CHAPTER THREE

3.0 LIGHT SOURCES, REQUIRED PROPERTIES IN PDT AND PHOTORADIATION TECHNIQUES

3.1 Introduction

One of the most important aspect in a therapeutic photoradiation is to bring sufficient light to reach a target site to bring about photoactivation of the sensitizer. Hence, appropriate light sources and light delivery techniques should be employed to allow for maximum tissue penetration of the appropriate wavelength of light. Doiron and Svaasand (1983) reported that light penetration is directly dependent on the wavelength and the power density of the incident light. It is therefore not surprising that the above parameters will determine the extent of tumour necrosis and the effectiveness of PDT as a treatment modality.

In the initial PDT investigations of Diamond *et. al.* (1972) and Kelly and Snell (1976), white light with a strong ultra-violet (UV) component from a mercury lamp was used to photosensitize HpD which has a major absorption peak at about 395nm (soret band) in the UV. However Dougherty *et. al.* (1978) and Dougherty (1981) was the first to use red light between 620-630nm to photoirradiate tumours. As a result of this, a filtered high powered xenon discharge lamp was used. Subsequently, argon ion pumped dye lasers were used and were found to yield good tumour responses. Henceforth, red light (620-630nm) was used to photoactivate HpD or Hp. This wavelength of light offers maximum tissue penetration although it is the least intense absorption peak of HpD and Hp.

Since the development of HpD-PDT programme at this centre, a simple and cost-effective red light source based on an overhead projector halogen lamp was constructed

(Low *et al,* 1986). It has been proven to be as effective as the laser in the treatment of superficial malignancies in animal models.

Subsequently, at the local center here, two other light sources were developed and modified from an overhead projector and a surgical head lamp (Low *et. al.*, 1994) were upgraded in design for practical use in the first phase of clinical trials for the treatment of superficial malignancies. These light sources showed much promise as a suitable alternative to the laser system because they were cost effective, easy to operate, rugged and reliable. In fact, for routine clinical use for treatment of superficial tumours, they proved superior to the laser systems in terms of manoeuvrability in the clinical environment. At the same time, a commercial He-Ne laser system was used for basic animal studies pending for endoscopic and interstitial applications for the second phase of clinical trials involving treatment of internal malignancies at this centre. The eutectic gold vapour laser which was constructed at this centre by Cheah (1992) will also be used for the second phase of clinical trials at this centre.

3.2 Review of Light Sources Used In PDT

3.2.1 General Review Of Light Sources And Lasers

Pioneering work in the treatment of numerous cutaneous and subcutaneous malignancies were carried out using incoherent light sources such as xenon and mercury discharged lamps and also tungsten-halogen incandescent lamps (Diamond *et. al.*,1972; Kelly and Snell., 1976; Dougherty *et. al.*, 1978; Forbes *et. al.*, 1980). It is interesting to note that in the treatment of recurrent breast carcinoma, the best results reported that the power density of 100mW/ cm^2 or more was effective for surface illumination of the lesions.

However, one of the problems encountered with incoherent lamps was that these lamps produced a lot of infra-red radiation that induced excessive thermal heating of tissue exposed to the light. Furthermore, the broad spectral output cannot be effectively coupled into optical fibres for endoscopic or interstitial application. These incoherent lamps cannot be used for the effective treatment of invasive malignancies at depths greater than about 5.0 mm from the surface. This is due to the limitation in penetration depth of red light at 630nm as determined by the absorption and diffuse scattering of light in human tissue (Doiron and Svaasand, 1983). It was logical then to substitute incoherent light sources with laser sources with an output in the red part (620-630nm) of the visible spectrum for HpD-PDT work. To date, most HpD-PDT clinical studies have been carried out with lasers because they are able to produce high output powers of red light of narrow bandwidth. These laser sources offer the further possiblity of flexible beam delivery to the tumour site or into the tumour mass (interstitial) by the use of optical fibres (200-600nm core diametres). The output from the laser is usually coupled into a single fibre or multiple fibres (2 to 4) using beam splitting devices. Lasers coupled to fibre optics enable endoscopic applications of PDT in the treatment of various neoplasms which occur internally or are too massive to be accessible through surface illumination. The next few sections review the most commonly used light sources in HpD-PDT. These are the incoherent light sources such as the incandescent and the gas discharge light sources. The other light sources are the coherent laser sources.

