J Oral Med Oral Surg 2022;28:4
https://doi.org/10.1051/mbcb/2021039

https://www.jomos.org

JOMOS
Journal of Oral Medicine and Oral Surgery

Original Research Article

Utility of nail enamel for inking of surgical margins in oral biopsy: a comparative study

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(Received: 3 September 2020, accepted: 1 September 2021)

Keywords:
Nail enamel / inking / surgical margins

Abstract – Background: India Ink is the most commonly used ink in surgical pathology. The main disadvantages of India Ink are longer drying time, monochrome, toxicity and cost. Because of these disadvantages, alternative materials have been suggested to replace India ink. The aim of this study is to evaluate the effectiveness of nail enamel for inking of surgical margin and to compare it with India ink. **Materials and methods:** $N = 20$, which included 10 mucosal and 10 skin samples. Each selected margin is divided into 2 equal halves and one is inked with India ink and the other with nail enamel (Vernis A Ongles: Dark green). After routine processing and staining, the effectiveness of nail enamel and India ink were compared based on macroscopic and microscopic parameters. **Results:** Less drying time and visibility on paraffin wax block were excellent for nail enamel. Microscopic visibility of nail enamel was comparable with that of India ink. However, processing fluids contamination is the main drawback of nail enamel. **Conclusion:** Nail enamel can be used as an alternative to India ink because of its less drying time, ease of application, good visibility on wax blocks and microscopically, availability in multiple colours, cost effectiveness and non-toxicity.

Introduction

Oral Squamous Cell Carcinoma (OSCC) is the most common malignancy of oral cavity and the 12th most common cancer in the world [1]. India has the highest cases of (approximately 20%) of oral cancers in the world [1]. Primary mode of management of OSCC is surgical resection followed by adjuvant radiotherapy and chemotherapy when needed [2]. Precise macroscopic and histopathological analysis of resected specimens and surgical margins by pathologists is necessary for final diagnosis [3].

Tumour involvement of surgical margins of resected specimens has significant therapeutic and prognostic implications. Malignant lesions can extend beyond the macroscopic limits of surgical resection, which if not diagnosed accurately can result in poor prognosis [3]. In examining surgically excised tissues, it is often desirable to identify the orientation of the specimen permanently for both further gross examination and microscopic identification of resected surgical margins [4]. Several methods have been used for proper identification and orientation of resected margins in surgical pathology. Inking of surgical margins is the more reliable method than sectioning techniques and stitches/suture marker methods for identification of surgical margins [5]. Stitches can damage microtome knives and sutures can cause artifacts with loss of tissues [6]. India Ink is the traditional marking ink in the field of surgical pathology because of its resistance to rigorous tissue processing schedule and good visibility under microscope [5]. The main disadvantages of India Ink are longer drying time, monochrome, toxicity and cost. Common alternatives to India Ink are acrylic pigments, alcian blue, coloured gelatin and starch [7].

In 2013, Haspeslagh *et al.* described a cheap method for dotting skin melanocytic lesions using nail enamel. They discovered that nail varnish can adhere to skin, be resistant to tissue processing and can be detected under microscope [6,8]. A new alternative method of Inking using nail enamel is discussed in this study. The aim of the study: to evaluate the effectiveness of nail enamel for inking of surgical margin and to compare the macroscopical and microscopical properties of India ink and nail enamel when used as a tissue marking dye.

Materials and methods

Tissue preparation

An *ex vivo* comparative pilot study was done with the approval from the Institutional Review Board (Scientific review board number-SRB/SDC/OPATH-1903/20/02). Total sample size

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of $n = 20$ surgical margins were selected, which was further divided into two groups. Group-I consisted of 10 mucosal margins ($n = 10$) and group-II consisted of 10 skin margins ($n = 10$). Surgical resected OSCC specimens fixed in 10% formalin were retrieved from the archives of Oral and Maxillofacial Pathology Department, Saveetha Dental College, Chennai India, during the period of 2018 to 2019. Surgical resected OSCC specimens were blotted dry with paper towels, following which representative skin and mucosal margins were cut using surgical blades. Each selected mucosal and skin margin were divided into 2 equal halves, following which India ink and nail enamel were applied.

