic amines is a typical mechanism leading to azo dye mediated genotoxiticy [34]. The hepatic cytochrome p-450 isozyme

Table 2: Definitions of substances containing chromophores in the literature

Term	Definition
Color additive	Includes any dye, pigment, or other substance that can impart color to a food, drug, or cosmetic or to the human body [35].
Dye	Any substance (natural or synthetic), used to change the color of a target [36].
Lake	A pigment manufactured by precipitating a dye with an inert binder, or "mordant", usually a metallic salt [37].

system in particular is thought to activate mutagenicity of azo dyes, forming a mutagenic metabolite/product through N-oxidation [38]. The N-hydroxylaryl amines are then either activated (glucuronidation) or inactivated (acetylation), which may influence their mutagenicity. In acidic pH, nitrenium ions are formed that can alkylate bases in DNA, especially guanine, possibly resulting in carcinogenesis [38]. It therefore appears that the products of azo dye metabolism, rather than the parent compound itself, are responsible for mutagenesis and subsequent (or perpetuating) carcinogenesis. Trypan blue is an example of an azo dye; it is used as a surgical aid in ophthalmic surgery .

3.2.4 Phenothiazine derivatives

The phenothiazine derivatives are positively-charged chromophore containing entities commonly composed of phenol sulphur and nitrogen. Within the three-ring chromophore of the thiazine dyes, nitrogen and sulphur make bridges between two six-carbon rings [39]. One such example is Methylene blue.

3.2.5 Cyanine derivatives

Cyanines are polymethine dyes, which can cover the spectrum from IR to UV. Their chemical structure consists of two aromatic rings separated by a polymethine chain. ICG is an FDA licensed example which is gaining increasing popularity. Flavonoids are naturally occurring cyanines [40].

3.2.6 Indigoid derivatives

Indigo and thioindigo dyes cover the NIR to UV spectrum, depending of their chemical composition and structure. These are typically insoluble, colored compounds that are solubilized by addition of sulfonate groups to the indigoid structure. Indigo carmine is one such example, with its color being both pH and reduction-oxidation sensitive. Reports indicate respiratory tract toxicity and hypotension as possible side effects [41].

3.3 Adverse effects.

Literature intensively reports the proposed and purported benefits of optically active adjuncts to surgical practice. However, an in-depth discussion of the potential risks and adverse effects is notably missing from recent, extensive reviews on the subject . It is imperative that the operating surgeon is well-aware of these adverse effects, both as ultimate guarantor of patient safety, and due to the risks related to the prescription and administration of off-label medicinals [42]. Analysis of the retrieved literature regarding chromophores used in current surgical practice reports four overarching themes: carcinogenesis, cytotoxicity, metabolic interference, and drug-drug interactions.

3.3.1 Carcinogenesis

A major concern with chromophore-containing molecules in current surgical practice is their carcinogenic potential. The mutagen can either be the chromophore-containing molecule itself, or its metabolites. In the former category, one finds triarylmethane derivatives, whilst azo derivatives feature prominently in the latter category.

Triarylmethane derivative Gentian violet has been associated with mutagenicity, carcinogenesis and clastogenicity (induction of chromosomal breaks) in bacterial and mammalian cells [43]. Brilliant green is considered to be as toxic as malachite green, which is known to be genotoxic and carcinogenic, resulting in its ban from food production in the European Union [44]. It has also been demonstrated that PBV, methylene blue and indigo carmine, which are commonly used in SNLB, cause DNA damage to breast epithelial cells in vitro (MCF-7 and HB-2 cells lines using the alkaline comet assay) [45]. Exposure of the methylene blue to ambient light has also been reported to cause DNA damage leading to cancer [46].

Azo dyes such as trypan blue have been associated to mutagenesis, including basal cell carcinoma [47]. Phenylenediamine and benzidine are the major mutagenic moieties of carcinogenic azo dyes. Literature shows that only those azo dyes which contain either of these molecular moieties possess mutagenic potential (see Section 3.2, describing azo dye mutagenic pathways).

3.3.2 Allergy and anaphylaxis

The occurrence of allergic and anaphylactic reaction following dye administration is well-documented. PBV dye is the commonest to be used in SNLB mapping, and it has been extensively studied. There is some evidence suggesting anaphylactic reactions to PBV are IgE mediated [6]. Based on retrospective studies when administered for SNLB, 0.56% patients develop allergic reactions and 0.06% of patients develop severe anaphylaxis [48]. However, one prospective study reported the overall incidence of allergy associated with PBV dye at 0.9% [49]. The use of other substitutes for PBV is not without risk. Methylene blue itself has been associated with severe skin necrosis upon intradermal injection, whilst IgE mediated anaphylaxis has been demonstrated for isosulfan blue injection [5].

