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# Are laryngeal papilloma virus-infected cells viable in the plume derived from a continuous mode carbon dioxide laser, and are they infectious? A preliminary report on one laser mode

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**Abstract**

The purpose of this study was to determine the potential risk of transmitting viable viral infected cells as well as viral infectivity of laryngeal papilloma in the plume derived from a continuous mode carbon dioxide laser. Each of 10 juvenile recurrent laryngeal papilloma specimens was divided into two equal parts, and one part was irradiated with a carbon dioxide laser employing a continuous mode at the power setting of 10 watts with 0.5 mm spot size and a power density of 1667 W/cm$^2$. The resultant laser plume was trapped and was cultured simultaneously with the other part of the specimen which served as the control. All irradiated specimens tested yielded negative culture results while all the control counterparts revealed viable cell growth. To detect the viral infectivity, laser plume was cultured with two separate cell systems, one was the porcine PS clone D cell line and the other normal mucosal cells obtained from the same patient, and to control these systems both cell lines were also designed to be infected with polio virus. Both cell lines in the viral infectivity testing systems revealed no sign of viral infection. The results suggest that papilloma virus-infected cells cannot survive the continuous mode of carbon dioxide laser irradiation. We primarily conclude that, to avoid airborne transmission of plume containing laryngeal papilloma viral-infected cells and infectious viral particles, the carbon dioxide laser parameters should be in a continuous mode with the power density equal to, or more than, 1667 W/cm$^2$.

**Key words: Laryngeal neoplasms; Papilloma; Laser surgery**

## Introduction

The carbon dioxide laser is the most versatile laser system used in surgical practice. The system usually produces a tremendous vapour-plume, especially when the continuous mode is employed. The plume, with its malodorous smell can cause nausea, dizziness and headache. The question of whether the laser plume contains viable cells is still not clearly answered. Some investigators have reported a lack of viability of cells in laser plume (Oosterhius *et al.*, 1982; Voorhies *et al.*, 1984; Mullarky *et al.*, 1985; Abramson *et al.*, 1990; Starr *et al.*, 1992), while others (Mihashi *et al.*, 1975; Mounts and Shah, 1984; Walker *et al.*, 1986; Kashima *et al.*, 1991) have found evidence of viable cells. However, no study has mentioned the significance of the laser parameters used in relation to the viability of cells in the plume. This study was carried out to further clarify this controversial issue. Laryngotracheal papilloma is well-known for its nature of long history of recurrence. To date, carbon dioxide laser vaporization of the disease via laryngoscopic or bronchoscopic approach, by virtue of its bloodless field is still the best treatment. In spite of generous use of the smoke evacuator, some of the smoke escapes into the operating room environment, creating a potential hazard for medical and paramedical personnel. This study was also designed to determine the infectivity of viral particles which may present in the laser plume.

## Materials and methods

Ten fresh specimens of papilloma tissue along with normal hypopharyngeal mucosa were obtained from known cases of recurrent respiratory papilloma. All of these cases were pathologically diagnosed after the initial operation. The specimens were removed at the time of operation by microscopy with cupped forceps. Every specimen was washed for at least three cycles with normal saline solution to reduce bacterial or fungal contamination. Specimens of

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normal hypopharyngeal mucosa were immersed in tissue culture media, fresh papilloma tissue specimens were divided into two portions: one portion was immediately placed in a bottle containing Dulbecco's Modified Eagle (D-MEM) tissue culture media, this served as a control and the other portion was lased by carbon dioxide laser (Sharplan model 1060), employing a continuous mode from a 125 mm handpiece with the power setting of 10 watts, spot size of 0.5 mm and at the power density of approximately 1667 W/cm$^2$. Each whole piece of specimen, which measured around 25 mm$^3$, was totally lased. The laser plume generated was collected by trapping with sterile 0.22 micron pore size filter paper attached to the end of the sterile suction tube. The end of the tube was held 2 cm away from the specimens. After the process of lasing, the filter paper was immediately detached and further divided into two equal halves, then immersed in the separate bottles containing Dulbecco's Modified Eagle tissue culture medium. The specimens were immediately transferred to the laboratory where they were crushed and treated with penicillin, streptomycin and amphotericin B. Each set of specimens which now composed of three specimens (one normal mucosa, one fresh papilloma and one plume-derived) was then placed in D-MEM media with 10 per cent foetal bovine serum albumin. The plume-derived specimens were then further divided into three portions, contained in different flasks. To the first plume-derived specimens no addition was made, this system was designed to detect the viable laryngeal papilloma infected cells. To the second plume-derived specimens porcine PS clone cell line was added, and to the third plume-derived specimens normal mucosal cells were added, these two systems were designed to detect viral infectivity. To control the last two systems, porcine PS clone D cells and normal mucosal cells were separately infected with polio virus in different flasks. All of these specimen systems were then incubated at 37° Celsius for 60 days. The culture methodology was a standard cell culture procedure for diagnostic virology as described by Schmidt (1989). Microscopic examination of the cultured specimens was then carried out every day for 45 days. Microscopic photography was also taken.