Inclusion criteria

Skin and mucosal resected surgical margins.

Exclusion criteria

Bone, adipose tissue and muscle were not included in the present study.

Inking procedure

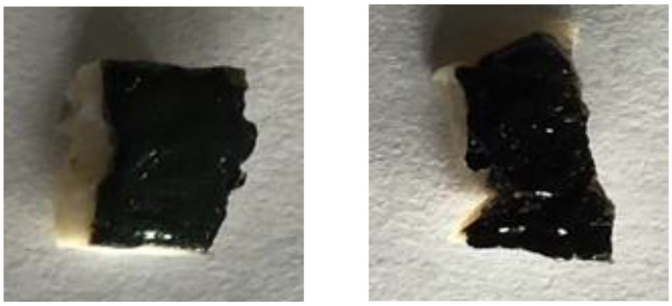
Indian Ink-Black color (NICE Indian ink, Nice chemicals private limited) and nail enamel-Dark Green (Vernis A Ongles) were applied to the external surfaces of each resected mucosal and skin margins. The major content of India ink is carbon molecules and which is suspended in a colloidal suspension medium. Commonly available color of India ink is black, hence used in this study. The brand and color of nail enamel used in this study was Vernis A Ongles and dark green. The selection of nail enamel color was randomly selected from the approaching colors of India ink. The selected nail enamel contains film forming agent-nitrocellulose and/or cellulose derivative, a plasticizer and a solvent — Polyphenylsilsesquioxane. Both India ink and nail enamel were applied with a hair paint brush at room temperature and allowed to dry and drying time was noted for both nail enamel and India ink (Fig. 1).

Tissue processing and staining

Tissue samples were then processed with 99.9%, 90% and 80% propranolol for 30 min each, followed by clearing in xylene for two changes, 30 min each and impregnation with molten paraffin wax for overnight. After processing, the tissues were embedded in the L-former using molten paraffin wax and cut at 3 μm on a rotary microtome (LEICA RM-2245) and mounted on a positively charged slide. Sections were stained with haematoxylin and eosin (H&E) and all slides were blinded for the type of ink and examined under light microscopy (Olympus CG20i) by independent observers.

Evaluation of inking procedure

The paired tissue samples were evaluated by Oral Pathologists blinded to the type of inking. Ease of application of ink, drying time, visibility of ink on wax blocks, slides macroscopically and microscopically, penetration into deeper tissues and interference with cell morphology were scored according to the criteria in Table I.



(a) (b)

Fig. 1. (A) Tissue inked with nail enamel. (B) Tissue inked with India ink.

Statistical analysis

Statistical analysis of the data was done using IBM SPSS statistics 23 software. Chi-square test was used to find out statistical significance of data. *P* value less than 0.05 is considered as statistically significant.

Results

Results obtained following the evaluation of parameters were represented in Table II. The application of both India ink and nail enamel were easy on the selected surgical margins. The drying time for India ink was found to be 17–20 min, which was comparatively higher when compared to drying time of nail enamel (7–10 min). Contamination of processing fluids was evident when the mucosal and skin specimens were inked with nail enamel. But India ink showed no contamination of processing fluids for both mucosal and skin specimens.

All the skin samples inked with both India ink and nail enamel showed good visibility on paraffin blocks (Fig. 2). In contrast, 90% of the mucosal samples inked with Nail enamel and 60% of the mucosal samples inked with India ink showed good visibility on paraffin blocks (Tab. II).

40% of the mucosal samples inked with India ink showed visibility on slides during naked eye examination (Tab. II). On the contrary, none of the mucosal samples inked with nail enamel showed visibility on slides during naked eye examination. For skin samples, 40% of samples inked with India ink and only 10% of samples inked with nail enamel showed good visibility on slides during naked eye examination. In mucosal samples, a statistically significant association was found ($p = 0.025$) between the type of ink used and the visibility of ink on naked eye examination (Tab. V). No statistical correlation was observed for skin samples.

