Non-invasive Detection of Retained Surgical Items Using Near-Infrared Fluorescence

ABSTRACT

This proposed research focuses on the preliminary investigation for developing a novel detection method for retained surgical items (RSIs), specifically surgical sponges. Several methods are currently available for the avoidance and detection of RSIs; however, they remain a significant problem. The proposed research utilizes the transparent near-infrared region (800 nm) which is devoid of fluorescence and absorption by the human body. This wavelength has significant penetration depth and can be effectively used to detect objects intra-operatively. In our preliminary studies, we have shown that the non-covalently linked NIR dye ICG enables quick and accurate identification of surgical sponges even in highly scattering media analogous to the surgical field. Additionally, the systems needed for the fluorescence imaging in this wavelength are both cheap and robust. We now seek to improve upon the initial method we have developed. We will do this in two ways: firstly by testing other classes of NIR fluorophores in order to optimize the stability and optical properties of the imaging agents and secondly by covalently linking a fluorophore to the polymer of the surgical sponges as a proof of concept. While we will only be testing surgical sponges, this method of tagging surgical materials can be applied to many other materials including plastics and other types of bandaging. This seed grant will be crucial for developing the data required for extramural funding toward developing a procedure for covalently labeling surgical sponges using our synthesized NIR dye and further clinical implementation of this technique.

1 INTRODUCTION

The main aim of this preliminary study is to address the reoccurring problem of retained surgical items (RSIs) following surgical procedures. When surgical items are left in patients their discovery can be difficult without additional surgical intervention: especially when they are not visible by X-ray. Even MRI can be inadequate in discovering items that can absorb moisture in the body. Hence it would be desirable to develop a new methodology that can be used in the discovery of RSIs during or immediately after surgery. The ideal method would be reliable, inexpensive, noninvasive, and fast. Specifically, near-infrared (NIR) light has several advantageous properties that satisfy these requirements and make it ideal for intraoperative RSI detection. Because absorption and scattering are low in the NIR wavelength range of 700-900 nm, photon attenuation is minimized and target detection below the tissue surface is possible [1]. Auto-fluorescence from the human body is greatly reduced in the NIR region and this characteristic allows a high signal-to background ratio for target identification. NIR light is non-ionizing radiation and is extremely safe at the fluence rates needed for *in vivo* imaging.

A new method that fulfills these requirements would be universally adaptable for every surgical procedure even if the expected probability of the presence of RSI is relatively low. The NIR window has been utilized for cancer imaging and other *in vivo* imaging applications using NIR fluorescent dyes that concentrate in the cancerous tissue or specific healthy organs [2, 3]. At that point NIR light is used to visualize cancerous tissue or specific organs. Since NIR dyes proved to be tissue specific imaging it seems logical to extend the use of NIR fluorescence to identify and significantly reduce or eliminate the number of RSI cases. Our Specific Aims are centered on the development of a novel, cost-effective method using NIR light to ensure accurate detection of RSIs before surgical closure, leading to a significant reduction in their incidence rates. This approach has been demonstrated in a series of preliminary experiments designed to test the effectiveness of NIR dye incorporation into surgical sponges and their subsequent detection in a biological medium and will be further developed by testing several classes of organic dyes to optimize RSI detection. Completion of these aims will greatly **impact** human surgery by empowering surgeons to avoid retained surgical items using a quick, reliable, inexpensive and harmless method. The unique properties of the proposed tagging agents should allow surgical tools (eg. sponges, retractors, staples, scalples, etc.) embedded with NIR dyes to be easily differentiated against the body's natural biomolecules when viewed with a NIR detector (NIR camera). Surgical sponges, gauze or similar surgical materials that are difficult to detect using conventional methods would be marked with appropriate NIR dyes that are covalently attached to the material. This covalent attachment, paired with the historic non-toxic nature of these compounds allows us to be confident in their ability to be safely utilized. Surgical sponges are made of a variety of materials, such as nylon, rayon, polyester, etc. Any synthetic material can be covalently labeled with NIR dye by adding a small amount of NIR dye labeled monomer before polymerization. The technology for this is well known and used to modify polymeric fiber properties [4]. NIR fluorescence then would be detected before and during the surgical closure. The dyes can be detected by using a simple stationary or handheld imaging detector that projects the image onto a computer monitor where the surgeon can check for the possible presence of RSI by a simple glance.

