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REVIEW

Laser Doppler flowmetry in endodontics: a review

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Abstract

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Vascular supply is the most accurate marker of pulp vitality. Tests for assessing vascular supply that rely on the passage of light through a tooth have been considered as possible methods for detecting pulp vitality. Laser Doppler flowmetry (LDF), which is a noninvasive, objective, painless, semi-quantitative method, has been shown to be reliable for measuring pulpal blood flow. The relevant literature on LDF in the context of endodontics up to March 2008 was reviewed using PubMed and MEDLINE database searches. This search identified papers published between June 1983 and March 2008. Laser light is transmitted to the pulp by means of a fibre optic probe. Scattered light from moving red blood cells will be frequency-shifted whilst that from the static tissue remains unshifted. The reflected light, composed of Doppler-shifted and unshifted light, is returned by afferent fibres and a signal is produced. This technique has been successfully employed for estimating pulpal vitality in adults and children, differential diagnosis of apical radiolucencies (on the basis of pulp vitality), examining the reactions to pharmacological agents or electrical and thermal stimulation, and monitoring of pulpal responses to orthodontic procedures and traumatic injuries. Assessments may be highly susceptible to environmental and technique-related factors. Nonpulpal signals, principally from periodontal blood flow, may contaminate the signal. Because this test produces no noxious stimuli, apprehensive or distressed patients accept it more readily than current methods of pulp vitality assessment. A review of the literature and a discussion of the application of this system in endodontics are presented.

Keywords: endodontics, laser Doppler flowmetry, pulp, vitality tests.

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Introduction

Before commencing root canal treatment, information is collected regarding the history, clinical examination and special tests to allow a diagnosis. There are several ways to obtain information about the condition of the pulp, including hot and cold thermal testing and electric pulp testing. It should be emphasized that vitality implies that blood supply is present within the tissues. Hence, only a test that actually measures or assesses pulp blood flow can be called a vitality test (Ingle *et al.* 2002, Berman & Hartwell 2006).

Each of the sensibility tests involves stimulating sensory nerve responses, at best, a proxy-marker of pulp health, so stimulation of the nerve fibres is not an ideal method to determine vitality status. Vascular supply and not innervation is the most accurate determinant of pulp vitality (Fratkin *et al.* 1999, Ingle *et al.* 2002, Radhakrishnan *et al.* 2002). As a result, teeth that have temporarily or permanently lost their sensory function (e.g. anaesthetized, traumatized, or teeth involved in orthognathic surgery) may be nonresponsive to these tests. However, they may have intact vasculatures (Ikeda & Suda 1998, Yanpiset *et al.*

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2001). Moreover, some nerve fibres may be highly resistant to necrosis and so may remain reactive long after the surrounding tissues have degenerated. Therefore, thermal and electric tests may give false-positive responses if only the pulp vasculature is damaged (Ingle *et al.* 2002, Radhakrishnan *et al.* 2002).

Vitality testing of teeth requires the measurement of pulpal blood flow (PBF), and several experimental methods have been used for its assessment (Ingle *et al.* 2002, Berman & Hartwell 2006). These include invasive methods such as radioisotope clearance (Hock *et al.* 1980, Kim *et al.* 1983), H_2 gas desaturation (Aukland *et al.* 1964, Tönder & Aukland 1975), and noninvasive techniques such as laser Doppler flowmetry (LDF) (Gazelius *et al.* 1986, Olgart *et al.* 1988, Ingólfsson *et al.* 1994a,b, Emshoff *et al.* 2000a,b, 2004a,b, Roeykens *et al.* 2002, Polat *et al.* 2004), pulse oximetry (Schnettler & Wallace 1991, Gandy 1995, Kahan *et al.* 1996, Noblett *et al.* 1996, Goho 1999, Radhakrishnan *et al.* 2002, Gopikrishna *et al.* 2006, 2007a,b, Jafarzadeh & Rosenberg 2009), dual wavelength spectrophotometry (Nissan *et al.* 1992), photoplethysmography (Shoher *et al.* 1973, Schmitt *et al.* 1991, Diaz-Arnold *et al.* 1994, Miwa *et al.* 2002), and measurement of surface temperature (Stoops & Scott 1976, Banes & Hammond 1978, Fanibunda 1986, Kells *et al.* 2000a,b, Smith *et al.* 2004, Jafarzadeh *et al.* 2008).

Since the usage of laser Doppler method by Yeh & Cummins (1964) to estimate the velocity of red blood cells in capillaries, it has been widely adopted for the measurement of blood flow specially in soft tissues (Stern 1975, Holloway & Watkins 1977, Baab *et al.* 1986, Baab & Oberg 1987, Boutault *et al.* 1989, Bystrova *et al.* 2001, Mayrovitz *et al.* 2001, Raamat *et al.* 2002, Sato *et al.* 2003). It is a noninvasive, objective, painless, semi-quantitative method, which has been proved to be a reliable method for PBF measurement (Gazelius *et al.* 1986, 1988, Olgart *et al.* 1988, Wilder-Smith 1988, Sasano *et al.* 1989, Ramsay *et al.* 1991a,b, Vongsavan & Matthews 1993a, Ingólfsson *et al.* 1994a, Ebihara *et al.* 1996, Mesaros & Trope 1997, Emshoff *et al.* 2000a, 2004d, Yanpiset *et al.* 2001). It is also reproducible and has become recognized as the gold standard in determining PBF (Gazelius *et al.* 1988, Olgart *et al.* 1988, Wilder-Smith 1988).

