UNIVERSITY OF LATVIA
FACULTY OF MEDICINE

# The assessment of microcirculation in oral mucosa by means of imaging photoplethysmography

DIPLOMA THESIS

Author: **Robert Andrianirina Muckle**
Student **ID: rm14027**
Thesis supervisor: **LU BF associated professor Dr.biol. Marcinkevičs Zbigņevs**

RIGA 2019

# TABLE OF CONTENT

ABSTRACT ..... 3
KOPSAVILKUMS ..... 5
INTRODUCTION ..... 6
1. LITERATURE REVIEW ..... 8
1.1 Oral Mucosa ..... 8
1.1.1 Structure ..... 8
1.1.2 Blood Supply ..... 13
1.1.3 Pigmentation of oral mucosa ..... 14
1.2 Local Anesthesia in Dentistry ..... 15
1.2.1 Application of local anesthesia ..... 15
1.2.2 Types of local anesthesia ..... 18
1.2.3 Side effects of local anesthesia ..... 22
1.2.4 Monitoring of local anesthesia ..... 23
1.3 Optical techniques for microcirculation monitoring in oral mucosa ..... 24
1.3.1 Laser Speckle Contrast Analysis: ..... 24
1.3.2 Laser Doppler ..... 26
1.3.3 Photoplethysmography ..... 27
2. METHODS AND MATERIALS ..... 30
2.1 Materials and Instrumentation ..... 30
2.2 Examination Protocol ..... 30
2.3 The algorithm of iPPG processing ..... 31
2.4 Statistical analysis ..... 33
3. RESULTS AND DISCUSSION ..... 34
3.1 Gingiva baseline perfusion ..... 34
3.2 Perfusion changes during anesthesia ..... 35
CONCLUSION ..... 37
AKNOWLEDGEMENT ..... 38
BIBLIOGRAPHY ..... 39
APPENDIX ..... 46

# ABSTRACT

Manipulations with mucosa has become a routine procedure in dentistry, demanding novel techniques for mucosa function assessment, as the clinical aspects of the mucosa do not always reflect the underlying morphofunctional features and can mask alterations. Local anesthesia is very common in dentistry, in order to avoid discomfort for the patients during routine dental procedures. However, anesthesia might cause some side effect-complications, because of many reasons (high dose, sensitivity to components, temporal disturbances of gingival and pulpal blood supply, neurotoxicity etc.). Reduction of dose is crucially important, but without causing discomfort for the patient. Currently there is no objective verification technique for anesthesia monitoring in dentistry, therefore the development of new techniques are crucially important.

**Aim:** To asses use of imaging photoplethysmography for oral mucosa perfusion monitoring during the local infiltration anesthesia containing epinephrine.

**Objectives:** To evaluate reliability of imaging photoplethysmography for recording of oral mucosa perfusion. Furthermore to asses oral mucosa baseline perfusion within the subject group. And to evaluate effect of anesthetic solution (articaine and epinephrine) on oral mucosa blood perfusion after infiltration injection.

**Methods and Materials:** The study was carried out on 12 subjects. Alterations of mucosal microcirculation was produced by administration of articaine with epinephrine on each subject. The imaging photoplethysmography system consists of LED light source, industrial monochromatic camera equipped with 540nm narrow-band filter. Video signal was continuously recorded for 12 minutes (6 minute baseline, and 6 minute local anesthesia post administration period) and processed offline to obtain perfusion index.

**Results:** Photoplethysmography demonstrate reliability in oral mucosa perfusion monitoring. The basal perfusion values moderately differ across the subjects. Administration of local anesthesia containing epinephrine produced remarkable decrease of perfusion in the anesthetized mucosa region.

**Conclusion:** Imaging photoplethysmography can be utilized as a reliable contactless technique for temporal and special assessment of oral mucosa perfusion. Various different subjects exhibited moderately different levels of baseline oral mucosa perfusion. Epinephrine containing infiltration anesthesia substantially decreased oral mucosa perfusion in anesthetized site.

**Keywords:** blood microcirculation, tissue perfusion, perfusion monitoring, imaging photoplethysmography, remote photoplethysmography, oral mucosa, gingival microcirculation, dental anesthesia

# KOPSAVILKUMS

Manipulācijas ar smaganām ir kļuvušas par standarta procedūrām zobārstniecībā rosinot nepieciešamību pēc smaganu funkcionālām izvērtēšanas metodēm. It īpaši tādēļ, ka smaganu klīniskā aina nevienmēr atspoguļo to funkcionālo stāvokli un morfoloģiskās īpatnības var nomaskēt funkcionālos traucējumus. Vietējā anestēzija ir neatņemama zobārstniecības procedūra, kas novērš pacienta diskomfortu manipulāciju laikā, taču dažreiz tā var radīt arī nevēlamās blakusparādības vai pat komplikācijas, kam ir vairāki iemesli (lielās devas, individuālā jutība uz kādu no anestēzijas komponentu, traucējumi pulpas un smaganu asins apgādē, vai pat neirotoksicitāte). Līdz ar to devas samazināšana ir ļoti svarīga, taču ne uz pacienta diskomforta rēķina. Diemžēl, līdz šim zobārstniecībā nav objektīvu metožu anestēzijas novērtēšanai.

**Darba mērķis:** Novērtēt attēlveides fotopletizmogrāfijas izmantošanas iespējas mutes gļotādas perfūzijas monitoringam epinefrīnu saturošas infiltrācijas anestēzijas laikā.

**Darba uzdevumi:** Novērtēt attēlveides fotopletizmogrāfijas pielietojumu mutes gļotādas perfūzijas reģistrācijā. Izmeklējamo personu grupā novērtēt mutes gļotādas perfūzijas bazālo līmeni . Novērtēt artikaīna ar epinefrīna ietekmi uz mutes gļotādas perfūziju.

**Materiāli un metodes:** Pētījumā iesaistījās 12 izmeklējamās personas. Gļotādas perfūzijas izmaiņs tika izraisītas injecējot artikaīna šķidumu ar epinefrīnu. Attēlveides fotopletizmogrāfijas sistēma sastāvēja no LED gaismas avota un industriālās monohromatiskās kameras kura tika aprīkota ar 540nm šaurjoslu interferences filtru. Smaganu video signāls tika nepārtraukti reģistrēts 12 minūtes (6 minūtes bazālais līmenis, un 6 minūtes pēc anestēzijas periods).Video apstrādi veica lai iegūtu perfūzija indeksu.

**Rezultāti:** Fotopletizmogrāfija ir piemērota mutes gļotādas perfūzijas reģistrācijai. Mutes gļotādas bazālā perfūzija dažādām izmeklējamām personām bija nedaudz atšķirīga. Lokālā anestētiķa ar adrenalīnu ievadīšana būtiski samazināja gļotādas perfūziju anestezētā apvidū.

**Secinājumi:** Attēlveides fotopletizmogrāfija var tikt izmantota kā uzticama metode telpiski un laikā vērtējot mutes gļotādas perfūziju. Dažādām izmeklējamām personām var būt nedaudz atšķirīgus mutes gļotādas bazālās perfūzijas vērtības. Epinefrīna saturoša anestētiķa ievadīšana būtiski samazina mutes gļotādas perfūziju anestezētā apvidū.

**Atslēgvārdi:** mikrocirkuklācija, audu perfūzija, perfūzijas monitorings, attēlveides fotopletizmogrāfija, lokālā anestēzija.

# INTRODUCTION

Oral mucosa is a unique tissue, with a complex structure and diverse functions and therefore can manifest different systemic and local pathologies at early stages, such as diabetes (Scardina et al., 2017), neuropathy (Jääskeläinen, 2017), sepsis (Top et al., 2011), potentially serving as a diagnostic indicator. Due to contemporary advancements in dentistry, neurology and surgery manipulations with mucosa has become a routine procedure, demanding novel techniques for mucosa function assessment, as the clinical aspects of the mucosa do not always reflect the underlying morphofunctional features and can mask alterations.

It is well known that microcirculation is a reliable indicator of tissue functioning and is deranged in pathological conditions. In this regard particularly promising are contactless optical methods due to measurement site accessibility as mucosa is well exposed and highly sensitive to contact compression, which can produce iterations and can due to rich population of bacteria cause infection concerns during contact diagnostic procedures.

Local anesthesia is very common in dentistry, in order to avoid discomfort for patients during routine dental procedures. Local anesthetic solution usually consists of two substances which are the local anesthetic and a vasoconstrictor. Generally in dentistry amides are used as local anesthetic, which act also as mild vasodilators. In order to negate the vasodilatory attribute of the amides vasoconstrictors are supplemented (Webb, 2016). The vasoconstrictor causes the blood vessels to constrict and leads to a lower absorption rate, reduction of systemic toxicity, longer activity time, and increased hemostasis (Peedikayil and Vijayan, 2013). However, anesthesia might cause some side effect complications, because of many reasons:high dose, sensitivity to components, temporal disturbances of gingival and pulpal blood supply and neurotoxicity (Malamed, 2013). Reduction of dosage is crucially important, but without causing discomfort for the patient. Conventionally the patient is asked to confirm numbness and loss of perception and is then tested by pricking (Baart and Brand, 2017) which is not objective therefore elderly patients and children might have difficulties reporting adequate situation due to fear, stress or various other factors, which may lead to repeated injection of anesthesia and a larger cumulative dose. Currently there is no objective verification technique for anesthesia monitoring in dentistry, therefore the development of new techniques are crucially important. Ideally this technique should be easy to apply, contactless, harmless, quick and economical. A competent alternative for anesthesia monitoring might be imaging photoplethysomography- simple and cost-effective technique for contactless microcirculation monitoring (Rubins et al., 2016; Sun et al., 2012; Verkruysse et al., 2008). This technique provides information regarding tissue perfusion, using simple instrumentation: a light source and a video camera, which allows detecting back-reflected light changes modulated by blood pulsations. Applying this technique could lead to a decreased usage of subjective and unreliable sensitivity tests. However, there are no studies evaluating reliability of iPPG for oral mucosa perfusion monitoring. And there are relatively few studies exploring effect of adrenaline containing local anesthetics – Articaine on oral mucosa blood flow during infiltration injection.

**The aim** of present study was to assess use of imaging photoplethysmography for oral mucosa perfusion monitoring during the local infiltration anesthesia containing epinephrine.

**The objectives** of the study were

1) To evaluate reliability of imaging photoplethysmography for recording of oral mucosa perfusion.

2) To asses oral mucosa baseline perfusion within the subject group.

3) To evaluate effect of anesthetic solution (Articaine and epinephrine) on oral mucosa blood perfusion after infiltration injection.

# 1. LITERATURE REVIEW

## 1.1 Oral Mucosa

### *1.1.1 Structure*

The oral mucosa is covered by stratified epithelium which is composed of several cell layers. These layers express between the surface and the deepest cell layer multiple patterns of differentiation. Areas who are exposed to mechanical forces due to mastication, like the gingiva and hard palate, have keratinized epithelium similar to the epidermis of skin. The differentiation process in which this surface layer of keratin is assembled is called keratinization or also cornification. Usually, the keratinized epithelium expresses multiple distinct layers or strata. Next to the basement membrane is the basal layer, or also called stratum basale, which is a layer of cuboidal or columnar cells. Above the basal layer are multiple lines of larger elliptical cells. This layer is called prickle cell layer or stratum spinosum. The prickle cell layer and the basal layer combined makeup half or up to two-thirds of the thickness of the whole epithelium. The next layer above is the granular layer, also known as stratum granulosum, in which resides larger flattened cells containing small granules that stain heavily with hematoxylin. The granules are known as keratohyalin granules. The final surface layer is made up of very flat squamous cells, which are known as squames, that stain bright pink with eosin. The squames do not contain any nuclei. That layer is called keratinized layer or also stratum corneum. The mechanically resilient stratum corneum is able to disperse the shearing forces during mastication. The differentiation pattern of these cells is often called orthokeratinization. Masticatory mucosa, for example in parts of the hard palate and of the gingiva, commonly displays a variation of keratinization which is called parakeratinization. In this variant the surface layer stains for keratin like mentioned before but shrunken nuclei are still present in abundant or all of the squames. Most commonly parakeratinization is seen in the gingiva and is considered a normal event which does not necessarily indicates disease. Another variant is the so called incomplete keratinization, or incomplete parakeratinization, in which with stains like Mallory’s triple stain a similar staining of the outermost squames of the keratinized or parakeratinized layer expresses a similar picture to that of deeper nucleated cells, which suggests that cells expressing this pattern have become rehydrated by up taking fluid from the oral cavity. Also these variants of keratinization do not imply disease (Squier and Brogden, 2011). In this so-called masticatory mucosa the attachment of the keratinized epithelium to the underlying tissues is facilitated by the lamina propria, a collagenous connective tissue. The mucosal lamina propria consists of cells, blood vessels, fibers, and neural elements installed in ground substance (Squier and Kremer, 2001). It can be divided into two layers. The superficial papillary layer is associated with the epithelial ridges or papillae and the deeper layer is known as the reticular layer. Many capillary loops are present and the collagen fibers are thin and loosely arranged in the papillary layer. In contrast to that the collagen fibers in the reticular layer are arranged in thick bundles which tend to lie parallel to the surface plane (Squier and Brogden, 2011).

Regions in which flexibility is needed in order to adapt to movements like swallowing of bolus, speech and chewing the coverage is facilitated by non-keratinizing epithelium (Squier and Kremer, 2001).This epithelium is in many regions thicker than keratinized epithelium and expresses also a different ridge pattern at the connective tissue interface. The non-keratinized epithelium has a basal and prickle cell layer which resemble those previously described for the keratinized epithelium. However, the cells of the non-keratinized epithelium are a little bit larger. The outer half consists of two zones which are called stratum intermedium that represents the intermediate zone and stratum superficiale that represents the superficial zone. A granular layer is absent and the cells of stratum superficiale express nuclei that are seldomly shrunken (Squier and Brogden, 2011). This so-called lining mucosa can be seen on the floor of the mouth and buccal areas. Its connective tissues have also higher elasticity and flexibility in comparison to the masticatory mucosa.

