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Using India Ink as a Sensor for Oximetry: Evidence of its Safety as a Medical Device

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Abstract

Clinical EPR spectroscopy is emerging as an important modality, with the potential to be used in standard clinical practice to determine the extent of hypoxia in tissues and whether hypoxic tissues respond to breathing enriched oxygen during therapy. Oximetry can provide important information useful for prognosis and to improve patient outcomes. EPR oximetry has many potential advantages over other ways to measure oxygen in tissues, including directly measuring oxygen in tissues and being particularly sensitive to low oxygen, repeatable, and non-invasive after an initial injection of the EPR-sensing material is placed in the tumor. The most immediately available oxygen sensor is India ink, where two classes of carbon (carbon black and charcoal) have been identified as having acceptable paramagnetic properties for oximetry. While India ink has a long history of safe use in tattoos, a systematic research search regarding its safety for marking tissues for medical uses and an examination of the evidence that differentiates between ink based on charcoal or carbon black has not been conducted.

Methods—Using systematic literature search techniques, we searched the PubMed and Food and Drug Administration databases, finding ~1000 publications reporting on adverse events associated with India/carbon based inks. The detailed review of outcomes was based on studies involving >16 patients, where the ink was identifiable as carbon black or charcoal.

Results—Fifty-six studies met these criteria. There were few reports of complications other than transient and usually mild discomfort and bleeding at injection, and there was no difference in charcoal vs. carbon black India ink.

Conclusions—India ink was generally well tolerated by patients and physicians reported that it was easy to use in practice and used few resources. The risk is low enough to justify its use as an oxygen sensor in clinical practice.

Keywords

Carbon particles; EPR sensor; India ink; Structured literature review; Adverse Events

The clinical value of repeated measurements of oxygen in tissues rests fundamentally on the central roles that oxygen levels play in a large array of physiological and pathophysiological

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processes. Despite this importance, oxygen in tissues is seldom measured directly. If measured at all, oxygen level is assessed in the vascular system, while the greater need for many clinical applications is to know the oxygen level in tissues. Diseases whose treatment strategies would benefit from taking tissue oxygen into account include tumors, peripheral vascular disease, and wound healing. For example, in radiation treatment of cancers, the level of oxygen in the tumor is the single most important variable that affects the treatment outcomes [1]. Developing treatment plans taking this into account, especially if the oxygen in the patient's tissues can be successfully manipulated to improve the therapeutic ratio, is a key motive for identifying oximetry methods than can provide this information to clinicians while being readily and noninvasively integrated into clinical care for these patient groups.

Several unique features of EPR oximetry have the potential to address these needs, i.e., after a paramagnetic sensor is initially injected or implanted into a tissue of interest, EPR oximetry can make repeated and noninvasive measurements that directly assess tissue oxygen. These EPR measurements can be made repetitively in two clinically important ways: (1) Because scans can be taken virtually continuously for several minutes while patients rest comfortably, minute by minute changes can be assessed, including evaluating whether the tissue is responsive to the patient's breathing enriched oxygen. (2) The patient can also be measured again at any time, over a period of up to many months if appropriate. This allows evaluating the tissue's response to therapy and monitoring progression of disease. Another clinically important advantage of EPR is that it is particularly sensitive to measuring very low levels of oxygen in tissues, i.e., at the hypoxic levels that are prognostic of a tumor being potentially more resistant to therapy and/or of its greater likelihood of being aggressive. In order to take advantage of these features of EPR, a paramagnetic sensor needs to be initially injected into tissue. India ink (reported here) is one such sensor.