Successful application of LDF in human teeth was first described by Gazelius *et al.* (1986). They were able to recognize regular heart beat oscillations in the LDF readings, and correlated them with simultaneous electrocardiogram (ECG) readings. The LDF produced regular signal fluctuations for teeth with vital pulp that were identical in rate to ECG waves. Teeth with necrotic pulps showed no synchronous signals, but produced irregular fluctuations or very steep spike traces that were attributed to movement artifacts.

The value of this method has been well documented, but its high cost and difficulty of use in clinical practice have delayed its wide-scale introduction (Sasano *et al.* 1989, Ramsay *et al.* 1991b, Ingólfsson *et al.* 1993, 1994b, Hartmann *et al.* 1996, Evans *et al.* 1999, Roeykens *et al.* 1999, Roebuck *et al.* 2000). This review will address the mechanism, indications, influencing factors, limitations and practical considerations in the use of LDF, as well as the reliability of such systems.

Search strategy

A literature search for relevant articles on LDF in the context of endodontics up to March 2008 was reviewed using PubMed and MEDLINE database search. Table 1 shows the keywords used and the search results. After removing duplicates, the remaining papers were retrieved and their reference lists checked to identify any other articles/textbooks relevant to the topic, which may have provided additional information.

Mechanism

The Doppler effect is the basis of LDF. The effect was first described in 1842 by Austrian physicist Christian Doppler in a paper entitled 'On the Colored Light of Double Stars and Some Other Heavenly Bodies'. It

Table 1 Keywords used in search strategy

Keywords	Number of publications	Earliest paper	Latest paper
Laser Doppler flowmetry jsubsetd	242	June 1983	March 2008
Laser Doppler flowmeter jsubsetd	49	June 1983	March 2008
Laser Doppler endodontics jsubsetd	36	January 2000	March 2008
Laser Doppler flowmetry root canal jsubsetd	23	May 1990	May 2007
Laser Doppler flowmetry endodontics jsubsetd	34	May 1990	March 2008
Laser Doppler flowmeter root canal jsubsetd	7	February 1996	May 2003
Laser Doppler flowmeter endodontics jsubsetd	6	November 1992	May 2003

explains the frequency shift that a wave undergoes when emitted from an object that is moving away from or towards an observer. It manifests itself, as example, in the increase in the pitch of the siren of an ambulance, when this vehicle moves towards an observer (Riva 2001).

Laser Doppler flowmetry is an optical measuring method that enables the number and velocity of particles conveyed by a fluid flow to be measured. The particles (1–20 μm) must be big enough to scatter sufficient light for signal detection but small enough to follow the flow faithfully (Durst *et al.* 1976, Durrani & Greated 1977, Drain 1980, Bonner & Nossal 1990, Albrecht *et al.* 2003).

The original technique used a light beam from a helium–neon (He–Ne) laser emitting at 632.8 nm. Other wavelengths of semi-conductor laser have also been used: 780 nm and 780–820 nm (Kimura *et al.* 2000). Laser light is transmitted to the dental pulp by means of a fibre optic probe placed against the tooth surface (Gazelius *et al.* 1986, Bonner & Nossal 1990, Rowe & Pitt Ford 1990, Roeykens *et al.* 1999, Roebuck *et al.* 2000, Albrecht *et al.* 2003). Two equal-intensity beams (split from a single beam) intersect across the target area. The scattered light beams from moving red blood cells will be frequency-shifted whilst those from the static tissue remain unshifted in frequency (Gazelius *et al.* 1986, Bonner & Nossal 1990, Rowe & Pitt Ford 1990, Roeykens *et al.* 1999, Albrecht *et al.* 2003). The reflected light, composed of Doppler-shifted (light reflected by a moving object is Doppler-shifted) and unshifted light, is returned by an afferent fibre within the same probe to photodetectors in the flowmeter and a signal is produced (Bonner & Nossal 1990, Roeykens *et al.* 1999, Roebuck *et al.* 2000) (Fig. 1). The photodetectors convert the interference pattern arising from the mixing of shifted and unshifted light into a semi-quantitative measurement of blood flow, termed the Flux signal, which is measured in arbitrary units. The received signal is calculated with a preset algorithm in the LDF machine (Roebuck *et al.* 2000, Berman & Hartwell 2006) (Fig. 2). The LDF output signal or Flux can be simplified as a function of the product of red blood cells' concentration as well as their mean velocity (Gazelius *et al.* 1986, Bonner & Nossal 1990, Rowe & Pitt Ford 1990, Roeykens *et al.* 1999, Kimura *et al.* 2000). In fact, Flux is the number of moving red blood cells per second times their mean velocities (Berman & Hartwell 2006). When used to assess the vitality of teeth, the size of the Flux signal obtained from a healthy vital control tooth can be compared with that

