

Ultraviolet disinfection with a novel microwave-powered device

D.A. Devine¹, A.P. Keech¹, D.J. Wood¹, R.A. Killington², H. Boyes¹,
B. Doubleday¹ and P.D. Marsh¹

¹Leeds Dental Institute and ²Division of Medical Microbiology, School of Biochemistry and Molecular Biology, University of Leeds, UK

812/5/01: received 7 March 2001, revised 2 May 2001 and accepted 4 May 2001

D.A. DEVINE, A.P. KEECH, D.J. WOOD, R.A. KILLINGTON, H. BOYES, B. DOUBLEDAY AND P.D. MARSH. 2001.

Aims: To evaluate the antimicrobial efficacy of a novel u.v. beaker, powered in a domestic microwave oven.

Methods and Results: Three beakers were compared, with most rapid killing obtained in the Neutra Plasma 50™. Ultraviolet light generated within the beakers efficiently killed planktonic and surface-associated *Streptococcus mutans*, *Pseudomonas aeruginosa*, vegetative *Bacillus stearothermophilus*, herpes simplex and polio viruses. *Candida albicans* and *Mycobacterium phlei* were less rapidly killed, and only 70% inactivation of *B. stearothermophilus* endospores was achieved. Irradiation for 45 s reduced viable bacterial counts in saliva by > 99%.

Conclusions: The u.v.-generating beakers efficiently reduced viable counts of bacteria, yeast and viruses. Kinetics of killing varied, reflecting the fact that lethal mechanisms are complex, and probably depend on interplay between u.v. and heat.

Significance and Impact of the Study: This novel method of generating u.v., using a cheap and widely available power source, provides a rapid, inexpensive and non-toxic method of disinfection with a wide range of applications in hospitals, clinics and the home.

INTRODUCTION

In many areas in which practitioners and patients are exposed to blood and other body fluids, improvements in cross-infection control are being sought. Effective disinfection and sterilization techniques protect patients from infection by organisms contaminating materials or instruments, and practitioners from cross-infection (Hovius 1992; Samaranayake 1993). There is a need for disinfection methods which are effective while also being cheap, rapid, non-toxic, suitable for a wide range of materials and easily put into practice. While guidelines for disinfection and sterilization procedures may be laid down, as for example by the British Dental Association, studies have found that good practice is not always followed (Treasure and Treasure 1994; Hudson-Davies *et al.* 1995; Lloyd *et al.* 1995). In some situations, efficient disinfection is especially important. For example, dental prosthetics laboratories receive a large

number of potentially hazardous materials which are contaminated by both saliva and blood; these should be disinfected, both coming into and leaving the laboratory, to prevent transmission of bacteria, yeasts, and viruses such as hepatitis B (Owen and Goolam 1993). Materials used for impressions cannot be heat sterilized and are often damaged by chemical disinfection (Blair and Wassell 1996). Also, chemical disinfectants may be toxic, deteriorate with storage time and organic matter can reduce their activity.

Ultraviolet (u.v.) irradiation has long been recognized as an effective method for killing microbes (Chang *et al.* 1985), and there has been a recent resurgence of interest in its application, particularly in the water industry (Abbaszadegan *et al.* 1997; Oppenheimer *et al.* 1997) and in disinfection of surfaces of food (Wallner-Pendleton *et al.* 1994; Kuo *et al.* 1997). Ultraviolet irradiation has advantages over many existing disinfection methods in not requiring chemicals or heat, and in being fast. However, limitations in current u.v. technology have restricted its use. Relatively expensive power sources and arc-lamps are needed to generate u.v. In addition, the likelihood of output

Correspondence to: D. Devine, Oral Microbiology, Division of Oral Biology, Leeds Dental Institute, Clarendon Way, Leeds LS2 9LU, UK.

degradation and shadowing leads to non-uniform exposure of articles.

A novel device has been developed for generating u.v. light for routine nucleic acid decontamination of plastics prior to PCR (Bonass *et al.* 1999). The device comprises a hollow-walled quartz beaker which can be placed within a domestic microwave oven; mercury vapour between the two walls allows excitation of microwaves into u.v. light. Thus, a cheap and widely available power source is used, targets are exposed to u.v. from all directions and the beaker gives no shadowing or output degradation.

There are many applications for this type of approach in disinfection and cross-infection control in dentistry and medicine, and for use in the home. The aim of this study was to evaluate the microbicidal efficacy of the novel u.v. beaker, prior to testing its suitability for specific applications within hospitals, clinics and in the home.