3.3.3. Cytotoxicity and histotoxicity

Inhibition of mitochondrial respiration is a major adverse effect that features prominently in the critique of triphenylmethane dyes (such as the components of Bonney's blue) [50]. In some countries, its use in food and cosmetics is prohibited . In addition, PBV has been found to affect intraoperative pulse oximetric recordings [51]. Koivusalo et al report a transient, false lowering of peripheral oxygen saturation values immediately after injection of PBV, which has a peak absorption at a wavelength of 640 nm. Although the effect is short-lived, arterial oxygen tension readings were not affected [52]. Cardio-respiratory arrest due to PBV administration has also been reported [5]. Similarly, perilesional injection of isosulfan solution (1%,

3 mL) caused a profound pulse oximetry desaturation, whether given sub-areolar, intradermal or intraparenchymally .

Literature also gives some evidence for dye-mediated integumentary toxicity. It has been reported that a commercial ink surgical marker pen containing Gentian violet causes cell and tissue death and reduced cell migration in tenocytes harvested *ex vivo* from (human) patients undergoing anterior cruciate ligament reconstruction [53]. Additionally, at surgically relevant concentrations and exposure times, ICG caused acute and chronic retinal pigment epithelium toxicity, whilst trypan blue exhibited chronic toxicity at all concentrations tested .

3.3.4 Drug-drug interactions

Methylene blue acts as a monoamine oxidase inhibitor, and if infused intravenously at doses exceeding 5 mg/kg, it may precipitate serious serotonin toxicity and serotonin syndrome, when combined with serotonin reuptake inhibitors (e.g., duloxetine, sibutramine, venlafaxine, clomipramine, imipramine). Methylene blue can also precipitate haemolytic anaemia in carriers of the G6PD (favism) enzymatic deficiency [54].
Finally, most of these molecules contain hydrophobic moieties, and are carried in complex with albumin. Therefore, the potential for displacement of drugs being carried around the body in this way (such as warfarin, and administered hormones and steroids) needs to be considered.

4. Rationalized development

Two broad trends sum up the current research efforts in chromophore-containing molecules as surgical adjuncts. The first trend is defined by working on native tissues, whilst the second involves the use of extrinsic contrast agents .

4.1 Development based on native tissues

When activated by energy in the UV-NIR spectrum, neoplastic tissues emit intrinsic fluorescence based on unique biochemical and morphological abnormalities. In particular, decreased green fluorescence is caused by collagen breakdown, whilst red fluorescence is increased due to higher endogenous porphyrin production. Both increase the red/green fluorescence ratio and have been reported as predictors of malignancy .

Two-photon fluorescence microscopy is another example which has been studied for dynamic imaging of tumor growth and invasion [55]. The potential advantages of this technique, compared to one-photon fluorescence techniques, include increased depth of penetration and axial resolution [56]. Common drawbacks of this technique include the complexity of determining the mechanisms that influence the signal.

Moreover, the relationship between the optical signature and disease progression is difficult to assess [57].

Ratios of intrinsic optical absorption of human tissues (water, lipid, blood) compared to haemoglobin saturation have been harnessed in NIR spectroscopy for the diagnosis and detection of cancer. Applications consist of three-dimensional parallel-plate diffuse optical tomography and time-domain optical mammography to evaluate differentiation between benign and malignant tumors [58]. However, such techniques remain significantly limited by practical considerations [59].

4.2 Extrinsic contrast agents

Several strides have been recently reported in the use of extrinsic contrast agents. Optical imaging with NIR fluorescence relies on a wavelength window (650-900 nm) where the tissues present the minimum absorption coefficient, resulting in increased tissue penetration. This feature allows the development of probes with high signal to background ratios (see Section 3.1 for examples of non-targeted contrast agents).

The enhanced permeability and retention (EPR) effect is a size-based phenomenon whereby large molecules and complexes benefit from enhanced vascular permeability and reduced/dysfunctional lymphatic drainage in the peritumoral vicinity [60]. Rosenthal et al assert that this effect is principally the result of the expression of growth factors that promote vascular permeability [61]. However, an extensive, well-established body of research clearly demonstrates that this is a Bradykinin-mediated effect , with striking similarities between infection and cancer [62]. This concept has formed the basis for several commercially successful therapeutics in clinical oncology .

