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LASER WAVELENGTHS IN OPHTHALMOLOGY: A CRITICAL REVIEW

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Laser-tissue interactions, including histological and molecular studies, associated with the solid state laser wavelengths (210-213nm) and the excimer laser wavelength (193nm) are compared and contrasted.

ABLATION CHARACTERISTICS: HISTOLOGICAL ANALYSIS

The pulsed ultraviolet radiation issued from 193nm excimer and 213nm solid-state lasers have been shown to cause limited collateral damage to surrounding tissues (Vetrugno et al., 2001), collagen lamellae remains well organised with minimal collateral damage in both 213nm (Gailitis et al., 1991; Ren et al., 1990) and 193nm studies (Krueger et al., 1985). Studies to date, have published pseudomembrane depths less than 1 µm following ablation with a solid state 213nm (Ren et al., 1990; Gailitis et al., 1991; Caughey et al., 1994) and excimer 193nm laser (Campos et al., 1993; Fantes et al., 1989; Trokel et al., 1983; Puliafito et al., 1985; Marshall et al., 1986).

An in vivo study reveals similar clinical course and histopatholgy results following laser ablations with the excimer 193nm and solid state 213nm laser

Ren, Q., G. Simon, et al. (1993)

COMPARATIVE STUDY: 213nm vs 193nm

PURPOSE:

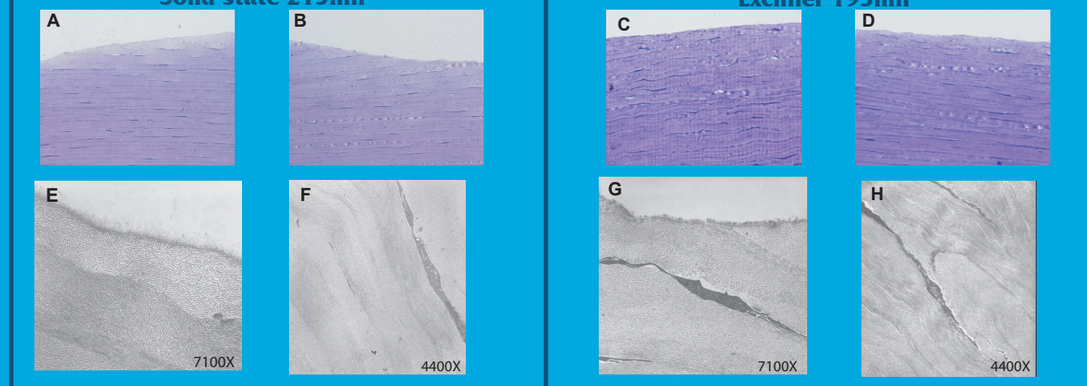
To compare ablation characteristics between laser ablations with solid state 213nm or excimer 193nm

METHODS:

In vitro porcine corneas were ablated with either the CustomVis Pulzar Z1 solid state laser or the Bausch & Lomb Technolas 217. Resin embedded, longitudinal sections were processed for Light Microscopy (LM) (stained with Toluidine Blue 1%) and Transmission Electron Microscopy (TEM) analysis.. Ablation characterisitcs were assessed qualitatively.

RESULTS:

LM: Sections (A, B, C, D) at 400X. TEM: Ablated corneal surfaces for Pulzar Z1 (E) and Technolas 217 (G). Ablated surfaces are smooth with no collateral or thermal damage. TEM micrographs from both laser systems show evidence of the electron dense 'pseduomembrane', measured <0.5µm wide. Analysis of regions in the deeper stroma following ablation with both laser types show no evidence of collateral damage. Deep stroma: Pulzar Z1 (F) and Technolas 217 (H).