India ink as a paramagnetic sensor has several advantages. For patients, by way of being similar to cosmetic tattoos, a mark made by superficially injecting paramagnetic India ink can be easily understood. Also, because of its similarity to cosmetic tattoos and medical ink markers already in use, the regulatory path to marketing it is relatively straightforward. The disadvantage for patients is its permanent cosmetic appearance, even when small or not publicly visible (such as on the bottom of the foot or inside the mouth, cervix or colon). For the currently available clinical applications using L-band EPR in vivo oximetry, the principal technical disadvantage is its capability to place and measure sensors in tissues only only within ~5–35 mm from body surfaces. In particular, because of the relatively broad line of India ink, the working depth is less than 10 mm; moreover, in order to get a representative depth in the tumor, we usually restrict the measurements to tumors that are within 5 mm of the surface. (In contrast, the working depth of L-Band for very narrow-lined oximetric materials such as sensors made from lithium phthalocyanine can be 30 mm; in this case, we restrict the measurements to tumors within 25 mm. Under advanced preclinical development and preparations for initiating clinical trials is an implantable resonator that would remove any restriction on depth [5, 6]).

For India ink to be successful as a sensor for EPR oximetry, it is necessary to ensure that its carbon particles have strong EPR signals that are sufficiently sensitive to the presence of oxygen. To that end, Bernard Gallez et al. [2] have investigated many candidate inks using

carbon black [3] and *charcoal* [4]. Swartz et al. [5,6] have successfully used two inks with the necessary EPR properties to measure tissue oxygen in vivo in 27 humans with 36 ink injections using L-band EPR. Spurred by the importance of conducting clinical studies of oxygen in tumors, this paper reports on a systematic review of the medical literature conducted to determine whether there are any safety concerns in using India ink for oximetry and whether there is evidence of any differences in inks based on charcoal or carbon black.

India ink is composed of carbon particles combined with a suspending agent (i.e., water and potentially other ingredients) to form an aqueous suspension. Carbon particles are a large class of compounds that may have chemisorbed oxygen complexes such as carboxylic and phenolic groups on their surfaces to varying degrees, depending on the conditions of their manufacture.

When carbon particles are used in humans, three attributes about carbon are most significant: its purity, whether it is activated, and how it was made:

Factor 1: *Purity* is relevant because, while carbon is basically inert with a several-thousand-year-long history of its safe use in humans, impurities associated with incomplete combustion during its production can have more, or more serious, side effects. For example, polycyclic aromatic hydrocarbons (PAHs) that come from incompletely combusted fuels may be carcinogenic.

Factor 2: *Activation* of carbon particles refers to being reheated and oxidized after their initial production, with the outcome that particles become highly porous. When carbon is prescribed to be ingested for people following consumption of a toxic substance, this property is desirable because it enhances carbon particle's adsorptive properties, making it more effective as a detoxifier. For this reason, *activated* forms of carbon are more likely to be available in pharmacies for human use. However, there is no evidence that this property impacts its safety or effectiveness when used for tattooing/medical marking or as a sensor for oximetry.

Factor 3: How it is made (and from what). In part because there are numerous variations of carbon, numerous synonyms of each variant, and an inconsistent use of these synonyms [7], the safety data sheets typically use a name and the unique numerical identifier of chemical substances assigned by Chemical Abstracts Service (CAS). This review refers to the two main classes of carbon particles used in India ink as *carbon black* (CAS 1333-86-4 [or -3 or -5]) or *charcoal* (CAS 7440-44-0).

While there is no evidence that one class of carbon is safer than the other when used in medical or cosmetic marking, there is a long history of cosmetic tattoo inks being more likely to be made from *carbon black*. In addition, US brands of inks used in medical marking usually use *carbon black*, while European, Asian and Australian brands for medical marking typically use *charcoal*. Therefore, this review has deliberately distinguished these two classes of carbon in reports of adverse events and will evaluate whether there is any evidence of differences between them.

As noted above, India inks also have a suspending component. There is only one ingredient common to all cosmetic tattoo inks and all medical marking inks found in our review: water. While charcoal based inks tend to not add anything else, most cosmetic tattoo inks and carbon black inks have other ingredients added for purposes of improving preservation, viscosity, pH, etc. (See Table 40.1 for details.) While most of these ingredients are food grade and listed as generally regarded as safe (GRAS), these additional ingredients have the potential to be the source of any side effects and so need to be considered when evaluating the safety of India ink for markers in oximetry.