Good microscopic visibility was observed for the entire samples of skin and mucosal specimens and was found to be independent on the type of ink used (Tab. II). 90% and 50% of the

Table I. Macroscopic and Microscopic scoring criteria for inking of surgical margins.

S:no	PARAMETERS EVALUATED	EVALUATION CRITERIA
1	Ease of application of ink	0 – Easy 1 – Difficult
2	Drying time of ink	0 – Less than 10 minutes 1 – Greater than 10 minutes
3	Visibility of ink on wax blocks	0 – No 1 – yes
4	Visibility of ink on naked eye in slides	0 – No 1 – yes
5	Visibility of ink on microscopy	0 – No visibility of ink 1 – Focal patches of ink 2 – Diffuse patches of ink 3 – Band of ink
6	Penetration in to deeper tissues	0 – No penetration 1 – Penetrated till epithelium 2 – Penetrated till mucosa 3 – Penetrated till submucosa
7	Interference with cell morphology	0 – No 1 – yes

Table II. Macroscopic and microscopic parameters of Indian Ink and nail enamel on mucosal and skin margins.

Parameters	Ink used	Mucosa samples	Skin samples
Ease of application	Nail Enamel	Easy	Easy
	India Ink	Easy	Easy
Drying time	Nail Enamel	7–10 minutes	7–10 minutes
	India Ink	17–20 minutes	17–20 minutes
Contamination of processing fluid	Nail Enamel	100%	100%
	India Ink	0%	0%
Visibility on paraffin blocks	Nail Enamel	90%	100%
	India Ink	60%	100%
Visibility on naked eye examination of slides	Nail Enamel	0%	10%
	India Ink	40%	40%
Visibility on microscopy	Nail Enamel	100%	100%
	India Ink	100%	100%
Penetration in to tissues	Nail Enamel	60%	0%
	India Ink	20%	0%
Interference with cell morphology	Nail Enamel	20%	0%
	India Ink	0%	0%

skin and mucosal specimens inked using nail enamel were observed as diffused patches under microscopic examination (Tab. III). On the contrary, India ink marked samples of both skin and mucosa were predominantly of band type (Figs. 3 and 4). Table III shows the statistical correlation ($p = 0.001$) between visibility on microscope and type of ink used in skin specimens.

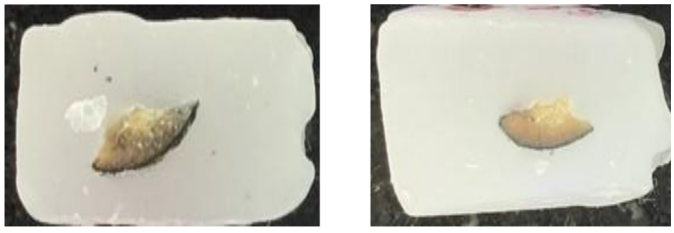
All the skin samples inked with both nail enamel and India ink showed no penetration into deeper tissues and no interference with cellular and nuclear morphology (Fig. 6). 80% of the mucosal samples inked with India ink showed no penetration into tissues. Remaining 20% of the samples showed penetration till submucosa level. In contrast to this, 40% of mucosal samples inked with nail enamel showed no penetration into tissues and 10%, 30% and 20% of samples were penetrated till epithelium, mucosa and submucosa respectively (Fig. 5 and Tab. IV).

Table III. Ink used vs visibility on microscope.

Specimen type	Ink used	Visibility on microscope			*p* value
		Focal patches	Diffused patches	Band	
Mucosa	Nail Enamel	40%	50%	10%	0.165
	India Ink	10%	50%	40%	
Skin	Nail Enamel	0%	90%	10%	*0.001
	India Ink	0%	30%	70%	

Table IV. Ink used vs penetration in to deep tissue.

Specimen type	Ink used	Penetration in to deep tissue				*p* value
		No penetration	Penetrated till epithelium	Penetrated till mucosa	Penetrated till submucosa	
Mucosa	Nail Enamel	40%	10%	30%	20%	0.149
	India Ink	80%	0%	0%	20%	
Skin	Nail Enamel	0%	0%	0%	0%	–
	India Ink	0%	0%	0%	0%	



(a) (b)

Fig. 2. Visibility of ink on wax blocks. (A) Inked with nail enamel, (B) Inked with India ink.

