

# Comparative Study of Acrylic Color and India Ink for Their Use as a Surgical Margin Inks in Oral Squamous Cell Carcinoma

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## ABSTRACT

**Objective:** To compare acrylic color and India ink for their use as a surgical margin. Since, acrylic paints are available in multiple colors, they can be used to mark different surgical margins with different specific colors.

**Materials and methods:** The study was carried out on 30 surgically excised and formalin fixed oral squamous cell carcinoma (OSCC) specimens. Four surgical margins of each 15 specimens were marked with India ink and the remaining were marked with acrylic colors. The grossed specimens were then processed using routine histopathology technique. The 4  $\mu$ m sections of tissue block were cut using microtome and sections were stained with hematoxylin and eosin stain. Various macroscopic and microscopic parameters were used to study the suitability of acrylic color as surgical ink.

**Results:** Three different technicians found equal ease of application for acrylic color and India ink. The drying time for acrylic color ( $1.30 \pm 0.009$  minutes) was significantly less than India ink ( $2.30 \pm 0.05$  minutes). Out of total 60 blocks made for acrylic colors, 52 (86.66%) displayed clear visibility on paraffin blocks (93.33%). In contrast, out of 60 blocks made for India ink, only 34 showed faint visibility on paraffin blocks (56.66%). All acrylic colors were visible very clearly and uninterruptedly under the microscope, while three (5%) surgical margins stained with India ink were interrupted. With the acrylic colors, no contamination or staining of other tissues, processing fluids and solutions were reported. However, with India ink, three (5%) samples reported contamination in alcohol and xylene. There was no interference with the microscopic interpretation of nuclear and cellular details for both acrylic color and India ink whatsoever.

**Conclusion:** Acrylic colors are more suitable as surgical ink over India ink because of its availability in different colors, ease of application, faster drying time, and no contamination with

processing fluids, easy availability and excellent visibility on paraffin blocks and under microscope. Moreover, if needed, painting with multiple colors could also help in reassessment of surgical margins of the gross specimen in the future.

**Keywords:** Histopathology, Marking ink, Oral cancer, Surgical margins.

**How to cite this article:** Sarode SC, Sarode GS, Patil S, Mahajan P, Anand R, Patil A. Comparative Study of Acrylic Color and India Ink for Their Use as a Surgical Margin Inks in Oral Squamous Cell Carcinoma. World J Dent 2015;6(1):26-30.

**Source of support:** Nil

**Conflict of interest:** None

## INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity which is usually preceded by variety of potentially malignant disorders.<sup>1,2</sup> Diagnostic delay has shown to be of significant factor in disease progression.<sup>3</sup> The pathologists make final diagnosis after careful and precise histopathological analysis and microscopic examination of the resected specimens. Malignant lesions, invasive or *in situ*, may extend beyond the macroscopic limits and approach or cross the boundaries of surgical excision which if not diagnosed accurately can render treatment ineffective. Tumor involvement of surgical margins of resected specimen has significant therapeutic and prognostic implications.<sup>4</sup>

The orientation of the specimen by the surgeon before receipt in the laboratory is usually achieved by inserting either clips or sutures.<sup>5</sup> Recently, inking of the margins has gained popularity due to its ease of application and clear demarcation of the borders.<sup>5</sup> Traditionally, India ink has been used to mark surgical resected margins.<sup>6</sup> However, when multiple surfaces/margins need examination or laterality of specimens need to be discernible even after specimen dissection, scarcity of varied colored ink is keenly realized. To add precision to the examination of margins, color them using different colors in the gross specimen itself. Three-dimensional microscopic reconstruction of the tumor along with the adjacent structures is possible. Postoperative comparison of tissue

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planes, which can be predicted by preoperative imaging, is also possible. Orientation of the specimen is maintained so that the pathologist can re-visit the grossed specimen, if needed.<sup>7</sup>

Although, surgical pathologists are aware of the numerous benefits of using colored ink in surgical and histopathological practice, these colored inks are unavailable in India and importing them is not an economical option. With this view in mind, we investigated the use of acrylic colors as a substitute to multicolored surgical ink and compared it with traditional India ink.

## MATERIALS AND METHODS

### Samples

The study was carried out on 30 surgically excised and formalin fixed OSCC specimens. Surgical margins of 15 specimens were marked with India ink (Loba Chemie Pvt Ltd, Lot no. LM04581210, maximum absorbance: 570-580 nm) and the remaining 15 were marked with acrylic colors (Camlin Limited, product no. 0805721). Mainly, four different contrasting acrylic colors were used to mark the different margins. Color-coding was given for each margin, e.g. red—anterior, green—posterior, blue—lateral, brown—medial. Thus, there were 60 (15 × 4) specimens in each for acrylic dye and India ink.