In the United States alone, the exact incidence rates of RSIs are unknown because of physician and/or hospital unwillingness to report such cases and confidentiality agreements resulting from RSI lawsuits and settlements [5, 6]. However, studies have estimated that RSIs occur in an estimated 1:1000 – 1:1,500 abdominal operations and 1:8000 – 1:18,000 inpatient operations [7, 8]. These numbers may seem innocuous, but when one considers that there are greater than 45 million inpatient surgeries per year in the United states and 234 million operations globally, the frequency of these potentially fatal errors is far too steep [9,10]. The health, monetary, and morale-related repercussions of RSIs can be so severe that the NoThing Left Behind® initiative was started in 2004 to help healthcare providers better understand the events that lead to RSI cases and to develop methods in an attempt to prevent them [11]. Additionally, the legal ramifications are quite significant, with RSI cases falling under the doctrine of *res ipsa loquitur* ("the thing speaks for itself"), wherein the surgeon is automatically declared negligent once an RSI has been detected. Thus, the plaintiff is virtually guaranteed compensation, which has been reported to be anywhere between $50,000-$150,000 depending on the severity of the case and the state in which it is filed [8, 12, 13]. In some cases, minor complications may occur relatively soon following a surgical procedure. Yet in others, serious complications such as fistula formation, sepsis, and even death can occur, with patients retaining the RSI inside of their body months or years after the procedure. In one recently documented case, a sponge left inside of a woman two years after she underwent a cesarean section resulted in the sponge traversing the abdominal wall causing an abscess and sepsis (Figure 1) [14]. Virtually all these cases could have been eliminated with the use of the proposed method in this seed grant application. A significant percentage of surgical sponges that were left behind could have been detected by using the invisible and harmless NIR illumination of the surgical area in conjunction with NIR fluorescence imaging.

Surgical sponges are by far the most common RSI due to their relatively small size, the frequency of their use (especially during emergency procedures), and because once saturated with blood they can become indistinguishable from various tissues and organs [6]. Strategic counting occurs prior to and following surgical procedures to ensure the correct number of sponges are recovered; however, despite the attempts of the OR staff, 80% of RSIs occur even after the count was called correct. Because of innate human error, three separate systems, x-ray, radio-frequency identification (RFID), and data-matrix-coded (DMC) sponges have been implemented in an attempt to limit retained surgical sponges (RSSs) (Figure 1,**right**) [15].

X-ray tags that are embedded in the sponges can be used as the sole detection method or in conjunction with one of the other two. However, even with this technology, false-negative results have been found in 10-30% of RSI cases [16]. Attributing factors to these false readings may be the negligence of the radiologist to correctly identify the x-ray tag in the x-ray scans, or the positioning of the x-ray tag within the patient's body cavity. Still, even when this method is successful in finding the lost sponge it is not an ideal approach as it exposes the patient to unnecessary and potentially harmful ionizing radiation. When RFID tags are used as a detection method, a chip or tag is placed on each sponge. If there is an incorrect count after surgery, a wand that detects radio-frequency waves is passed over the surgical site. Because of the high cost of producing a large number of RFID chips/tags this method is not the most ideal [17]. DMC sponges, while they offer some improvement, are still a basic counting technique and require accurate counting before and after surgery. Additionally, if more materials are needed during the operation, it can slow down critical procedures if each item must be scanned before use [18]. Although these three methods aid in limiting the cases of RSIs, incidences still seem to slip through the cracks. It is for this reason that we are proposing a fourth detection method. Our proposed method can be used in combination with any of the other currently used methods or as a standalone method.

2 QUESTIONS

In order to fully develop this technology, we propose the experiments to address a series of pertinent questions, and they include: (1) Can an additional, safe, effective, and cost-conscious detection method be created using NIR dyes? (2) Can the NIR dye from our proof of concept studies be covalently linked to the surgical tools? (3) Will other NIR dyes offer improved performance in terms of optical properties or stability during covalent attachment to the surgical tools and in biological media? (4) Can the proposed method be easily incorporated into the current surgical procedures of all hospitals?