MATERIALS AND METHODS

Organisms

The panel of organisms included *Candida albicans* NCTC 3153, *Streptococcus mutans* NCTC 10449 and *Pseudomonas aeruginosa* NCTC 10332. *Bacillus stearothermophilus* and *Mycobacterium phlei* were kindly provided from the culture collection of Dr K. Kerr (Division of Microbiology, School of Biochemistry and Molecular Biology, University of Leeds). Free endospore suspensions and bio-indicator vials, containing 10^5 – 10^7 endospores of *B. stearothermophilus* ATCC 7953, were purchased (Merck, Poole, UK). Vegetative cells were removed from endospore suspensions by incubation in phosphate-buffered saline (PBS: pH 7.4), containing $50 \mu\text{g ml}^{-1}$ lysozyme (Sigma), for 30–45 min at 37°C and centrifuging at 5000 g for 15 min. Endospores were washed 10 times in PBS prior to use. Herpes simplex virus type 1 (HSV-1) was strain HFEM (Watson *et al.* 1966), and an attenuated polio virus strain (Sabin type 3) was provided by the National Institute of Biological Standards, London. Whole saliva samples, collected from three individuals who had not eaten or drunk for at least 2 h, were pooled and vortexed, with 3 mm glass beads to disrupt clumps and co-aggregated bacteria, and used as a source of complex communities of micro-organisms.

Growth conditions

Bacteria and yeast. The liquid medium used to grow all strains of bacteria and *C. albicans* was Brain Heart Infusion (BHI) broth (Oxoid), with or without 30% (v/v) sterile horse serum (Oxoid). The solid medium for growth of *C. albicans* was Sabouraud agar (Oxoid), and nutrient agar (Oxoid) was

used for *B. stearothermophilus*. The remaining bacterial strains were grown on BHI containing 1.2% (w/v) agar (Oxoid). Saliva was inoculated onto Columbia agar (Oxoid) containing 5% (v/v) sterile horse blood. *Bacillus stearothermophilus* was incubated aerobically at 55°C. *Streptococcus mutans* was grown in an anaerobic Work Station (Mark III, Don Whitley Scientific Ltd, Shipley, UK) at 37°C under an atmosphere of 80% nitrogen, 10% hydrogen and 10% carbon dioxide. *Pseudomonas aeruginosa*, *Myc. phlei* and *C. albicans* were grown aerobically at 37°C. For determinations of total counts in saliva, Columbia blood agar plates were incubated at 37°C in 5% CO₂. Organisms were stored at –80°C in 30% (v/v) sterile glycerol.

Viruses. Baby Hamster Kidney Cells (BHK-21) and HEp-2 cells were grown in the Dulbecco modification of Eagle's medium (DMEM; Flow Laboratories) as previously described (Watson *et al.* 1966). HSV-1 was grown and assayed in BHK-21 cells, polio virus in HEp-2 cells. Virus stocks were grown to a multiplicity of infection of 0.1 pfu per cell. HSV-1 was assayed by the suspension plaque assay method of Russel (1962) and polio virus by the agar overlay monolayer method (Killington *et al.* 1974). Assays were fixed in formol saline, stained with gentian violet and the readily-discernible plaques counted with the aid of a stereomicroscope. All assays were performed in duplicate.

Ultraviolet beakers

Ultraviolet beakers (Fig. 1) were supplied by Nimbus Innovations Ltd. (Monmouth, UK). Beakers A and B, used in most experiments, comprised hollow-walled quartz beakers with lids of similar construction. The hollow walls contain mercury vapour. The Neutra Plasma 50™ (Merck) beaker, which became available later in the study, was also used in some experiments; this is a quartz beaker, identical to B, encased in a plastic jacket to protect the user from heat and ozone and to lower the output of u.v. light to the exterior of the beaker.

Irradiation of microbes

Bacterial and yeast suspensions. Broth cultures (18–24 h incubation) were washed in quarter strength Ringer's solution (RS; Lab M) and adjusted to an optical density (O.D.) at 540 nm corresponding to 10^4 , 10^5 or 10^6 colony-forming units (cfu) ml⁻¹. Endospore suspensions were diluted to concentrations of 10^4 – 10^6 endospores ml⁻¹ in RS, or commercially-prepared vials were placed directly into the beaker. Aliquots (1 ml) of bacterial or yeast suspensions were placed in 7 ml polypropylene bottles; these were placed in the u.v. beaker and irradiated on a rotating turntable for 15–120 s in a commercial microwave oven on high output



Fig. 1 The microwave-powered beakers used in the study to generate ultra-violet light. From the left, Beaker A (internal and external diameters 8.5 and 10.5 cm, respectively, and height 13.7 cm), Beaker B (internal and external diameters 5.0 and 7.0 cm, respectively, and height 17.0 cm) and the Neutra Plasma 50™ (internal and external diameters 5.5 and 7.0 cm, respectively, and height 17.0 cm. The quartz beaker is encased in a plastic jacket)

setting (Tricity model MV621, Tricity Bendix, Slough, UK or Toshiba model ER 7820, Toshiba, Camberley, UK). Ultraviolet beakers were fully cooled to room temperature prior to irradiation, unless otherwise stated. One of the potentially important and unique properties of the beakers is that u.v. light is generated from all directions. To determine the importance of this characteristic, in some experiments the beaker lid or the 7 ml polypropylene bottle lid was removed.

After irradiation, organisms were suspended in RS and left to recover at room temperature for 30 min, prior to further dilution in RS and determination of viable counts using spread plates. Dose–response curves were plotted from means of at least three determinations carried out in duplicate, allowing calculation of decimal reduction values (D : time taken to kill 90% of the inoculum) and lethal doses (LD: time taken to reduce counts to undetectable levels, i.e. > 99.9% killing). Regression analysis was used to determine whether killing curves were linear, indicating single-hit killing kinetics, or non-linear, indicating multi-hit or multi-component killing.