### Methodology

The surfaces of fixed specimens were dried with blotting paper. The painting of the specimens with acrylic colors and India ink was done using commercially available brush (Camlin Limited, white bristle brush, size: 4). The painted specimens were immersed in alcohol for a few minutes or air dried to fix the color on the surface (Fig. 1). Excess color was washed off and then the specimen was grossed as done routinely. Four surgical margins (anterior,

posterior, medial and lateral) were grossed from each specimen and then processed using routine histopathology technique, i.e. formalin fixed—paraffin embedding technique. Every specimen was processed separately, especially to examine the contamination of processing fluids. Sections of 4 μm were cut from each block and stained with hematoxylin and eosin stain.

## Interpretation of Results

The following features were used to study the suitability of acrylic color as surgical ink:

1. Ease of application: present or absent
2. *Drying time*: It is the time measured from the application of dye to drying of the dye. For accurate and precise measurement of time a stopwatch was used.
3. Visibility on paraffin blocks: This was checked with naked eyes.
4. Visibility on microscopic sections: Using binocular compound microscope.
5. Staining or contamination of other tissues, processing fluids and solutions: Processing fluids and tissue specimens were checked with the naked eyes for staining and contaminations. The processing fluids tested were formalin, alcohol and xylene.
6. Interference with microscopic interpretation of nuclear and cellular detail: Checked using on the hematoxylin and eosin-stained slides using binocular compound microscope.

Three different technicians performed the experiments and three different investigators did interpretations of results. The degree of agreement was calculated using Kappa test to authenticate the results.

## RESULTS

The interpretations of results of all aforementioned parameters are shown in Table 1.

### Ease of Application

The specimens were painted with acrylic colors (60) and India ink (60) using commercially available brush by three different technicians. All the 120 specimens were equally divided among three technicians. All of them noticed equal ease of application for both the samples. (Table 1).

### Drying Time

The time required for the drying of the acrylic dye ( $1.30 \pm 0.009$  minutes) was significantly lesser than India ink ( $2.30 \pm 0.05$  minutes). The similar drying period was observed for the different colors of acrylic dye. The results are shown in Table 1.



Fig. 1: Painting of the surgical margins with acrylic colors

**Table 1:** Analysis of acrylic dye and India ink on different parameters

S. no.	Parameter		Result
1	Ease of application	Acrylic dye (n = 60)	100%
		India ink (n = 60)	100%
2	Drying time	Acrylic dye (n = 60)	1.30 ± 0.009 min
		India ink (n = 60)	2.30 ± 0.05 min
3	Visibility on paraffin block	Acrylic dye (n = 60)	52 (86.66%)
		India ink (n = 60)	34 (56.66%)
4	Visibility on microscopic examination	Acrylic dye (n = 60)	60 (100%)
		India ink (n = 60)	60 (100%)
5	Contamination of solutions	Acrylic dye (n = 60)	None
		India ink (n = 60)	3 (5%)
6	Interference with nuclear and cytoplasmic details	Acrylic dye (n = 60)	None
		India ink (n = 60)	None

(n = 60; Total number of surgical margin samples, i.e. 15 specimens × 4 margins = 60)

### Visibility on Paraffin Blocks

Out of total 60 blocks made for acrylic colors, 52 displayed clear visibility on paraffin blocks (86.66%) (Fig. 2). In contrast, out of 60 blocks made for India ink only 34 showed clear visibility on paraffin blocks (56.66%) (Fig. 3).

### Visibility on Microscopic Sections

The surgical margins were clearly visible on the slides for both acrylic colors and India ink (Figs 4 and 5). But, three specimens (5%) marked with India ink showed

interrupted staining on microscopic examination. Most importantly, all acrylic colors (100%) were visible very clearly and uninterruptedly under the microscope (Fig. 4).

### Staining or Contamination of Other Tissues, Processing Fluids and Solutions

With the acrylic colors, no contamination or staining of other tissues, processing fluids and solutions were reported. However, with India ink three samples (5%) reported contamination in alcohol and xylene.