The proposal focuses on developing the chemistry, analysis and methods toward producing the NIR-labeled tools for direct surgical use. We propose several **specific aims** for this project in order to gather additional preliminary data.

Specific Aim 1 – Synthesize novel classes of dyes for RSI detection including BODIPY, oxazine, and perylene dyes, among others, and determine their optical properties

Specific Aim 2 – Non-covalently bind the novel dyes to surgical sponges to analyze their performance in RSI detection as done in our proof of concept studies to determine which classes offer improved detection and stability for clinical translation.

Specific Aim 3 –Prepare a covalently linked surgical sponge as a proof of concept and determine leeching in biological materials.

Specific Aim 4 – Determination of *in vivo* performance of hit compounds in mouse models by Dr. Jeffrey Olson at Emory Healthcare (EHC).

3 STUDY METHODOLOGIES

3.1 AIM 1: Synthesize novel classes of dyes for RSI detection and determine their optical properties – Our proof of concept experiments were performed using indocyanine green (ICG), a well characterized and FDA approved cyanine dye [19]. While this dye has been proven useful in a number of biomedical applications, it is far from the only NIR organic dye which we can explore. There are multiple classes of fluorophores which we have a wealth of experience working with (see the PI, Dr. Henary's biosketch). These classes include penta- and heptamethine cyanines, squaric acid dyes, phenoxazines, BODIPY dyes, and perylene dyes (Fig. 2). Each of these classes of compounds shows absorption and emission in the NIR range and can be modified for incorporation in polymeric fibers.

Cyanines – Cyanine dyes are a vast and highly tunable family of compounds. They consist of two nitrogen containing heterocycles connected by a conjugated polymethine bridge. Both the bridge and heterocycles can be modified to imbue these dyes with any desired chemical properties. Various cyanine dyes have found use in many medical procedures for labeling both healthy and cancerous tissues [20]. ICG, used in our preliminary studies, is a member of this family.

Squaric acid dyes – Squaric acid dyes are structurally related to cyanines, but contain an oxybutene ring. While they are traditionally less stable than cyanines, we have recently developed a new type of squaric acid dye which shows remarkable stability by incorporating positively charged ammonium cations to interact with the central ring (manuscript in preparation). These squarylium dyes display excellent molar absorptivity and fluorescence quantum yield and were incorporated in this proposal to lower the limit of detection for the marked sponges.

Phenoxazines – We have selected this class of compounds to mimic methylene blue: another FDA approved imaging agent. We have substituted oxygen for the sulfur of methylene blue to improve the molar absorptivity and quantum yield of the fluorophore [21]. These structures are more compact than the cyanine and squaric acid dyes and are much more stable in solution.

BODIPY dyes – BODIPY dyes are a well-known and thoroughly investigated class of compounds. They are a more compact dye, similar to the phenoxazines, and are quite stable in solution.

Perylenes – Perylenes have been shown to be highly chemically stable and thermally stable up to 350°C [22]. Recently, water soluble perylenes have been described in the literature, which has allowed for the use of these compounds in aqueous media.

The rationale behind these dye selections is that key characteristics of these NIR dyes will make them effective for intra-body detection, specifically the absorbance and fluorescence $\lambda_{max,}$ the Stokes shift, and the quantum yield. These dyes and their modifications are well known, and we have a wealth of experience in the synthesis, purification, and characterization of NIR dyes; as such, we will only prepare representative members of each class to test in non-covalently linked surgical sponges. Compounds which show good optical properties both when free and when incorporated into the sponges will be earmarked for further study in covalent polymer attachment. The majority of these dyes are also non-toxic and have high elimination rates when injected *in vivo* [1-3, 19-21].