In vivo conditions often result in micro-organisms growing in the presence, for example, of blood components. This may result in morphological or physiological variations. Therefore, experiments were repeated using organisms grown in BHI broth containing 30% (v/v) sterile serum. Additionally, many potential applications of this technique would require disinfection of materials or equipment that may have been bathed in organic fluids, such as saliva or blood. To simulate this, in some experiments organisms were suspended in RS containing 30% (w/v) sterile horse serum (Oxoid) prior to irradiation.

Bacteria and yeast dried onto surfaces. Microbes in fluids, bathing equipment or materials, may also be subject to drying prior to disinfection. This was simulated by placing 100 μ l aliquots of pure cultures or of pooled saliva on the surfaces of glass slides or blocks of silicone dental impression material (Aquasil base, Dentsply Ltd, Weybridge, UK; prepared according to manufacturers' instructions). Slides and impression material blocks in Petri dishes were placed to dry in a 37°C incubator for 3 h. Dried organisms were harvested from surfaces prior to irradiation (100% counts), and after irradiation for up to 120 s, using moistened calcium alginate swabs (TSC Ltd, Heywood, UK). Following re-suspension in RS, viable counts were determined as above. All experiments were carried out in duplicate on at least three occasions.

Viruses. Virus suspensions (1 ml) in 7 ml polypropylene bottles were placed in the u.v. beaker and irradiated in a Matsui M196T (950 Watt) (Matsui, Hemel Hempstead, UK) domestic microwave oven for 30–120 s, at high power. The virus suspensions were allowed to recover for 30 min at room temperature, prior to dilution in growth medium, and assayed as described earlier.

Viruses were dried onto glass surfaces by adding 1.0 ml suspensions in DMEM medium to a glass Bijoux bottle. Bottles were placed into an air-tight box overnight at 37°C to dry. Prior to and following irradiation, virus particles were allowed to recover at room temperature for 30 min and were resuspended in 1 ml PBS prior to assay. All experiments were carried out on at least three separate occasions. As for bacteria and yeast (above), dried non-irradiated samples were enumerated as controls.

Measurement of beaker temperature

Ultraviolet beakers were cooled to room temperature and a temperature measurement strip (RS Components) placed on the internal wall, 4 cm from the top. Beakers were exposed to microwaves for timed intervals up to 90 or 120 s. The maximum temperature reached by the internal walls of the beakers was indicated by a colour change of the temperature measurement strip. The procedure was repeated with a 500 ml beaker full of water placed in the microwave oven alongside the u.v. beaker. These experiments were repeated on three occasions and the median maximum temperature recorded.

RESULTS

Irradiation of microbes in suspension

Preliminary experiments showed that rates of killing of *Ps. aeruginosa* and *Strep. mutans* were very similar in beakers A and B. These were used interchangeably in most experiments, and the results presented in Table 1 were obtained using these beakers. The Neutra Plasma 50™ beaker killed most rapidly, achieving > 90% killing of *Ps. aeruginosa* within the first 10 s of u.v. generation (data not shown).

Killing of bacteria and *C. albicans*. Suspensions in plastic bijoux bottles were exposed to u.v. light in beaker A or B for up to 2 min at inoculum levels of 10^4 and 10^6 cfu ml⁻¹ (Table 1). When organisms were grown in BHI broth without added serum, u.v. light generated within the beakers efficiently (> 99.9% reduction in number) killed planktonic

Strep. mutans, *Ps. aeruginosa* and vegetative *B. stearothermophilus*. *Candida albicans* and *Myc. phleii* were less affected. Inoculum concentration had little effect on *D*-values of microwave-generated u.v. light against bacteria, while *C. albicans* was most efficiently killed at low inoculum levels. The inclusion of 30% (v/v) serum in the growth medium resulted in a reduction in sensitivity of *Strep. mutans* and *Ps. aeruginosa* to u.v. inactivation (Table 1). For *C. albicans*, the presence of 30% (v/v) horse serum in the growth medium resulted in increased sensitivity to u.v. killing when the inoculum was 10^6 cfu ml⁻¹, but decreased sensitivity at 10^4 cfu ml⁻¹.

Dose-response curves indicated that single-hit (linear), multi-hit and multi-component killing occurred within the beakers (Fig. 2), dependent on the organism, inoculum concentration and growth medium. For example, while killing of *Strep. mutans* was probably single-hit when BHI was the growth medium, multi-hit kinetics were indicated when the organism was grown in the presence of serum (Fig. 2c). Similarly, at the lower inoculum level, killing of *Ps. aeruginosa* became multi-hit (Fig. 2b). *Mycobacterium phleii* exhibited multi-component killing, especially at low inoculum concentration. In most experiments, killing of *C. albicans* appeared to be single-hit, but the killing curve of the 10^4 cfu ml⁻¹ inoculum grown in BHI + serum was steeper (Fig. 2e). Multi-component killing was indicated for *C. albicans* (10^4 cfu ml⁻¹) grown in BHI containing 30% serum.