## Results

After 45 days of incubation, there was no cell growth in any culture of the first plume derived specimens (Figure 1) while there was evidence of cell growth in every control fresh specimen group (Figure 2). The growing cells showed evidence of cytopathic effects typical of viral infection, i.e. large size, clear cytoplasm, cytoplasmic vacuolization and nuclear chromatolysis. However, all of the growing cultures were dead after eight consecutive subcultures. In the second and third plume-derived specimen to which PS clone D and normal mucosal cell were added, there were no morphological changes suggestive of viral infection, while viral cytopathic effects were demonstrated in every polio virus-infected control co-culture.

## Discussion

The plume usually generated from tissues being lased by a high power continuous mode laser has been found to be a hazard in animal experiments (Freitag *et al.*, 1987). It increases bronchial secretion, (Freitag *et al.*, 1987) as well as neutrophils in the secretion, and a temporary state of hypoxia has also been noted (Freitag *et al.*, 1987). There has, however, been no report of laser plume-induced disease in humans. Matchette *et al.* (1993) and Ediger and Matchette, (1989) conducted an *in vitro* study and reported viable bacteriophage in laser plume. Kashima *et al.* (1991) and Sawchuk *et al.* (1989) demonstrated that human papillomavirus DNA was present in the laser plume. The lasers employed in these studies were in the pulse mode with a pulse duration of 0.05-0.10 second, and the power was set at 1-15 watts with a power density of 1270 W/cm$^2$ or below. In contrast, Nezhat *et al.*

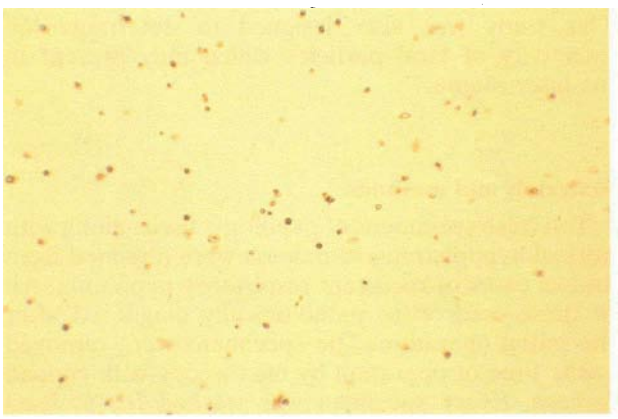

FIG. 1

Photomicrograph showing negative culture of specimen obtained from laser plume in D-MEM media. Note the carbon particles of different sizes, and absence of viable cells. (× 100)

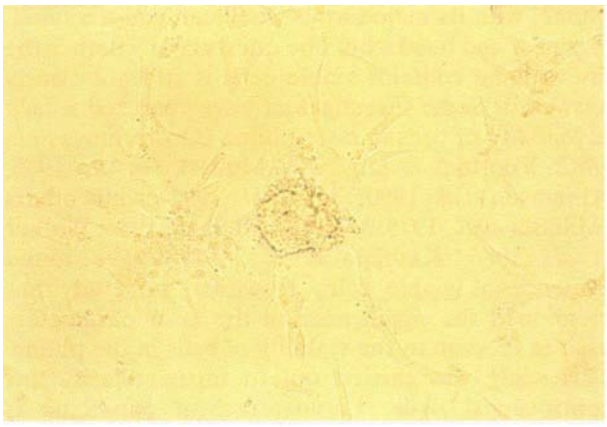

FIG. 2

Photomicrograph showing the pattern of growth of papilloma cells from a control specimen in D-MEM tissue culture media. Note the characteristic viral cytopathic effects. (× 100)

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(1987) and Voorhies *et al.* (1984) conducted *in vivo* studies and found no viable cells in the laser plume. The lasers used in the latter studies were in continuous mode with power settings of 15-30 watts and power density not less then 6000 W/cm$^2$. In the work reported here, we used a carbon dioxide laser in continuous mode with power set at 10 watts, and a power density of not less than 1667 W/cm$^2$. The results from this study suggested that, no viable cells nor infectious viral particles are found in the laser plume. It is noteworthy that there were differences in laser parameters used in the studies that gave positive and those that gave negative results. Use of a laser in continuous mode and with a power density of more than 1667 W/cm$^2$ probably is required to kill all the living cells or organisms being lased, including infectious viral particles. A pulse or repeat mode and fluence of less than 1200 W/cm$^2$ is probably not sufficient to kill the cells in the laser plume. To test this idea, a controlled study comparing these two modes as well as a superpulse mode, and differences in power density, will follow. It is probably safe here to mention that, in order to avoid a health hazard regarding tumour cell implants in humans, as well as a risk of infectivity from viral DNA, particularly from the laryngeal papilloma, the laser used should be in continuous mode with the power setting of more than 10 watts and the irradiance of not less than 1667 W/cm$^2$. Nevertheless this does not preclude one from wearing a surgical mask, since even sterile plume particles may have deteriorative effects on human. The idea of employing a continuous mode of carbon dioxide laser in the laryngeal area is probably against a conventional recommendation which advises an intermittant 0.1 second or superpulse pulse mode. However Weisberger (1991) recommended a continuous mode with a power density of 1200-1500 W/cm$^2$ for laryngeal papilloma, he found no increase in adverse effects employing this technique.