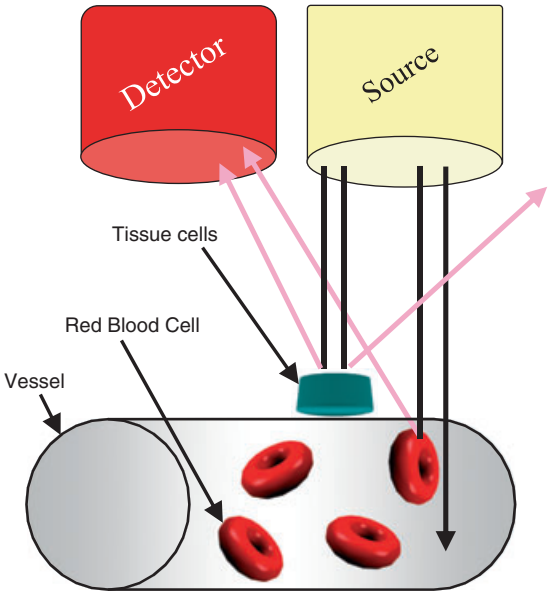

Figure 1 Doppler principle.

of the suspected nonvital tooth. The Flux signal from a tooth with a vital pulp should be greater than from a tooth with a nonvital pulp (Roebuck *et al.* 2000). It should be emphasized that the optical properties of a tooth change when the pulp becomes necrotic and this can produce changes in the LDF signal that are not due to differences in blood flow, as discussed by Soo-ampon *et al.* (2003).

In fact, as red blood cells represent the vast majority of moving objects within the tooth, measurement of the Doppler-shifted backscattered light serves as an index of PBF. LDF evaluates dynamic changes in blood flow by detecting blood cell movement in a small volume of tissue (about 1 mm^3) (Oberg 1990, Vongsavan & Matthews 1993a, Ketabi & Hirsch 1997, Perry *et al.* 1997, Emshoff *et al.* 2004d).

Most current laser Doppler devices give readout, in addition to the Flux, in perfusion units (PUs). These PUs are arbitrary and calculated by the software that accompanies each device. No current laser Doppler device can present absolute perfusion values of blood flow (e.g. mL min^{-1} 100 g^{-1} tissue), so the PUs are never comparable between various types of devices, and even for the same device they could vary at different times unless the device is calibrated frequently using special suspensions of particles in liquid that have a known inherent vibration (Berman & Hartwell 2006).

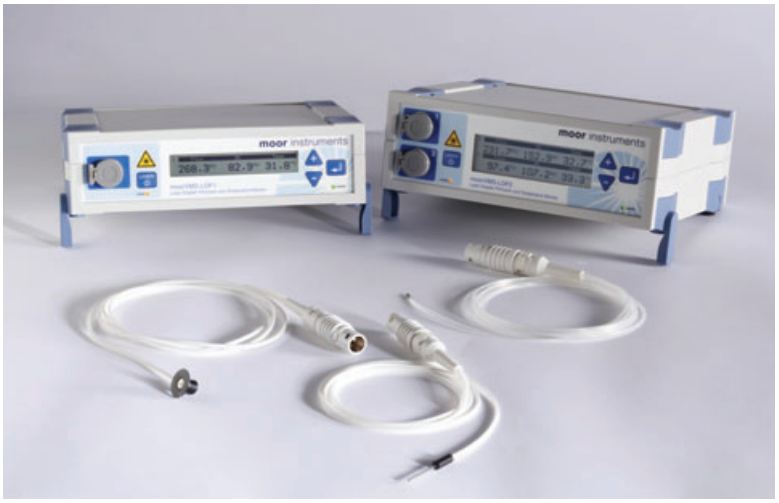

Figure 2 The main body of the system and probes can be used in endodontics (with official permission of http://www.moor.co.uk).

The penetration depth of a LDF beam in teeth has been investigated. In extracted teeth, with various density of laser, laser light achieved approximately 4–6 mm depth of root densely and 13 mm depth less densely (Ikawa *et al.* 1999, Polat *et al.* 2005a). In some teeth, the laser beam may even reach to apex. Contact or noncontact of the probe on tooth surface did not significantly influence penetration of the light (Polat *et al.* 2005a).

Indications

Laser Doppler flowmetry has been used in PBF measurements for:

1. Estimation of the pulpal vitality: In treatment planning, it is important to assess the pulpal status of individual teeth when making a differential diagnosis of dental pain. Moreover, the diagnosis of a tooth with a necrotic pulp may be difficult particularly when referred pain is present. In these situations, a suitable test and its precise interpretation are of paramount importance (Gazelius *et al.* 1986, Schmitt *et al.* 1991, Ingólfsson *et al.* 1994a).