Note: Unpublished Data

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CORNEAL ABLATION & DNA DAMAGE: IN VIVO STUDY

- One significant type of DNA damage caused by nuclear absorption of UV radiation is the generation of cyclobutyl pyrimidine dimers between adjacent thymidine residues on the DNA strand, causing local deformation of its structure (Jones et al., 1987).
- Living cells use an excision repair process to correct this damage.
  The technique of autoradiography is used to quantify unscheduled DNA synthesis (UDS), which is interpreted to be an indicator of the excisiore-pair process.
- Sparsely labelled cells (SLC) indicate DNA repair (i.e. indicates UDS) due to damage, while heavily labelled cells (HLC) represent the 'D' phase of standard DNA replication, and are not thought to be indicators of DNA damage (Nuss et al., 1987).
- The 193-nm excimer laser does not produce significant DNA damage (Nuss & Puliafito, 1987) whereas 266-nm is a known carcinogenic wavelength.
- The 213 nm wavelength was investigated by van Saarloos et al., 2000 (unpublished data). Unscheduled DNA synthesis (UDS) was measured in corneal epithelial and stromal cells surrounding corneal incisions produced by 193nm, 213nm and 266nm laser irradiation using autoradiography as described below. van Saarloos, P.P., Dair, G.T., Paz Linares, S.M., Lloyd, D.J. (1999) Investigative Ophthalmology and Visual Science Conference Proceeding. 40 (4), S340.

METHODS

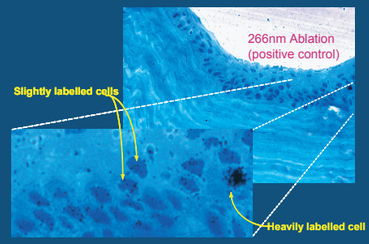
- Rabbits were exposed to corneal laser irradiation by different laser sources:
  1) Nd:YAG 213 nm wavelength
  2) Excimer 193 nm wavelength (negative control)
  3) 266 nm wavelength (positive control)
- Autoradiography was used to detect the amount of UDS occuring in surrounding cells immediately after ablation. This method entails cell incubation with labelled thyamine, thus allowing visualisation of DNA replication.

DNA ANALYSIS

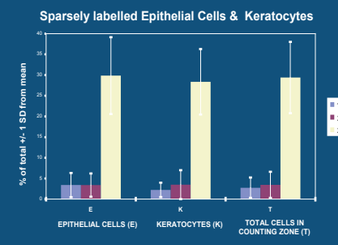
- Immediately after ablation, eyes were enucleated and incubated in calf serum with radioative thyamine for 3 days.
- Corneas were fixed and sectioned (2-3um)
- Sections were dipped into photographic emulsion, incubated for 2 weeks, fixed and stained.

CELL COUNTS

- Epithelial cells and keratocytes were counted:
  - Heavily labelled > 15 grains in nucleus (represent normal DNA replication)
  - Slightly labelled cells > background & < 15 (represent DNA repair).
- Expressed as % of total cell counts



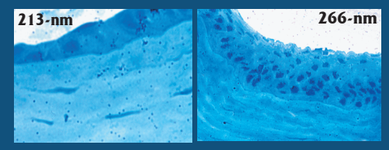
RESULTS



- No significant difference of SLCs level between 213nm and 193nm [p<0.05].
- For 193 & 213 nm: less than 5% (av.) of cells revealed UDS
- Highly significant difference of SLCs level between the 266-nm treated cells to that of both the 193-nm and the 213-nm treated cells [p<0.05].

CONCLUSION

- Corneal ablations with 193 or 213 nm produce minimal DNA damage.
- Whereas, the 266 nm wavelength causes significant DNA damage.



One Explanation:

213nm is relatively close to 193nm on the spectral scale; they fall within the 190 - 220 nm 'window of ablation'.
It is thought that nuclear DNA is protected from this range of UV light by the surrounding cytoplasmic components (Heida and Ito, 1986).

ABLATION RATES

- Stromal water content is a major factor in the effectiveness and predictability of laser ablation.
- Feltham et al (2002) studied the excimer 193nm ablation rate across a range of hydrated and dry hydrogel materials. The ablation rate is signficantly affected during excimer laser ablation on fully hydrated materials.
- However, in a controlled environment, the 213nm and 193nm ablation rate of procine cornea and PMMA samples behave similarly (Shen & Joos et al., 1997).