Beyond the ingredients of the 'India ink' per se, other factors may impact whether any problems are reported in each study, e.g., the sterility of the manufacturing process, the hygienic practices of the tattooist or the tattoo facilities, the training and qualifications of the injector, or any problems encountered during its administration (like unintentionally perforating an anatomical border during injection of a polyp in the colon). Similarly, medical use studies may report on the efficacy of the marker for its intended use, e.g., if the intent is to mark areas for future resection, the authors may focus on how visible the marker was during surgery. These problems may not have any bearing on the marker as used in oximetry. Consequently, we will consider these additional factors as important contexts in evaluating the evidence of the safety of India ink for oximetry.

India inks deliberately injected as permanent tattoos have a long history of usage in humans dating back at least several thousand years [8, 9]. The ~5300-year-old mummified 'iceman' whose tattooed remains were recovered in 1991 from a glacier provides evidence of the long use of carbon markings in Europe, also suggesting their likely association with medical treatment [10, 11]. More importantly, there is an extensive body of evidence, based on millions of people from all races many of whom have had their tattoos for a lifetime, that carbon tattoos are generally very safe and not life-threatening.

The object of carbon marking of the skin, whether for cosmetic, punitive or medical reasons, is to leave a permanent and visible marking that is otherwise relatively inert. Because it is administered using needles to push or inject the particles below the epidermal layer, acute biological side effects such as superficial pain and bleeding may occur, which should resolve within ~2 days [12].

The intended uptake and long-term storage of ink particles by the dermal and sub-cutaneous fibroblasts are based on a specific non-inflammatory defense mechanism that protects the living body against injuries and invasions by non-toxic foreign bodies without necessarily involving immune reactions. Studies of the basic pathology of tattoos done in animals [13] and humans [14] substantiate the body's response to carbon as an inert foreign object. These studies show that, within 24 h after injection, carbon particles are endocytosed by fibroblasts as well as macrophages in the dermis and subcutis. Biopsies of human tattoos at 1 month showed no sign of inflammation, scarring or necrosis. Biopsies at 1, 3, 40 years all found stable carbon particles within cell membranes. Macrophages, present in the epidermis, were observed to take up larger particles and were at least partially responsible for initial removal of some of the ink from the epidermis. With the reformation of the basement membrane as recovery from the trauma from the needle is completed, there is no further removal by

macrocytes. Because the fibroblasts tend to remain in place and live a long time, the carbon particles they contain remain permanently embedded in the body and in their original location.

Of particular relevance for this review are medical uses of carbon particles, particularly as used for marking, which parallel the intended use for oximetry. Seven uses of inks for medical marking have been reported in the literature: (1) marking the gastro-intestinal tract for future surgical biopsy or removal, e.g., marking polyps or tumor sites in the colon for rectal and sigmoid resection [15–24]; (2) marking nonpalpable tumors in the head and neck, e.g., marking recurrences, detectable only by imaging methods after prior thyroidectomy, for surgical resection [25–27]; (3) marking nonpalpable breast tumors for surgical removal [28–31] or for stereotactic marking of the track during needle biopsy to guide anticipated future surgery [32–35]; (4) marking the field of radiation for radiotherapy delivered in multiple fractions [36]; (5) marking the periphery of breast cancer tumors for future surgery following neoadjuvant chemotherapy which might change the original margins [37]; (6) identifying the sentinel lymph node of primary tumors where the route of lymphatic drainage is ambiguous such as head and neck, breast cancers in the medial third of the breast, or periclitral vulvar tumors, which may have unilateral or bilateral lymphatic drainage [38]; and (7) marking superficial tissues to use as a paramagnetic sensor for EPR oximetry [5, 6, 39].