2. Pulp testing in children: sensibility tests are not reliable in children, because they are subjective and rely upon the patient response. LDF may be a better choice. Moreover, it has been shown that LDF is a suitable method for the measurement of PBF in deciduous incisors (Fratkin *et al.* 1999, Goho 1999, Ingle *et al.* 2002).

3. Periapical radiolucencies may have nonendodontic origins, so application of vitality tests, such as LDF, can help in differential diagnosis of these radiographic views (Chandler *et al.* 1999).

4. Laser Doppler flowmetry can help monitor age-related changes in PBF. Using this system, it has been shown that the haemodynamics in the human pulp is reduced with age (Ikawa *et al.* 2003).

5. Monitoring the effect of exercise on PBF is another indication of using LDF. It has been indicated that PBF varies during exercise, with a mean percentage change of 38% from the level at rest. The mean percentage increase in gingival blood flow is 65%. Also, the pulse rate increases during exercise. There is no direct relationship between increased pulse rate and PBF. It shows that the mechanisms controlling pulpal and gingival blood flows are different. LDF readings were only reproducible in individual teeth provided that the patient was at rest, and this should be considered in clinical or research measurements (Watson *et al.* 1992).

6. Monitoring of reactions to local and systemic pharmacological agents (including local anaesthetic solutions) may be undertaken with LDF (Odor *et al.* 1994a,b, Premdas & Pitt Ford 1995, Chng *et al.* 1996, Ketabi & Hirsch 1997, Ahn & Pogrel 1998, Öztürk *et al.* 1998, Fernieini *et al.* 2001, Shimada *et al.* 2006, Terakawa *et al.* 2007).

Using this device, it has been shown that 2% lidocaine with 1 : 100 000 epinephrine, as used for buccal infiltration, significantly reduces blood flow pulpally and gingivally. PBF reduction is more than gingival blood flow reduction, which may be critical for compromised pulps with already reduced blood flow in

teeth undergoing invasive treatment (Ahn & Pogrel 1998). Also, it has been reported that the use of vasoconstrictors with prilocaine anaesthetics had less pronounced effects on PBF than those reported for lidocaine with epinephrine (Chng *et al.* 1996).

Fernieini *et al.* (2001), using LDF, compared the haemodynamic effects of local anaesthetic administration. The greatest change was associated with anxiety and occurred just before the injection. This investigation has confirmed the sensitivity of LDF as an investigational device for assessing haemodynamic changes associated with anxiety and the administration of local anaesthesia.

7. Laser Doppler flowmetry can be used for monitoring of reactions to electrical (Raab *et al.* 1988) or thermal pulp stimulation (Andersen *et al.* 1994, Mavropoulos *et al.* 1995, Goodis *et al.* 2000).

Raab *et al.* (1988) examined the responses of rat incisor microcirculation to electrical stimulation. A fast initial decrease was followed by a long lasting increase in PBF. Goodis *et al.* (2000) showed that as tooth temperatures were lowered, PBF slowed but did not stop completely.

Andersen *et al.* (1994) used LDF to study the changes in PBF evoked by application of cold or heat to the teeth. Switching from a temperature of 33 °C to 5 °C induced a slow decrease of PBF to about 80% of control, and also warming to 39 °C evoked a small reduction. Both cooling and warming sometimes triggered a rise in PBF. Mavropoulos *et al.* (1995) used LDF to study the effect of mandibular nerve block on changes in PBF evoked by application of cold or heat to the teeth. PBF showed a 7% increase in response to heat and 20% decrease when the tooth was exposed to cold. Neither response was affected by a mandibular nerve block.

8. Another indication of LDF is in monitoring pulpal reactions to orthodontic procedures (McDonald & Pitt Ford 1994, Barwick & Ramsay 1996, Brodin *et al.* 1996, Ishikawa *et al.* 1998, 1999, Ikawa *et al.* 2001, Sano *et al.* 2002, Konno *et al.* 2007).

Many studies have been reported on the effect of intrusive orthodontic forces on PBF. Ikawa *et al.* (2001) described how intrusive force significantly reduced PBF. Sano *et al.* (2002) revealed that PBF was significantly reduced during continuous intrusive force application, which was followed by recovery after wire removal. Brief intrusive force produced a significant reduction of PBF, but the reduction rate did not differ significantly during the observation periods. However, Barwick & Ramsay (1996) indicated that PBF is not altered during the application of a brief intrusive force. Also, Brodin *et al.* (1996) showed that orthodontic intrusion of teeth evoked a temporary reduction in the PBF, whereas extrusion had no effect.

9. Laser Doppler flowmetry can be used in measuring of PBF after orthognathic surgery. Amongst patients who undergo a segmental maxillary osteotomy or Le Fort I osteotomy, significant reduction of pulpal sensibility has been noted in the teeth in the osteotomized segment or maxilla (Hutchinson & MacGregor 1972, Pepersack 1973, Summers & Booth 1975). Several authors reported the use of flowmetric values to demonstrate this (Ramsay *et al.* 1991a, Okada 1992, Dicerbo 1993, Dodson *et al.* 1994, 1997, Aanderud-Larsen *et al.* 1995, Geylikman *et al.* 1995, Buckley *et al.* 1999, Emshoff *et al.* 2000a,b, Justus *et al.* 2001, Sato *et al.* 2003, Harada *et al.* 2004a,b). In patients undergoing segmental maxillary osteotomies, the postoperative occurrence of tooth nonresponsiveness to electronic tests has incidences ranging from 6% to 43%. LDF is thus of great value in securing more reliable diagnostic information (Johnson & Hinds 1969, Leibold *et al.* 1971, Hutchinson & MacGregor 1972, Pepersack 1973, Theisen & Guernsey 1976).