3.2.2 Incoherent Light Sources Used In PDT

Incandescence is obtained by resistive heating of a filament contained in an inert gas which emits a great amount of radiation. Its output gives a broad spectrum of polychromatic light whose exact shape is dependent on the temperature of the material heated. A significant part of the output spectrum is in the infrared region which may produce great heat in the material to which it is exposed. This infrared component needs to be removed by filtering to minimize heating and it will usually not directly affect photochemical reaction.

In 1980, Forbes *et al.* used a quartz halogen lamp (250W) to treat cutaneous tumour deposits. The light from the lamp was focussed by a spherical reflector and an ellipsoidal reflector onto a dichroic infrared reflector (Kodak 301A) and an infrared absorbing glass (Corning I-75) to eliminate infrared component. The beam was then allowed to pass through a colour glass filter (Corning 2-61) to obtain a beam of light with a narrow range of frequency between 620nm to 640 nm. This red light was finally collimated with a lens. The flux density of light was approximately 40 mW/cm^2 over a circle of 4.5 cm in diameter at a distance of 4.5 cm from the front of the instrument.

The other commonly used light source was the gas discharge lamp. High pressure xenon and mercury arc lamp were the two most used in PDT. They rely on ionized gas (plasma) to produce light emission. The spectral output of the lamp is characterized by the gas and its molecular structure. The output spectrum is generally very broad with a strong spectral lines. These lamps require significant filtering due to the broad spectral shape and large infrared component.

Dougherty *et al* (1979) used a 5000W xenon arc lamp (Schoeffel Ins. Corp.) which was fitted with a water filter (6 inches in diameter), an IR reflecting mirror (34-01-2; Baird Atomic, Inc.) and a 2 IR absorbing filters (Corning CSI-75) to absorb and filter infrared radiation. A red cutoff filter (Corning CS2-6) was used to obtain the spectral range of the emitted light at 600-700 nm. The light was then directed downward through a 60^0 mirror and then through a pair of lenses to produce a uniform beam of approximately 7 cm in diameter at a point 12 cm from the lens. The effective red light intensity which correspond with an HpD absorption band between 620-640nm was $25mW/cm^2$. Both the incandescent light sources mentioned above have low flux intensity which required very long exposure times in order to deliver the required amount of light flux at the target site.

Section 3.3 describes two incoherent light sources (a modified overhead projector and a surgical head lamp) developed at the University of Malaya for clinical use.

3.2.3 Coherent Laser Sources Used In PDT

A laser has properties significantly different from the incandescent and gas discharge sources that make it highly useful in porphyrin detection and activation. However, only sufficient high powered laser emitting at the range of 620-640nm were found to be of practical use. To date, most HpD-PDT works have been carried out using a 5 to 20 W argon ion laser to pump a dye laser using Rhodamine B or R6G dyes to produce visible red light. These systems are capable of producing 1 to 4 W of output power.

Alternative laser sources are the Helium Neon (He-Ne) laser and the gold vapour laser (GVL). These lasers have emissions at 632.8nm and 628nm respectively. Therefore, these lasers do away with the necessity of the dye laser for HpD-PDT application. The output power of the He-Ne is typically 1mW-30mW. On the other hand, the GVL has output power of up to 5 W. Both of these lasers offer the added advantage of posing less technical difficulties to PDT users such as having to ensure proper wavelength tuning which is required with a dye laser system due to its broad spectral bandwidth.

Diode lasers should have a major impact on the future applications and accessibility of clinical PDT. The diode systems are compact, inexpensive, and reliable, and are able to generate in excess of 1 W of power. In addition, these systems do not require water cooling. Currently, these systems are generating light at 700-800nm, and the development of systems which emit at shorter wavelengths is proceeding rapidly.

The next section describes the He-Ne laser used extensively in the animal model studies at this centre. The GVL was also described, as an extension for the second phase of clinical trials. It also explains their smooth running and the inherent limitations.