There are four primary ways that medical uses differ from cosmetic tattooing (in addition to being more likely to use inks manufactured or prepared in-house under more controlled circumstances that adhere to pharmacopeia standards). First, medical marking uses a hollow needle for injecting the ink rather than a tattoo machine, with the intent to place the ink superficially but below the dermis or mucosal surface. Second, medical uses may target tissues not used in cosmetic tattoos, e.g., the submucosa of the colon or oral cavity or deeper tissues including tumors. Third, although pigments other than carbon-particle-based may be used in medical marking, the purposes are not cosmetic and so a more restricted set of pigments (green, blue or carbon particles) are used. Fourth, injections of pigment at a single site may be larger (e.g., the volume of pigment injected deep into the submucosa and transmural regions prior to colorectal neoplasm resections [40]).

One very pertinent result of the very lengthy and widespread use of India ink in human subjects was that the Food and Drug Administration (FDA) does not claim regulatory jurisdiction over its manufacture and use. (Note: worldwide, in the EU, Asian countries, and New Zealand/Australia among others, governments have also opted not to regulate the inks injected into humans.) Instead, the FDA has chosen to designate all such inks (whether used for cosmetic or medical marking) as ‘cosmetics’ [41] which do not require FDA approval based on evidence of its safety and efficacy before being marketed. Manufacturers may optionally choose to apply for a 510(k) premarketing notification, e.g., two US carbon black inks (Spot® and Endomark™) are marketed as an approved ‘medical device’ for marking.

While the peer-reviewed literature and marketing brochures often contain claims about the safety of using India ink as a medical marker, the contents are not based on a systematic review of the available evidence and may be oriented toward enhancing sales or

demonstrating its use. Therefore, we undertook a systematic search of the published literature and other key databases to examine available evidence about the safety of India ink used in medical marking and to obtain data about the inks that would allow differentiating between carbon black and charcoal.

The databases used in the systematic search were PubMed (for peer-reviewed articles) and the FDA's database for reporting post-marketing events and [ClinicalTrials.gov](https://www.clinicaltrials.gov) (for other official but non-peer-reviewed sources). A keyword and title search resulted in 975 articles, dating from 1893 through July 2016, that reported clinical studies of adverse events. ABF and VAW independently reviewed the titles and abstracts of all 975, eliminating those reporting only on tattoo *removal*, nonmedical or chemical analyses, medical uses other than for marking (e.g., detoxification), nanoparticles, or animal studies. All studies cited in the manufacturer's website for brand name medical marking inks were included. Among the remaining 178 articles reviewed in detail, 46 were dropped after reading of the article revealed that they were not pertinent. In addition, where articles did not specify the type of carbon used, the safety data sheets for named manufacturers were consulted to distinguish charcoal and carbon black. Nonetheless, the majority of articles about cosmetic tattoos and 42% of those reporting medical uses did not provide enough information to distinguish carbon black from charcoal ink.

Indeed, among cosmetic articles, most pigments were unidentified beyond their color: 63.4% reporting on 'black' pigment were case studies involving one or two people with tattoos, and 24.4% were broad overviews about tattooing and its general potential for side effects (i.e., were not systematic reviews). Among the remaining 12.2%, three were surveys about the experience of people with cosmetic tattoos and two were studies of cadaver tissue containing cosmetic tattoos.

As reported in the three surveys and the overviews of cosmetic tattoos, the overall rate of experiencing a problem associated with black ink is very low (~2%). Although there are rare serious complications [42], the overwhelming majority of problems, if any, were not serious, i.e., would not be reportable adverse events in a clinical study. (Of note, the most common 'problems' associated with cosmetic tattoos, infection due to poor hygienic practices during or following the tattooing, 'regret' and desire to remove the tattoo, are not associated with a pigment type per se. Likewise, transient bleeding and soreness are associated with the tattooing process, not the pigment.)

Potential problems associated with black ink in cosmetic articles included:

- a. Acute reactions (i.e., usually resolving in a few hours or days): local reactions, bleeding, pain and local inflammation (lasting longer with infection). Local reactions reported include local itchiness, redness, swelling, stinging.
- b. Chronic reactions (i.e., late onset and/or lasting for several weeks or longer): continuing or late onset local reactions, color fading, granulomas (i.e., non-painful raised protuberances at the edge or on the surface of the tattoo), scar formation, excessive reaction to foreign bodies, and sensitivity to light.