10. Measuring of PBF after traumatic injuries: Traumatized teeth may have their innervation damaged and give a negative response to pulp tests although their blood circulation, and thus their true vitality, is functional. Only generalized impressions may be gained from thermal and electric tests subsequent to trauma. Usually 1 to 8 weeks elapse before a normal pulpal response can be elicited (Andreasen & Andreasen 1994), but longer observation periods may be required. Regeneration of damaged nerves is slow, and it may be many months before positive nerve responses return. If endodontic treatment is initiated whilst blood vessels are attempting to regrow through the apical foramen, the pulp healing is arrested and there is no further development of the root in immature teeth. LDF is an accurate and objective technique for assessment of pulpal vitality in these teeth (Gazelius *et al.* 1988, Olgart *et al.* 1988, Andreasen 1989, Heithersay & Hirsch 1993, Andreasen & Andreasen 1994, Ebihara *et al.* 1996, Mesaros & Trope 1997, Evans *et al.* 1999, Lee *et al.* 2001, Roeykens *et al.* 2002, Strobl *et al.* 2003, 2004a,b, 2005, Emshoff *et al.* 2004a,b,c,d, Emshoff *et al.* 2008, Winzap-Kälin *et al.* 2005).

11. Monitoring of revascularization of replanted teeth (Mensdorff-Pouilly *et al.* 1995, Yanpiset *et al.* 2001, Ritter *et al.* 2004): LDF readings correctly

predict the pulp status (vital versus nonvital) in 84% of replanted teeth (74% are correct for the vital teeth and 95% are correct for the nonvital teeth). There is no significant association between the efficacy of LDF and tooth type (Yanpiset *et al.* 2001).

Factors influencing the results

The LDF assessment of PBF may be highly susceptible to environmental and technique-related factors. Variables such as probe design (Ingólfsson *et al.* 1993, Hartmann *et al.* 1996), probe holder characteristics (Gazelius *et al.* 1988, Olgart *et al.* 1988, Wilder-Smith 1988, Ramsay *et al.* 1991b, McDonald & Pitt Ford 1994, Hartmann *et al.* 1996, Musselwhite *et al.* 1997), gingival isolation devices (Hartmann *et al.* 1996, Vongsavan & Matthews 1996, Soo-ampon *et al.* 2003, Polat *et al.* 2004, 2005b), flowmeter characteristics (Ingólfsson *et al.* 1994b, Hartmann *et al.* 1996, Odor *et al.* 1996a,c, Roebuck *et al.* 2000), mineralization of enamel and dentine (Vaarkamp *et al.* 1995), the temperature of the environment, the position and the resting status of the patient, the position of the probe (Edwall *et al.* 1987, Shimazaki *et al.* 1989, Oberg 1990, Ramsay *et al.* 1991b, Ingólfsson *et al.* 1994b, Hartmann *et al.* 1996, Odor *et al.* 1996b, Akpinar *et al.* 2004), heartbeat-synchronous oscillations, tooth discolouration, stress, intake of drugs, age-related changes, etc, may significantly influence LDF results (Okabe *et al.* 1989, Olgart *et al.* 1989, Heithersay & Hirsch 1993, Matthews & Vongsavan 1993, Chng *et al.* 1996, Musselwhite *et al.* 1997, Verdickt & Abbott 2001, Ikawa *et al.* 2003). These factors can be categorized as below.

Characteristics of the laser beam

Many variables regarding the characteristics of laser beams have been found to significantly affect the Flux signal recorded from dental pulps. These include the choice of bandwidth filter (used to reduce signal noise) (Odor *et al.* 1996a,c), the wavelength of the laser source (Odor *et al.* 1996a), and the fibre separation within the probe (Ingólfsson *et al.* 1993, Odor *et al.* 1996c). Studies comparing some of these variables have supported the use of a narrow, 3 kHz bandwidth filter rather than a 20 kHz bandwidth filter (Odor *et al.* 1996a,c, Roebuck *et al.* 2000) and the use of a 810 nm (Odor *et al.* 1996a) or a 633 nm laser source (Roebuck *et al.* 2000). The use of probes with a relatively wide fibre separation of 500 μm was supported by two studies (Odor *et al.* 1996c, Roebuck *et al.* 2000), whilst others recommended a narrower fibre separation of 250 μm (Ingólfsson *et al.* 1993, 1994b). However, some of these studies (Odor *et al.* 1996a,c) used root filled teeth as the nonvital sample. The optical properties of root filled teeth, whose pulp chambers will be filled with opaque material, may differ from those of nonvital, nonendodontically treated teeth, whose pulp chambers may be filled with necrotic debris or may even be empty.