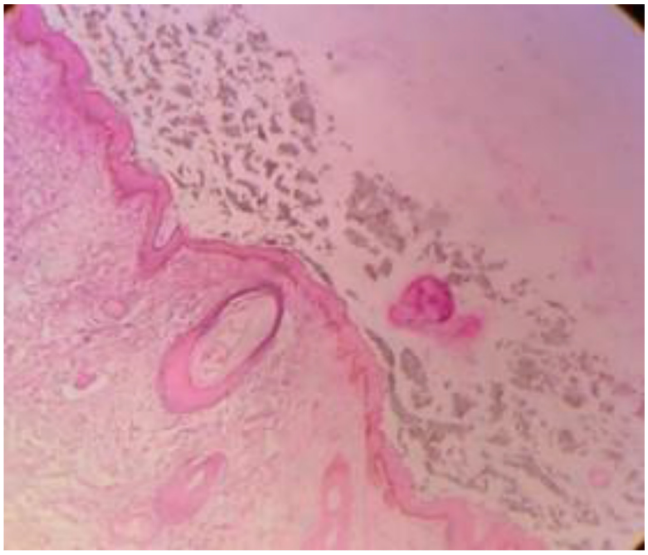


Fig. 3. Photomicrograph of skin section inked with nail enamel showing band appearance.

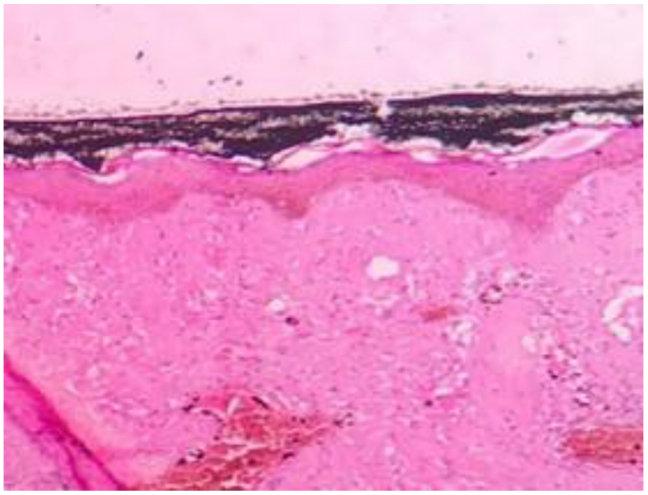


Fig. 4. Photomicrograph of skin section in which India ink showing band appearance.

Discussion

Histopathological evaluation of surgical margins of a resected tumour specimen can give an understanding about the extent of tumour spread. Errors in proper identification and orientation of resected tumour margins can lead to treatment failure and poor prognosis. Inking of resected margins is the most reliable and safe method for proper identification and correct orientation of surgical margins. India ink is the most commonly used inking material in surgical pathology and it is a colloidal suspension of inert carbon black obtained from burning of bones, tar pitch *etc.* It fulfils most of the qualities required in a surgical marking ink like survival during tissue

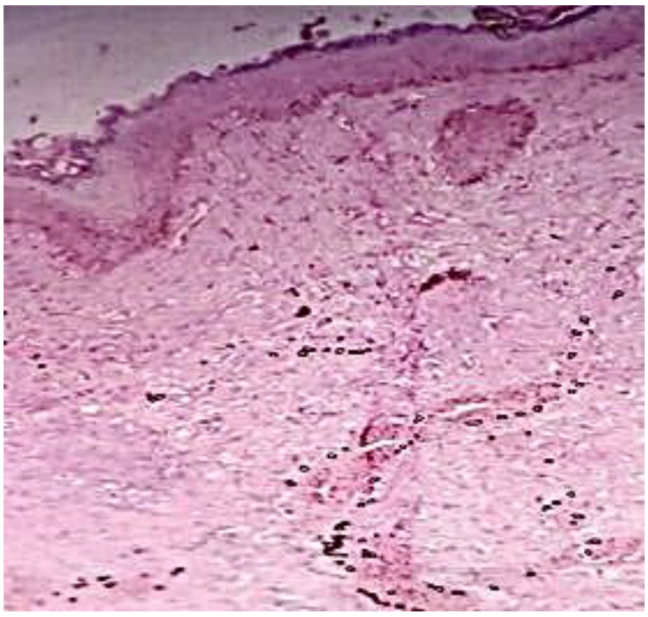


Fig. 5. Photomicrograph of mucosal section inked with nail enamel showing penetration into deeper connective tissue.