Before further study, we will fully evaluate the various optical properties of the key NIR dyes synthesized, including absorbance, fluorescence, molar absorptivity, quantum yield and solvatochromic/hydrophobicity experiments to fully determine the characteristics in serum. Briefly, absorption measurements will be achieved with a Perkin-Elmer Lambda UV/VIS/NIR (Lambda 50) Spectrophotometer (Norwalk, CT). Laser Induced Fluorescence (LIF) emission spectra will be acquired using a K2 Spectrofluorometer (ISS, Champaign, IL) equipped with a R298 Hamamatsu Photomultiplier Tube (Bridgewater, NJ) with laser excitation (Laser Max, Rochester, NY). Slit widths will be set to 2 mm and integration time of 3 s. All optical measurements will be

performed at 37 ◦C in 100% fetal bovine serum (FBS) buffered with 50 mM HEPES, pH 7.4. The hydrophobic characteristics of the dyes will be evaluated by acquiring absorbance and emission spectra of the dyes in varying ratios of methanol-nanopure grade water mixture (in the range of 0–100% methanol/water) until there was no further discernible spectral changes. After analyzing the synthesized compounds using all of the methods described above, the compounds with most promising optical properties corresponding to high molar absorptivity and fluorescence quantum yield that display limited aggregation in serum will be subjected to further evaluation as outlined below.

3.2 AIM 2: Non-covalently bind the novel dyes to surgical sponges to analyze their performance in RSI detection as done in our proof of concept studies to determine which classes offer improved detection and stability for clinical translation – Migrate and entrap the most promising NIR dyes into surgical sponges to produce NIR-marked surgical sponges then analyze the fluorescence intensity changes that correspond to the migration of the NIR fluorophores into the material

In our preliminary studies, we entrapped various concentrations of ICG (1 nM, 1μM and 1mM) in surgical sponges as a proof of concept for the use of NIR dyes in RSI detection. Using surgical sponges which had been treated with ICG, we found that the dyes could be easily detected even in highly scattering biological media (Figs. 3-5). In order to determine which class will show the optimum physicochemical and optical properties for use in labeling RSIs, we will prepare representative compounds from each class and, if they display promising optical properties, test them as we did in our preliminary studies with ICG. NIR dyes can be entrapped in the interior of polymeric fibers using suitable solvents that migrate the hydrophobic NIR dye into the polymer where it is entrapped upon solvent evaporation. This method successfully mimics the covalent attachment method and to achieve this we plan to use a technology similar to the one we developed earlier to entrap dyes into polymeric microspheres [23]. Briefly, surgical sponges will be soaked in boiling water for several hours to allow the polymer to swell; a slow swelling process is required not to damage the fibers' mechanical integrity. Solutions of the dyes dissolved in water or suitable organic solvents, depending on the solubility of the dye, will then be added dropwise to the sponge solution with vigorous stirring. Following addition of the dye, the sponge will be removed from the solution and rinsed with cool water until it rinses clear at which point it will be dried using a commercial hot-air dryer. While non-covalent absorption of the compounds into the polymers is impractical for use in actual medical products, this will give us a quick and easy means to determine which dye families we should investigate for covalent attachment.

Once the dyes have been incorporated into the sponge fibers, we will perform *in vitro* NIR fluorescence imaging to ensure that the sponge exhibits a strong contrast to the blood. To do this, dye-infused sponges will be soaked in blood-filled receptacles for several hours prior to imaging. Porcine blood and organs will be used due to their similarity to human tissues. To detect the presence of the dye-infused sponges in a highly scattering medium in our preliminary studies, we used a NIR night vision monocular and a suitable laser (700-900 nm depending on the dye) to irradiate the samples. While this imaging system used in our preliminary studies is non-ideal, it is simple, commercially available, and cheap; all desirable qualities for a novel RSI detecting system. The presence of leached dye will be detected using extraction followed by simple spectroscopic or HPLC-MS methods.