Although lasers with longer wavelengths give higher Flux readings, probably due to their greater penetration through tooth tissue, it may increase the risk of including nonpulpal blood flow within the signal, and so reduce the vital to nonvital signal ratio (Pettersson & Oberg 1991, Vongsavan & Matthews 1993a,b, Odor *et al.* 1996a).

Probe angulation

Few studies have assessed the effect of the probe angulation on the results of LDF. One study reported that probe angulation did not affect the pattern of light transmission within the tooth (Odor *et al.* 1996b).

Probe position

Some studies (Ramsay *et al.* 1991b, Hartmann *et al.* 1996) have noted higher Flux values being obtained from vital teeth as the probe was moved closer to the gingival margin, but moving the probe closer to this area may increase the possibility of including nonpulpal signals from the periodontal tissues. Akpinar *et al.* (2004) placed the probe on the cervical third of tooth crown, taking the crown sizes of the teeth into account. One reason for this sampling site is that the output signal for transmitted laser light is minimal because of the tooth thickness in this region, or because of pulpal backscattering. Also, Ingólfsson *et al.* (1994b) showed that the output signals from the incisal position were significantly lower than the output signals from gingival, mesial, or distal positions.

In contrast with this, Odor *et al.* (1996b) showed that probe position did not affect the pattern of light transmission. Edwall *et al.* (1987) found only minor variation in blood flow estimates obtained from adjacent sites on the same tooth.

Probe design

Laser Doppler flowmetry probes have been produced in various designs, according to their usage. The difference

between probes may be in their external features which suits them for different applications, or may be in their internal characteristics including their fibre separation (Ingólfsson *et al.* 1993, Roebuck *et al.* 2000).

Probe holder characteristics

Laser Doppler flowmetry is complicated by the fact that the laser beam must interact with moving cells within the pulpal vasculature, so to avoid the artifacts, a suitable holder should be produced to fit over the teeth, and thus provide stabilization for the probe, maintain its true contact with the tooth as well as creating a reproducible positioning at follow-up to assess progress. Although measurements can be obtained with hand-held probes (Wilder-Smith 1988), the best traces will be obtained when the probe is supported in a dental putty splint.

Table 2 shows various kinds of holders, which have been used for LDF probes. As this table shows, most of studies have used rigid splints fabricated with different materials such as silicone, plastic, acrylic, self-curing resins, etc, or exceptionally by using rubber dam clamps or even manual holding of the probe. Dental splints can be made using dental putty, which is moulded over the teeth before drilling a small hole to accommodate the probe 2–3 mm from the gingival margin.

Few studies have compared various kinds of holders; however, Hartmann *et al.* (1996) showed that a silicone splint probe holder resulted in significantly higher values than a polyurethane splint. The combination of the polyurethane splint with isolation devices decreases the Flux values. A polyurethane/rubber dam combination is the most efficient in individualizing the PBF.

Nonpulpal signals

Nonpulpal signals, principally from periodontal blood flow, can significantly contaminate the Flux signal recorded from the pulp, so it has been claimed that signals obtained from human teeth do not necessarily indicate PBF and may be confounded by signals obtained from nearby tissues (Amess *et al.* 1993, Vongsavan & Matthews 1993b, 1996, Ikawa *et al.* 1999, Roebuck *et al.* 2000, Soo-ampon *et al.* 2003, Akpinar *et al.* 2004, Polat *et al.* 2004, 2005a,b) (Table 3). Also, it has been shown that the output signal from a nonvital tooth is significantly lower than that from a vital tooth, but those from nonvital teeth do not usually register as zero. For this reason, it has been suggested that part of the signal recorded from the enamel surface actually derives from blood flow in tissues outside the pulp (Matthews & Vongsavan 1993, Matthews *et al.* 1994, Hartmann *et al.* 1996). In fact,

Table 2 Systems have been proposed as probe holder

Used/proposed holding system	Authors/year
Modified rubber dam clamp	Gazelius *et al.* (1986)
Manual holding	Wilder-Smith (1988)
Custom fabricated jig	Musselwhite *et al.* (1997)
Silicone splint	Gazelius *et al.* (1988), Olgart *et al.* (1988), McDonald & Pitt Ford (1994), Ahn & Pogrel (1998)
Plastic splint	Ramsay *et al.* (1991b), Watson *et al.* (1992), Norer *et al.* (1999), Emshoff *et al.* (2000b), Yanpiset *et al.* (2001), Strobl *et al.* (2003), Emshoff *et al.* (2004a,b,c,d), Strobl *et al.* (2004a,b, 2005), Sasano *et al.* (2005), Konno *et al.* (2007)
Green rubber base splint	Roeykens *et al.* (1999), Evans *et al.* (1999), Roebuck *et al.* (2000)
Acrylic splint	Akpinar *et al.* (2004), Polat *et al.* (2004)
Self-cured resin splint	Vongsavan & Matthews (1996), Ikawa *et al.* (2001), Sato *et al.* (2003), Soo-ampon *et al.* (2003), Harada *et al.* (2004a,b)
Polyurethane splint	Hartmann *et al.* (1996)
Polyvinyl siloxane stents	Justus *et al.* (2001), Verdickt & Abbott (2001)
Individual resin cap	Ikawa *et al.* (2003)
Individual resin plate	Sano *et al.* (2002)