## Conclusions

The plume derived from larygneal papilloma tissue being lased by a continuous mode carbon dioxide laser with irradiance of more than 1667 W/cm$^2$ is void of viable cells and infectious DNA or viral particles. The conclusions drawn from this study could probably apply to other types of tumour, both benign and malignant, being treated by carbon dioxide laser.

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## References

Abramson, A. L., Dilorenzo, T. P., Steinberg, B. M. (1990) Papillomavirus disturbance in the plume of laser-treated laryngeal papilloma? *Archives of Otolaryngology, Head and Neck Surgery* **16:** 604–607.

Ediger, M. N., Matchette, L. S. (1989) *In vitro* production of viable bacteriophage in a laser plume. *Lasers in Surgery and Medicine* **9:** 296–299.

Freitag, L., Chapman, G. A., Sielczak, M., Ahmed, A., Russin, D. (1987) Laser smoke effect on the bronchial system. *Lasers in Surgery and Medicine* **3:** 283–288.

Kashima, H. K., Kessis, T., Mounts, P., Shah, K. (1991) Polymerase chain reaction identification of human papillomavirus DNA in $CO_2$ laser plume from recurrent respiratory papillomatosis. *Otolaryngology-Head and Neck Surgery* **104:** 191–195.

Matchette, L. S., Vegella, T. J., Faaland, R. W. (1993) Viable bacteriophage in $CO_2$ laser plume. *Lasers in Surgery and Medicine* **13:** 18-22.

Mihashi, S., Jako, G. J., Incze, J., Strong, M. S., Vanghn, C. W. (1975) Laser surgery in Otolaryngology. Interaction of $CO_2$ laser and soft tissue. *Annals of the New York Academy of Science* **267:** 263–294.

Mounts, P., Shah, K. (1984) Respiratory papillomatosis: etiological relation to genital tract papillomaviruses. *Progress in Medical Virology* **29:** 90–114.

Mullarky, M. B., Norris, C. W., Goldberg, I. D. (1985) The efficacy of the $CO_2$ laser in the sterilization of skin surface and in the plume emissions. *Laryngoscope* **95:** 186–187.

Nezhat, C., Winer, W. K., Nezhat, F., Nezhat, C., Farrest, D., Reeves, W. G. (1987) Smoke from laser surgery: Is there a health hazard? *Lasers in Surgery and Medicine* **7:** 376–382.

Oosterhius, J. W., Verschveren, R. C. J., Eibergen, R., Oldhoff, J. (1982) The viability of cells in the waste products of $CO_2$ laser evaporation of cloudman mouse melanomas. *Cancer* **49:** 6 1–67.

Sawchuk, W. S., Weber, P. J., Lowy, D. R., Dzubow, L. M. (1989) Infectious papillomavirus in the vapour of warts treated with carbon dioxide laser or electrocoagulation: Detection and protection. *Journal of the American Academy of Dermatology* **21:** 41–49.

Schmidt, N. J. (1989) Cell culture procedures for diagnostic virology. In *Diagnostic procedures for viral, rickettsial and chamydial infections.* 6th Edition (Schmidt, N. J., Emmons, R. W., eds.) American Public Health Association Inc., Washington, pp 51–100.

Starr, J. C., Kilmer, S., Wheeland, R. G. (1992) Analysis of the carbon dioxide laser plume for Simian Immunodeficiency Virus. *Journal of Dermatologic Surgery and Oncology* **8:** 297–300.

Voorhies, R. M., Lavyne, M. H., Strait, T. A., Shapiro, W. R. (1984) Does the $CO_2$ laser spread viable brain-tumor cells outside the surgical field? *Journal of Neurosurgery* **60:** 819–820.

Walker, N. P. J., Matthews, J., Newsom, S. W. B. (1986) Possible hazards from irradiation with the carbon dioxide lasers. *Lasers in Surgery and Medicine* **6:** 84–86.

Weisberger, E. C. (1991) Laryngology. In *Lasers in Head and Neck Surgery.* 1st Edition. (Weisberger, E. C., ed.), Igaku-Shoin, New York, pp 197–220.

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