The specialized epithelium is covering the dorsum of the tongue. This specialized epithelium is characterized by a mosaic pattern of keratinized and non-keratinized epithelium It is tightly attached to the muscle of the tongue. A layer of glandular and loosely organized fatty connective tissue encompassing some of the major nerves and blood vessels which supplies the tissue and divides it from the bone or muscles lying underneath, represents the submucosa in areas like the lips, cheeks and regions of the *palatum durum*. In other parts such as gingiva and other regions of the hard palate, no submucosa is present and direct attachment of oral mucosa and the periosteum is facilitated. This direct attachment of oral mucosa and periosteum is defined as mucoperiosteum, providing non-elastic and tight attachment.

In several areas nodules of lymphoid tissue can be found. By invaginating into the lamina propria this epithelium forms crypts. Due to the large infiltration rate by lymphocytes and plasma cells these areas are very important in fighting infections of the oral cavity.

In order to establish a moisturized surface area minor salivary glands are situated in the submucosa. Through small ducts the secretion of the glands containing mucins, several antimicrobial substances and epidermal growth factor (EGF) are discarded.

The moistening of the surface and the missing of appendages are elements which differentiate the oral mucosa from the skin (Squier and Kremer, 2001).

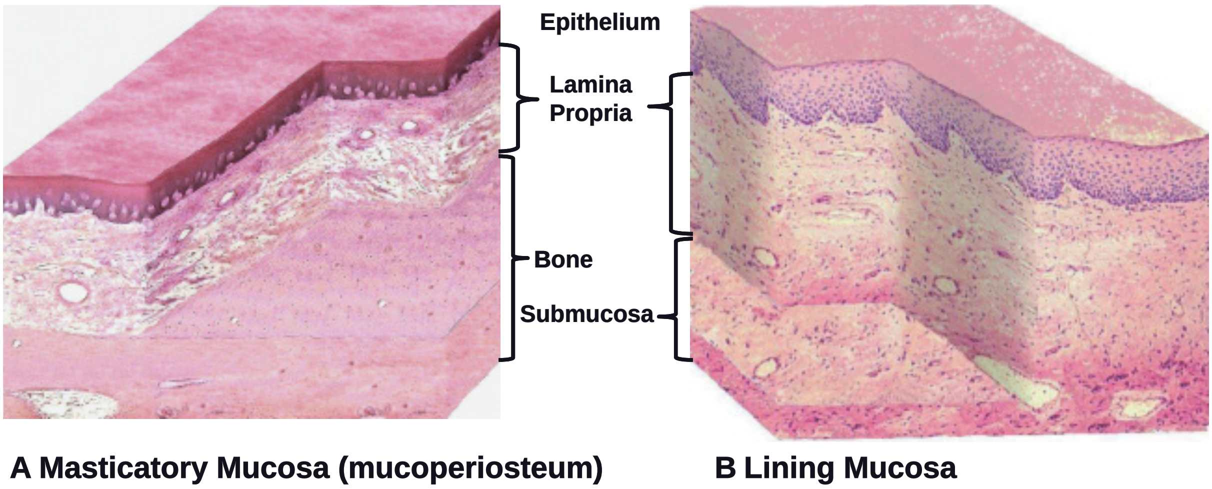


Figure 1 A) shows the arrangement of the masticatory mucosa. Figure 1 B) shows the arrangement of the lining mucosa (Squier and Brogden, 2011).

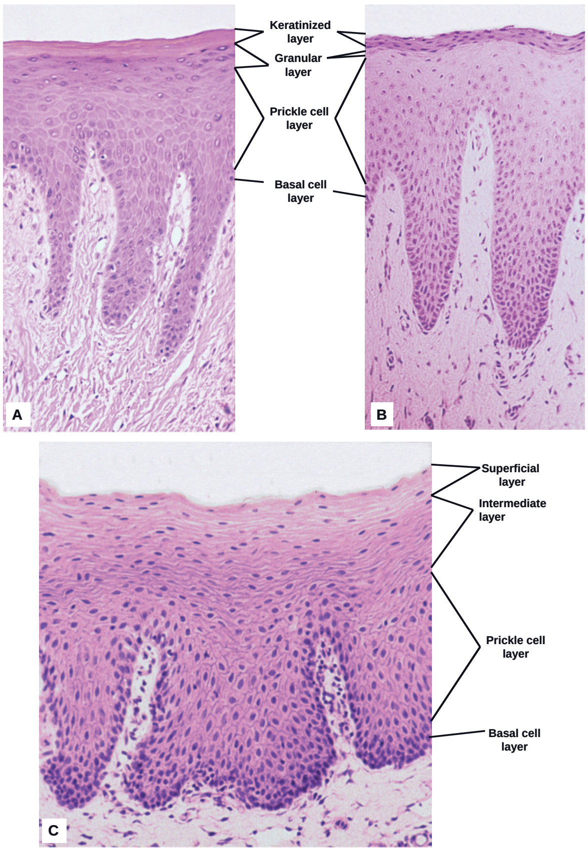


Figure 2 A) shows orthokeratinized epithelium of the gingiva expressing a dark stained granular layer. Figure 2 B) shows parakeratinized epithelium of gingiva expressing keratin squames which have kept their shrunken nuclei and a granular layer containing a few scattered granules.

Figure 2 C) shows non-keratinized epithelium of the buccal mucosa which expresses no clear difference between the cell layers and the occurance of cell nuclei in the surface layer (Squier and Brogden, 2011).

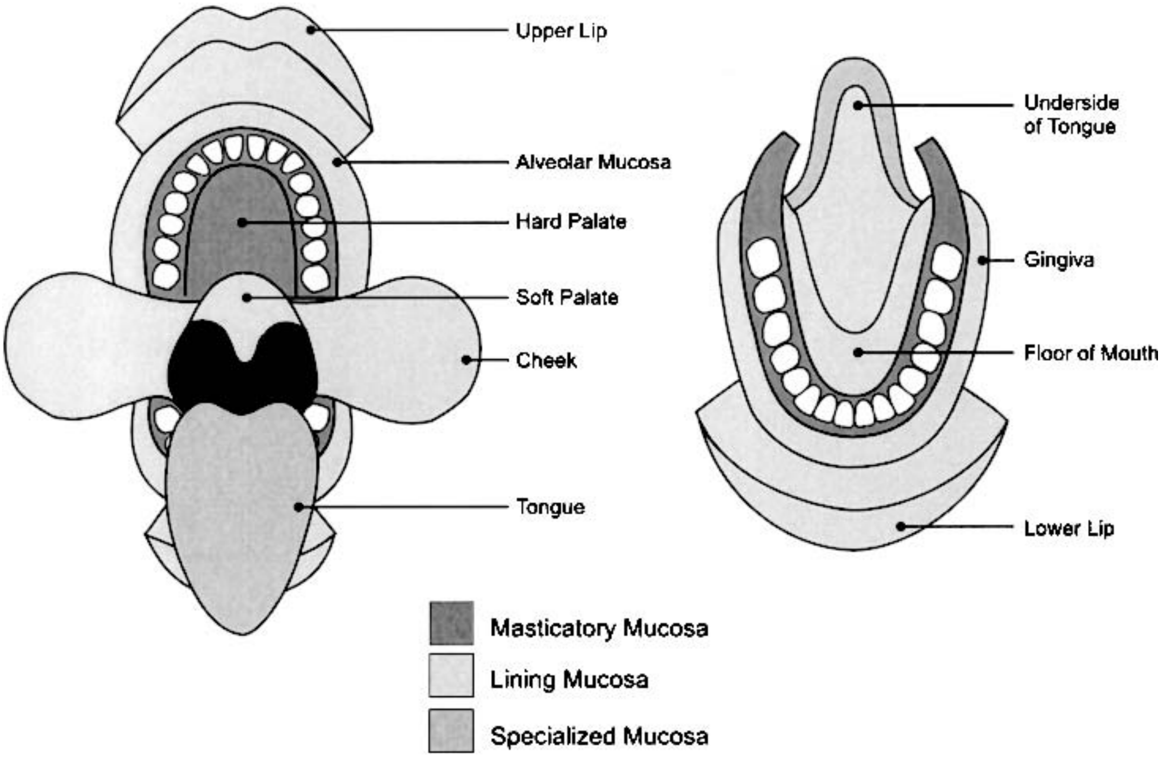


Figure 3 demonstrates the areas which the three different types of mucosa occupy (Roed-Petersen and Renstrup, 1969).

Collin and Dawes (1987) measured the distribution of the three different types and came to the result that. Approximately 60% of the oral mucosa in the oral cavity is occupied by lining mucosa, approximately 25% by masticatory mucosa and 15% by the specialized mucosa exclusively found at the dorsum of the tongue.

Table 1 characterizes the layers of the masticatory and specialized mucosa (Bergmeier, 2018).

| Regional variation in the oral mucosa: 1. Masticatory and specialised mucosa | | | |
|---|---|---|---|
| Masticatory mucosa | Covering epithelium | Lamina propria | Submucosa |
| Gingiva | Thick, orthokeratinised or parakeratinised. Stratified squamous epithelium often showing stippled surface | Long narrow papillae; dense collagenous connective tissue; not highly vascular but have long capillary loops with numerous anastomoses | No distinct layer, mucosa firmly attached by collagen fibres to cementum and periosteum of alveolar process (mucoperiosteum) |
| Hard palate | Thick, orthokeratinised (or parakeratinised in parts), stratified squamous epithelium with transvers palatine ridges (rugae) | Long papillae; thick dense collagenous tissue, especially beneath rugae, moderate vascular supply with short capillary loops | Dense collagenous connective tissues attaching mucosa to periosteum, fat and minor salivary glands packed into connective tissue in regions where mucosa overlies lateral palatine neurovascular bundles |
| Specialised mucosa | | | |
| Dorsal surface of the tongue | Thick keratinised and non-keratinised, stratified squamous epithelium: forms three types of lingual papillae—some bear taste buds | Long papillae: minor salivary glands in posterior portion; richly innervated especially near taste buds. Capillary plexus in papillary layer. Large vessels lying deeper | No distinct layer; mucosa is bound to connective tissue surrounding musculature of the tongue |

Table 2 characterizes non keratinized lining mucosa and the keratinized lip zones (Bergmeier, 2018).

| Regional variation in the oral mucosa: 2. Lining mucosa | | | |
|---|---|---|---|
| | Covering epithelium | Lamina propria | Submucosa |
| Soft palate | Thin, non-keratinised stratified squamous epithelium taste buds present | Thick with numerous snort papillae elastic fibres forming elastic lamina. Highly vascular, well-defined capillary network | Diffuse tissue containing many minor salivary glands |
| Ventral surface of the tongue | Thin, non-keratinised stratified squamous epithelium | Thin numerous short papillae and some elastic fibres; a few minor salivary glands: capillary network in suprabasal layer; reticular layer relatively avascular | Thin and irregular; may contain fat and small vessels: where submucosa is absent, mucosa is bound to connective tissue surrounding the tongue musculature |
| Floor of the mouth | Vary thin, non-keratinised stratified squamous epithelium | Short papillae; some elastic fibres; extensive vasculature with short anastomosing capillary loops | Loose fibrous connective tissue containing fat and minor salivary glands |
| Alveolar mucosa | Thin, non-keratinised stratified squamous epithelium | Short papillae, connective tissue contains many elastic fibre capillary loops close to surface supplied by vessels running superficially to the periosteum | Loose connective tissue with thick elastic fibres attaching it to periosteum of alveolar process: minor salivary glands present |
| Labial and buccal mucosa | Vary thick, non-keratinised stratified squamous epithelium | Long slender papillae; dense fibrous connective tissue with collagen and soma elastic fibres: rich vasculature giving off anastomosing loops into papillae | Mucosa firmly attached to underlying muscle by collagen and elastin; dense collagenous connective tissue with fat, maw salivary glands (sometimes sebaceous glands) |
| Lips: vermillion zone | Thin, orthokeratinised stratified squamous epithelium | Many narrow papillae. capillary loops close to the surface of papillary layer | Mucosa firmly attached to muscle; some sebaceous glands in vermillion border |
| Lips: intermediate zone | Thin, parakeratinised stratified squamous epithelium | Long, irregular papillae elastic fibres and collagen fibres in connective tissue | Minor salivary glands and fat in intermediate zone |

### *1.1.2 Blood Supply*

The oral mucosa has a rich blood supply which is provided from parallel to the surface running arteries of the submucosa or, when mucoperiosteum is present in the deep part of the reticular layer. These vessels give off continuously smaller branches that anastomose with neighboring vessels in the reticular layer of the lamina propria, which is also called submucosal plexus, before forming a broad capillary network in the papillary layer (Lindeboom et al., 2005; Squier and Brogden, 2011). Capillary loop, which derive from this network pass into the connective tissue papilla. The capillary loops lie close to the basal layer of the epithelium however they never enter it. Each capillary loop in the gingiva has one ascending arterial limb and one descending venular limb (Lindeboom et al., 2005). The oral mucosa has a higher concentration of capillaries, because its vascular network contains mainly capillary loops whose characteristic shape reminds of a hairpin or horseshoe (Lindeboom et al., 2005), than in comparison to skin where they are only found around hair follicles.

Different patterns can be seen in different regions. For example only one capillary loop enters into each papilla in the cheek area. Whereas multiple capillaries are suppling every filiform papilla in the tongue.

The venules in the reticular layer collect the blood from the capillary beds. The venules are connected with veins which are situated beneath the mucosa. The major part of the venous blood is then transported by the internal jugular vein.

The lamina propria contains also lymphatic capillaries which occurs as blind vessels in the papillae which eventually drain into bigger vessels in the submucosa. All of the lymphatic vessels conclusively drain into the dep cervical lymph nodes.

Comparing all regions within the oral mucosa the greatest blood flow occurs in the gingiva. However, in comparison to the skin all regions of the oral mucosa have a greater blood flow at average temperatures. Unlike the skin, which has an impact in temperature regulation, human oral mucosa misses arteriovenous shunts, but it does have plentiful anastomoses of arterioles and capillaries, which without doubt commit to its ability to heal more quickly than skin set side by side after injury (Squier and Brogden, 2011).