To determine the effects of protein in the suspending medium on u.v. killing, *Ps. aeruginosa*, *Strep. mutans* and *C. albicans*, grown in BHI without serum, were washed and resuspended in RS containing 30% (v/v) horse serum.

Table 1 Antimicrobial activity of microwave-generated u.v. light

Organism	Growth medium	Value against inoculum (suspended in ¼ Ringer's solution)					
		10 ⁴ cfu ml ⁻¹			10 ⁶ cfu ml ⁻¹		
		<i>D</i> *	LD†	<i>R</i> ² ‡	<i>D</i> *	LD†	<i>R</i> ² ‡
<i>Pseudomonas aeruginosa</i>	BHI	28	52	0.92	24	67	0.99
	BHI + 30% serum	38	120	0.98	42	> 120	0.97
<i>Streptococcus mutans</i>	BHI	20	72	0.96	17	64	0.96
	BHI + 30% serum	32	72	0.96	32	84	0.98
<i>Mycobacterium phleii</i>	BHI	> 120	> 120	0.92	> 120	> 120	0.65
<i>Bacillus stearothermophilus</i> §	BHI	53	100	0.95	56	> 120	0.99
<i>Candida albicans</i>	BHI	21	74	0.96	110	> 120	0.97
	BHI + 30% serum	108	> 120	0.83	87	> 120	0.97

**D*-value: time of exposure to u.v. light (in s) taken to kill 90% of the inoculum.

†LD value: time of exposure to u.v. light (in s) taken to reduce viable counts by > 99.9%.

‡*R*² value derived from linear regression analysis.

§Vegetative cells.

