L929 cell cytotoxicity associated with experimental and commercial dental flosses

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Abstract. This aim of the study was to investigate the cytotoxicity of two commercial and two experimental dental flosses. Two commercial, Oral B® Essential Floss (nylon-waxed) and Thai Silk Floss (silk-waxed), and two experimental, Floss X (nylon-waxed) and Floss Xu (nylon-unwaxed) dental flosses were used. The cytotoxic assay was performed by using cell cultures (L929) which were subjected to cell viability test with methyl-tetrazolium. Each floss specimen (0.4 g) was placed in 1 ml of Minimum Essential Medium at 37 oC with 5% CO₂ at 100% humidity in an incubator for 24 hours. After incubation, the cell mitochondrial activity was evaluated for detecting viable cells using optical density as per the guidelines of ISO 10993-5:2009(E). Cytotoxic effects were evaluated by measuring percentage of cell viability at 3 points of time- 5 mins, 30 mins, and 1 hr. The results showed that two commercial dental flosses and Floss X had cell viability about 90% at the three time points; however, the experimental Floss Xu presented 80% cell viability at 5 min and <70% cell viability at 30 min and 1 hr. The results concluded that the commercial dental flosses and the experimental dental floss with wax tested in this study were acceptable for clinical use.

1. Introduction

Conventional brushing has been found to be inadequate for the complete removal of entrapped food particles and plaque from the tooth structures. Their removal is important as it reduces the incidence of caries, gingivitis, and halitosis. The use of adjunctive cleaning aids, such as dental floss, is suggested for good dental hygiene practice as it provides easy access to interdental and proximal areas, where conventional tooth brushes may not reach. Dental flosses were traditionally made from threads which were coated with wax that provided a certain degree of lubrication, and it made the dental floss less traumatic for the patients.

Currently, dental flosses are fabricated using various polymers, such as nylon, silk, etc. [1] and vary extensively in terms of structure, size, and additives. Very few studies are conducted on the efficacy of dental floss. Although Terézhalmye et al found no clinically relevant differences in plaque-removing efficacy between woven, shred-resistant, and unwaxed dental flosses when combined with manual tooth brushing, the results were significantly better as compared to only brushing [2]. The findings...
were also in conjunction with a meta-review by Sälzer, where a small but significant effect on gingivitis was noted when tooth brushing was combined with flossing [3]. Although some studies have noted no additional benefit of flossing [4, 5], recommendation for its usage are often made by dental practitioners. Furthermore, information should be available regarding the safety of the materials and the additives used for the fabrication of these dental flosses. Therefore, the objective of this study was to evaluate cytotoxicity associated with two experimentally fabricated dental flosses and to compare them with commercial ones.

2. Materials and Methods
Four different types of dental flosses were used in this study and were as follows:
(1). Oral B® Essential Floss, a waxed dental floss made up of nylon (Fig.1).

![Fig. 1: Oral B® Essential Floss](image1)

(2). Thai Silk Dental Floss®, a waxed dental floss fabricated from natural silk. It is a product of “The Foundation of the Promotion of Supplementary Occupations and Related Techniques of Her Majesty Queen Sirikit of Thailand”(Fig.2).

![Fig.2: Thai Silk Dental Floss®](image2)

(3). Experimental dental Floss X, an unwaxed dental floss fabricated from nylon. It composition was evaluated with FTIR, a process that measures the range of wavelengths in the infrared region that are absorbed by a material, before subjected to further studies (Fig.3).

![Fig.3: Experimental dental floss Xu without wax](image3)

(4). Experimental dental Floss Xu, a waxed dental floss which was compositionally identical to Floss X but was further coated with microcrystalline wax (Fig.4).

![Fig.4: Experimental dental floss X after coating with wax](image4)

3. MTT assay for cell viability
The cytotoxic assay was performed using L929 cell cultures which were subjected to the cell viability test with methyl-tetrazolium. Each floss specimen (0.4g) was placed in 1 ml of Minimum Essential Medium at 37°C with 5% CO2 at 100% humidity 24 hrs in an incubator. L929 cells were seeded into 96-well plates and maintained in culture for 24 hrs (1 doubling period) into form a semi-confluent monolayer [6]. They were then exposed to the test compound over a range of concentrations. After 24 hrs exposure, the formazan formation was determined for each treatment
concentration and compared to that determined in control cultures. For each treatment, the percentage inhibition of growth was calculated.

The cell viability was assessed at 5 mins, 10 mins, and 1 hour by tetrazolium bromide reduction (MTT) assay for mitochondrial activities in all of the test and control groups. The dental flosses were removed from the wells. The cells were cleansed three times with PBS solution. One hundred microliters of the MTT (Hi Media) solution (0.1 mg/ml of tetrazolium bromide salt dissolved in Basal Medium Eagle, GibcoBRL) was added to each well, and the plates were incubated overnight at 37 °C in a 5% carbon dioxide incubator.

During incubation, the yellowish extracellular MTT salt was converted into purplish intracellular formazan by metabolic enzymes in the mitochondria. Propanol in 0.04 mol/L HCL was used to lyse the Vero cells, and the purplish lysate was read using an ELISA reader (Lab Systems, Multiscan EX) with a 570-nm filter. This procedure was done for each of the four dental flosses. In addition, a similar assay was also done for control group.