### 1.1.3 Pigmentation of oral mucosa

The color of the oral mucosa is the end result of multiple factors, including the presence and thickness of epithelial keratinization, pigmentation and the vascularity of the underlying connective tissue of the oral mucosa.

The pigmentation can be categorized into two types: endogenous and exogenous.

Endogenous pigmentation origins from certain cells due to ordinary physiologic processes. Melanin and hemoglobin of the red blood cells are the main contributors in the endogenous pigmentation.

Melanocytes, pigment cells in the basal layer, derive from pluripotent cells of the neural crest ectoderm during embryogenesis and produce melanin. Within these melanocytes melanin is synthesized in form of a small structure known as melanosomes. Melanin is composed of copolymers which are responsible for different colors. Eumelanin is a copolymer for brown and black. Pheomelanin is a copolymer for yellow and red. Both copolymers are derived from tyrosine and are discarded on protein filaments.

In every area of skin or oral mucosa of each person despite pigmentation variations approximately the same number of melanocytes are present. The color difference depends on the activity of the melanocytes in producing melanin and the breakdown of melanosomes within keratinocytes.

People with heavy melanin pigmentation have a lot of cells in the connective tissue which contain melanin. Most likely these cells are macrophages that took up melanosomes. People who are light skinned in contrast to the former mentioned express rarely oral melanin pigmentation.

In skin, melanocytes have a major role in protection against ionizing radiation, and melanin production is associated with such exposure.

Melanin pigmentation in oral mucosa is clinically commonly seen in regions like lips, buccal mucosa, gingiva, tongue and hard palate.

Exogenous pigmentation origins from foreign materials inducted into the body systemically or locally.

An example for most common exogenous pigmentation are the so called amalgam tattoos, which are bluish gray spots of discoloration. During systemical presence certain metals like lead or bismuth and carbon black can lead to coloration of the gingival margin and may be indicators of systemic poisoning (Squier and Brogden, 2011).

## 1.2 Local Anesthesia in Dentistry

Local anesthesia in dentistry is typically applied when pain during dental treatment is expected to occur and would be otherwise uncomfortable or too painful to endure for the patient. It is also applied when during treatment pain occurs and the patient is not able to stand the pain any further.

Generally preventative anesthesia is applied in dental practices. It helps to build up trust into the dentists abilities and treatment measurements (Baart and Brand, 2017).

### *1.2.1 Application of local anesthesia*

Local anesthesia is applied via injection into the tissue.

After the needle penetrates shallowly the oral mucosa a small amount of anesthetic solution is injected. A few seconds later deeper insertion of needle till wished depth is done. Aspiration should be done in order to avert intravascular injection which could potentially lead to a systemic reaction to the anesthetic agent or vasoconstrictor.

After negative aspiration the anesthetic solution is then fully deposited. The administration of the anesthetic solution should be made always as gently and slowly as possible (Peedikayil and Vijayan, 2013).

Local anesthesia can furthermore inhibit the local inflammatory response to injury which usually sensitizes nociceptive receptors and leads to pain and hyperalgesia (Whiteman et al., 2011).

Nowadays multiple techniques in administering the anesthetic solution are available in dentistry.

Infiltration technique is the first choice in order to anesthetize the maxillary and mandibular incisors, canines and all teeth in children till age five.

Especially in the upper jaw the infiltration technique is effective due to the circumstances that the cortical bone in the upper jaw is comparably narrow. This allows the anesthetic solution to diffuse and anesthetize the buccal roots. In order to anesthetize the palatal roots a second infiltration of anesthetic solution to the greater palatine nerve and nasoplatine nerve branches is made (Jung et al., 2017).

Main infiltrative techniques are the posterior superior alveolar (PSA) infiltration, middle superior alveolar (MSA) infiltration and anterior superior alveolar (ASA) infiltration.

Posterior superior alveolar (PSA) infiltration is targeting maxillary molars and the adjacent buccal soft tissue, the periodontal ligament, bone and periosteum. Only the mesiobuccal facet of the first molar is poorly anesthetized by application of this technique. The needle is inserted at the height of the buccal vestibule and a little bit distally in regards to the zygomatic process.

Middle superior alveolar (MSA) infiltration is targeting the mesiobuccal facet of the first molar, the premolars and the adjacent buccal soft tissue, periodontal ligament, bone and periosteum. The needle is inserted laterally in regards to the maxillary second premolar at the height of the buccal vestibule. The needle penetrates roughly five millimeter and after negative aspiration, administration of one milliliter of anesthetic solution is made.

Anterior superior alveolar (ASA) infiltration is targeting the canines and the incisors till the midline, the adjacent buccal soft tissue, periodontal ligament, bone and periosteum. ASA is similar to the MSA technique except that the needle in this case is inserted over the maxillary canine. Also in this technique after negative aspiration, administration of one milliliter of anesthetic solution is made (Reed et al., 2012).

Regional nerve blocks anesthetize a larger area than other techniques. It is preferred over others in the mandible because of its higher bone density (Jung et al., 2017).

Basic nerve block techniques are the maxillary nerve blocks which can be subcategorized in high tuberosity anesthesia and greater palatine foramen block. Other basic nerve blocks are inferior alveolar nerve block and the Gow-Gates nerve block.

Maxillary nerve blocks are targeting to anesthetize half of the maxilla. This grants several types of surgical procedures to be done on the maxillary sinus and maxillae.

In the high tuberosity anesthesia a 35 millimeter long 25 gauge needle is inserted at a 45 degree angle superior posteriorly in regards to the maxillary tuberosity. More precisely it is inserted into the pterygopalatine fossa. After negative aspiration one cartridge of anesthetic solution is injected. One half of the upper jaw should be anesthetized after this procedure.

In the greater palatine foramen block technique the needle penetrates into the greater palatine foramen. The foramen is generally located one centimeter palatally between the second and third molar. Usually it can be palpated and verified as a soft spot on the hard palate. Before injection the needle is bent to a degree of circa 45 degrees and then introduced into the foramen. After negative aspiration 9.5 milliliter of anesthetic solution is deposited. The hard plate tissues laterally till the midline and anteriorly till the distal facet of the canine should be anesthetized after this procedure.

One side effect which occurs on patients with small maxilla is that the anesthetics may get through to the parasympathetic sphenopalatine ganglion, leading to blurred vision or diplopia.

The inferior alveolar nerve block gives anesthesia in the mandible from the third molar to the midline, chin and buccal soft tissue anteriorly to the premolars, lower lip, periosteum and periodontal ligament. The lingual nerve is also anesthetized while the needle is being retracted halfway during injection. A 35 millimeter long 27 gauge needle penetrates from the opposite lying premolars circa 1.5 centimeter high from the mandibular occlusal plane in between the pterygomandibular raphe and the deep tendon of the temporal muscle. The needle is inserted until it contacts the bone. The needle is after that contact then one to two millimeters retracted and if negative aspiration occurs 1.5 milliliter of anesthetic solution is administered.

In comparison to the standard alveolar nerve block the Gow-Gates technique has a higher success rate and lower possibility of positive aspiration. This technique anesthetizes the inferior alveolar, buccal, lingual as well as the auricotemporal and myeloid nerves. The patient is asked to open the mouth as wide as possible so that the condyle rotates and translates forward. The practitioner then palpates with the thumb the condyle and the needle is inserted from the opposite lying canine at the occlusal plane height of the maxillary second molar. The needle penetrates 25-30 millimeter deep until it contacts the bone. The needle is then one to two millimeters retracted and if negative aspiration occurs one cartridge of anesthesia is administered.

The intraligamentary injection technique is administered in order to anesthetize deciduous dentition and permanent dentition as well. Th needle penetrates through the gingival sulcus on several points. On the buccal and lingual or palatal facet each as distally and as medially as possible an injection is made. Roughly 0.2 milliliter of anesthesia is administered. The injection should take at least 20 seconds. In this technique the anesthetic solution diffuses through the alveolar wall into the intraosseal space and consequently blocks the nerve.

Generally, the intrapulpal injection technique is applied during endodontic treatment when the usually administered anesthesia techniques do not have that much of impact.

Roughly 0.2-0.3 milliliter of anesthetic solution is deposited with a short needle into the pulp chamber and into the root canals. This technique is uncomfortable for the patient however is very quick and effective (Jung et al., 2017).

The intraseptal injection technique is handy when aesthesia of soft tissue, bone, homeostasis for surgical flap, scaling and root planing is aimed for. The needle is entering the interdental papilla adjacent to the tooth which is going to be treated in a 90 degree angle. It penetrates roughly two millimeter into the interdental septum. The injection of 0.2 to 04 milliliter should take at least 20 seconds (Reed et al., 2012).

The intraosseous injection technique is only used when the most commonly used injection techniques like infiltration and nerve block have no effect.

In order to enter the interseptal bone a hole with a round bur is drilled. Nowadays special devices are on the market which help to apply this technique. When the hole is finished in the cortical plate, the needle is able to pass through the drilled tunnel and roughly 0.6 to 1.2

milliliter is deposited. The anesthesia is effective immediately and usually works for 15 to 45 minutes (Jung et al., 2017).

### *1.2.2 Types of local anesthesia*

Local anesthetic solution usually consists of two drugs which are the local anesthetic and a vasoconstrictor. Both drugs have their own specific dose limitation.

In administering local anesthetics the dosage which is described in milligram should be considered more relevant than the volume which can be recorded in either milliliter or cartridges. Because the serum concentrations are linked to the total dosage rather than the concentration of the solution. As an example, administering 20 milliliter of two percent or 10 milliliter of four percent anesthetic agent produces in the end an identical serum concentration.

Every local anesthetic solution has their individual specific maximum recommended dose (MDR) which is depicted in mg/kg. However the MDR for the drugs is determined differently from literature to literature and varies from 4.4 mg/kg to 6.6 mg/kg (Moodley, 2017).

In order to have better effectiveness and prolonged duration of local anesthetics at the site of infiltration the tumescent analgesia technique is used.

A high volume but low concentration of a local anesthetic together with dilute epinephrine is infiltrated at the side where anesthesia is needed, causing the tissues to become firm. Because of the increase in hydrostatic pressure in the so-called tumescent tissue, blood vessels are compressed and reduced removal of local anesthetic from the site of injection can be observed. Also is the anesthetic action prolonged by epinephrine which acts as a vasoconstrictor.

Due to the delayed systemic absorption the peak plasma concentration of local anesthetic is reduced and postponed, so that larger doses can be safely used.

The reason local anesthetics usually work only for short period of time is because of their ability to cross through blood vessel walls and are hence quickly evacuated from the injection site (Whiteman et al., 2011).

Ideally the local anesthetic agent should be immediately effective, facilitate profound anesthesia, should be free of risk of local or systemic toxicity and should not give rise to allergic responses (Overman, 2007).

Injections into an infection prone area has a delayed or even absent onset. This is caused by the low pH of the extracellular tissue caused by the inflammatory process in the area of infection. The normal pH is 7.4 however due to infection it can be lowered to five, six or even lower. Because of the pH drop only a small part of the free base form of the anesthetic can transverse into the nerve sheath in order to inhibit nerve impulse conduction. Furthermore could a needle insertion into an active infection area promote the spread of the infection (Peedikayil and Vijayan, 2013).

Nowadays amide have replaced the esters and are the mainly used local anesthetic agents.

Amides are local agents which are considered safe and nonallergenic. in comparison to the ester amides have a quicker onset and longer duration. Especially the lack of allergenicity makes the amides preferable over the ester agents (Overman, 2007). The liver metabolizes them and excretion of it is done by the kidneys. Amides act also as mild vasodilators. This leads to a higher absorption rate of the anesthetic into the bloodstream which leaves the patient on higher risk of systemic toxicity, reduced duration of activity and more bleeding at the site of injection. In order to negate the vasodilatory attribute of the amides vasoconstrictors are supplemented (Webb, 2016).

Mainly used amides are articaine, lidocaine, mepivacaine and prilocaine.

Articaine is considered an amide which also contains on its aromatic ring an ester side chain. So far it is known as the only amide anesthetic which has an ester group. This ester group allows biotransformation in the plasma by hydrolyzing it by blood cholinesterase, which is quicker in breaking down articaine than its metabolization the liver by hepatic microsomal enzymes (Moodley, 2017; Overman, 2007). Due to that its half life of 20 minutes is quite short in comparison to the 90 minutes of lignocaine which needs total hepatic clearance. Furthermore it has consequently a lower risk probability of systemic toxicity at long lasting appointments where further injections and therefore higher doses of anesthetic solution are made (Moodley, 2017).

The thiphene ring raises the lipid solubility of articaine. This leads to better diffusion across tissues and boosts the ability of passing through lipid membranes. The maximal dosage of articaine in a healthy adult patient is considered to be seven cartridges. Despite having an ester linkage articaine is not linked to higher allergenicity rates like ester anesthetic solutions. Articaines allergy profile is comparable to the other amides. Hypersensitivity towards amide agents is considered to be extremely rare. Allergic reactions to articaine do not exclude administration of different amide agents. Which means that in case of articaine allergy the administration of lidocaine or any other amide is not necessarily excluded. (Overman, 2007)

Articaine is quite popular due to its higher injection success rate as a result of the higher lipid solubility and quicker diffusion across hard and soft tissues which leads to better anesthesia of the palatal root with a buccal injection. Usually articaine anesthesia is offered in four percent articaine and 1:100,000 epinephrine and four percent articaine and 1:200,000 epinephrine concentrations. It is also the most popular anesthetic agent in Europe. However, in the U.S. it holds the second place with 35.6 % of the market share.