Of particular interest here are the 53 articles that discussed any type of medical use for marking *and* identified the pigment as either carbon black (62.3%) or charcoal (37.7%). For the details reported in this summary, we also excluded the case studies, studies with <16 patients, and the overviews from this group, leaving (a) 11 studies involving a total of 547 patients with 1 injections of carbon black ink (Table 40.2) and (b) 12 studies, totaling 3850 patients with 1 injections using charcoal ink (Table 40.3). All 23 of these larger studies involved using carbon-based inks for pre-surgical marking, albeit used in a variety of organs, e.g., colon, breast, esophagus, stomach. Each study was carried out in a different medical center located in 11 different countries.

This final, most pertinent group of 23 large studies reported either no or only a few minor problems attributable to either carbon black or charcoal ink. No acute symptoms related to hypersensitivity or granulomas were reported for either type of ink.

There were some apparent differences between charcoal and carbon black inks: The charcoal studies were more likely to mention acute pain or bleeding, but the rate of experiencing any pain was well under 1% and usually described as well tolerated or mild and transient, i.e., associated with the injection. One patient's lesion was temporarily enlarged. For carbon black inks, three studies reported local dispersion of the ink. One study using ink to mark sentinel lymph nodes reported that one *non*-sentinel node was marked. However, these few differences in reporting outcomes do not appear to be attributable to the ink so much as to the medical use and the absence of any standards for clinical studies to report outcomes for procedures that are adjuvant to other therapies. Because the ink was only ancillary, authors if discussing the ink at all tended to focus on whether the ink performed adequately as a marker to correctly and readily identify lesions, whether patients tolerated the injections well, and the advantages for clinicians, i.e., how easily the injection was performed and how little time or few financial resources it necessitated.

Because many medical uses involved surgical resection, postsurgical specimens could be examined. For such studies, for both charcoal and carbon black ink, the reported histology was generally consistent with the expected low-grade biological response to ink as an inert foreign object, i.e., most were mild and consistent with the ink being labeled as 'well tolerated'. The typical time between injection and obtaining the specimen was ~14 days when used for pre-surgical marking. However, for other uses, or when the lesion was not resected, the follow-up period reported was up to 14 months for carbon black and 7 years for charcoal, although no long-term problems were reported.

In summary, the overwhelming body of evidence suggests that both carbon black and charcoal inks used in medical marking are well tolerated by patients and easily performed with minimal resources of time or supplies in a clinical setting. Based on this systematic search of studies over several decades of worldwide experience, we conclude that the evidence based using charcoal and carbon black inks for medical marking supports the safety of its use as a paramagnetic sensor for oximetry.

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Table 40.1

Black dye and other ingredients in brand name India inks, by reviewed use in cosmetic tattooing, medical marking or both

Use for ink (brands)	Dye (CI & CAS codes)	Other ingredients ^a
Cosmetic Tattoo Only (Demco Black, Makkuro Sumi Blackout Tattoo, True Black, Kabuki Outlining Ink, Wilson's Black Tattoo Ink)	Carbon Black CI#77266 CAS#1333-86-4 Black Pigment 7 CI#77266	Water Ethyl alcohol Isopropyl alcohol Shellac Propylene glycol Hamamelis virginiana (Witch Hazel) Glycerin (glycerol) PEG-200 glyceryl stearate PVP (polyvinylpyrrolidone) Rheology modifier acrylic monomer
Medical Marking and Non-Medical Uses (Kor-i-Noor, Pelikan, Higgins Black Magic, Defco/BD India Ink)	Carbon Black CAS#1333-86-3	Water Ammonia hydroxide Phenol Ethylene glycol Shellac Natural resins Undisclosed nonhazardous ingredients
Medical Marking Only (<i>carbon black</i> : Accu-Tatt, Steritatt, Endomark, Spot, Black Eye, Printex) (<i>charcoal</i> : Sterimark, Carbo-Rep, Charcotrace, Carlo Erba)	Carbon Black CI#77266 CAS#1333-86-5 Charcoal CAS#7440-44-0 Activated Charcoal CAS#7440-44-0	<i>Both</i> : Water, Sodium chloride <i>Carbon black inks only</i> : Hamamelis virginiana/Witch Hazel Glycerin/Glycerol Isopropyl alcohol polysorbate Benzyl alcohol Simethicone Carboxymethylcellulose sodium salt <i>Charcoal inks only</i> : Gum Arabic ^b Sodium hydroxide ^b Hydrochloric acid