3.3 Light Sources And Lasers Developed And Used In This Study

3.3.1 Development Of Two Alternative Simple Red Light Sources For Clinical Use

Two simple red light sources, based on an overhead projector and a surgical head lamp were developed and modified as an alternative to the high power lasers for PDT. These simple red light sources were found to be as effective as the Helium-Neon laser in the treatment of superficial malignancies which will be presented in the following chapter. The output optics in the overhead projector was replaced by a cold mirror to filter out IR light that generates heat. A red paper filter to match the bandwidth of the HpD and Hp absorption band was placed on the fresnel lens of the overhead projector and at the tip of the fibre of the surgical head lamp. The advantage of the alternative red light source over other incoherent light sources used by earlier investigators is primarily its simplicity and cost-effectiveness. The light sources have an output of sufficient power for effective PDT to be administered.

3.3.1.1 Modification Of The Overhead Projector Lamp

The red light source was modified from an overhead projector as shown in Fig. 3.1a and the schematic diagram in Fig. 3.2. The overhead projector (Sinon 270A) used a 400W, HLX projector halogen lamp. It consisted mainly of a voltage step down circuit for the halogen bulb, a cooling fan, a collimating lens and a 14 inch plastic fresnel lens. The output optics which consisted of a beam folding mirror and lens fixture were replaced by a cold mirror (no. 42414, Edmund Sc.Co.). The cold mirror is an inexpensive standard optics which filters out the infrared radiation of the spectral output. It was found that it transmits in the IR beyond 700nm and reflects visible light shorter than 700nm in wavelength. Without modification of the internal electrical and optical arrangement of the overhead projector, a common red plastic paper filter, normally used for stage illumination was placed across the plastic fresnel lens to further narrow the output wavelength of the light source which cuts off visible light with wavelength shorter than 600nm. The spectral output from the bare lamp and subsequently, the filtering with the red paper filter to obtain red light between 600-650nm, are shown in Fig.3.3. Analysis of the optical spectrum showed that 30% of the red spectrum of the filtered red light coincided with the absorption peak at 600-650nm of the HpD and Hp. The total output power of the light source, measured with a power and energy metre (Scientech 365) was found to be 3.2 W of filtered red light with approximately 1 W of output between 600-650nm of the effective red light for therapy. By altering the distances from the lens, spot size varying from a minimum of 3.0 cm diameter to as large as 10.0 cm were obtained. With an effective output power of 1 W, larger superficial lesions can be treated. The modified projector light source is also portable and can be conveniently adjusted to provide either a horizontal or a vertical beam.

Fig. 3.1a Modified overhead projector lamp

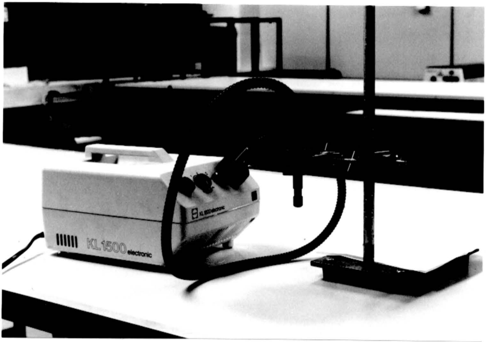

Fig. 3.1b Surgical headlamp with optical fibre

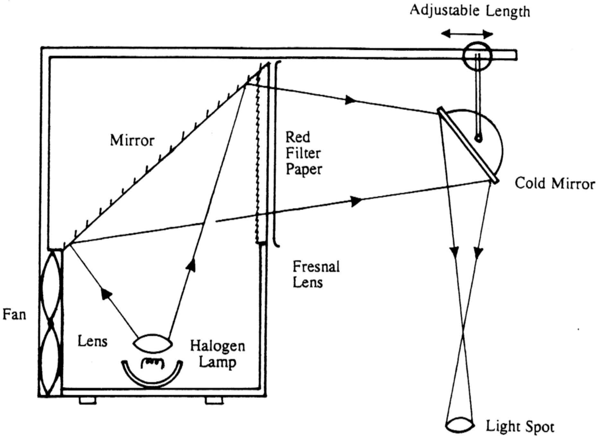

Fig. 3.2 Schematic diagram of the red light source modified from the overhead projector

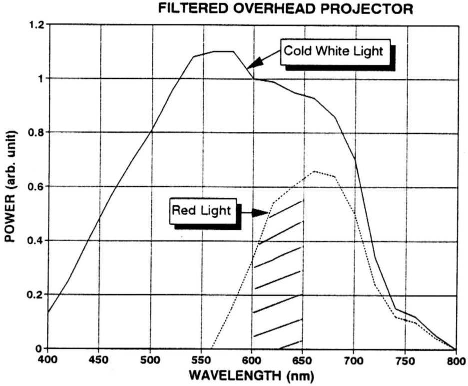

Fig. 3.3. Output spectra of the overhead projector showing the cold white light with a red plastic paper filter. The shaded area shows that 30% of the red light is within 600-650nm of the absorption bandwidth of HpD and Hp