Lidocaine is the most popular anesthetic agent in the U.S. with a market share of 49%. It was introduced in 1948 as the first amide in local anesthetics and introduced a big improvement over the former used ester anesthetics. Usually lidocaine anesthesia is offered in two percent lidocaine and 1:100,000 epinephrine and two percent lidocaine and 1:50,000 epinephrine concentrations (Webb, 2016). The lower solution concentration of lidocaine in comparison to articaine is due to the fact that articaine has equal analgesic efficacy accompanied by lower systemic toxicity which conclusively allows the usage of articaine in higher concentrations than other amide anesthetic solutions like lidocaine (Johansen, 2004). If the rare case of allergy to lidocaine occurs or the patient has bisulfite allergy usage of it is contraindicated.

Mepivacaine is offered in form of two percent mepivacaine and 1:20,000 levonordefrin and three percent plain mepivacaine. Due to its comparably mild vasodilatory effect in regards to other amides it comes in handy for patients with contraindications for usage of vasoconstrictors and or usage of four percent plain prilocaine. Nonetheless the duration of anesthesia is comparatively short. Administration is contraindicated when the patient expresses allergy to it, which is rare, or if vasoconstrictors are added and the patient admittedly has a bisulfite allergy or is taking tricyclic antidepressants.

Prilocaine expresses less toxicity and potency however a bit longer duration of action than lidocaine or mepivacaine. It is offered as four percent prilocaine with 1:200,000 epinephrine and as plain four percent prilocaine. It is a milder vasodilator than the major part of other amides. It is considered a good choice in patients where administration of vasoconstrictors is contraindicated. Because prilocaine administered in higher doses is reducing the bloods oxygen- carrying capacity it is contraindicated in patients at risk for methemoglobinemia, patients having problems with oxygenation like sickle-cell anemia, cardiac/ respiratory failure, and also for patients who are receiving acetaminophen or phenacetin due to increased methemoglobin levels.

Due to the fact that its metabolization not only takes in the kidneys but also in the lungs place, the metabolization in the liver is more effortless. Furthermore, the kidneys are more faster cleansed of prilocaine than other amides. Administration is contraindicated when the patient expresses allergy to it, which is rare, or if a vasoconstrictor is also added and the patient admittedly has a bisulfite allergy.

As formerly already mentioned the vasoconstrictor causes the blood vessels to constrict and leads to a lower absorption rate, reduction of systemic toxicity, longer activity time, and increased hemostasis. By using vasoconstrictors the total maximum dosage of the anesthetic agent can be elevated up to almost 40%. The most effective vasoconstrictor so far is epinephrine (Peedikayil and Vijayan, 2013).

Local anesthesia without vasoconstrictors should only be used when there are potent contraindications in using local anesthesia containing vasoconstrictors.

Epinephrine and levonredefrin are the commonly used vasoconstrictors. Usually epinephrine is added to dental anesthetics like artisan, lidocaine, prilocaine and bupivacaine. The concentration of epinephrine is displayed on the cartridge in 1:50,000, 1:100,000 and 1:200,000.

Because the duration of anesthetic activity is identical in all the former mentioned concentrations in anesthetizing pulpal and soft tissue it is advised to use in these cases the smallest concentration. Patients with a relative contraindication for vasoconstrictors often may receive the minimum possible dosage of epinephrine, in order not to exceed the cardiac maximum recommended dose of 0.04 mg per appointment, which is also displayed on Table 3.

In all dental local anesthetic solutions containing vasoconstrictors in cartridges a preservative called bisulfite is added. Bisulfates can be found often in food and beverages, too. It has been reported that bisulfate can cause hypersensitivity particularly in asthmatics. If Patients express allergic reactions to bisulfites anesthetic solution free of vasoconstrictors should be administered (Webb, 2016).

Table 3 shows the maximum recommended dose per appointment of epinephrine for healthy adult patients and adult patients with significant cardiovascular disease (Webb, 2016).

| EPINEPHRINE MAXIMUM RECOMMENDED DOSE PER APPOINTMENT | | | | |
|---|---|---|---|---|
| | HEALTHY ADULT PATIENT | | ADULT PATIENT WITH SIGNIFICANT CARDIOVASCULAR DISEASE | |
| Concentration | Dose | Cartridges | Dose | Cartridges |
| 1:50,000 Epinephrine | 0.2 mg | 5.5 | 0.04 mg | 1.1 |
| 1:100,000 Epinephrine | 0.2 mg | 11.1* | 0.04 mg | 2.2 |
| 1:200,000 Epinephrine | 0.2 mg | 22.2* | 0.04 mg | 4.4 |

### *1.2.3 Side effects of local anesthesia*

Local anesthesia is frequently used. Side effects and complications in regards to how often these occur when local anesthesia is administered is quite rare.

The complications which can occur can be divided into local and systemic complications.

Local side effects can be described as complications which only occur locally at the area where local anesthesia got injected.

One side effect which can occur is needle breakage. The needles used in dentistry can be bent quite easy and are very flexible. They can be bent before injection if needed. However multiple attempts of bending can lead to higher risk of needle breakage (Baart and Brand, 2017). Another local side effect is prolonged anesthesia or paresthesia. Local anesthesia can be present for several hours in soft tissue anesthesia. It can sometimes endure longer than anticipated. As long as it persists only for a few hours it is considered fine. However, if the anesthesia is present for several days, weeks or months it can give rise to further complications like tongue biting, drooling, loss of taste and speech impediment.

Paresthesia is considered as one of the most occurring accidents of dental malpractice litigation (Malamed, 2013).

Local anesthesia has neurotoxic attributions. But experts assert that paresthesia is most often associated with mechanical trauma and not chemical trauma. The major part of paresthesia, approximately 95%, happens in the mandible when the lingual nerve is anesthetized. However occurrence of paresthesia after administration with the Gow-gates and also the Vazirani-Akinosi technique and in the medical area have yet to be reported (Webb, 2016).

Previously it was believed that articaine had an increased risk of paresthesia but however the study by Pogrel (2012) came to the conclusion that the incidents of paresthesia from articaine were in proportion to its market share (Pogrel, 2012).

Due to the administration soft tissues are anesthetized too and this can lead subsequently to patients unconsciously biting or chewing on these tissues.

Facial nerve paralysis, trismus , hematoma, pain on injection burning on injection, infection, edema and sloughing of tissues are also categorized as local side effects (Malamed, 2013).

Systemic side effects are usually toxic reactions which are happening due to the fact that local anesthetic solution got recklessly injected intravenously.

Children are in higher risk to have systemic reactions due to overdosage. Usually the dosage is determined in regards to the physical status of the patient, the site of anesthesia, the vascularization of the oral tissues and the injection technique. However, in children maximum dosage recommendations vary due to age and weight. Toxicity mainly manifests in the cardiovascular and central nervous system. Here it either stimulates or depresses the central nervous system. Stimulation of the central nervous system can cause a toxic vasoconstrictor reaction, and the signs and symptoms are tachycardia, sweating, apprehension, and hyperactivity. Depression of the central nervous system may follow, causing bradycardia, hypoxia, and respiratory arrest.

Allergic reactions as a response to administration of local anesthesia is considered rare. The agent with the highest allergic reaction accidents is procaine. Reactions due to allergy include anaphylaxis, photosensitivity, fever, angioedema, dermatitis and urticaria (Peedikayil and Vijayan, 2013). As in an previously mentioned chapter also Bisulfite, which acts as an conservant of epinephrine, can give rise to allergic reactions (Webb, 2016).

Table 4 lists adverse reactions of commonly used local anesthetics which are categorized under psychogenic, allergic and toxic(Peedikayil and Vijayan, 2013).

| |
|---|
| Psychogenic |
| Syncope (most common) |
| Hyperventilation |
| Nausea, vomiting |
| Alterations in heart rate or blood pressure |
| Mimicking of an allergic reaction |
| Allergic (potential allergens) |
| Esters (true amide allergy is very rare) |
| Metabisulfite (present with epinephrine and with levonordefrin) |
| Methylparaben (no longer added to dental cartridges) |
| Toxic effects |
| Primarily neurologic signs may initially manifest as sedation, light-headedness, slurred speech, mood alteration, diplopia, sensory disturbances, disorientation, muscle twitching |
| Higher blood levels may result in tremors, respiratory depression, tonic–clonic seizures |
| If severe, may result in coma, respiratory arrest, cardiovascular collapse |
| Methemoglobinemia |
| Associated with prilocaine, articaine, benzocaine |
| Paresthesia |
| Apparently more common with articaine and prilocaine |

## *1.2.4 Monitoring of local anesthesia*

In order to check if the local anesthesia is working accordingly the physician asks first the patient open questions like: Do you already notice any change yet? What do you feel? Could you indicate where it is tingling or where it feels numb?

Is the patient unable to answer these questions clearly the physician can furthermore ask: What about the lip? And the tongue? and can additionally ask about divergences in feeling: Does it have the same felling as this side? If I touch here does it feel the same as there? It should be avoided to ask closed questions which can lead to unreliable answers.

Before starting the treatment usually the dentist pricks with for example a dental probe to check the anesthesia (Baart and Brand, 2017).

## 1.3 Optical techniques for microcirculation monitoring in oral mucosa

### *1.3.1 Laser Speckle Contrast Analysis:*

Laser Speckle flowmetry is a vascular imaging tool which could be potentially used during surgery (Eriksson et al., 2014; Hecht et al., 2009; Yuan et al., 2015) and evaluation of wound healing (Lindahl et al., 2013; Milstein et al., 2016).

It is measuring blood flow velocity by quantifying the decrease in speckle contrast as a result of blurring of dynamic speckles within a fixed camera exposure time (Nadort et al., 2016). Laser speckle can be defined as a random granular pattern produced when laser light illuminates a rough object (Briers and Webster, 1996). It happens due to high coherence rate of laser light.

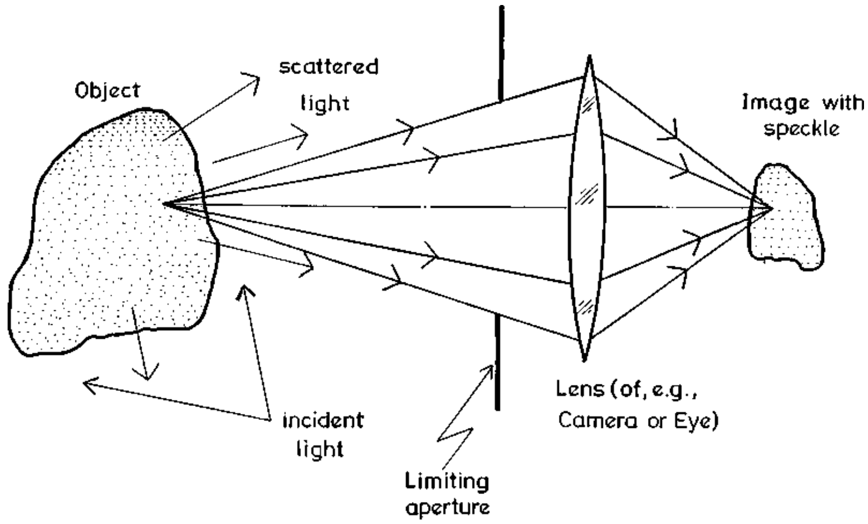


Figure 4 shows the formation of image speckle (Briers et al., 1999).

In time varying speckle the object is moving and the speckle pattern subsequently changes. In case of a solid object, the speckles will move along with the object. This phenomena is called decorrelation, which is also happening when the light is scattered from a big amount of individual scatterers. That is also the case in fluids where particles are situated in. Here a speckle individually will appear to twinkle like a star.  It is assumed that fluctuation frequency spectrum is dependent on the velocity of the motion. Which consequently gives us then the possibility to get information about the scatterers motion.

LASCA is the modern digitalized evolution of former laser speckle imaging. The former basic technique was to take photos under laser illumination. The exposure time needed to be the same as the correlation time of the intensity fluctuation, which is very short und subsequently would freeze the speckle and give a high contrast speckle pattern. The longer the exposure time is the more the moving speckles would average out and would give rise to a low contrast rate. The downfall of this method is that there are two stages in processing. First a photo needs to be taken. Then it has to be developed and then processed. Consequently no real time operations can be done with this technique. Recently a single-exposure speckle photography method which is fully digital and can map the blood flow in real time is developed which is called Laser speckle contrast analysis (LASCA). The mechanism is founded on the basic method in which a camera is recording the speckle patterns which are produced by whatever vascular system is recorded and is then digitalized frame by frame and then the contrast is computed. A minimum in this contrast is considered as an indicator of the occurrence of a heartbeat. The method utilizes a CCD camera, a frame grabber and a specific software to compute the local contrast and also a conversion of it into a false color map of contrast. The exposure which is usually 20 milliseconds is short enough so that the rendering and application of the technique can be done in real time. Currently light from a laser which is usually a Helium-Neon laser with a wavelength of 633mm illuminates the wanted area which is needed to be investigated (Briers et al., 1999).