Exploitation of this phenomenon has recently been explored using ICG complexed to plasma protein . However, this method relies on complexing of ICG to plasma proteins for the size effect, and therefore suffers from the inherent instability of such complexes. NIR imaging with ICG complexed to albumin is not helpful in situations where there is significant peritumoral inflammation [63].

The EPR effect is well-established to be a phenomenon of most solid cancer [5]. However, complexed ICG does not seem to benefit from this effect, such as in the case of renal cell carcinoma [64]. In particular, ICG used in this fashion is susceptible to instability in aqueous solution, concentration-dependent aggregation and rapid degradation [64]. Therefore, research has focused on increasing stability in biological fluids by altering its formulation in poly lactic glutamic acid nanoparticles and liposomes, to increase stability and prevent aggregation [65]. Unfortunately, such formulations do not alter the potential for toxicity (see Section 3.3).

4.3 Targeted contrast agents

Non-targeted contrast agents have been extensively explored in the field of cancer, yet some tumors present up-regulated proteolytic enzymes, allowing their detection

to be correlated with specific characteristics. The notion of targeted products is for administration in their inactive state, only to be activated when in contact with a localized source of such an enzyme [5]. These techniques present the possibility of lower background noise. Commercially available exponents of such technology include NIR probes that are activated by proteases, such as cathepsin and matrix metalloproteinase [66]. Since cathepsins and matrix metalloproteinases are also produced by macrophages and neutrophils, they are also copiously produced in inflammatory tissue, rheumatoid arthritis and cerebral ischemia [5].

Targeted organic fluorophores exploit the increased metabolism, angiogenesis, growth signaling receptors and replicative behavior of tumor cells. NIR fluorescent agents conjugate to specific moieties, such as epidermal growth factor receptor, Her2/Neu receptor, vascular endothelial growth factor, folate receptors, or monoclonal antibodies; providing significantly less background noise whilst possessing longer periods of usability when compared to radioactive tracers .

Intensive research effort has been afforded to the field of quantum dot nanoparticles, which are small inorganic crystals of semiconductor materials. Their attractive qualities include high signal intensity, size tunability and photo stability [57]. However, quantum dots present a significant toxicity hazard, because of their heavy metal cores (such as cadmium), their tendency toward aggregation, and stability of their surface chemistry [5]. No clinical applications of quantum dots have been reported to date. Silica nanoparticles have been presented as a potential biocompatible alternative to quantum dots [5]. However, their subcellular distribution, prolonged residence in several tissues, and hepatotoxicity in mouse models suggest that clinical usefulness is still elusive [67].

5. Future directions

Several publications in the basic sciences report limited usefulness of conventional fluorescence, because of the difficulties caused by non-specific background light and absorption of visible light by biological chromophores, limiting depth penetration . In contrast, this review reports that such chromophore-containing molecules make up the majority of optically active products used in clinical practice. The advantages of extra-visible wavelength chromophores have been widely reported, but their adoption is also dependent on the development of suitable intra-operative imaging systems. Conventional single-band camera systems do not distinguish autofluorescence from the probe signal, and they do not compensate for geometric and intensity distortions produced by photon-tissue interaction [67]. More recently, systems for filtering NIR fluorescence in real time, as well as real-time stereoscopic imaging systems combining visible with NIR light, have been described [68].

Cost and convenience are practical and important considerations. For example, in SLNB, the cost of conventional dye plus radiopharmaceutical techniques, and the limited availability of this technology, are often cited as limiting factors [69]. However,

NIR techniques are limited by the obligatory requirement for high cost, high maintenance equipment [70]. Moreover, the necessity of intraoperative imaging systems, whether head-wear or stand-alone, limits operator comfort and tolerance. Scope exists for further miniaturization and ergonomic improvement before such systems become accepted in the mainstream.

4. Conclusion

This review appraised the applications of chromophore-containing molecules in contemporary operative surgery, including controversies regarding safety and efficacy, as well as directions of current research. Advancement of surgery as a specialty requires development of techniques that reduce tactile sensory input, as well as demanding significantly higher degrees of tissue discrimination than can be afforded by the traditional “knife and fork” approach.