Table V. Visibility of nail enamel and India ink on mucosal specimens.

Parameters compared in mucosal specimens	*p* value
Visibility on paraffin blocks	0.121
Visibility on naked eye examination of slides	*0.025
Interference with cell morphology	0.136

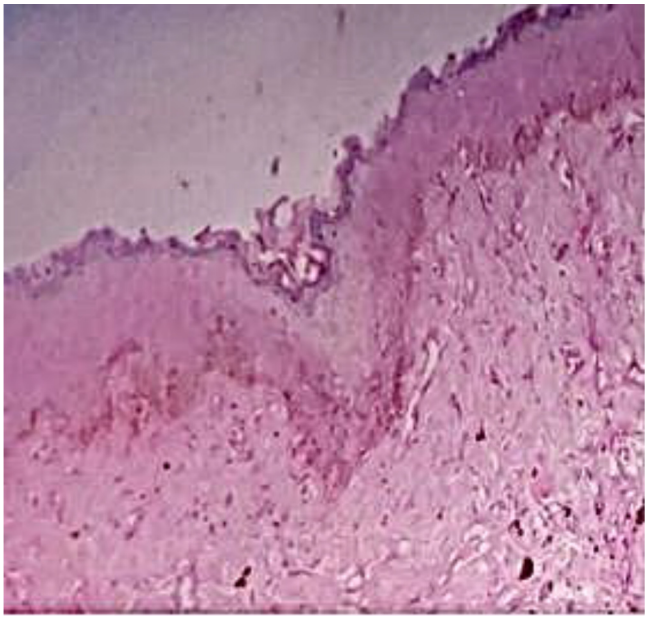


Fig. 6. Photomicrograph of mucosal section inked with nail enamel interfering with cellular morphology.

processing, staining procedures and good visibility under microscope [9]. Nail enamel consists of a film forming polymer dissolved in organic solvent, which can form a covering upon drying.

In the present study, the application of India ink and nail enamel were found to be equally easy. Several studies have stated the easy application of India ink [3,5,7]. No similar works were found in literature to support the ease of application of nail enamel. Haspeslagh *et al.* and J. Van Hevele *et al.* stated the utility of nail enamel as surgical ink in intra oral tissues, however, the ease of use of nail enamel was not explained in the above mentioned studies [8,10]. The increased ease in utility of nail enamel could be due to its lesser drying time and ability to form a thick resistant layer which is smudge free and easy to handle.

When considering the drying time, several studies were available to assess the drying time of India Ink [3,5,11]. However, to the best of our knowledge, no studies evaluated the drying time of nail polish. In the present study, it is observed that nail polish had a lower dying time than India ink. Increased drying time of India ink will make the grossing procedure more time consuming and can often lead to spreading of the ink when processed without proper drying [8,10,11]. Van Hevele *et al.* also suggested the utility of nail enamel in frozen sections and found it to be more acceptable due to its lesser drying time [10]. This may be attributed to chemical composition of nail polish containing film forming polymers dissolved in solvents. Application of nail enamel on to the tissue surface causes the evaporation of the solvents leaving behind the coloured polymer. Decreased drying time is the main advantage of nail enamel over India ink.