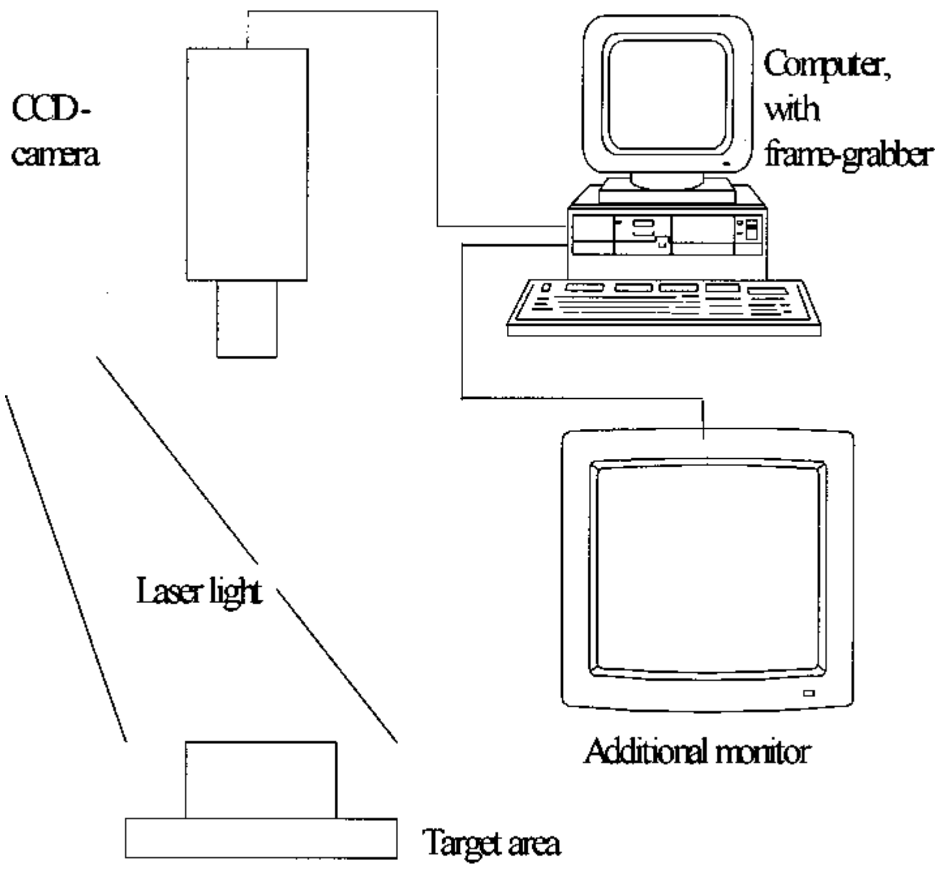


Figure 5 shows the hardware setup of LASCA (Briers et al., 1999).

### *1.3.2 Laser Doppler*

The Laser Doppler is also a noninvasive measurement method which gives the possibility to examine blood flow. It can be therefore also used to observe changes in blood flow related to diseases and drugs. It was introduced in the 1980s. Here a red helium-neon laser ray having a 632.8mm wavelength, which are well absorbed by the erythrocytes, is applied perpendicular towards the surface on the gingiva. The application is done via a probe who has two optical fibers. One fiber emits a laser beam. The laser beam that traveled through the gingiva and hits the erythrocytes is bounced off with a modified frequency. This phenomena is called Doppler effect. The frequency from stationary objects will remain unshifted (Evans et al., 1999). The reflected beams are then received by the second so called receiving optical fiber. Conversion of the light signals into electric signals and their amplitude variations are then graphically recorded. This enables the possibility to compute the blood flow in a living tissue (Ambrosini et al., 2002). One of the shortcomings in this method is that it can not accurately calculate the absolute blood flow. It produces only a relative value of blood flow (Vongsavan and Matthews, 1993). This Technique finds application in various fields like retina (Riva et al., 2010), skin (Svalestad et al., 2010), intestine (Hoff et al., 2009), bone (Hellem et al., 1983) and kidney (Babos et al., 2013). Additionally, in the orofacial area (Retzepi et al., 2007; Verdonck et al., 2009), the appliance has been also used to point out the microcirculation of the tongue (Singh et al., 2008), the buccal mucosa (Hirai, 2005), human dental pulp (Chen and Abbott, 2009; Gazelius et al., 1986), periodontal tissue (Cho et al., 2013), luxated teeth (Gazelius et al., 1988) and the masseter muscle (Curtis et al., 2012). Reduction of the use of objective and unreliable sensitivity tests by using LDF is thereby possible. One of the shortcomings of LDF is that micro movements can not be prevented. Also the pressure and the angle of the probe placed by the operator can not repeatedly reestablished when the procedure is done in handheld (Kouadio et al., 2018). The distance should be kept less than or equal to three millimeter in order to get reliable measurements of gingival blood flow (Matsuki et al., 2001).

The light scattered by moving RBCs goes through a Doppler frequency shift and intermits with light that is scattered by static tissue, which are without a Doppler shift, consequently provides a frequency spectrum between roughly 20 Hz–20 kHz (Sun et al., 2016).

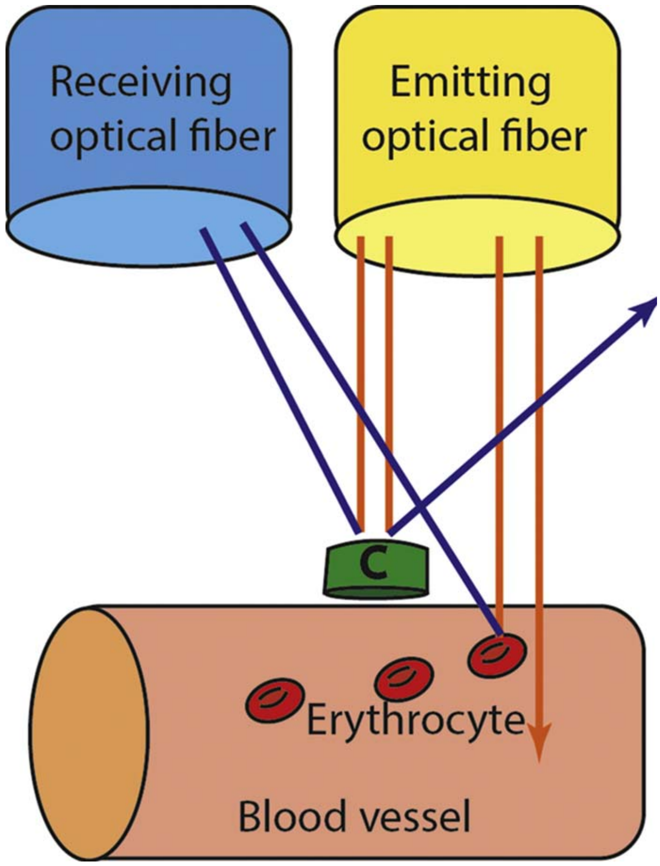


Figure 6 shows the effect of doppler applied to laser radiation. C displays an immobile cell (Kouadio et al., 2018).

### *1.3.3 Photoplethysmography*

iPPG is a noninvasive method which can map the cardiac synchronous pulsation. The PPG waveform is composed of a pulsatile so-called AC physiological waveform associated with cardiac synchronous changes in the blood volume with each heartbeat, and is overlaid on a slowly varying DC baseline with numerous lower frequency components associated with sympathetic nervous system activity, thermoregulation and respiration. The AC component is the pulsatile component of the PPG waveform and its principal frequency, which is generally around 1 Hz, depends on the heart rate. The underlying DC component is linked to the tissues and to the average blood volume. The DC component varies leisurely because of respiration, vasoconstrictor waves and vasomotor activity, Traube Hering Mayer waves and thermoregulation. Characteristics of both components varies from body site to body site. With proper electronic filtering and amplification both components can be extracted for consecutive pulse wave analysis (Allen, 2007). The method is used generally in the reflection mode. The illuminating light source and the video camera instead of the former photoplethysmograph method where still a photodetector was needed, are here placed alongside each other.

The general consensus is that the PPG waveform derives from pulsatile variation in the tissue optical density caused by arterial pulsations which are the most notable (Kamshilin et al., 2015). A video of the illuminated area is recorded usually by a monochromatic CCD camera. And the light intensity variations due to the pulsing of the blood is captured. To have an appropriate tradeoff between sampling often enough to capture the slender structure of the PPG signals and granting the camera enough time to incorporate and compute each frame, a frame rate of 30 frames per second is used. Every frame of the video is then divided into boxes or groups of adjacent pixels. For every box or group the pixel value of each frame is calculated. Calculating the average pixel value of each box for each frame against the time produces a PPG signal. The differences in each frames pixel values are influenced by the changes in absorption by the tissue as the blood flows through it and also because of changes in the ambient light (Zheng and Hu, 2007). The video is usually recorded under ambient light. Under the premise that green color signal transports the maximal amount of pulsatile information and the red color signal carries little relevant information ,compensation of artifacts which are common for both color channels the green-red difference (G-R) method is used. Consequently iPPG is computed as a difference of green and red color signals. Theoretical studies and simulations imply that the best signal to noise ratio in iPPG should be gathered with wavelengths in the ranges of 420-455mm and 525-585mm, with peaks at 430mm,540mm and 580mm corresponding to violet, green, and yellow light respectively. Green with the wavelength 510-570mm, red with the wavelengths 710-770mm and NIR light with the wavelength of 770-1400mm are optimal for crossing through epidermis and bloodless skin. Nonetheless hemoglobin's absorption peaks for green and yellow light which has the wavelength of 570-590mm, which consequently gives rise to a better signal-to-noise ratio for these wavelengths. Combination of multiple color channels when the iPPG is extracted from the RGB video is considered more effective than computing the data from an individual wavelength. It also desensitizes the iPPG extraction from RGB to movements and lighting variations. Shortcomings of this method are when the patients tissues have high melanin concentrations. Visible light with wavelengths below 600mm are heavily absorbed by melanin. This can lower the quality of PPG. Because of this circumstance usage of red or NIR-light for PPG were recommended in the past (Unakafov et al., 2018).

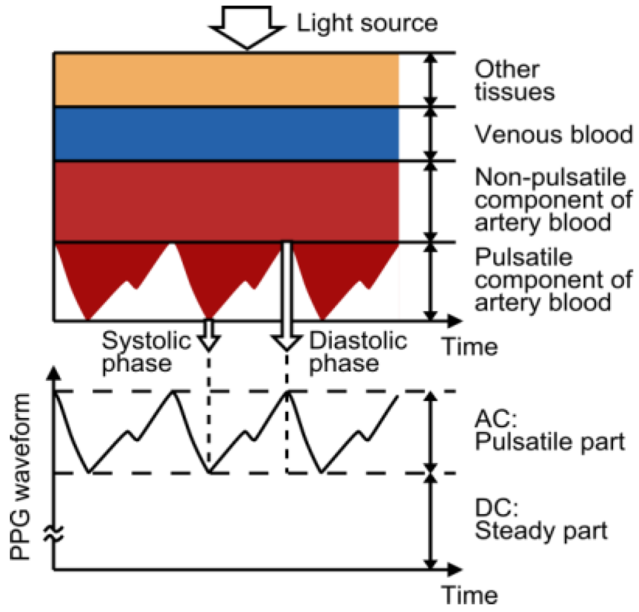


Figure 7 shows the principle of photoplethysmography (Tamura et al., 2014).

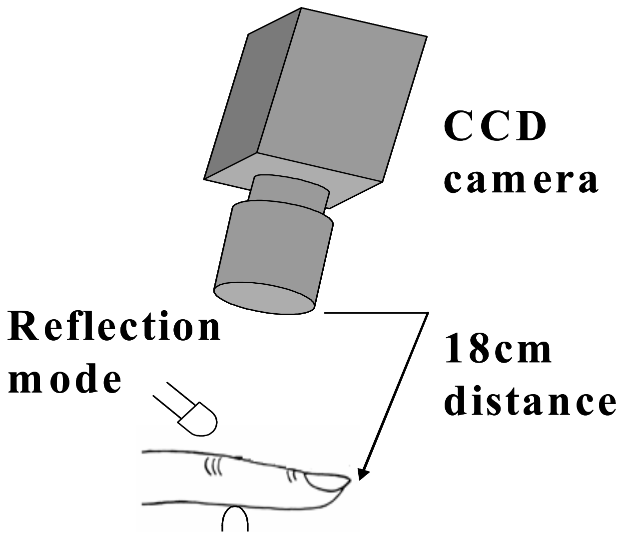


Figure 8 shows the setup of an imaging PPG System (modified from Zheng and Hu, 2007).

# 2. METHODS AND MATERIALS

## 2.1 Materials and Instrumentation

The iPPG setup was developed for contactless acquisition of gingival microcirculation. The setup consists of a white LED light source (100W electric power), an industrial camera (Ximea-xiQ USB-3.0, ADC 8-12-bits, resolution 648x488 pix.) with mounted lens (Edmund Optics, C-mount f=25mm) and 540nm CWL 10nm FWHM narrow-band filter (Edmund Optics). The light source was powered by stabilized power supply and provided uniform illumination of the measurement site. To reduce glare of gingiva, crossed polarizers were placed in front of the camera lens and light source (Figure 9). Camera exposure was adjusted optimally to fit camera dynamic range and avoid pixel saturation for the area of interest. Video acquisition was performed at 50 frames per second.

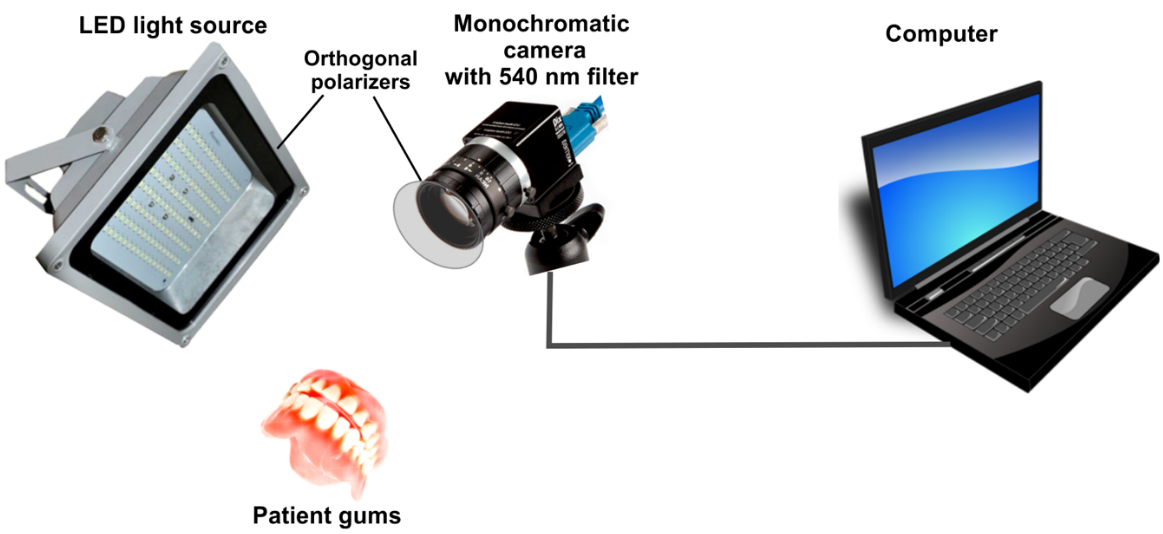


Figure 9 explains the iPPG setup including Hardware and subject of interest.