Table 3 Nonpulpal signals from tissues other than pulp

Authors/year	Nonpulpal signal origin	Nonpulpal signal recording	Proposed isolation	Effect of isolation	Sample size	Type of teeth	Comments
Hartmann *et al.* (1996)	Periodontium Lip	Not stated	Heavy-gauge, Hygienic black rubber dam Cotton roll between lip & tooth Metal foil	69% 8% 20%	27	Central & lateral maxillary incisors	They proposed the use of rubber dam in combination with a rigid splint to enhance the validity of recordings
Vongsavan & Matthews (1996)	Supporting tissues	7–15%	Aluminum foil	45%	8	Deciduous mandibular incisors	The major part of the signal recorded from an intact tooth is from the pulp but a significant component is from tissues outside the tooth
Roebuck *et al.* (2000)	Periodontium	Unclear	Not stated	Not applicable	11	Maxillary incisors	Studies on PBF should quantify the proportion of nonpulpal signal for the recording method used
Soo-ampon *et al.* (2003)	Periodontium	57%	Black rubber dam	73%	22	Maxillary incisors	LDF is not reliable for assessing the vitality of teeth in adults
Akpinar *et al.* (2004)	Labial gingiva Palatal gingiva	Not stated	Periodontal paste	46% (labial gingiva) 10% (palatal gingiva) 63% (both)	20	Maxillary central incisor	Contribution of labial gingiva to the signal is more than palatal gingiva
Polat *et al.* (2004)	Unclear	Unclear	Rubber dam	Not applicable	26	Maxillary anterior teeth	Beams reflected from canal filling materials and coronal restoration may reach the gingiva (especially labial) and turn back to probe
Polat *et al.* (2005a)	Periodontium	Unclear	Periodontal paste Rubber dam	Unclear	51	Maxillary and mandibular single-rooted teeth	It is impossible to eliminate the contamination from periodontal tissues completely
Polat *et al.* (2005b)	Vestibular mucosa Palatal mucosa Adjacent teeth Lips Tongue Tip of the nose	Not stated	Rubber dam	Not applicable	12	Maxillary central incisor	Together with the isolation of the gingiva, the crown should be isolated as well

laser light on the tooth surface scatters widely outside the tooth, in part reaching the periodontal tissue, and then returns to the detector, resulting in contamination (Ikawa *et al.* 1999). Some studies suggest that between 45% and 60%, depending on the recording conditions (Ingólfsson *et al.* 1994a), or as much as 82% (Amess *et al.* 1993) of the signal recorded from an intact incisor may not be from the dental pulp.

When the crown is not isolated, the unwanted tissues may contribute to the signal, so when measuring PBF, it is necessary to isolate the crown (Amess *et al.* 1993, Hartmann *et al.* 1996, Soo-ampon *et al.* 2003, Akpinar *et al.* 2004, Polat *et al.* 2005b). Some ways for removing or decreasing the contribution of neighbouring tissues to the signal are application of cotton rolls (Hartmann *et al.* 1996), periodontal paste (Akpinar *et al.* 2004, Polat *et al.* 2005a), and most importantly rubber dam (Hartmann *et al.* 1996, Soo-ampon *et al.* 2003, Polat *et al.* 2004, 2005a,b). Regarding the usage of rubber dam, it should be emphasized that opaque heavy-gauge dams are the most protective against signed contamination (Hartmann *et al.* 1996, Soo-ampon *et al.* 2003).

Even with these precautions, a large proportion of the signal may be due to the supporting structures (Hartmann *et al.* 1996, Vongsavan & Matthews 1996, Soo-ampon *et al.* 2003, Akpinar *et al.* 2004), so without taking precautions, such as rubber dam application, results may be inconsistent (Polat *et al.* 2004). Polat *et al.* (2005a) believe that, for the present, it is impossible to eliminate the contamination from periodontal tissues completely.

Tooth type

Most of studies on LDF have been undertaken on anterior teeth including central and lateral incisors and canines. However, a few studies have included premolar teeth (Aanderud-Larsen *et al.* 1995, Ahn & Pogrel 1998, Norer *et al.* 1999, Emshoff *et al.* 2000a,b, Yanpiset *et al.* 2001, Sasano *et al.* 2005, Konno *et al.* 2007) or molars (Odor *et al.* 1994a,b, Norer *et al.* 1999).

It has been reported that an increase in laser power increased PBF detection from the thicker teeth (incisors but not from premolars) (Sasano *et al.* 2005). Also, Yanpiset *et al.* (2001), working on incisors and premolars, reported that there was no significant association between the efficacy of LDF and tooth type when vital and nonvital teeth were grouped. There was no significant difference for the nonvital teeth. However, when the vital teeth were evaluated, significant difference was found. They also showed a significant difference between first premolar and second premolar teeth with the second premolar being the least accurate tooth type.