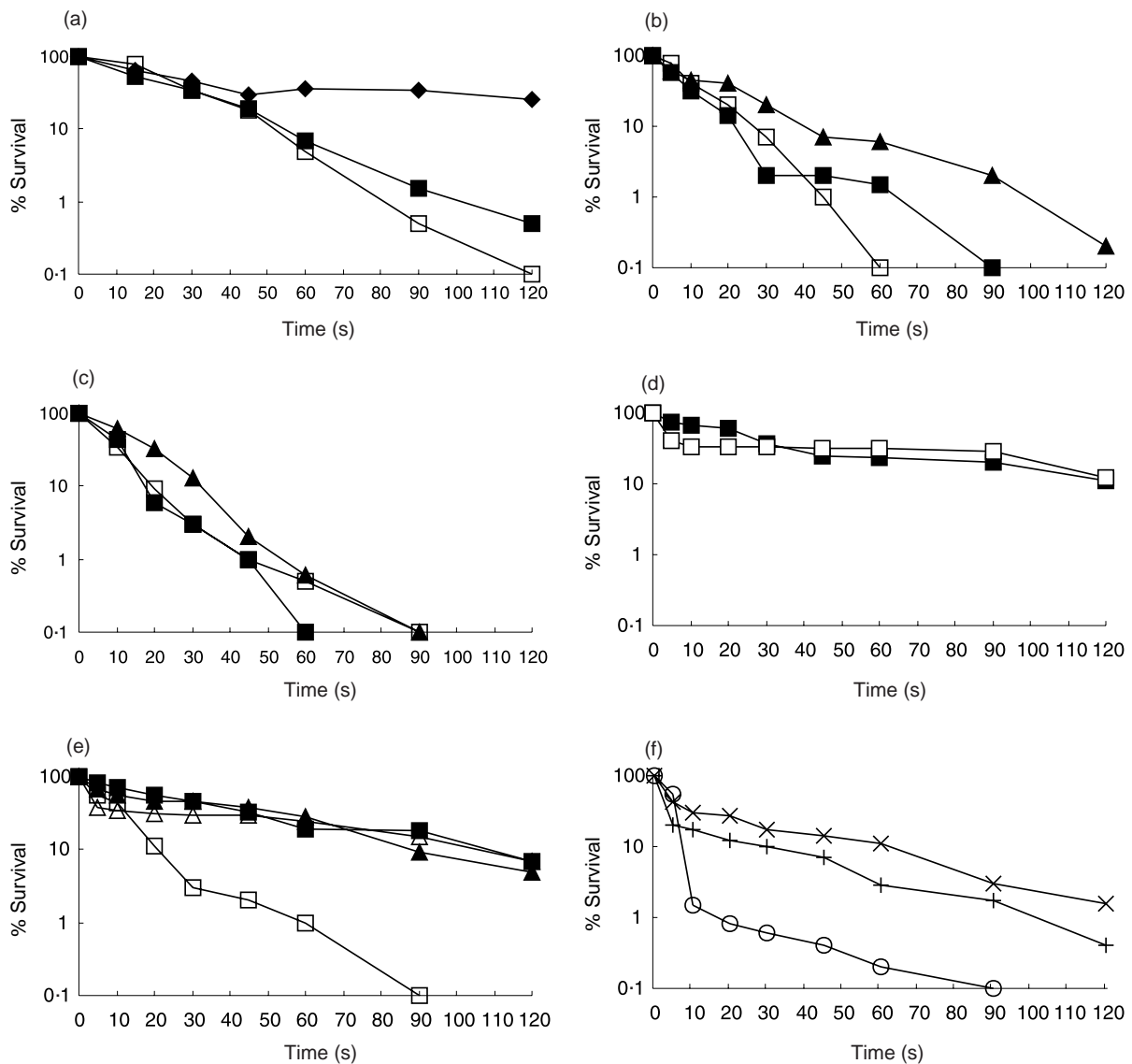


Fig. 2 Dose-response curves of bacteria and *Candida albicans* to u.v. light generated within the u.v. beakers. (a) *Bacillus stearothermophilus*, (b) *Pseudomonas aeruginosa*, (c) *Streptococcus mutans*, (d) *Mycobacterium phlei*, (e) *Candida albicans*; 10^6 (■) and 10^4 (□) cfu ml⁻¹ grown in BHI broth, 10^6 (▲) and 10^4 (△) cfu ml⁻¹ grown in BHI broth plus 30% (v/v) serum, 10^6 endospores ml⁻¹ (◆). (f) Organisms dried onto the surface of silicone dental impression material; *Pseudomonas aeruginosa* (○), *Streptococcus mutans* (×) and *Candida albicans* (+)

These suspensions were markedly less susceptible to killing by u.v. light than when suspended in RS alone; 90% killing was not achieved within 120 s for any of the three organisms at any inoculum concentration (Table 2).

Exposure to u.v. in the beaker from all directions resulted in the most efficient killing. Lids of 7 ml polypropylene bottles were opaque to u.v. light, and when they were omitted from bottles placed in the u.v. beaker, both *D*- and *LD* values were reduced to < 5 s for killing *Ps. aeruginosa* (10^6 cfu ml⁻¹), and to 8 s (*D*) and 102 s (*LD*) for *C. albicans*. Similarly, when the lid of the u.v. beaker was omitted,

resulting in u.v. generation in the beaker only from the sides and bottom, much lower levels of killing were observed than when the lid was in place.

Saliva. Ultraviolet irradiation within the beaker was sufficient to reduce total counts in whole pooled saliva by 68% in 15 s, and > 99% in 45 s, but a few isolated colonies were detected after irradiation for 120 s.

Endospores. After irradiation for 90 s, 70% inactivation of endospore suspensions (10^6 ml⁻¹) was achieved (Fig. 2a)

Table 2 Influence of protein in the suspending medium on microbial inactivation by microwave-generated u.v. light

Organism	Inoculum concentration (cfu ml ⁻¹)	% Killing after exposure to u.v. light for 120 s when suspended in:	
		RS*	RS + 30% (v/v) serum
<i>Pseudomonas aeruginosa</i>	10 ⁴	> 99.9	87
	10 ⁶	> 99.9	77
<i>Streptococcus mutans</i>	10 ⁴	> 99.9	59
	10 ⁶	> 99.9	48
<i>Candida albicans</i>	10 ⁴	> 99.9	68
	10 ⁵	93	67

*1/4 Ringer's solution.

and this only increased to 75% following exposure for 180 s. When bio-indicator vials were exposed to u.v. in the beaker for 4 min, sugar fermentation was detected after subsequent incubation of the vials for 24 h. After 6 min irradiation, detectable sugar fermentation was further delayed and required incubation for 48 h.

Viruses. Inactivation of suspensions containing 9.9×10^9 pfu ml⁻¹ HSV-1 was rapid, with a 98.6% reduction in infectious virus particles seen after 30 s. No infectious virus particles were detected after irradiation for 120 s (Table 3). Similarly, 96.5% inactivation of polio virus suspensions (9.2×10^8 pfu ml⁻¹) occurred within 30 s. Small numbers of infectious virus particles were detected after irradiation for 120 s.

Irradiation of microbes associated with surfaces

Streptococcus mutans, *Ps. aeruginosa* and *C. albicans* exhibited 77% (± 9), 4% (± 1) and 17% (± 6), survival of drying, respectively, when grown and suspended in BHI and dried onto impression material. Survival of bacteria in pooled human saliva after the drying process was 12% (± 5). Organisms that survived drying were exposed to u.v. in the beakers, and dose-response curves for each species indicated multi-component killing (Fig. 2f); *D*-values were 8 s (*Ps. aeruginosa*), 30 s (*C. albicans*) and 63 s (*Strep. mutans*). These curves were quite different to those obtained for the same organisms in suspension at a comparable inoculum level of 10⁴ cfu ml⁻¹ (Fig. 2b,c). Concentrations of salivary bacteria were reduced by 99% after irradiation for 5 s and > 99.9% after 20 s. Those infectious HSV-1 particles which survived the drying process (0.5%) and were irradiated were extremely susceptible; no survivors were detected after 15 s (Table 3). Conversely, polio virus (8.5% of the original inoculum survived drying) was less susceptible to u.v. when surface-associated, compared with susceptibility when in suspension (Table 3).

Table 3 Inactivation of herpes simplex and polio virus particles by microwave-generated u.v. light

Virus preparation	Percentage inactivation after irradiation for (s):			
	30	60	90	120
Herpes virus – suspension*	98.63	99.84	99.83	100
Herpes virus – dried†‡	100	100	100	100
Poliovirus – suspension*	96.50	99.86	99.47	99.89
Poliovirus – dried†§	66.67	99.08	99.64	99.99

*Infectious virus particles irradiated while suspended in growth medium.