3.3 AIM 3: Prepare a covalently linked surgical sponge as a proof of concept and determine leeching in biological materials – NIR dyes have already been used to detect authentic drugs and clothing [4]. We can incorporate NIR dyes into the rayon, and/or polyester fibers of surgical grade sponges using copolymerization of labeled and unlabeled monomers and can develop this method using standard chemical technologies. Medical applications of the dyes cited above indicate that the detection of NIR marked sponges inside of a patient's body prior to the surgical team closing the surgical site or even after the closure is made is also feasible. The dye must be covalently bound to the polymer that constitutes the sponge in order to ensure the dye will not leach into the patient's body. To show that our proposed compounds can be covalently linked to the final surgical materials, we will prepare a single covalently linked NIR tagged polymer as a proof of concept (Fig.6). While we cannot prepare the final sponge from the polymers, preparation of labeled polymers will suffice to prove the feasibility of this method. As shown in Fig. 6, we will functionalize the fluorophores with a conjugating group (C.G.) as discussed in Fig. 2 this will either be attached to the fluorophore directly or through a short linker. The conjugating group will be selected to have a complimentary reactivity to functional groups present on the polymer. The functionalized dye can then be coupled either with the monomer for subsequent copolymerization or with the full polymer. Each path offers different advantages and disadvantages. Reaction of dye with monomer will ensure higher loading of the fluorophore on the final polymer, but the harsh reaction

conditions of the polymerization could decompose some of the less stable dyes. Reaction of dye with the polymer will make it more difficult to achieve high loading, but will allow us to use milder conditions to ensure that the dye does not decompose. Both reaction pathways will be investigated to determine the optimum method. We will test that the polymers retain fluorescence comparable to the non-covalently tagged surgical sponges by measuring the relative fluorescence of equal masses of each to ensure that the covalent link will allow for the excellent detectability that we achieved in our preliminary studies. If this aim is successful, we will begin approaching the companies that make the surgical sponges to further test the applicability of this method by incorporating the tagged polymers into surgical sponges.

3.4 AIM 4: Determination of *in vivo* performance of hit compounds in mouse models by Dr. Jeffrey Olson at Emory Healthcare – Through our collaboration with Dr. Olson, we will test the effectiveness of the dye labeled surgical sponges which show the most promise for RSI detection. Dr. Olson is an experienced neurosurgeon at EHC and has a wealth of experience in clinical effectiveness studies (see letter of support). After the best dye for RSI labeling has been identified, we will tag a set of surgical sponges to be used by Dr. Olson in *in vivo* validation of the method in mouse models. Dr. Olson will test the non-covalently tagged sponges to ensure that they remain fluorescent even after extended use that may be expected in surgery and that they will be detectable during normal operative use. This will provide the link that we need to show that this method can be successfully translated to the clinic to improve surgical outcomes by reducing RSI incidences and will lead to future clinical effectiveness studies.

4 ANTICIPATED RESULTS OF THE STUDY

In our preliminary studies, we showed that non-covalent labeling of surgical sponges with ICG allowed for facile differentiation between the surgical materials and biological media using a cheap, commercially available imaging system. In aims 1 and 2, our goal is to explore other classes of dyes to determine if they will offer improvement over ICG. We expect that, by using other classes of dyes, we can obtain compounds with better optical properties and improved stability relative to ICG. We will investigate the different classes to determine which offer the best all-around performance for RSI detection using non-covalently labeled surgical sponges, and we expect that we will be able to improve on the imaging performance we obtained with ICG. In aim 3, we seek to prepare a proof of concept covalently labeled surgical sponge polymer. We expect that the dye used will retain its original properties after incorporation into the polymers and will enable their detection similar to what we achieved in the non-covalently labeled sponges. Lastly, in aim 4, we seek to prove the applicability of this strategy in *in vivo* mouse models. We expect that even after extended exposure to biological material, the NIR dyes will allow clear differentiation between surgical materials and biological material. The funds requested in this research proposal will help the PI and co-I to initiate a new independent research project, and establish a pathway for obtaining major grants. Upon successful completion of the proposed technology and using the data we will obtain from the current research Seed Grants in Health Care Innovation we plan to seek additional funding for the development of NIR covalently labeled surgical sponge material from either federal (NIH R01), Department of Defense (DoD) and private (sponge manufacturers) sources for full clinical effectiveness studies.

5. SIGNIFICANCE

The significance of the proposed research is the following: (**1**) The development of a novel method for detecting RSIs prior to surgical closure, (**2**) the new method will work synergistically with current methods to allow accurate RSI detection, (**3**) the new method allows for the detection of RSIs with cheap and available imaging systems, and (**4**) the new method uses harmless NIR light as opposed to current methods which use harmful radiation.