## 2.2 Examination Protocol

The Study comprised 12 subjects undergoing routine dental procedures in the Dental clinics of Medical faculty. Before all procedures subjects were informed about possible risks of local anesthesia and gave their written informed consent. All procedures were approved by University local Ethics committee, and were in accordance to the Declaration of Helsinki. The subject was comfortably seated in the dental chair in supine position and the head was fixed by a vacuum support pillow (Figure 10). A soft dental disposable rubber dam cheek retractor was placed so that the anterior maxillary gingiva was laid bare. The light source and camera were focused on the recording site, and a video was continuously recorded for 12 minutes which consists of a 6 minute baseline period and a 6 minute post administration of local anesthesia period.

0,85 ml (half a cartridge) of 4% articaine-hydrochloride with 1/100.000 epinehphrine (Septodont UK Ltd, Units R & S - Orchard Business Centre - St Barnabas Close -Allington, Maidstone, Kent ME16 0JZ – UK) was used on each subject. The injection was administered with a 30 Gauge 12 millimeter short needle. Buccal infiltration was facilitated by placing the needle subgingivally into the alveolar mucosa close to the mucogingival margin over the estimated root of the right lateral incisor (D12). The local anesthetic solution was slowly infiltrated after negative aspiration.

Figure 10 shows how the subject is seated in the dental chair with the head fixed in a vacuum support pillow and the iPPG setup.

## 2.3 The algorithm of iPPG processing

The acquisition of the video was performed by *Ximea CamTool* software. The video was stored in uncompressed *avi* format and processed offline using custom-developed dedicated Matlab software(Marcinkevics et al., 2016). The processing consisted of several stages: 1) the video was stabilized, to compensate motion artifacts, and the region of interest (RoI) was manually selected; 2) the iPPG signal was computed from video and the fast-varied (AC 0.7-4 Hz) and slow-varied (DC 0-0.3 Hz) components were obtained, 3) the amplitudes of AC and DC components were calculated from iPPG signal (Figure 11).

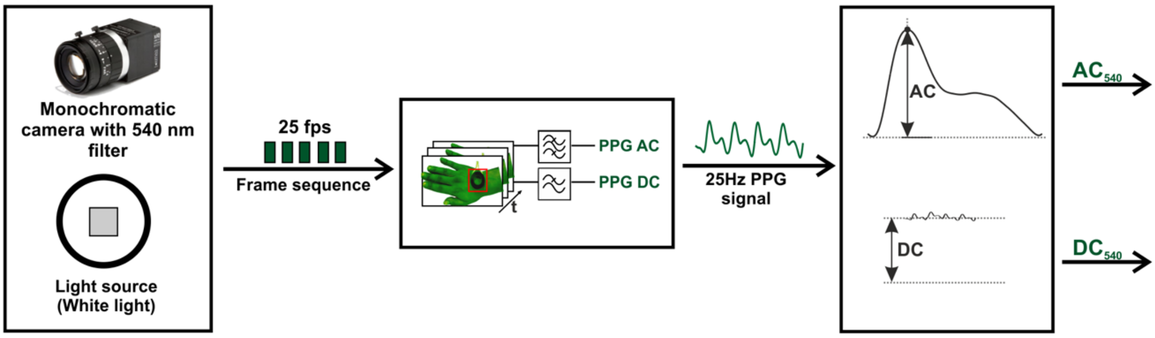


Figure 11 shows the acquisition of the AC and DC signal.

To obtain perfusion parameter, a single-period iPPG waveform was computed in beat-per-beat manner. The feet of AC signal are detected, by finding the local signal minimum. The beat positions, pulse rate, DC and AC amplitude were found in every cardiac cycle. Perfusion index (*PI*) is related to both AC and DC signals, and calculated as follow:

$$PI(\%) = 100\, AC/DC \quad (1)$$

The *PI* characterizes microcirculatory perfusion in tissue. To obtain hi-resolution perfusion map, the *PI* was calculated in every pixel of the video, using algorithms described in previous works of Marcinkevics et al. (2016) and Rubins et al. (2016). The *PI* map was normalized, using simple equation: $PI_{norm}=(PI_i-PI_{min})/(PI_{max}-PI_{min})$, where $PI_{min}$ is obtained from baseline (where perfusion is not obtained, e.g. teeth), $PI_{max}$ is obtained from the site having maximum circulation (Figure 12).

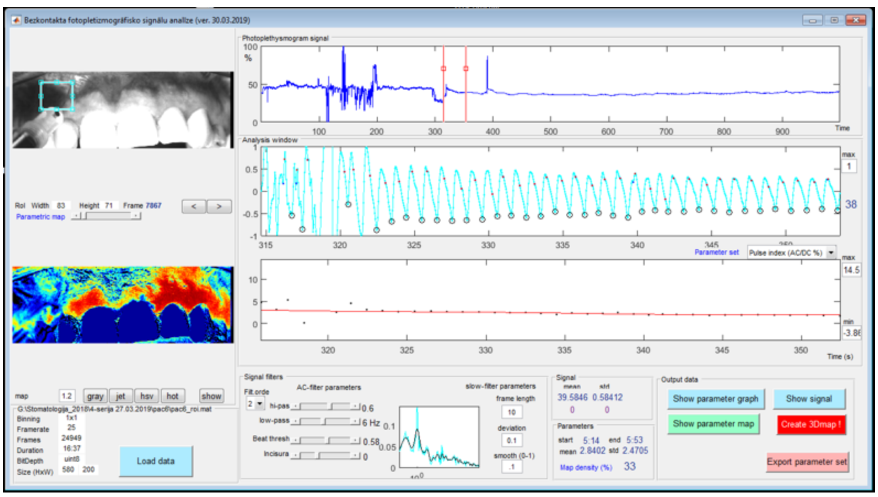

Figure 12 shows the application of the PI and the PI mapping.

## 2.4 Statistical analysis

In order to assess anesthesia triggered perfusion changes in the subject group, the subjects individual PPG perfusion index values were selected during baseline (first 6 minute time period), first, second, third and fourth minute following administration and at the end of recording, where values did not change (plato phase). Arithmetic mean and standard deviation was calculated and group mean perfusion values were compared in different stages using Kruskal-Wallis One Way ANOVA with Tukey post-hoc test for multiple comparison at the significance level $p<0.05$. The data is expressed as mean±standart deviation.

# 3. RESULTS AND DISCUSSION

## 3.1 Gingiva baseline perfusion

A relatively high amplitude iPPG waveform was observed in all subjects during baseline conditions, implying adequate basal perfusion, which substantially exceed the so-called “biological zero” signal (Fig 1A). As depicted in iPPG PI maps, perfusion in the anterior gum region expressed symmetrical bilateral distribution of perfusion (see.Fig.1A).

The perfusion values moderately varied (3.7-1.4 a.u.) across the subjects, see Fig.2.B, possibly due to other influencing factors such as different levels of emotional stress and arterial blood pressure or differences during registering procedure, although similar variance of gingival perfusion has been previously observed in studies of Ahn et.al and Ketabi et al. during Laser Doppler measurements (Ahn and Pogrel, 1998; Ketabi and Hirsch, 1997). The comparison of the obtained gingiva baseline perfusion is rather difficult as several principal differences exist between Laser Doppler Imager obtained perfusion which is a product of red blood cell velocity and concentration and photoplethysmography derived perfusion index which is determined mainly by magnitude of the AC component.

Still debatable is interpretation of imaging photoplethysmography AC component (waveform) as there is no unified consensus regarding reflection mode photoplethysmogramme genesis and its provided information. It has been proposed that iPPG AC waveform obtained during green light illumination, reflects partly complex dynamics of red blood cell orientation and changes in RBC aggregation in small caliber vessels (Njoum and Kyriacou, 2017), partly on viscoelastic properties of the dermis (dermal deformation) as the dominant mechanism of PPG formation (Moço et al., 2018), and depends on pulsatile transmural pressure of the arteries, which mechanically compress capillaries in the dermis, thus modulating the blood volume in the capillary bed, which consequently modulates the power of remitted light (Kamshilin and Margaryants, 2017). For this reason photoplethysmography signal largely depends on tissue structure and may exhibit differences between gingiva and skin.

However observations indicate some similarities between cutaneous iPPG and iPPG signal acquired from gingiva despite of tissue differences. However, in contrast to cutaneous, gingival iPPG has lower AC amplitude, and larger distortions, probably because of non-uniform illumination of gum surface and motion artefacts during signal recording and adequate motion correction algorithms may potentially improve signal quality.

### 3.2 Perfusion changes during anesthesia

In the moment of anesthetic administration gingiva slightly moved, leading to production of a sharp and short artefact in the signal for one to four seconds, consequently this fragment was excluded from offline analyses. Rapid and statistically significant ($p<0.05$) decrease of perfusion (approx. 50% from baseline) was observed adjacent to injection site one, to three minutes post administration. Low perfusion region gradually spread in lateral direction reaching the midline and remain low for entire recording time, as seen in the map acquired at the end of recording during plato phase, Figure 13. The same trend is depicted in ROI averaged perfusion curve recorded from one subject, Figure 14A.

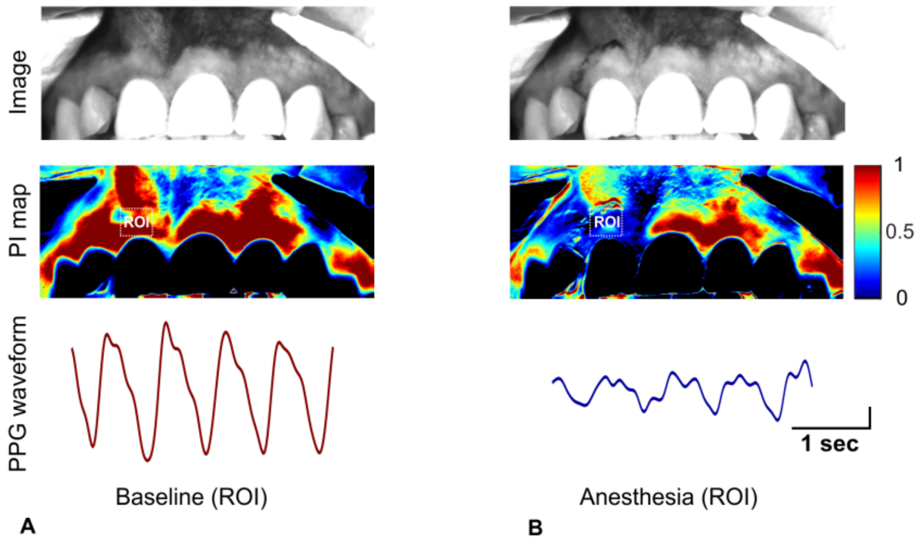


Figure 13 displays the image of gingival, perfusion map and waveforms, obtained from region of interest (RoI) during the baseline (A) and 5 minutes following administration of local anesthetics (B).

In the first minute following administration, group mean perfusion decreased by 36.66±16% , at second 51.88±11.56%, at third 56.80±12.39% and at fourth minute 59.57±11.06% from the baseline. While the largest perfusion was observed during plato phase 62.16±12.96%, five to six minutes after the injection.

Substantial alterations were noticed in iPPG waveform, the amplitude diminished and distortions increased during plato phase, where the gingiva perfusion in the anesthetized region was substantially reduced reaching close to "biological zero" level as Hb absorption decreased due to eliminated functional capillary density, produced by arteriolar vasoconstriction at anesthetic affected site(Figure 13B).

Similar perfusion alterations were observed by Laser Doppler Imaging after administration of adrenaline containing anesthetics in (Ahn and Pogrel, 1998; Ketabi and Hirsch, 1997). Nevertheless in this studies effect of lidocaine and lignocaine containing adrenaline on gingival blood flow has been evaluated but information regarding the effect of articaine with adrenaline is not studied yet. The comparison of present data is challenging as to the best of our knowledge, this is the first pilot study exploring use of remote photoplethysmography for assessment of gingiva perfusion in human subjects during local infiltration anesthesia and particularly using artecaine with adrenaline.

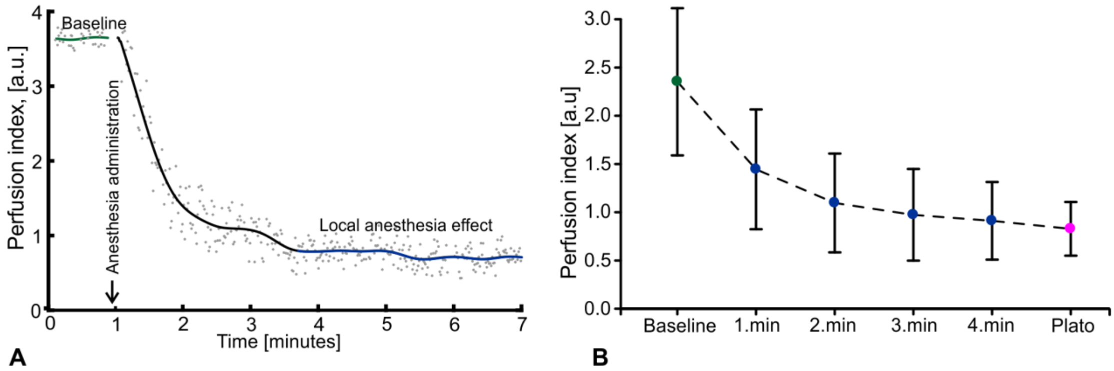


Figure 14 presents the characteristic trend of the gingiva perfusion index at the baseline and following anesthesia, (A) , data from one subject. Group (n=12) mean perfusion values during baseline, and following anesthesia (mean±std) (B).