†Infectious virus particles suspended in growth medium and allowed to dry on the surfaces of glass bijoux bottles prior to irradiation.

‡Number of HSV-1 infectious virus particles surviving drying was 5.08×10^7 (0.5% of original 9.90×10^9 pfu ml⁻¹ inoculum placed on the surface), and this was taken as the 100% value prior to irradiation.§Number of polio virus particles surviving drying was 7.80×10^7 (8.5% of original 9.20×10^8 pfu ml⁻¹ inoculum placed on the surface), and this was taken as the 100% value prior to irradiation.

Effects of beaker temperature on microbial killing

The u.v. beakers only require relatively low power microwave ovens to generate u.v. and domestic ovens are essentially overpowered. The excess is converted into heat, and this was measured using temperature strips placed on the internal walls of beaker B and the Neutra Plasma 50TM (Table 4). While the Neutra Plasma 50TM remained slightly cooler than beaker B for the first minute of irradiation, temperatures > 104°C were reached after irradiation for 90 s, compared with between 88 and 93°C for beaker B.

The increased temperature of the u.v. beakers was moderated by placing a 500 ml beaker of water on the microwave oven turntable (Table 4). This water absorbed excess microwave energy which would otherwise have gone towards heating the u.v. beakers, hence reducing the internal wall temperatures of both the Neutra Plasma 50TM and beaker B. Increased temperature within the beakers also

Table 4 Temperatures reached within u.v. beakers exposed to microwaves

Ultraviolet beaker	500 ml beaker of water*	Maximum internal temperature (°C) (median, <i>n</i> = 3) after irradiation for (s):					
		15	30	45	60	90	120
Beaker B	–	< 37	> 60 < 65	> 77 < 82	> 82 < 88	> 88 < 93	> 99 < 104
	+	< 37	> 44 < 46	> 49 < 54	> 49 < 54	> 65 < 71	> 77 < 82
Neutra Plasma 50™	–	< 37	> 54 < 60	> 62 < 65	> 71 < 77	> 104 < 110	nd†
	+	< 37	> 40 < 42	> 49 < 54	> 54 < 60	> 71 < 77	nd

*500 ml beaker of water placed on turntable in the microwave oven alongside the u.v. beaker.

†Not determined.

affected bactericidal u.v. output (personal communication, C. Wenlock, Ambalamps Ltd), lowering the output of light at 254 nm by increasing the wavelength of the generated light.

To investigate the influence of the temperature of the u.v. beakers, killing of *Ps. aeruginosa* (10^6 cfu ml⁻¹) within beaker B which was hot at the start of irradiation was compared with killing when beaker B was fully cool. Beaker B was heated by being irradiated empty for 60 s, and a suspension of *Ps. aeruginosa* was immediately placed within the hot beaker, irradiated for 15 s and the surviving viable count determined. This was repeated with the beaker being cooled for 5, 10, 15 and 20 min before the *Ps. aeruginosa* suspension was exposed to u.v. The temperature of the beaker was also manipulated by repeating the above experiment with a 500 ml beaker of water placed on the microwave turntable alongside the u.v. beaker, thereby reducing the maximum temperature reached within the u.v. beaker (Table 4). The temperature of the u.v. beaker at the start of a period of irradiation had a minimal effect on the total percentage kill achieved (Fig. 3). However, when a beaker of water was placed within the microwave oven to reduce the maximum temperature reached in the u.v. beaker, killing of *Ps. aeruginosa* was less efficient (Fig. 3).

DISCUSSION

The aim of this study was to determine whether the novel u.v. beakers described were capable of killing or inactivating a range of micro-organisms, with a view to assessing their potential for development as a rapid method of disinfection or sterilization for use in hospitals, laboratories and clinics. Total counts of bacteria, yeast and viruses, and bacterial counts within saliva, were rapidly reduced to very low levels after exposure to u.v. within the beakers. However, *Myc. phleii* and endospores of *B. stearothermophilus* were less sensitive. Not surprisingly, inoculum concentration influenced the rate of killing in some cases, and the presence of high concentrations of organic matter in the suspending medium reduced killing in all cases. A pulsed power source has been shown to increase the bactericidal efficiency of u.v.