COLLABORATION PLAN

Dr. Maged Henary, the PI, is a synthetic organic chemist at Georgia State University with expertise in heterocyclic chemistry. He has a wealth of experience in the design and synthesis of NIR fluorophores for the detection and image guided surgery of tumors. Dr. Olson is a clinical neurosurgeon at EHC with vast experience in brain tumor treatment and in the translation of research projects to clinical use. His expertise will be invaluable in performing animal testing of the labeled surgical instruments and in preparing the methods for clinical translation. Mr. James Todd, a Registered Staff Nurse ECC (Emergency Care Center) from Grady Memorial Hospital (GMH), will be operating in a consultant capacity for the current research project. Because this research is centered on eliminated cases of retained surgical sponges, Mr. Todd will educate us in current methods being used at GMH, pros and cons with the current methods, and as a liaison between the lab and clinical applications.

Figures:

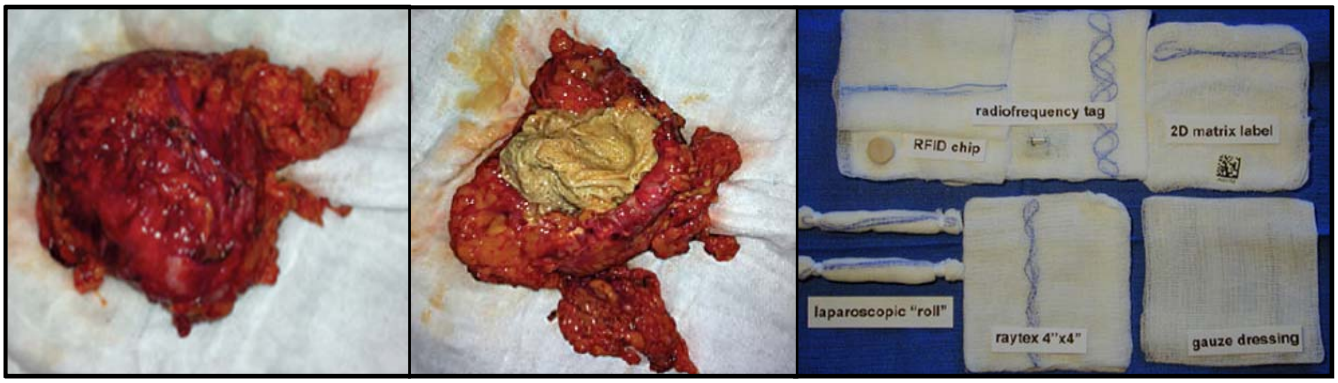

Fig. 1. (**left**) An excised sponge from a two year old surgery surrounded by scar tissue (**middle**) sponge with scar tissue removed [13] (**right**) various surgical sponges with x-ray tags (blue), RFID chip and tag, DMC, and no detection method (gauze). [[1]4]