Previous imaging PPG studies on cutaneous circulation demonstrated close similarity between iPPG perfusion and Laser Doppler obtained perfusion trend (Marcinkevics et al., 2016), advocating perfusion measurement similarities, which is rather speculation. The direct comparison of perfusion obtained by these two photonic techniques is problematic in the framework of present study as mucosal and cutaneous structure differs. We could conclude that in the light of present knowledge the observed vasoconstriction during infiltration anesthesia seems consistent as adrenaline produce substantial vasoconstriction at injection site which prevents further spread of anesthetics prolonging its effect (Moço et al., 2018).

Taken together present pilot results provide first insight in the use of remote photoplethysmography technique for oral mucosa assessment but further studies are required to assess reliability of this method using different provocation tests.

# CONCLUSIONS

- Imaging photoplethysmography can be utilized as a reliable contactless technique for temporal and special assessment of oral mucosa perfusion.
- Various different subjects exhibited moderately different levels of baseline oral mucosa perfusion. Epinephrine containing infiltration anesthesia substantially decreased oral mucosa perfusion in anesthetized site.

- Epinephrine containing infiltration anesthesia substantially decreased oral mucosa perfusion in anesthetized site .

# AKNOWLEDGEMENT

I would like to thank University of Latvia Biology Faculty associated Professor Zbignevs Marcinkevičs, for his support and contribution and giving me the opportunity to execute the research.

I would also like to thank Udis Rubins from Institute of Atomic Physics and Spectroscopy for his support in data collection and analysis.

Furthermore I owe my gratitude to Professor Ieva Henkuzena of the Dentistry Faculty of the University of Latvia for valuable advice and contributing in carrying out the experiment.

I am grateful and indebted by the University of Latvia Dentistry Faculty by allowing me to carry out the research at their facilities and using their dental equipment.

I would like to express my gratitude to my fellow 5th year dentistry students at the University of Latvia for their continuous help and cooperation during the whole study period.

I would like to grab this opportunity to express my wholeheartedly thanks and gratitude to my family for sharing my ups and downs and supporting me throughout the whole studies.

Lastly I want to thank all those persons who participated in the research by letting me collect the research data.

# BIBLIOGRAPHY

Ahn, J., and Pogrel, M.A. (1998). The effects of 2% lidocaine with 1:100,000 epinephrine on pulpal and gingival blood flow. Oral Surgery, Oral Med. Oral Pathol. Oral Radiol. Endodontology *85*, 197–202.

Allen, J. (2007). Photoplethysmography and its application in clinical physiological measurement. Physiol. Meas. *28*, R1-39.

Ambrosini, P., Cherene, S., Miller, N., Weissenbach, M., and Penaud, J. (2002). A laser Doppler study of gingival blood flow variations following periosteal stimulation. J. Clin. Periodontol. *29*, 103–107.

Baart, J.A., and Brand, H.S. (2017). Local Anaesthesia in Dentistry, Second Edition (Cham: Springer International Publishing, ISBN 978-3-319-43704-0), 52,56,65-66,148-171.

Babos, L., Járai, Z., and Nemcsik, J. (2013). Evaluation of microvascular reactivity with laser Doppler flowmetry in chronic kidney disease. World J. Nephrol. *2*, 77–83.

Bergmeier, L.A. (2018). Oral mucosa in health and disease: A concise handbook (Cham: Springer International Publishing, ISBN 978-3-319-56064-9), 1-9.

Briers, J.D., and Webster, S. (1996). Laser speckle contrast analysis (LASCA): a nonscanning, full-field technique for monitoring capillary blood flow. J. Biomed. Opt. *1*, 174–179.

Briers, J.D., Richards, G., and He, X.W. (1999). Capillary Blood Flow Monitoring Using Laser Speckle Contrast Analysis (LASCA). J. Biomed. Opt. *4*, 164–175.

Chen, E., and Abbott, P. V (2009). Dental pulp testing: a review. Int. J. Dent. *2009*, 365785.
Cho, K.-H., Yu, S.-K., Lee, M.-H., Lee, D.-S., and Kim, H.-J. (2013). Histological assessment of the palatal mucosa and greater palatine artery with reference to subepithelial connective tissue grafting. Anat. Cell Biol. *46*, 171–176.

Collins, L.M.C., and Dawes, C. (1987). The Surface Area of the Adult Human Mouth and Thickness of the Salivary Film Covering the Teeth and Oral Mucosa. J. Dent. Res. *66*, 1300–

1302.

Curtis, D.A., Gansky, S.A., and Plesh, O. (2012). Deep and Superficial Masseter Muscle Blood Flow in Women. J. Prosthodont. *21*, 472–477.

Eriksson, S., Jan, N., Gert, L., and Sturesson, C. (2014). Laser speckle contrast imaging for intraoperative assessment of liver microcirculation: a clinical pilot study. Med. Devices Evid. Res. *7*, 257–261.

Evans, D., Reid, J., Strang, R., and Stirrups, D. (1999). A comparison of laser Doppler flowmetry with other methods of assessing the vitality of traumatised anterior teeth. Endod. Dent. Traumatol. *15*, 284–290.

Gazelius, B., Olgart, L., Edwall, B., and Edwall, L. (1986). Non-invasive recording of blood flow in human dental pulp. Endod. Dent. Traumatol. *2*, 219–221.

Gazelius, B., Olgart, L., and Edwall, B. (1988). Restored vitality in luxated teeth assessed by laser Doppler flowmeter. Dent. Traumatol. *4*, 265–268.

Hecht, N., Woitzik, J., Dreier, J.P., and Vajkoczy, P. (2009). Intraoperative monitoring of cerebral blood flow by laser speckle contrast analysis. Neurosurg. Focus *27*, E11.

Hellem, S., Jacobsson, L.S., Nilsson, G.E., and Lewis, D.H. (1983). Measurement of microvascular blood flow in cancellous bone using laser Doppler flowmetry and 133Xe-clearance. Int. J. Oral Surg. *12*, 165–177.

Hirai, H. (2005). [Application of a laser Doppler perfusion imaging Periscan PIM II for measuring the blood flow of the oral mucosa]. Nihon Hotetsu Shika Gakkai Zasshi *49*, 26–35.

Hoff, D.A.L., Gregersen, H., and Hatlebakk, J.G. (2009). Mucosal blood flow measurements using laser Doppler perfusion monitoring. World J. Gastroenterol. *15*, 198–203.

Jääskeläinen, S.K. (2017). Is burning mouth syndrome a neuropathic pain condition? Pain *159*, 1.

Johansen, Ø. (2004). COMPARISON OF ARTICAINE AND LIDOCAINE USED AS DENTAL LOCAL ANESTHETICS. University of Oslo.

Jung, R.M., Rybak, M., Milner, P., and Lewkowicz, N. (2017). Local anesthetics and advances in their administration – an overview. J. Pre-Clinical Clin. Res. *11*, 94–101.
Kamshilin, A.A., and Margaryants, N.B. (2017). Origin of Photoplethysmographic Waveform at Green Light. Phys. Procedia *86*, 72–80.

Kamshilin, A.A., Nippolainen, E., Sidorov, I.S., Vasilev, P. V., Erofeev, N.P., Podolian, N.P., and Romashko, R. V. (2015). A new look at the essence of the imaging photoplethysmography. Sci. Rep. *5*, 10494.

Ketabi, M., and Hirsch, R.S. (1997). The effects of local anesthetic containing adrenaline on gingival blood flow in smokers and non-smokers. J. Clin. Periodontol. *24*, 888–892.

Kouadio, A.A., Jordana, F., Koffi, N.J., Le Bars, P., and Soueidan, A. (2018). The use of laser Doppler flowmetry to evaluate oral soft tissue blood flow in humans: A review. Arch. Oral Biol. *86*, 58–71.

Lindahl, F., Tesselaar, E., and Sjöberg, F. (2013). Assessing paediatric scald injuries using Laser Speckle Contrast Imaging. Burns *39*, 662–666.

Lindeboom, J.A.H., Mathura, K.R., and Ince, C. (2005). The Microcirculation during Wound Healing after Oral and Maxillofacial Surgical Procedures. In Yearbook of Intensive Care and Emergency Medicine 2005, (New York: Springer, ISBN 987-3-540-23476-0), pp. 519–527.

Malamed, S.F. (2013). Handbook of local anesthesia, 6th Edition (St. Louis: Elsevier, ISBN 978-0-323-07413-1), 292-334.

Marcinkevics, Z., Rubins, U., Zaharans, J., Miscuks, A., Urtane, E., and Ozolina-Moll, L. (2016). Imaging photoplethysmography for clinical assessment of cutaneous microcirculation at two different depths. J. Biomed. Opt. *21*, 35005.

Matsuki, M., Xu, Y.B., and Nagasawa, T. (2001). Gingival blood flow measurement with a non-contact laser flowmeter. J. Oral Rehabil. *28*, 630–633.

Milstein, D.M.J., Ince, C., Gisbertz, S.S., Boateng, K.B., Geerts, B.F., Hollmann, M.W., van Berge Henegouwen, M.I., and Veelo, D.P. (2016). Laser speckle contrast imaging identifies ischemic areas on gastric tube reconstructions following esophagectomy. Medicine (Baltimore). *95*, e3875.

Moço, A. V., Stuijk, S., and de Haan, G. (2018). New insights into the origin of remote PPG signals in visible light and infrared. Sci. Rep. *8*, 8501.

Moodley, D.S. (2017). Local anaesthetics in dentistry - Part 2: Choice of local anaesthetic agent . South African Dent. J. *72*, 128–130.

Nadort, A., Kalkman, K., van Leeuwen, T.G., and Faber, D.J. (2016). Quantitative blood flow velocity imaging using laser speckle flowmetry. Sci. Rep. *6*, 25258.

Njoum, H., and Kyriacou, P.A. (2017). Photoplethysmography for the Assessment of Haemorheology. Sci. Rep. *7*, 1406.

Overman, P.R. (2007). Articaine: A New Alternative in Dental Hygiene Pain Control. Am. Dent. Hyg. Assoc. *81*.

Peedikayil, F., and Vijayan, A. (2013). An update on local anesthesia for pediatric dental patients. Anesth. Essays Res. *7*, 4.

Pogrel, M.A. (2012). Permanent nerve damage from inferior alveolar nerve blocks: a current update. J. Calif. Dent. Assoc. *40*, 795–797.

Reed, K.L., Malamed, S.F., and Fonner, A.M. (2012). Local anesthesia part 2: technical considerations. Anesth. Prog. *59*, 127–136; quiz 137.

Retzepi, M., Tonetti, M., and Donos, N. (2007). Gingival blood flow changes following periodontal access flap surgery using laser Doppler flowmetry. J. Clin. Periodontol. *34*, 437–443.

Riva, C.E., Geiser, M., Petrig, B.L., and Beijing 100193, PR China Ocular Blood Flow

Research Association (2010). Ocular blood flow assessment using continuous laser Doppler flowmetry. Acta Ophthalmol. *88*, 622–629.

Roed-Petersen, B., and Renstrup, G. (1969). A topographical classification of the oral mucosa suitable for electronic data processing. Its application to 560 leukoplakias. Acta Odontol. Scand. *27*, 681–695.

Rubins, U., Miscuks, A., and Lange, M. (2016). Simple and convenient remote photoplethysmography system for monitoring regional anesthesia effectiveness. IFMBE Proc. *65*, 378–381.

Scardina, G., Citarrella, R., and Messina, P. (2017). Diabetic Microagiopathy of Oral Mucosa Depends on Disease Duration and Therapy. Med. Sci. Monit. *23*, 5613–5619.

Singh, D.B., Stansby, G., and Harrison, D.K. (2008). Assessment of Oxygenation and Perfusion in the Tongue and Oral Mucosa by Visible Spectrophotometry and Laser Doppler Flowmetry in Healthy Subjects. In Oxygen Transport to Tissue XXIX, (Boston: Springer, ISBN 978-0-387-74910-5 ), pp. 227–233.

Squier, C., and Brogden, K.A. (2011). Human Oral Mucosa: Development, Structure and Function (West Sussex, UK: John Wiley & Sons, Ltd., ISBN 978-0813-81486-5), 9-16, 20-23, 42-46, 59-70, 80-83, 88-90, 93-94.

Squier, C.A., and Kremer, M.J. (2001). Biology of oral mucosa and esophagus. J. Natl. Cancer Inst. Monogr. 7–15.

Sun, S., Hayes-Gill, B.R., He, D., Zhu, Y., Huynh, N.T., and Morgan, S.P. (2016). Comparison of laser Doppler and laser speckle contrast imaging using a concurrent processing system. Opt. Lasers Eng. *83*, 1–9.

Sun, Y., Papin, C., Azorin-Peris, V., Kalawsky, R., Greenwald, S., and Hu, S. (2012). Use of ambient light in remote photoplethysmographic systems: comparison between a high-performance camera and a low-cost webcam. J. Biomed. Opt. *17*, 1–10.

Svalestad, J., Hellem, S., Vaagbø, G., Irgens, Å., and Thorsen, E. (2010). Reproducibility of transcutaneous oximetry and laser Doppler flowmetry in facial skin and gingival tissue. Microvasc. Res. *79*, 29–33.

Tamura, T., Maeda, Y., Sekine, M., Yoshida, M., Tamura, T., Maeda, Y., Sekine, M., and Yoshida, M. (2014). Wearable Photoplethysmographic Sensors—Past and Present. Electronics *3*, 282–302.

Top, A.P.C., Ince, C., de Meij, N., van Dijk, M., and Tibboel, D. (2011). Persistent low microcirculatory vessel density in nonsurvivors of sepsis in pediatric intensive care*. Crit. Care Med. *39*, 8–13.

Unakafov, A.M., Möller, S., Kagan, I., Gail, A., Treue, S., and Wolf, F. (2018). Using imaging photoplethysmography for heart rate estimation in non-human primates. PLoS One *13*.

Verdonck, H.W.D., Meijer, G.J., Kessler, P., Nieman, F.H., de Baat, C., and Stoelinga, P.J.W. (2009). Assessment of bone vascularity in the anterior mandible using laser Doppler flowmetry. Clin. Oral Implants Res. *20*, 140–144.

Verkruysse, W., Svaasand, L.O., and Nelson, J.S. (2008). Remote plethysmographic imaging using ambient light. Opt. Express *16*, 21434.