Norer *et al.* (1999), using LDF, studied the effect of tooth morphotype on intraindividual and interindividual variations of baseline data as well as on temporal variations between testing intervals in the assessment of PBF. Intraindividual comparisons of tooth morphotype-related PBF values revealed significant differences only for the first molar, whereas significant interindividual differences were found for the lateral incisor, canine, premolars and first molar. PBF values for the second premolar were significantly higher and those for the central incisor significantly lower than those for the lateral incisor and first molar. In contrast, measurements at the lateral incisor, first premolar, and first molar did not differ significantly. Multiple testing showed no significant effect on tooth morphotype-related PBF measurements.

Application of medications

Pulpal blood flow can change in response to the application of capsaicin to the adjacent gingival or alveolar mucosa (Verdickt & Abbott 2001) or consuming antihypertensive medications and nicotine (Musselwhite *et al.* 1997).

Limitations/considerations

Initially, there were some concerns that the power of the laser light would be hazardous to living tissues and could possible cause permanent damage, such as tissue burning. That fear has been shown to be unfounded as this light in all currently used medical devices is far below the maximum limit permitted for clinical applications and temperature changes in the illuminated tissue (Tozer 1979, Berman & Hartwell 2006).

The information provided by LDF can be ambiguous and must be interpreted with care (Matthews & Vongsavan 1993, Vongsavan & Matthews 1993a,b, Roebuck *et al.* 2000). Although this technique has sometimes been described as semi-quantitative, the device cannot measure blood flow in absolute units. Because of a nonlinearity between the signal output and the blood flow rate for all volume fractions less than 1%, a nonabsolute unit calibration may be found. For example, in acute pulpitis, in which the vascularity of the pulp is increased and the concentration of red

cells is above 1%, an increase in PBF can result in a decrease in the signal. This indicates that LDF values must be interpreted with caution (Vongsavan & Matthews 1993a,b).

There are other limitations to the use of LDF. It is susceptible to extraneous noise, such as loud sounds (i.e. vibrations) or movement near or in the apparatus itself. It may also be sensitive to contamination from blood flow in adjacent tissues. Furthermore, the cost of the equipment makes it unlikely that LDF will become a popular or widely used technique in the near future (Amess *et al.* 1993, Vongsavan & Matthews 1993b, 1996, Ikawa *et al.* 1999, Roebuck *et al.* 2000, Sooampon *et al.* 2003, Akpinar *et al.* 2004, Emshoff *et al.* 2004d, Polat *et al.* 2004, 2005a,b).

Although LDF has met with some success in medical applications, its use in dentistry has been hampered by various limitations (Ramsay *et al.* 1991b, Matthews & Vongsavan 1993, Emshoff *et al.* 2004d). A major problem is the presence of mineralized tissues that limit the penetration of the laser beam into the tooth, even though the penetration depth in teeth may be more than skin. The incident light must reach moving blood cells in the coronal pulp chamber. Although this limitation can be surmounted in animals by cutting a cavity and positioning the probes closer to the pulp, this solution cannot be applied in humans. Moreover, this invasive procedure may induce the liberation of various bioactive substances able to interfere with blood flow regulation (Hartmann *et al.* 1996). Also, the flow in individual microvessels, the number of vessels with active flow, and vessel diameter cannot be analysed by LDF (Matheny *et al.* 1993, Suda & Ikeda 2002).

It should be added that this method may be contraindicated in some heavily restored teeth and teeth with vital apical pulp tissue, because LDF probes detect only coronal PBF (Edwall *et al.* 1987).

Reliability of LDF

Whilst LDF has proved effective and reliable for some body tissues (Belcaro *et al.* 2000, Braverman 2000, Tabrizchi & Pugsley 2000), the limited translucency and multiple reflectance of teeth have cast doubt upon its validity to assess the condition of the pulp (Ikawa *et al.* 1999). Some workers have found this technique to be highly reliable, but only under specific and carefully controlled conditions (Evans *et al.* 1999).

Several studies have suggested a reliability of greater than 80–90% for LDF assessment of vitality (Wilder-Smith 1988, Ingólfsson *et al.* 1994a, Hartmann *et al.* 1996, Evans *et al.* 1999, Roeykens *et al.* 1999, Roebuck *et al.* 2000). Others have observed lower levels of reliability or technical difficulties, including the potential confounding factor that some signals originate from the periodontal tissue. The use of a dual-probe LDF system has been suggested to increase reliability (Roeykens *et al.* 1999, Roebuck *et al.* 2000).

Conclusion

Laser Doppler flowmetry promises an objective measurement of pulpal vitality. Because this test produces no noxious stimuli, apprehensive or distressed patients accept it more readily than sensibility methods. When the costs of the device decrease and their clinical application improves, this technology could be used for patients such as children or those who cannot communicate effectively or whose responses to conventional tests may not be reliable.

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