3.3.1.2 Surgical Headlamp With Optical Fibre

As an extension of the simple alternative red light source modified for routine clinical applications as described above, a second light source modified from a surgical headlamp with an optical fibre output was used. It was used for the purpose of treatment of smaller lesions and particularly to less accessible malignant site. The surgical head lamp (Schott KL 1500-2) consisted of a halogen lamp, an IR cut-off filter, a cooling fan, a convex lens, an adjustable attenuator and an end on adapter for the fibre optic cable

(Fig.3.1b). A schematic diagram is shown in Fig. 3.4. The attenuator has five lamp settings which used a 150W(Philips) halogen lamp. The minimum lamp settings had an effective total output power of 5.7mW and the maximum was 460mW. The IR cut-off filter, filters out the infrared radiation with wavelength longer than 700nm from being transmitted by the fibre. The same red filter paper which was used for the modified overhead projector was placed at the tip of the fibre optic cable to obtain red light. The spectral output of the bare halogen lamp and subsequently filtering with the red filter is shown in Fig.3.5. Thirty percent of the spectrum of the filtered red light coincided with the absorption peak at 600-650nm of the HpD and Hp. The fibre optic cable had a diameter of 6mm, which then defines the minimum beam diameter and the maximum power density of 500 mW/cm^2. The output from the optical fibre bundle with an output protective claddings and sheath is 1 cm in diameter but is sufficiently flexible for light irradiation of tumour masses normally not accessible using the projector light output.

Besides using this surgical headlamp for clinical treatment, it has also been used in the animal model work in determining skin reactions to light (refer to section 6.7.2). In this case, blue light was employed. A sharp cut blue glass filter (Schott) which has a wavelength between 400 to 525nm (Fig.3.6) was fitted in the mounting slot inside the surgical headlamp. The same fibre optic cable was used, which had a minimum diameter of 6mm. The maximum output power of the blue light was 32.8mW and the minimum was 0.13mW, with a maximum power density of 120 mW/cm^2.

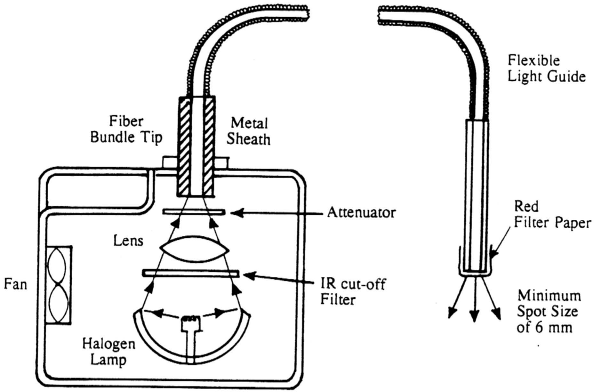

Fig. 3.4 Schematic diagram of the red light source from a surgical headlamp

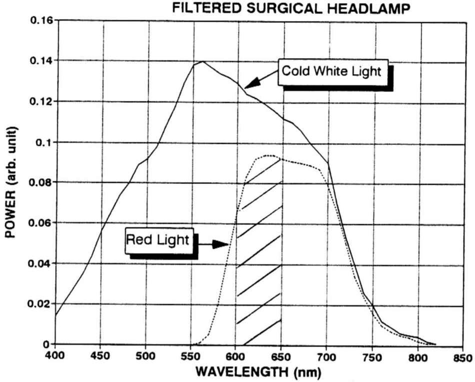

Fig. 3.5 Output spectra of the surgical headlamp showing the cold white light with a red plastic paper filter. The shaded area shows that 30% of the red light is within 600-650nm of the absorption bandwidth of HpD and Hp

3.3.2 Helium Neon laser

A commercial available Helium-Neon laser (200 He-Ne) from Shanghai Institute of Laser Technology was used for animals studies in the laboratory of the University of Malaya (Fig. 3.7).