In the present study, India ink showed no contamination of processing fluids for both mucosal and skin specimens. This was in concurrence with the previous literature [3,5,11]. Contamination of processing fluids were evident in this study when the mucosal and skin specimens were inked with nail enamel. This could be due to the chemical composition of nail enamel consisting of nitrocellulose polymers soluble in organic solvents like acetone and alcohol. In the present study grades of alcohol were used for dehydrating the tissue and the temperature will also aid in the increased dissolving capacity of alcohol. The contamination of tissue processing fluids may lead to increase in the tissue processing fluid wastage rate and it will reduce the cost effectiveness of nail enamel. No other previous literature was available to the best of our knowledge to ascertain the contamination of processing fluids by nail enamel. Haspeslagh *et al*. and Van Hevele *et al.* stated that nail enamel is adherent to skin and resistant to tissue processing.

When the visibility on paraffin wax blocks were considered, nail enamel performed better compared to India ink (Fig. 3 A&B). The good visibility of nail enamel could be due to the presence of inorganic pigments and the good visibility also implied the improved adherence of nail enamel to the tissues despite the dissolution by processing fluids. Even though the nail enamel performed better than India ink in the present study, the association between the type of ink used and visibility on paraffin wax blocks was not statistically significant.

Indian ink had better visibility on slides compared to nail enamel. This implied that sectioning and subsequent staining procedure using alcohol could be the reason for the separation of nail enamel from the tissue surface despite its adhesiveness to tissue macroscopically. The better visibility of India ink compared to acrylic colours on the slides during naked eye examination was also stated by Divya *et al.* [5]. The organic nature of pigments present in India ink has better adhesion to tissues than inorganic pigments found in nail enamel and acrylic colours [12,13]. The association between the type of ink used and the visibility on naked eye examination of slides was found to be statistically significant.

Even though both nail enamel and India ink showed microscopic visibility in all the samples selected, the visibility for mucosa and skin specimens differed for both the inks. Both the inks performed better in skin samples than in mucosal samples. This might be due to the rougher surface of the skin with skin appendages like hair, which will help the ink to adhere easily. Among mucosal samples, the association between the type of ink and the microscopic visibility of ink was found to be not significant, however that of skin samples were found to be significant with p value of 0.001. Skin and mucosal samples inked with nail enamel and India ink were in the form of band and focal patchy areas on the surface of the tissue. Both nail enamel and India ink showed no penetration into skin samples. For mucosal samples, nail enamel penetrated into the tissues and interfered with cellular morphology only in two samples. This could be attributed to the composition of nail enamel. India Ink neither interfered with cellular and nuclear morphology of skin specimens nor with mucosal specimens. Limitations of this study include smaller sample size and monochrome of nail enamel used. Utility of nail enamel was not evaluated in this study for IHC procedures. In *ex vivo* dotting using nail enamel, the samples used were already formalin fixed. Hence It should be assessed in vivo before replacing the India ink because in frozen section the tissues will be unfixed and fixing of the nail enamel to the tissue surface by formalin will not occur.

Nail enamel can be used as an alternative to India ink because of its less drying time, ease of application, good visibility on wax blocks and microscopically, availability in multiple colours, cost effectiveness and non-toxicity. However, more analysis needs to be carried to cash in the advantage of less drying time of nail enamel to be used in frozen sections. Inking with multiple nail enamel colors could also help in reassessment of surgical margins of the gross specimen in the future.

Conclusion

From the present study it is concluded that nail enamel can be used for inking the surgical margins and is yet to be on par with Indian ink, to be used for inking of surgical margins in the long run. This pioneer idea needs further research in area of immunohistochemistry and frozen sections to expand its application.

Authors contribution

Suvarna Kizhakkoottu: Conceptualization, Methodology, Writing original draft. Archana Santhanam: Methodology, Supervision, Editing. Herald J Sherlin: Supervision, Review and Editing. Gifrina Jayaraj: Review and Editing. Kanchi Ravi Don: Review and Editing.

Conflict of interest

The authors declare that they have no conflicts of interest in relation to this article.

Informed consent

The authors declare that informed consent not required.

Ethical committee approval

Ethical committee approval number: SRB/ SDC/ OPATH-1903/22/03.

Source of funding

This research did not receive any specific funding.

Acknowledgements. The Authors would like to acknowledge the help and support from Thamizhselvi, non teaching staff for their constant support with the research.

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