### Interference with Microscopic Interpretation of Nuclear and Cellular Details

On detailed microscopic examination of interphase between the dye and surgical margin revealed very minimal penetration of acrylic and India ink dye into the tissue (Figs 4 and 5). Moreover, such penetration was only seen at the margins of the tissue section, leaving all other areas unaffected. Hence, there was no interference with the microscopic interpretation of nuclear and cellular details.

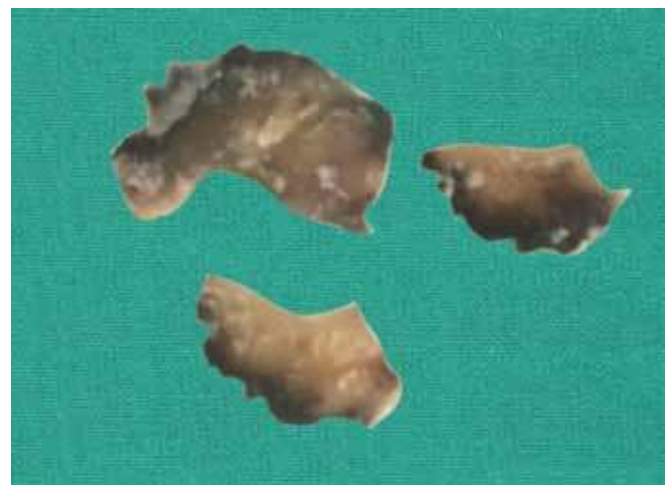
### DISCUSSION

Microscopic evaluation of the margins of excised OSCC is of paramount importance for determining that the tumor tissue does not extend to the excision borders. Use of dye or pigments are more reliable than sectioning techniques or suture markers for identifying the surgical margins before and after tissue processing.<sup>7</sup> In the present study, we evaluated acrylic paint that could be rapidly applied, and readily identified on histologic section by the pathologists.

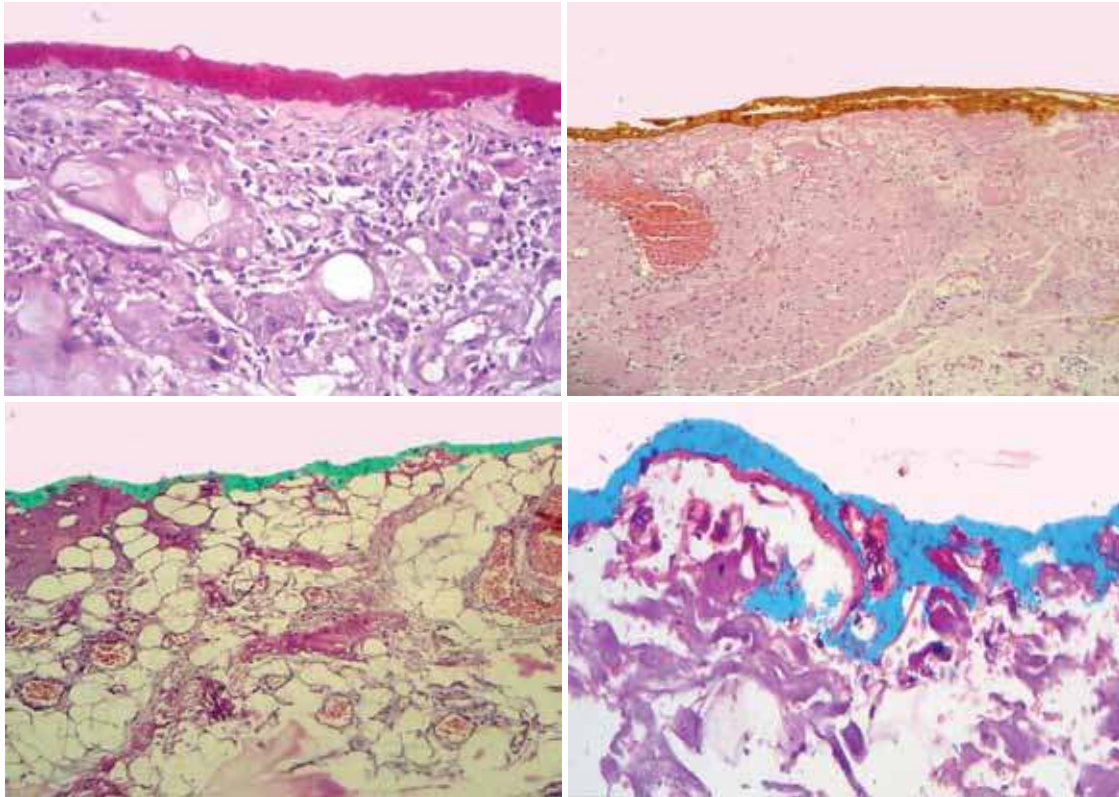
Acrylic paints were first used in the 20th century in Mexico. Acrylics are the most popular paint today because they are cost-effective, easy to dilute with water, they dry quickly and they are resistant to aging