The He-Ne laser is the commonest atomic laser. It is a relatively simple laser that consists of a laser tube and a power supply. The operation of the He-Ne laser is dependent

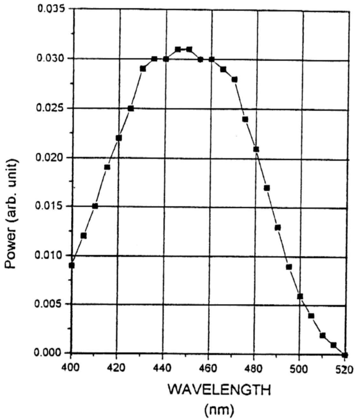

Fig. 3.6 Output spectra with the blue glass filter

on an electrical discharge through a mixture of ten parts of helium to one part of neon gases in a plasma tube. The discharge energy, through an intermediate helium metastable state, raises electrons of the neon atoms to an excited energy state. When more neon atoms are presented at the excited state, a condition known as 'population inversion' exists. Optical radiation at the energy (wavelength) separation of these states can then add to itself or amplifies by stimulated emission from the excited neon atoms. In order to achieve continuous-wave laser oscillation, a reflector is placed at each end of the plasma tube to form a resonator cavity which stores most of the optical radiation (photons) by reflection back and forth along the axis of the tube. This will then generate a spatially coherent and monochromatic output beam from the laser system.

The main part of the laser consists of a plasma tube, reflectors supporting and adjusting mechanical assembly. The plasma tube is terminated at each end with an optical-quality fused-silica Brewster's angle window. Direct current energy is supplied to the tube through the cathode and the anode located at each end of the tube, with the discharge length of 2.0m. The plasma tube is supported within the high Q optical cavity resonator which is formed by a high reflecting coating spherical mirror and a flat mirror with a specific reflectivity of around 98%. The laser mirrors are prealigned and can be fine adjusted to yield the maximum output. The output power of this He-Ne laser is 100mW initially but reduced to 65mW subsequently. It emits red light at a wavelength of 632.8nm which matches the absorption band of Hp and HpD. It has a minimum diameter of 3 mm and the beam could be enlarged by using appropriate lenses.

Unlike the gold vapour laser and the argon ion pumped dye laser, the He-Ne laser is inexpensive and easy to manage and operate. Due to its relatively low power output, the He-Ne laser represents no major hazard to the user. There is also no hyperthermic effect on the skin because of its specific wavelength. The above characteristics pose a very ideal situation for a clinical environment. The only limitation is its low power output which only enables to treat small lesions. It is known that He-Ne laser output power cannot be readily scaled up. Therefore the He-Ne laser is not a suitable laser for PDT application.

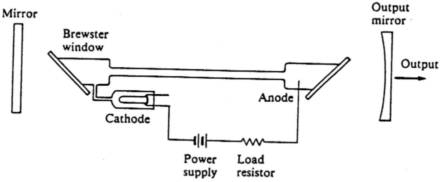

Fig. 3.7 Schematic construction of He-Ne laser

3.3.3 Gold Vapour Laser System

The GVL has received some attention as a possible source of intense optical pulses of wavelength 627.8nm, which may be optimum for the photosensitization of Hp and its derivatives in PDT. GVL belongs to the class of metal vapour lasers where the active medium is not a permanent gas. Instead the active medium is mono-atomic gold vapour obtained by heating a gold wire in a ceramic plasma tube by applying a longitudinal electrical discharge at a high repetitive rate. Neon is normally used as the buffer gas. The heat generated during the discharge raises the temperature of the tube sufficiently so as to vapourize partially the gold wires. When the required vapour pressure reaches about 0.1-0.3 torr at around 1650°C, the electrical discharge causes excitation of a sufficient number of gold vapour atoms leading to laser emissions.

The schematic operation of a GVL is shown in Fig.3.8. This laser system is developed at the University of Malaya (Cheah, 1992). GVL operates in pulsed mode. The pulse repetition frequency is typically in the range of 5 kHz to 15 kHz which enables the pulsed output to be treated in the same way as a continuous wave laser used in many medical applications. The laser pulsed duration is about 20-50ns. The GVL laser system operating at about 10kHz can produce 50ns laser pulses of 100μJ giving a 2 kW peak power or the equivalent of more than 1W of continuous red light at 628nm.