^aOther ingredients include any used for suspension, preservatives, improved viscosity, medical preventive uses, etc. Most MSDS indicate, or use CAS indicating, ingredients are food safe or cosmetic safe and some add 'sterile' or 'medical grade'

^bListed as being used for pH only as needed (only for Charcotrace)

Table 40.2
Review of 11 studies with >16 subjects, using carbon black based inks for medical marking

Author (ref #) Year country	Ink brand or homemade in pharmacy	Medical use	# of study participants and areas injected	Adverse events/reactions	Other problems/comments?
Sun [24] 2009 China	Pelikan	Long-term localization of atrophic gastritis	53 patients	None	None
Fennerty [17] 1992 USA	Pelikan,	localization of colonic lesions	26 patients with 32 india ink tattoos in colonic mucosa	Biopsies showed carbon particles in the mucosa, but no inflammatory reactions.	The mean follow-up time was 14 months. All follow-up visits showed the mucosa still darkly tattooed
Shafter [22] 1999 USA	Difco India Ink (now BD)	localization of Barrett's mucosa in esophagus	19 patients	None	None
Shatz [23] 1991 USA	Higgins	Localization of colonic lesions	64 patients	None	One patient had ink injected through the wall of the sigmoid into the peritoneal cavity, but was asymptomatic. Leakage may occur and obscure the polypectomy site.
Choy [38] 2015 USA	Spot	Localization of axillary lymph nodes (breast cancer)	28 patients	Inadvertent staining of lymphatic channels proximal to the node, migration of ink between nodes causing inadvertent staining of additional nodes.	One tattoo wasn't visualized. Authors believe that may be due to the injection being a small volume. For 3 patients the pigment was detected during surgery, but not during histology. Staining of a lymph node that was not a sentinel node occurred in one patient.
Hwang [18] 2010 Korea	Spot	Localization of colonic lesions	20 patients	Local leakage was seen with one patient (without peritonitis or abdominal pain). No other adverse events.	India ink does not diffuse through the mesentery and is thus permanent.
Park [20] 2008 Korea	Spot	Localization of colorectal tumors	63 patients	1 patient had ink diffused extensively, 6 patients had localized diffusion of ink; 1 patient had chills.	Patient with extensive diffusion made lesion hard to see.
Cipe [16] 2016 Turkey	Spot	Localization of distal surgical margin for rectal cancer	40 patients received marking; 25 patients were controls.	None	None
Aboosy [15] 2005 Netherlands	Rotring	Localization of soft colorectal lesions	19 patients with small lesions	None	5 patients had India ink particles present in lymph nodes
McArthur [19] 1999 USA	Koh-I-Noor,	Localization of colonic lesions	195 patients; 50 marked before surgery and 145 marked for future localization	No patients reported any incidence of fever or persistent abdominal pain and no examinations revealed evidence of abdominal tenderness.	Ink was injected through the bowel wall in a few cases.

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Salomon [21] 1993 USA	Koh-I-Noor (& homemade)	Localization of colonic lesions	20 patients; 12 had undergone resection.	2 patients had pigmented histiocytes and mild chronic inflammation. Others had superficial mucosal ulceration, granulation of tissue containing ink stained histiocytes, ink staining of macrophages in local lymph nodes.	They were unable to keep the homemade ink in suspension so only Koh-I-Noor was used.