EXCIMER LASER:

↑ CORNEAL HYDRATION = ↓ EXCIMER LASER (193nm) CORNEAL ABLATION RATE

Feltham et al. (2002)

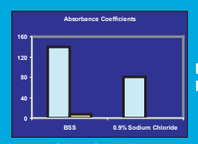
ABSORPTION CHARACTERISTICS

Dair et al., (2002) investigated the absorbance coefficients of laser light through balanaced salt solution (BSS) and sodium chloride (NaCl).

RESULTS

Absorption Coefficients (cm-1)

	BSS	NaCl
193nm	140	81
213nm	6.9	0.05



Dair et al., (2002)

- The absorbance of 213nm light in BSS & NaCl is much lower than 193nm
- A wavelength not significantly affected by aqueous solutions may provide more reliable and predictable treatment outcomes

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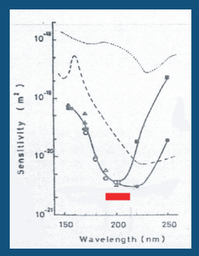
CYTOTOXICITY & MUTAGENICITY

- Clinical acceptance of ultraviolet laser radiation depends upon assessment of potential laser induced carcinogenesis and mutagenesis.
- Only a small number of studies have investigated the potential mutagenicity, cytotoxicity and free radical production of the 213nm laser for refractive surgery.
- In vitro and cell culture assays have been used to compare the solid state 213nm laser to the traditional 193nm excimer laser.
- A study by Munakata et al. (1986) plus supplementary data (Hieda & Ito, 1986) demonstrated the action spectra for cell inactivation and mutagenesis for bacterial and yeast cells after exposure to radiation in a vacuum (see graph).
- Conflicting results have been obtained; Kaido et al. (2002) found that 213 nm radiation was more cytotoxic and mutagenic than the 193 nm on cultured mammalian cells.
- Matchette et al. (1996) and Ediger et al. (1997) also found the 213nm to have a greater DNA damaging ability and cytotoxic action than the excimer 193nm, after irradiation of bacterial cells and corneas.
- Validity of results is disconcerting due to the number of technical issues facing these in vitro experiments.
- In vivo experiments may be more clinically significant, as performed by van Saarloos et al. (1999) summarized above. (top right)



Broken Line: inactivation of wet yeat cells (Ito et al., 1983). Dotted Line: absorption spectrum of DNA (Inagaki et al., 1974).

- A "deep valley" was shown at 190-220nm, illustrating that cells exposed to these wavelengths are least susceptible to cellular inactivation and mutation.
- An explanation for this valley is that there is screening by an intervening layer of cytoplasm before DNA located in the core.

TECHNICAL ISSUES:

ABSORBANCE CHARACTERISTICS:

- 213nm is weakly absorbed by aqueous solutions (Dair et al. 2001), whereas the efficiency of the 193 nm is greatly affected by water content (Feltham et al. 2002).
- The Kaido et al. (2001) method would have left a fluid layer over the cells to be ablated, effectively shielding the cells from 193nm radiation, but would have had little shielding effect for 213nm radiation.
- Similarly, Matchette et al. (1996) irradiated cells that resided in agar, a substance that contains up to 99.5% water content (Garrett & Grisham, 1999) and Ediger et al. (1997) irradiated cells residing in cuvettes of saline solution.
- To not account for the different absorbance abilities of the wavelengths in these studies may have increased the perceived mutagenic and cytotoxic effects of 213nm.

213nm SEPARATION TECHNIQUE:

- Kaido et al. (2001) used a narrowband 213nm mirror to separate the beams, which can also reflect up to 5% of the cytotoxic 266nm wavelength.
- Given the energy in the 266nm beam would have been much higher than the energy in the 213nm beam, the cells are likely to have been exposed to significant levels of 266nm radiation.
- The poor separation technique may have increased the mutagenic and cytotoxic effects.

FREE RADICAL PRODUCTION

- Free radicals are highly reactive molecules that have been linked to cancer.
- A normal balance of free radicals is required for cell viability and prevention of tissue inflammation and function (Kasetsuwan et al., 1999).
- Comparable levels of free radicals have been reported for both 193nm and 213nm lasers (Ediger et al., 1997).

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