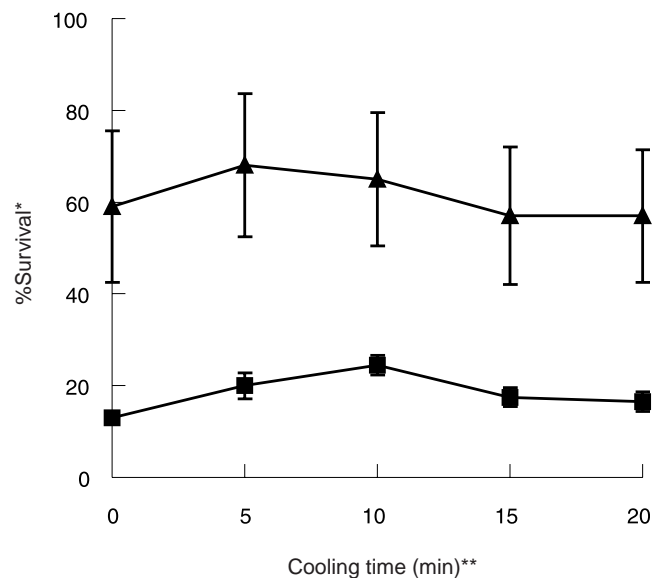


Fig. 3 Effect of beaker temperature on killing of *Pseudomonas aeruginosa*. *Percentage survival was calculated after *Ps. aeruginosa* had been irradiated for 15 s. **Cooling time: Beaker B was irradiated empty for 60 s and then allowed to cool for 5–20 min prior to irradiating *Ps. aeruginosa* (10^6 cfu ml⁻¹). The experiments were carried out in the presence (▲) or absence (■) of a 500 ml beaker of water on the turntable alongside the u.v. beaker

light (MacGregor *et al.* 1998) and this may be worth considering in the further development of this device.

Both linear (single-hit), multi-hit and multi-component killing occurred within the beakers, and kinetics of killing varied according to the target organism, inoculum concentration and growth medium. For example, at a concentration of 10^4 cfu ml⁻¹, *C. albicans* grown in BHI plus serum was less rapidly killed than when grown in BHI. The dose-response curves were also significantly different. The culture grown in BHI + serum probably followed multi-component kinetics because *C. albicans* produces germ tubes when grown in the presence of serum, thus providing a mixture of susceptible targets. Both *Ps. aeruginosa* and *Strep. mutans* were less rapidly killed when they had been grown in BHI +

serum compared with cultures grown in BHI broth alone. Serum in growth media is known to produce significant alterations in the cell walls of Gram-negative bacteria (Allan and Poxton 1994), and such phenotypic alterations may have accounted for the reduced susceptibility of *Ps. aeruginosa* grown in BHI plus serum. The influence of serum and other proteins on phenotypic properties may be significant *in vivo*; saliva contains 140–640 mg protein 100 ml⁻¹ (Jenkins 1978), but organisms growing in the gingival crevice or a bleeding periodontal pocket may be exposed to higher concentrations of serum.

Variations in dose responses may also have been because the mechanism of killing within these beakers is complex. The maximum temperature reached in the beaker had a marked effect on killing of *Ps. aeruginosa*. As bactericidal u.v. output in the beakers is known to decrease with increased irradiation time, and therefore probably also increased temperature, there appears to be a complex relationship between these parameters. Additionally, ozone is generated alongside u.v. light, and this may act in synergism with u.v. and heat. Synergistic killing has been observed between u.v. and hydrogen peroxide (Bayliss and Waites 1980; Gardner and Shama 1998), and similar interactions between oxidative and u.v. stresses may occur in the u.v. beakers used in the present study. This may also have been the reason why the Neutra Plasma 50™ beaker killed more rapidly than the other two beakers; more heat was generated by this beaker and the plastic jacket prevents users being exposed to ozone by delaying or preventing its escape from the beaker. An additional major advantage of the u.v. beakers is that targets are exposed to u.v. from all directions. The contribution of this to killing was demonstrated by the increased killing levels achieved when u.v.-opaque lids were removed from polypropylene bottles, and by the fact that much reduced killing rates were seen when the lid of the beaker was left off.

These experiments indicate that the u.v. beakers may be useful for disinfection but not sterilization; in many cases, a few cfu could be detected after irradiation for 2 min. Ultraviolet light is known to penetrate materials poorly (Gardner and Shama 1998) and is therefore most useful for surface disinfection. Organisms dried onto surfaces were very rapidly killed, but further assessment of killing of organisms deposited onto materials for specific applications, or those which have grown as biofilms, may be necessary. On-going studies in these laboratories are assessing the effects of microwave-generated u.v. light in the beakers on organisms on dental materials and equipment which become contaminated by blood and saliva. Saliva contains up to 10⁸ cfu ml⁻¹, and can harbour transient bacterial pathogens and a certain range of pathogenic viruses (Marsh and Martin 1999). High levels of killing of salivary bacteria were achieved in the u.v. beakers. Separate experiments showed

that enveloped and non-enveloped viruses are susceptible to killing by this method.

Ultraviolet light has been investigated for disinfection applications outside the water industry, e.g. in the disinfection of surfaces of food (Wallner-Pendleton *et al.* 1994; Kuo *et al.* 1997). Ultraviolet has also been shown to kill fungi on dental impression materials (Ishida *et al.* 1991), and bacteria on implant materials (Delgado and Schaaf 1990) and dental hand-pieces (Eakle *et al.* 1986). However, limitations in current u.v. technology have restricted its use. Development of the novel u.v. beakers used in this study, which have the advantages of a cheap power source and no output degradation or shadowing, could result in significant improvements in practice, and cross-infection control in certain contexts and with certain materials. The use of such beakers may also be extended to particular disinfection practices in the home, using domestic microwave ovens as power sources.

ACKNOWLEDGEMENTS

This work was supported by the NHS Primary Dental Care R&D Programme, grant number RDO/90/14. The authors are grateful to Jenton International plc and Nimbus Innovations plc for provision of u.v. beakers, and to C. Wenlock, Ambalamps Ltd, for information concerning u.v. output of the beakers.