The high operating temperature of up to 1700°C has been the reason for the short operating lifetime of a GVL laser tube and making it unsatisfactory as a commercial product. The present GVL has specific requirements that need considerations for routine clinical applications. It requires regular maintenance such as the replacement of the metal fill which has a lifetime of 300 hrs under standard operating conditions. Molybdenum foil electrodes, alumina, pyrex and quartz tubes also needs to be changed if the laser tube cracks. Another main limitation faced by GVL is the long start-up time (≈ 1 hr) required for the electrical discharge heating of gold until it produces sufficient vapour pressure to cause lasing action. For routine clinical application, it has to be installed on a single chasis which requires more compact laser system layout and design. The triggering system has to be re-designed to suit this purpose. The water-cooled coaxial return may be installed if the present laser system is required to operate at higher input power. However, if these problems are overcome, the GVL is suitable for PDT application because it produces an emission at 627.8nm which matches the absorption band of Hp and HpD exactly.

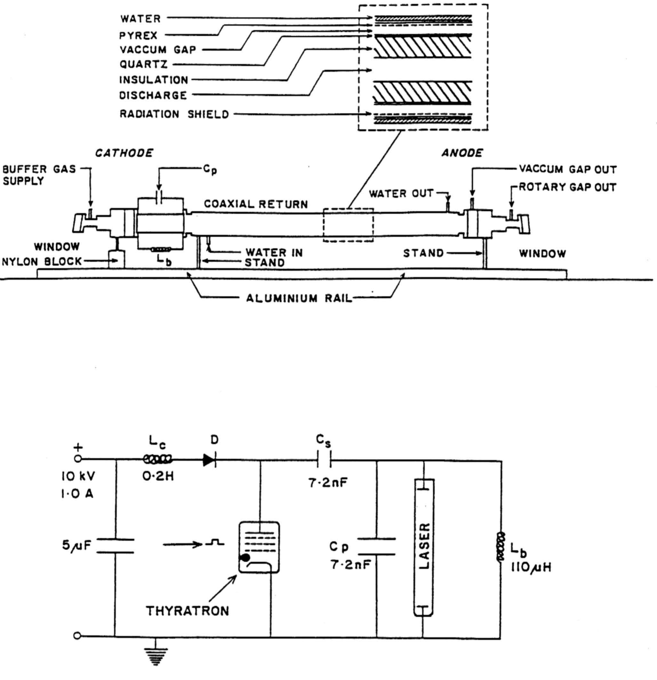

Fig. 3.8 Schematic operation of a gold vapour laser

3.4 Required Properties In The Treatment Dosimetry In PDT

Availability of oxygen may be a rate-limiting factor in PDT which should not be performed in severely hypoxic conditions. Oxygen needs energy of 94 kJ/mole to raise it from the triplet ground state to the excited singlet state (Stewart, 1994). In PDT, this energy is obtained from the sensitizer, which itself has been raised to a high-energy state. Only sensitizers which can emit more energy than 94kJ/mole are capable of activating singlet oxygen. This corresponds to a wavelength of approximately 850nm. It follows that only those compounds which absorb below this wavelength are suitable for PDT. In practice the light-absorbing compound loses some energy during its conversion to a metastable triplet state before energy is transferred to oxygen. The degree of energy loss varies from compound to compound but it is found that only those absorb light at 850nm or lower are efficient sensitizers. Other photophysical properties too are important. Compounds with a short triplet lifetime do not have an opportunity to transfer their energy to oxygen efficiently. The most efficient sensitizer is one which yields one molecule of singlet oxygen for each photon (quantum yield 1) absorbed. HpD absorbed at 630nm has a quantum yield of around 0.5. Whereas, the quantum yield of triplet photogeneration is about 0.8 for monomeric Hp. Another constraint placed upon a sensitizer's suitability is the depth to which the activating light can penetrate tissue. Longer wavelength (red) light can penetrate much more deeply than shorter wavelength (blue/green) light. Red light of around 650nm for example can penetrate 1 cm tissue, while light of 400nm can only penetrate tissue superficially. In practice, sensitizers which absorb in the region of 600-850nm are likely to be the best as they possess the right energetics for generating singlet oxygen and also absorb light at wavelengths which can penetrate tissues to sufficient depth to allow treatment of thick tumours.