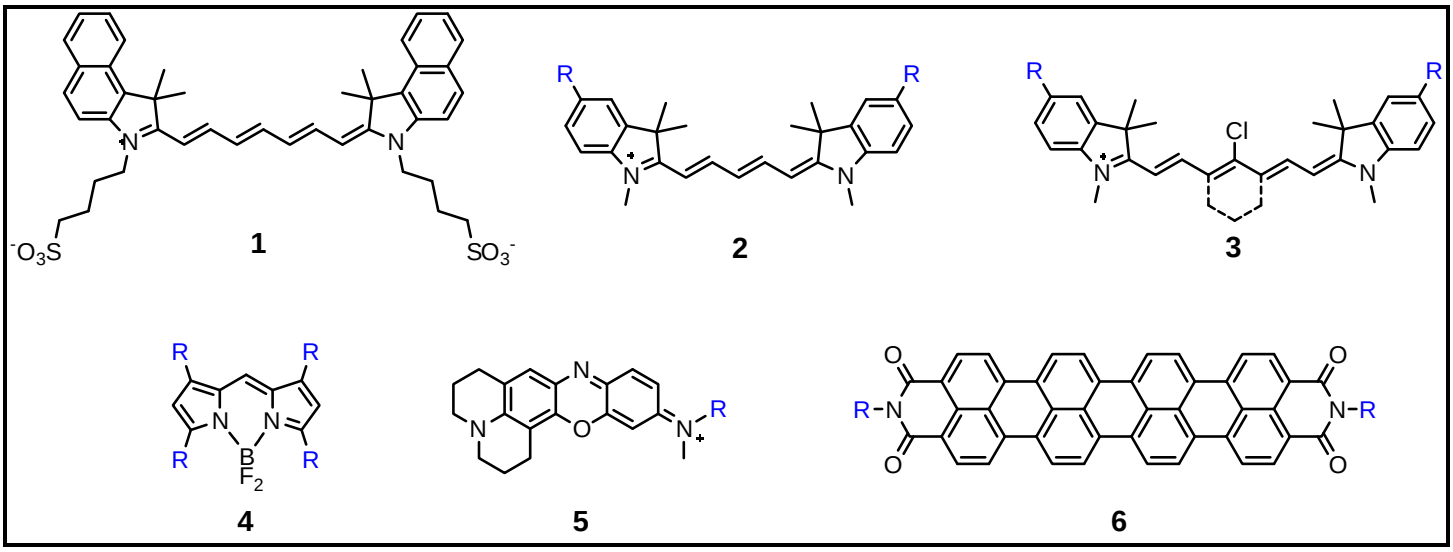

Fig. 2. (**1**) Structure of ICG and general structures of the proposed classes of dyes: (**2**) pentamethine cyanines (**3**) heptamethine cyanines (**4**) BODIPYs (**5**) phenoxazines (**6**) perylenes. R groups can be OH, NH_2, or COOH depending on the desired binding functionality and, for some dyes, can be linked to the dye through a short spacer (eg. $(CH_2)_n$ where n = 2-5.

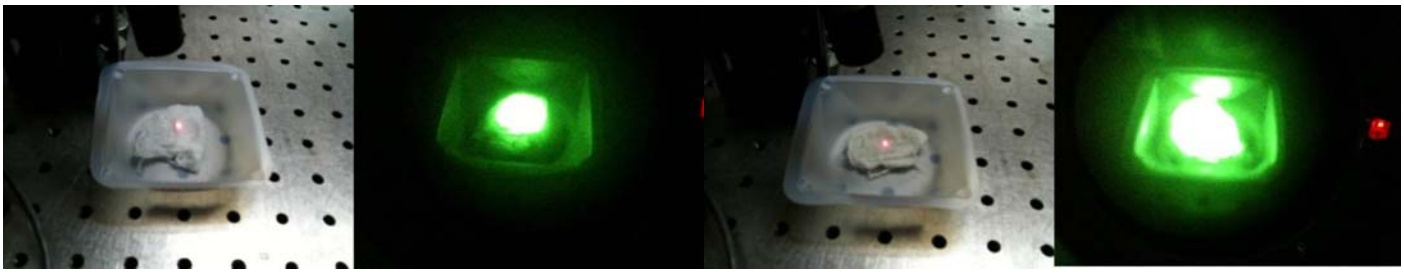

Fig. 3. (**left**) Lap pad treated with 1 µM ICG and irradiated with a 700 nm laser; Irradiated 1 µM lap pad viewed with a NIR night vision scope (**right**) Lap pad treated with 1 mM ICG and irradiated with 700 nm laser; 1mM lap pad viewed with NIR night vision scope [22].

Fig. 4. (Left) I µM-treated 0.64 x 0.64 cm cottonoid **(Middle)** I µM-treated cottonoid soaked in blood and placed in a section of porcine intestine **(Right)** I µM-treated cottonoid irradiated with a 700 nm laser and viewed with a NIR night vision monocular [22].

Fig. 5. (Left) I mM-treated 0.64 x 0.64 cm cottonoid **(Middle)** I mM cottonoid soaked in blood and placed in a section of porcine intestine **(Right)** I mM cottonoid irradiated with a 700 nm laser and viewed with a NIR night vision monocular [22].