REFERENCES

- Abbaszadegan, M., Hasan, M.N., Gerba, C.P. *et al.* (1997) The disinfection efficacy of a point-of-use water treatment system against bacterial, viral and protozoan waterborne pathogens. *Water Research* **31**, 574–582.
- Allan, E. and Poxton, I.R. (1994) The influence of growth medium on serum sensitivity of *Bacteroides* species. *Journal of Medical Microbiology* **41**, 45–50.
- Bayliss, C.E. and Waites, W.M. (1980) The effect of hydrogen peroxide and ultraviolet irradiation on non-sporing bacteria. *Journal of Applied Bacteriology* **48**, 417–422.
- Blair, F.M. and Wassell, R.W. (1996) A survey of the methods of disinfection of dental impressions used in dental hospitals in the United Kingdom. *British Dental Journal* **180**, 369–375.
- Bonass, W., Wood, D., Devine, D. and Marsh, P. (1999) A novel method for the inactivation of DNA contaminants in PCR. *Journal of Dental Research* **78**, 1070.
- Chang, J.C., Ossoff, S.F., Lobe, D.C. *et al.* (1985) UV inactivation of pathogenic and indicator organisms. *Applied and Environmental Microbiology* **49**, 1361–1365.
- Delgado, A.A. and Schaaf, N.G. (1990) Dynamic ultraviolet sterilization of different implant types. *International Journal of Oral and Maxillofacial Implants* **5**, 117–125.
- Eakle, W.S., Kao, R.T., Gordon, M. and Pelzner, R.B. (1986) Microbiological assessment of ultraviolet sterilization of dental handpieces. *Clinical Preventive Dentistry* **8**, 10–14.

- Gardner, D.W.M. and Shama, G. (1998) The kinetics of *Bacillus subtilis* spore inactivation on filter paper by u.v. light in combination with hydrogen peroxide. *Journal of Applied Microbiology* **84**, 633–641.
- Hovius, M. (1992) Disinfection and sterilisation: the duties and responsibilities of dentists and dental hygienists. *International Dental Journal* **42**, 241–244.
- Hudson-Davies, S.C.M., Jones, J.H. and Sarll, D.W. (1995) Cross-infection control in general dental practice: dentists' behaviour compared with their knowledge and opinions. *British Dental Journal* **178**, 365–369.
- Ishida, H., Nahara, Y., Tamamoto, M. and Hamada, T. (1991) The fungicidal effect of ultraviolet light on impression materials. *Journal of Prosthetic Dentistry* **65**, 532–535.
- Jenkins, G.N. (1978) Saliva. In *The Physiology and Biochemistry of the Mouth* 4th edn. pp. 284–359. Oxford: Blackwell Scientific Publications.
- Killington, R.A., Lee, D., Stott, E.J. and Osborne, J. (1974) Studies on the lysosomes of L132 cells infected with either rhinovirus type 2 or poliovirus type 1. *Journal of General Virology* **22**, 303–307.
- Kuo, F.L., Ricke, S.C. and Carey, J.B. (1997) Shell egg sanitation: UV radiation and egg rotation to effectively reduce populations of aerobes, yeasts and moulds. *Journal of Food Protection* **60**, 694–697.
- Lloyd, L., Burke, F.J. and Cheung, S.W. (1995) Handpiece asepsis: a survey of the attitudes of dental practitioners. *British Dental Journal* **178**, 23–27.
- MacGregor, D.J., Rowan, N.J., McIlvaney, L., Anderson, J.G., Fouracre, R.A. and Farish, O. (1998) Light inactivation of food-related pathogenic bacteria using a pulsed power source. *Letters in Applied Microbiology* **27**, 67–70.
- Marsh, P.D. and Martin, M.V. (1999) The Resident Oral Microflora. In *Oral Microbiology*. 4th edn. pp. 17–57. Oxford: Wright.
- Oppenheimer, J.A., Jacangelo, J.G., Laine, J.M. and Hoagland, J.E. (1997) Testing the equivalency of ultraviolet light and chlorine for disinfection of wastewater to reclamation standards. *Water Environment Research* **69**, 14–24.
- Owen, C.P. and Goolam, R. (1993) Disinfection of impression materials to prevent viral cross contamination: a review and a protocol. *International Journal of Prosthodontics* **6**, 480–494.
- Russel, W.C. (1962) A sensitive and precise plaque assay for herpes virus. *Nature* **249**, 360–361.
- Samaranayake, L. (1993) Rules of infection control. *International Dental Journal* **43**, 578–584.
- Treasure, P. and Treasure, E.T. (1994) Survey of infection control procedures in New Zealand dental practices. *International Dental Journal* **44**, 342–348.
- Wallner-Pendleton, E.A., Sumner, S.S., Froning, G.W. and Stetson, L.E. (1994) The use of ultraviolet radiation to reduce *Salmonella* and psychrotrophic bacterial contamination on poultry carcasses. *Poultry Science* **73**, 1327–1333.
- Watson, D.H., Sheddon, W.I.H., Elliot, A. *et al.* (1966) Virus specific antigens in mammalian cells infected with Herpes simplex virus. *Immunology* **11**, 399–408.