Table 40.3

Review of 12 studies with >16 subjects, using charcoal India inks for medical marking

Author (ref #) Year country	Brand name or 'Homemade' in pharmacy	Medical use	# of study participants and areas injected	Adverse events/reactions	Problems not related to pigment/comments
Mathieu [37] 2007 France	Sterimark	Localization of breast carcinoma	109 total patients:	Pain from injection in 6 pts. No other pain, inflammation, or redness observed	none
Chani [25] 2005 France	Sterimark	Ultrasound guided localization of recurrent thyroid lesions	101 lesions from 53 patients	3 patients had marked pain without other complications (one revealed neuroma).	Thirteen lesions had been injected but were not found intraoperatively. Carotid artery was injured in two cases. Histological analysis was difficult for one granuloma. Ink sometimes blocked in needle.
Cavalcanti [33] 2012 Brazil	Homemade	localization of breast lesions using ultrasound or mammography	135 specimens from 109 patients	No symptoms reported. In all specimens, foreign body granulomas: Acute inflam: 57 mild, 15 mod, 5 severe with abscess. Chronic inflam: 111 mild, 17 mod, 12 fat necrosis. 1 displacement artifacts	Comfortable for the patient and easy to perform. No impairment to histological analysis from injection.
Kang [26] 2009 Korea	Homemade	Ultrasound guided localization of recurrent thyroid lesions	83 lesions in 55 patients	No clinically important complications. No intolerable pain or bleeding.	Two pts. had visible dots. 3 lesions could not be injected due nearness to major vessels. 2 lesions could not be detected by surgeons due to fibrosis.
Ko [28] 2007 Korea	Homemade	Ultrasound guided localization of breast lesions	164 lesions in 134 patients	No adverse events noted other than initial pain and bleeding. Histopathology showed only low-grade foreign body reactions.	Large excision had to be made in one case where needle was blocked and not enough ink was injected. Two patients had a visible mark from the injection. Blockage of needle in 8 cases, but strong pressure allowed ink to be injected. Assistant continually agitated solution.
Langlois [29] 1991 Australia	Homemade	Localization of breast lesions using stereoscopic images	56 lesions (53 patients)	None	None
Mazy [34] 2001 Belgium	Homemade	Localization of breast lesions using stereoscopic images or ultrasound	221 lesions in 153 patients	Ink was 'well tolerated' by patients; In 79% of specimens no significant histological modification. Deposits were isolated in fat tissue or in the neoplastic proliferations without dissemination of the product. Local inflammatory reaction was noted in 21%, primarily consisting of a lymphoplasmacytic infiltration, a histiocytic and giant cell response or a neutrophilic infiltration.	Some discomfort from injections. The histological reactions when present did not prevent microscopic analysis, except in one small lesion (4 mm) that was partially masked by the charcoal
Mullen [35] 2001 USA	Homemade	Localization of breast lesions using stereoscopic images	376 patients (237 lesions marked, 200 followed for study)	None	Injected ink spread to chest wall; discharge of black liquid from biopsy tract several days after. Followed for up to 7 yrs with no additional problems

Author (ref #) Year country	Brand name or 'Homemade', in pharmacy	Medical use	# of study participants and areas injected	Adverse events/reactions	Problems not related to pigment/comments
Svane [31] 1983 Sweden	Homemade	Localization of breast lesions using stereoscopic images	56 lesions (31 removed at other hospitals)	None	Injection displaced one fibroadenoma (~5mm) and it was not marked as a result
Tirelli [27] 2016 Italy	Homemade	localization of parotid masses	23 patients 219 patients with carbon marking	2 cases had transient increase in lesion size,	In 4 cases the charcoal was above the lesion rather than in it. believed due to the density of the tissue.
Rose [30] 2003 Australia	Charco-trace	Localization of breast lesions	vs 292 with hookwire marking	None	Carbon tracks resist slicing and carbon can distort or obscure the lesion
Azavedo [32] 1989 Sweden	Homemade	Localization of breast lesions	2510 women	None	none