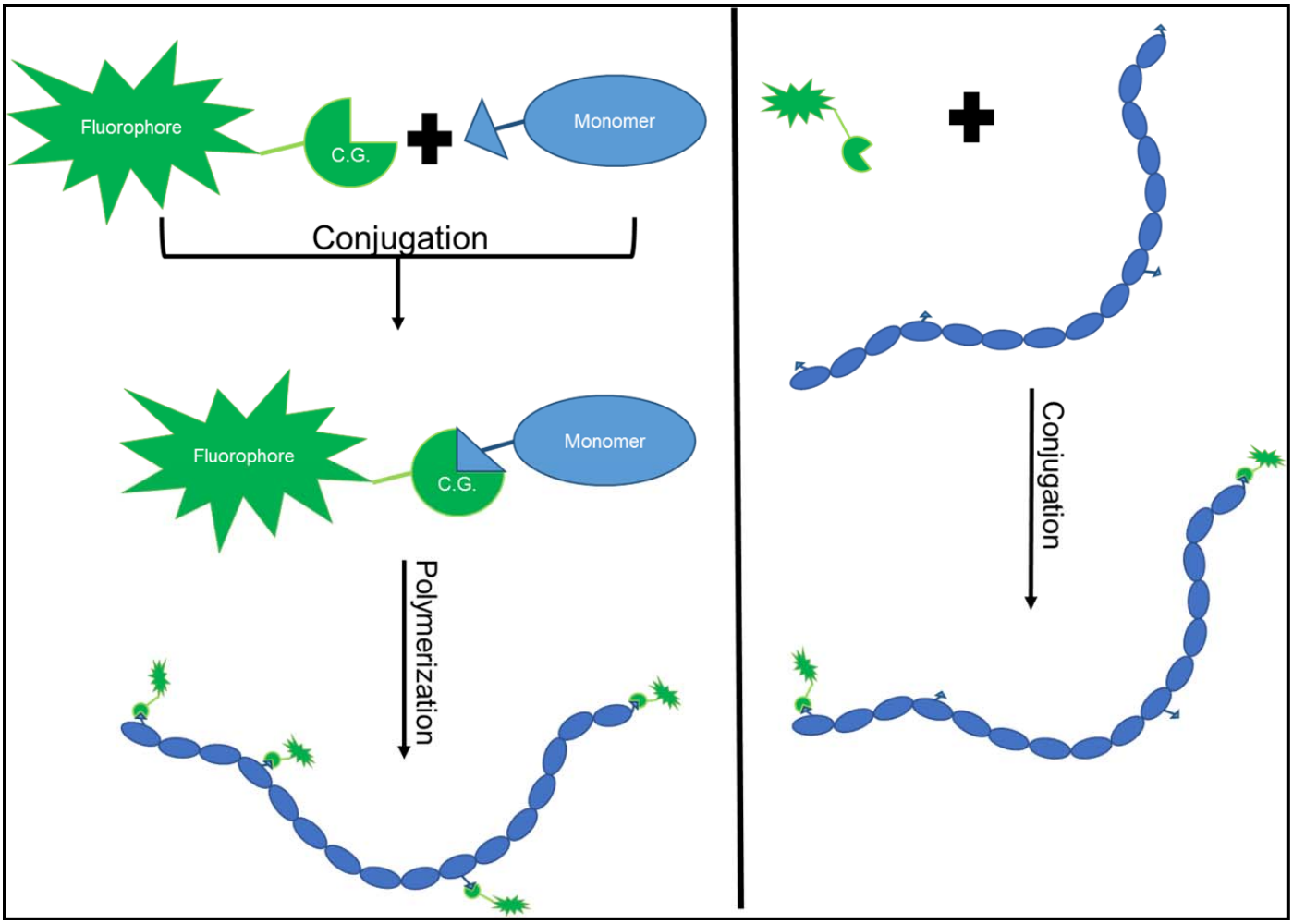

Fig. 6. Schematic representation of the two pathways for incorporation of the synthesized dyes into polymeric materials. (**left**) conjugation of the dye to monomer before copolymerization. (**right**) Conjugation of dye to full polymer.

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