These findings suggest that the research effort in this field is undergoing a “pivot shift” toward extrinsic contrast agents (autofluorescence) but away from UV emission. However, clinical application of chromophore-containing molecules emitting in the extra-visible part of the spectrum is dependent on parallel development of appropriate, affordable and convenient detection devices.

Literature emphasizes the limited usefulness of extrinsic chromophore-containing molecules relying on conventional (visible light) fluorescence. We report that such chromophore-containing molecules make up the majority of optically active products used in clinical practice. The use of most of these agents is based on historical precedent, often occurring off-label. Ignorance is no defense at law [71], therefore it is imperative that operating surgeons availing themselves of such technology are able to appraise the risk involved and share this knowledge in the process of informed consent. Widespread availability of agents with dubious characteristics suggests that the current surgical community does not perceive this threat to patient safety to be actual or significant.

It is clear that the use of chromophore-containing, optically active surgical adjuncts has the potential to significantly improve pathophysiological selectivity towards diseased tissue, and consequently, surgical outcomes. Development of cost-effective, properly trialed, custom-engineered products, rationally tailored toward their application is essential for these potential benefits to be translated to clinical practice.

7. Financial Disclosures.
The authors have no conflicts of interest to declare.

Figure Legend

Figure 1: The electromagnetic spectrum and its relation to visible, ultraviolet and infrared light, wavelength, frequency and tissue penetration. EM = electromagnetic; nm = nanometer.

Figure 2: Chemical structure of (a) porphyrin ring and (b) melanin, illustrating the classical electron delocalization system of single and double bonds.

Figure 3: Chemical structure of commonly used exogenous chromophores.

Figure 4: The main types of chemical structure for chromophore-containing molecules in current clinical use, with examples.

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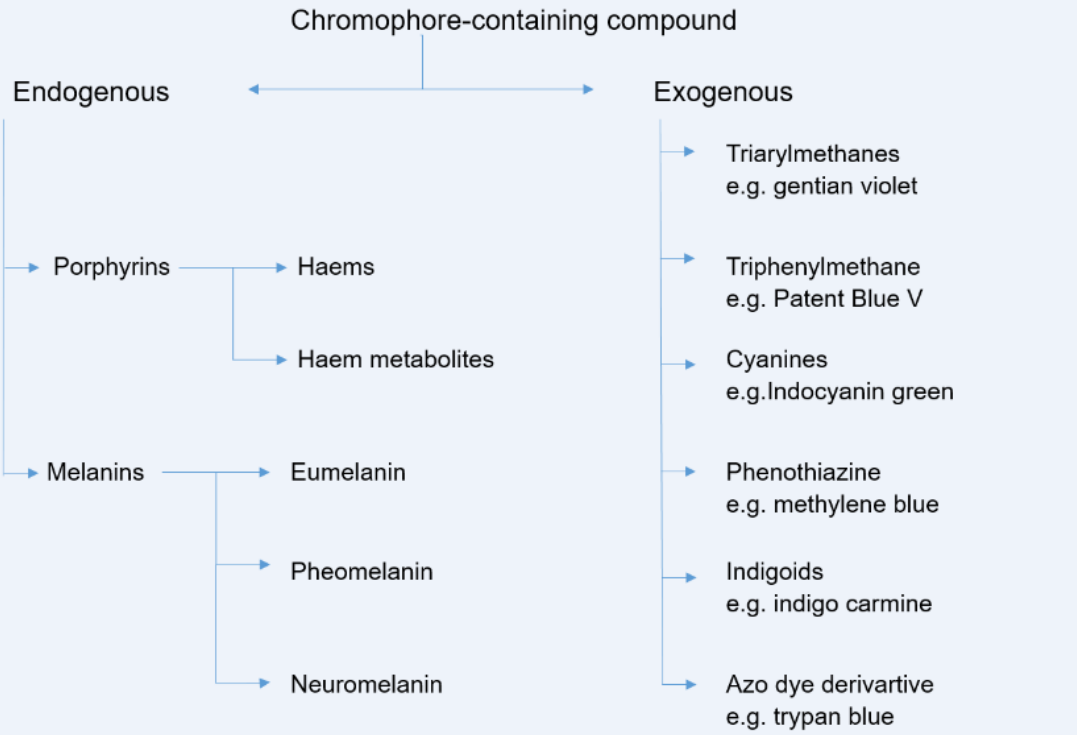
Graphical abstract



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