Another important limiting factor placed upon the photosensitizer is the drug dose which may be profoundly important in the therapeutic gain. Other requirements for a photosensitizer includes rapid body clearance to minimize unwanted photosensitization long after the patient has received treatment.

An important consideration to make while determining light dose requirements is that the dose rate will affect localized skin temperature rise. It is important to apply a suitable power density that does not induce localized over heating of the tissue so as to cause eschar formation. The irradiated optical power density utilized during clinical procedures with HpD-PDT is in the range of 50-400 mW/cm^2. Temperature rise is insignificant for optical dose rates in the lower part of this range. However, dose rates corresponding to the intermediate and upper part of the temperature range might easily result in tissue temperatures to reach between 40-50°C. These temperatures will typically be obtained after 15 mins of exposure. A typical treatment protocol with an incident irradiation 200-400 mW/cm^2 and 20 to 30 mins exposure may result in a 5-15 mins of pure PDT followed by a 15-30 mins period of simultaneous PDT and hyperthermia treatment (Svaasand *et. al.*, 1983). Unless a steady state temperature distribution of 41-45^{0}C (6-10^{0}C) above basal temperature is reached the hyperthermic contribution can be ignored.

3.5 Techniques Used In Photo-Irradiation

The light delivery technique employed here was the surface treatment technique. The exposed tumour surface was illuminated superficially with red light from the laser beam or the modified light sources as shown in Fig.3.9. The light beam, with a Gaussian profile was made to enclose the entire tumour mass and 2 mm beyond the tumour margin along its periphery with near uniform illumination. For the present studies, this non-invasive technique was adopted for murine tumours between 0.3-1.0 cm in size measured along the longest and the shortest diameter of the tumour surface. This was also employed in the case of the patients on clinical trial who had various sizes of tumour masses. As for the case of the modified overhead projector, the beam was expanded by altering the distances from the focal spot of the lens. On the other hand, for the modified surgical headlamps, the beam was adjusted by altering the distances of the fiber from the surface of the tumour. As in the case of the laser, in order for the light to cover the area of the tumour mass, the beam size could be increased by using a planar-convac lens which was mounted on a retort stand to enable easy manoeuvrability.

Fibre optical delivery systems for laser radiation will be employed in the second phase of the clinical trial of internal and deep seated malignancies. In this case, the laser beam can be focused into the optical fibre as shown in Fig.3.10 by the use of a microscope objective lens. The fibre can be brought directly into the tumour mass if deeply located tumours using the lumen of a needle (Laws *et al,* 1981; Dougherty *et al,* 1978). On the other hand, the flexibility of the fibre allows inserting of the fiber into internal organs for endoscopic PDT. The combination of fibre-optics delivery and endoscopic techniques give unique access into the cavities of the body for the treatment of internal malignancies.

Fig. 3.9 A mouse being treated using the surface irradiation technique

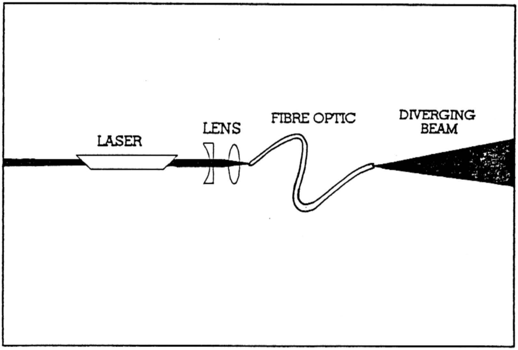

Fig. 3.10 A schematic diagram showing a laser and fibre optical coupling system

3.6 Conclusion

In conclusion, it has been found that for basic animal investigations and for routine clinical applications, a variety of red light sources can be applied to photoactivate HpD in tumours. Laser sources such as gold vapour laser and helium neon laser are suitable laser systems that can be used for HpD and Hp-PDT. They provide the added advantage of the interstitial treatment technique and the endoscopic application of fibre-optical coupling systems in the treatment of bulky and invasive malignancies. The two modified halogen light sources as alternatives to the laser in clinical application were found to be effective. They are easily manoeuvred, cost effective and provide straight forward surface irradiation when compared with any laser system.

Care should be taken in applying a suitable power density that does not affect localized skin temperature rise. The temperature rise should be minimum so as not to overheat the tumour tissue as well as the adjacent normal tissue from forming eschar formation.