Different models of dental floss were obtained and tested with elution test method onto mouse fibroblast cultures and incubated for 24 hours. Cellular viability was assessed using Dimethylthiazol diphenyltetrazolium bromide test (MTT assay) to determine the cytotoxicity level, according to MTT cytotoxicity test: ISO 10993-5: 2009(E)[7]. Cellular viability was assessed at 3 periods of time: 5 minutes, 30 minutes, and 1 hour. The lower the viability % value, the higher the cytotoxic potential of the test item. If viability was reduced to <70% of the blank, it was considered to have potential cytotoxicity.

### 4. Results

The results of the cytotoxicity test for the 4 dental flosses were shown in Table 1.

| Types of Dental Floss | Time   | Cell Viability [%] |
|-----------------------|--------|--------------------|
| Oral B                | 5 min  | 93.41              |
|                       | 30 min | 91.32              |
|                       | 1 hour | 85.45              |
|                      |        |                    |
| Floss X               | 5 min  | 93.68              |
|                       | 30 min | 90.76              |
|                       | 1 hour | 88.26              |
|                      |        |                    |
| Floss Xu              | 5 min  | 87.26              |
|                       | 30 min | 62.44              |
|                       | 1 hour | 56.33              |
|                      |        |                    |
| Thai Silk             | 5 min  | 103.67             |
|                       | 30 min | 93.71              |
|                       | 1 hour | 90.04              |

For all tested dental flosses, the highest percentage of cell viability was observed at 5 min and the least was at 1 hr. The percentage of cell viability decreased when the time was increased. The experimental dental floss Xu had the least and Thai silk had the highest percentage of cell viability. The floss Xu at 30 mins and 1 hr showed cell viability of <70%.

### 5. Discussion

Biocompatibility of materials is an important consideration for patients, clinicians, laboratory technicians, and manufacturers, especially if the material is intended to used intraorally. Therefore, it is of upmost importance that the material is devoid of any toxic, leachable, or diffusible substances that can be absorbed into the circulatory system, as they can cause local and systemic responses, including teratogenic or carcinogenic effects. As a result, cytotoxic studies should be conducted before dental materials are selected for use [8]. By definition, the cytotoxicity of an agent means the toxicological risks caused by a material or its extract in a cell culture [9].
A variety of commercial dental flosses are available in waxed, unwaxed, monofilament, or multifilament forms. Monofilamentous dental floss coated with wax can easily glide between teeth and does not fray, but it is generally higher in cost as compared to uncoated ones. However, studies have shown that there is no difference in terms of effectiveness between waxed and unwaxed dental floss, but some have added antibacterial agents such as chlorhexidine [10]. Factors to consider in choosing a floss include the space between teeth and user preference. Dental tape is also a type of floss that is wider and flatter than conventional floss and recommended for larger tooth surface area [8] [11]. The experiments in this study were performed according to the ISO 1999, which leaves some flexibility in specimen fabrication. Two aspects require further standardization for the ability of different types of dental floss to remove dental plaque. Toxicity is also an aspect that was considered in this study. The cytotoxicity results of the two commercial and two experimental dental flosses used in this study showed that the experimental unwaxed dental floss (Floss X) had the least of percentage of cell viability, but the other waxed dental flosses had similar percentage of cell viability. This could have been due to two possible factors. Firstly, the two commercial dental flosses (Oral B and Thai silk) were sealed in marketing packages before distribution for sales. Therefore, it can be assumed that they were sterilized, and the experimental dental floss with wax (Floss Xu) required coating by hot melting wax, which could have bactericidal effects on any adherent micro-organisms. On the contrary, the unwaxed experimental dental floss (Floss X) did not undergo any sterilization procedure and was only sterilized prior to the cytotoxicity test. Furthermore, the amount of specimen by weight for cytotoxicity test was more for Floss X than the other dental flosses. These factors could have contributed to higher toxicity of Floss X as compared to the other dental flosses. Studies have shown that the ability of different types of dental floss to remove dental plaque does not vary significantly [13]; the least expensive floss has essentially the same impact on oral hygiene as the most expensive one. Factors to be considered when choosing the right floss or whether the use of floss is appropriate as interdental cleaning aid may be based on [11]:
- Tightness of the contact area; to determine the width of floss
- Contour of gingival tissues
- Roughness of the interproximal surface
- Manual dexterity and preference of the patient: to determine if supplemental device is required.

Although flossing is commonly used to disrupt the oral biofilm between the teeth and to prevent gingival diseases, its effectiveness is greatly determined by patient’s preference, technique, and motivation to floss daily. Flossing is considered to be a more difficult method of interdental cleaning than using an interdental brush. Interdental brushes are preferred due to their one-handed usage and time efficiency as compared to flossing [14]. A groove in the gingival margin, known as a floss cleft, can also form after repeatedly using floss incorrectly along the mesial and distal surfaces of the tooth [15]. Traumatic flossing immediately after the placement of amalgam fillings can also result in an amalgam tattoo [16]. Moreover, the both waxed and unwaxed dental flosses are also found to have common problems due to misuse of product. But the benefits of flossing in lowering the risk of gingivitis and possibly interproximal dental caries outweigh their drawbacks.

6. Conclusion
Flossing is essential aid for oral health maintenance as the toothbrush bristles may not reach all the surfaces in between teeth or under gum line. However, materials used for the fabrication of dental flosses should be non-toxic and safe for usage. The results of the cytotoxicity tested conducted on L929 fibroblast cell line using MTT assay showed that the two commercial dental flosses and Floss X had cell viability of approximately 90% at 5 mins, 30 mins and 1 hour following exposure to the material. However, the experimental Floss Xu presented a lower cell viability, observed at <70% at 30
As per the results of the study the two commercial dental flosses and the waxed experimental dental floss were considered to be acceptable for clinical use.

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