**Fig. 2:** Visibility of acrylic color on paraffin block



**Fig. 3:** Visibility of India ink on paraffin block

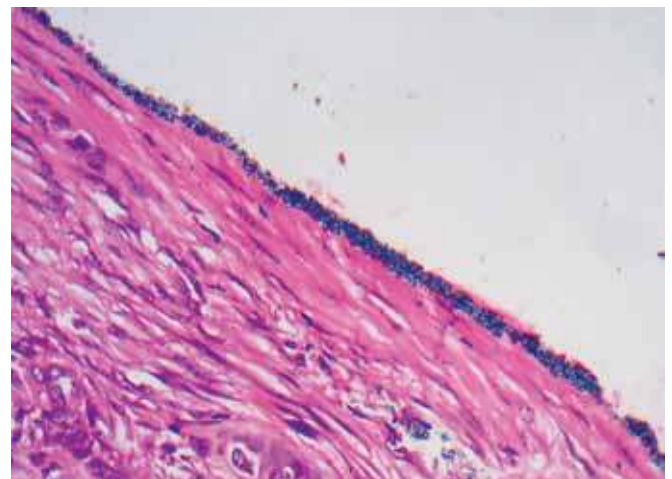


**Fig. 4:** Photomicrographs showing clear and uninterrupted visibility different acrylic colors at the surgical margin under compound microscope (hematoxylin and eosin stain: 100x)

and smudging. Acrylic paints are generally pigments suspended in acrylic polymer emulsion, that is, color pigments in a mixture of multiple chemicals as a thick emulsion. Acrylic color pigments are not transparent, so we can paint in layers and the previous color won't see through. One can clean acrylic paintbrushes, hands and other equipment with water and soap for multiple usages.<sup>8</sup>

A small pilot study was conducted on different acrylic colors for selection in the present study. After trial we short listed red, blue, green and brown colors, which fulfill the criteria for ideal surgical margin ink. In the present study, it was observed that these acrylic colors fix on fresh and formalin fixed tissue easily. They sustain the overnight processing and the staining procedures and can be seen on the paraffin blocks as well as on the sections. Similar results were reported by Tampi C<sup>7</sup> and Chiam HW et al.<sup>9</sup> India ink also showed similar results, but three cases displayed interrupted visibility on microscopic examination and none on paraffin blocks. Drying time for acrylic colors was  $1.30 \pm 0.009$  minutes as compared to India ink, which took  $2.30 \pm 0.05$  minutes to dry. The difference between them was statistically significant. Such analysis was not done in the literature previously; hence, comparison with previous studies is not possible.

Acrylic colors do not unduly color the processing fluids, or spread with in the tissue sections. In contrast, India ink displayed contamination of processing fluids in



**Fig. 5:** Photomicrograph showing visibility of India ink at the surgical margin under compound microscope (hematoxylin and eosin stain: 100x)

three cases. The big advantage of acrylic color is its availability in most stationery shops, it can be bought in single units of variable colors, have long shelf life, more cost effective and are nontoxic. The India ink is not available across the counter, available in single color, and is toxic.

The acrylic pigments were easily visualized on paraffin blocks and microscopically at the surgical margins. Different colors were distinctly stands out at all the levels. These results are in accordance with the study conducted by Tampi C<sup>7</sup> and Chiam HW et al.<sup>9</sup> Thus,

they fulfill all the criteria of being a reliable, easy to use, inexpensive, safe and stable substitute to India ink.

At present, these specific acrylic hobby colors are in regular use in laboratory in our department to mark the different surfaces in wide excision specimens of soft-tissue tumors with significantly good and consistent results. One biggest advantage of acrylic color over India ink is that we can designate different colors for different margins. This will help the pathologist in reconsideration of grossed specimen very effectively and he can identify the surgical margins without any difficulty. The same is true for microscopic examination; the microscopic visibility of particular color gives us the idea of the type of margin being examined.

In conclusion, acrylic colors are more suitable as surgical margin ink over India ink because of its availability in different colors, ease of application, faster drying time, no contamination of processing fluids, easy availability and excellent visibility on paraffin blocks and under microscope. Hence, we strongly recommend its use as a routine dye for marking surgical margins.

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