Vongsavan, N., and Matthews, B. (1993). Some aspects of the use of laser Doppler flow meters for recording tissue blood flow. Exp. Physiol. *78*, 1–14.

Webb, L. (2016). Dental anesthesia: Overview of injectable agents useful for nonsurgical periodontal therapy, RDH, https://www.rdhmag.com/articles/print/volume-36/issue-1/contents/understanding-available-agents.html .

Whiteman, A., Bajaj, S., and Hasan, M. (2011). Novel techniques of local anaesthetic infiltration. Contin. Educ. Anaesth. Crit. Care Pain *11*, 167–171.

Yuan, L., Li, Y., Li, H., Lu, H., and Tong, S. (2015). Intraoperative laser speckle contrast imaging improves the stability of rodent middle cerebral artery occlusion model. J. Biomed. Opt. *20*.

Zheng, J., and Hu, S. (2007). The preliminary investigation of imaging photoplethysmographic system. J. Phys. Conf. Ser. *85*.

# APPENDIX

1.pielikums LU KRMI Zinātniskās izpētes
Ētikas komisijas nolikumam.
Apstiprināts: LU KRMI ZP sēdē
31.08.2016.

# LATVIJAS UNIVERSITĀTES KARDIOLOĢIJAS UN REĢENERATĪVĀS MEDICĪNAS INSTITŪTA ZINĀTNISKĀS IZPĒTES ĒTIKAS KOMISIJA

## PIETEIKUMS

## I. PROJEKTA VADĪTĀJS

Vārds, uzvārds Zbignevs Marcinkevičs Zinātniskais grāds Dr.biol. Amats vad. pētnieks
Zinātniskās iestādes nosaukums LU BF
Nodaļa Cilvēka un dzīvnieku fizioloģijas katedras asinsrites laboratorija Adrese Rīga, Jelvagas iela 1
Tālr. _______ 67033920 Fakss 67033920 E-pasts zbigis@latnet.lv

## II. PROJEKTA NOSAUKUMS :" Mutes gļotādas mikrocirkulācijas novērtējums, izmantojot attēlveides fotopletizmogrāfiju"

## III. PROJEKTA IZPILDĪTĀJI

Vārds, uzvārds Robert Andrianirina Muckle Zinātniskais grāds Zobārstniecības profesionālās studijas, students
Zinātniskās iestādes nosaukums LU Bioloģijas fakultātes, Cilvēka un dzīvnieku fizioloģijas katedras Asinsrites laboratorija, LU Medicīnas fakultātes, Zobārstniecības klīnika.
Adrese: Jelgavas iela 1, Aspazijas bulvāris 6. Tālr. 67033920 Fakss 67033920

## IV. PROJEKTA KOPSAVILKUMS

A. PĒTĪJUMA OBJEKTI
Iezīmēt: **Cilvēki**

B. PĒTĪJUMA PROTOKOLA VĒSTURE UN PAMATOJUMS (iesk. izskaidrojumu **vienkāršā, saprotamā valodā**, uzrādot zinātnisko jautājumu, kuru šis pētījuma protokols risinās).

Pētījuma mērķis ir noskaidrot bezkontakta fotopletizmogrāfijas iespējas novērtēt infiltrācijas anestēzijas izraisītās smaganu mikrocirkulācijas izmaiņas.
Zobārstniecībā vietējā anestēzija ir kļuvusi par neatņemamu rutīnu procedūru, kas parasti ir nekaitīga un nerada paliekošas sekas, tomēr ir gadījumi, kad tā var izraisīt dažādas nevēlamas pacienta organisma reakcijas. Biežākā no komplikācijām ir nerva ilgstoša parēze, kas var ilgt pat vairākas dienas. Literatūrā esošā informācija liecina, ka novēroto blakņu iemesls ir lielas anestētiķa devas. Klīniskajās vadlīnijās veiksmīgas atsāpināšanas nodrošināšanai ieteiktās devas varētu

mazināt, taču trūkst objektīvu metožu anestēzijas efekta novērtēšanai, tādēļ praksē tiek lietotas ieteicamās optimālās devas, kas balstās uz empīriskiem novērojumiem un daudziem indivīdiem var būt pārāk lielas. Ikdienā anestēzijas kontrolē tiek izmantotas subjektīvas metodes- atsāpināto gļotādas rajonu vai zobu mehāniski stimulē, jautājot pacientam par sajūtām. Nereti satraukumā pacients nespēj objektīvi izvērtēt situāciju un viņam liekas ka atsāpināšana nav iestājusies, tādēļ tiek palielināta anestētiķa deva, kas var izraisīt nevēlamas blakusparādības. Īpaši grūti ir novērtēt atsāpināšanu vecāka gada gājuma pacientiem vai bērniem.
Līdz ar to anestēzijas pakāpes optimālai kontrolei zobārstniecībā trūkst objektīvu anestēzijas novērtēšanas metožu.
Ievadot anestētiķi, tas parasti izraisa lokālu vazokonstrikciju, un šis efekts atspoguļojas gļotādas mikrocirkulācijā anestēzijas skartajā apgabalā. Tādejādi gļotādas mikrocirkulācijas perfūzija varētu tikt izmantota par savdabīgu objektīvu anestēzijas indikatoru.
Tradicionāli gļotādas perfūzijas novērtēšanai izmanto lāzera Doplera flovmetriju, taču šai metodei ir vairāki būtiski ierobežojumi, un tā nav piemērota praktiskajā zobārstniecībā.
Attēlveides fotopletizmogrāfija ir samērā jauna un perspektīva metode, kas veiksmīgi tika izmantota ādas mikrocirkulācijas novērtēšanā, taču literatūrā nav minēts neviens pētījums par šīs metodes pielietojumu mutes gļotādas perfūzijas noteikšanā.
Šis pilot-pētījums ir nozīmīgs gan no praktiskā viedokļa klīniskajā zobārstniecībā, gan arī no mutes gļotādas mikrocirkulācijas mehānismu izpētes viedokļa.

**Pētījuma vispārējie uzdevumi:**

1. Noskaidrot bezkontakta fotopletizmogrāfijas metodes iespējas, reģistrējot mutes gļotādas perfūziju.
2. Izvērtēt lokālās infiltrācijas anestēzijas efektu uz smaganu mikrocirkulāciju.

C. PĒTĪJUMA PROTOKOLA ĪSS APRAKSTS (iesk. informāciju **vienkāršā, saprotamā valodā** par metodiku un tehnoloģiju, piem., paredzamais pētījumu objektu skaits un to vecums, asins daudzums, ievadītās zāles un medikamenti, aptaujas lapas, testi utt.).

Pētījumā tiks izmantota eksperimentālā fotopletizmogrāfijas sistēma, kas sastāv no monohromas augstas izšķirtspējas kameras un gaismu emitējošu diožu starotāja (LED), kas izstaro nelielas intensitātes gaismu ($< 10\ W \cdot m^{-2}$) redzamajā un tuvējā infrasarkanajā diapazonā. Izstrādātās ierīces darbība balstās uz ādas difūzās atstarošanas secīgu attēlu uzņemšanu, kam vēlāk seko attēlu automatizēta apstrāde. No attēla viena punkta intensitātes attiecīgajā viļņu garumā iegūst signāla amplitūdu, kas ir proporcionāla perfūzijai.

Pētījumā paredzēts iesaistīt brīvprātīgās personas: LU Medicīnas Fakultātes Zobārstniecības klīnikas pacientus, kuriem tiek nozīmētas standarta zobārstniecības procedūras, pirms kurām atbilstoši vadlīnijām veic standarta atsāpināšanu. Kopā pētījumam var būt nepieciešamas līdz pat 20 izmeklējamās personas. Pirms katra pētījuma protokola veikšanas nepieciešama cilvēka informēšana par pētījuma mērķiem, t.sk. zināmajiem riskiem un komplikācijām, un rakstiski apstiprināta piekrišanas saņemšana (informētās izmeklējamās personas piekrišanas forma).

Šajā pētījumā netiek iegūta un uzkrāta izmeklējamo personu identificējošā informācija (personas dati), jo katras pētījuma sesijas sākumā pēc nejaušības principa tiek automātiski ģenerēts pētījuma sesijas kods, kas nav saistīts ar konkrētās personas identitāti. Turpmāk sistēmā glabātie mērījumu dati ir anonīmi, jo ir piesaistīti sesijas kodam. Visas turpmāk minētās manipulācijas veic atbilstoši likumos un normatīvajos aktos noteiktajām prasībām.

Ierodoties klīnikā, pacientiem tiek piedāvāta dalība pētījumā un īsi izskaidrota pētījuma būtība, un piekrišanas gadījumā saņemts rakstisks apliecinājums.
Visas pētījumā paredzētās procedūras tiks veiktas atsevišķā, labi vēdināmā telpā. Reģistrācijas procedūras ilgums nepārsniedz 15 minūtes. Ja pieraksta laikā izmeklējamā persona pēkšņi nevēlas piedalīties, tā jebkurā brīdī var atteikties no reģistrācijas procedūras.

**Bezkontakta fotopletizmogrāfiskā signāla pieraksts**

Pirms plānotās atsāpināšanas mutes apvidū izmeklējamā persona atrodas ērtā pozā zobārstniecības krēslā ar atvērtu muti un īpašām aptumšojošām aizsargbrillēm. Mutē tiek ievietots vienreizējais gumijas gredzens smaganu paplešanai, lai bezkontakta fotopletizmogrāfijas sistēmas kamerai atsegtu anestezējamo smaganu zonu. Signāla reģistrācijai ir paredzēts izmantot augstāk minēto bezkontakta fotopletizmogrāfijas sistēmu, novietojot to 25-40cm no gļotādas virsmas. Reģistrāciju sāk ar smaganu bazālās perfūzijas pierakstu (3-5 min), kam seko anestētiķa injekcija un signāla pieraksts turpinās vēl 10-15 minūtes. Pēc anestēzijas iestāšanās fotopletizmogrāfijas sistēma (kamera un gaismas avots) tiek izslēgta un zobārsts veic visas plānotās procedūras (zoba labošana, ekstrakcija, vai citas).

D. PĒTĪJUMA IZPILDES TERMIŅI

Sākums: 2018.gada novembris

Beigas: 2021. gada oktobris

E. PĒTĪJUMA NORISES VIETA (-AS) LU Medicīnas fakultātes, Zobārstniecības klīnika. Adrese: Jelgavas iela 1, Aspazijas bulvāris 6, LU Bioloģijas fakultātes, Cilvēka un dzīvnieku fizioloģijas katedras Asinsrites laboratorija.

F. DAĻĒJA ATKLĀTĪBA: Ja pilna informācija pētījuma gaitā cilvēkiem kā pētniecības objektiem netiek sniegta, izskaidrot šāda protokola nepieciešamību, kā un kad objekti tiks informēti.

Izmeklējamās personas tiks informētas par pētniecības projekta mērķiem un konkrēto pētījumu.

## V. RISKI PRET IEGUVUMIEM

1. Izskaidrot būtību un riska pakāpi iespējamiem ievainojumiem, sāpēm, stresa, diskomforta, cilvēka neaizskaramības pārkāpumiem un citām blakus parādībām, kas izraisītas cilvēkiem vai dzīvniekiem protokola izpildes gaitā.

- Mērījumos izmantotā aparatūra cilvēka veselībai ir droša. Pētījumā tiek izmantotas gaismas diožu apgaismojums ar viļņa garumiem no 450-800 nm un intensitāti, kas nav kaitīga cilvēka ādai un acīm (< 10 $W \cdot m^{-2}$). Redzamās gaismas diodes ir drošas, jo bieži tiek lietotas arī ikdienas apgaismojumiem. Izvēlētās diožu starojuma jaudas ir izvēlētas atbilstoši lāzeru drošības standartiem (*Safety of Laser Products – Part 1: Equipment Classification and Requirements* (IEC 60825-1:2007)) un nepārsniedz ikdienas LED apgaismojuma jaudu.

Turklāt, aizsargbrilles nepieļauj šī LED apgaismojuma nonākšanu ne pētnieka (ārsts), ne izmeklējamā (pacients) acīs.

- Pētījumā tiek iesaistīti Zobārstniecības Klīnikas pacienti, kas tur pārstājas un ir aizpildījuši visas tam nepieciešamās veidlapas, un līdz ar to ārstējošais zobārsts ir informēts par pacienta stāvokli.
- Šajā pētījumā piedalās pacienti, kam tiek veikta zobu plānveida ārstēšana, tādēļ paredzētā atsāpināšana ir neatņemama ārstēšanas sastāvdaļa.

2. Izskaidrot veiktos pretpasākumus, lai mazinātu traumas risku un aizsargātu pētniecības objektu tiesības un labklājību.

Procedūra tiks veikta atbilstoši likumos un normatīvajos aktos noteiktajām prasībām, un to zinātniskajā iestādē veiks kvalificēts personāls. Pirms procedūras veikšanas potenciālā izmeklējamā persona tiek aptaujāta par veselības stāvokli saskaņā ar zobārstniecības vadlīnijām.

3. Izskaidrot šo pētījuma potenciālos ieguvumus (i) pētījuma objektiem, (ii) sabiedrībai un cilvēcei.

(i) Izmeklējamās personas varēs iegūt informāciju par saviem gļotādas mikrocirkulāciju raksturojošajiem parametriem.

(ii) Šis pētījums sniegs būtisku informāciju, kas nepieciešama, lai nākotnē izstrādātu objektīvas lokālās anestēzijas efekta novērtēšanas metodes zobārstniecībā, potenciāli samazinot atsāpināšanas nevēlamās parādības, palielinot pacientu aprūpes kvalitāti. Šajā pētījumā iegūtie dati paplašinās esošo priekšstatu par smaganu mikrocirkulācijas vadības mehānismiem.

**VI. APLIECINĀJUMS** Es, ______ (projekta vadītājs),

esmu pilnībā iepazinies (-usies) ar informāciju, kas attiecināma uz pētījumu. Es ievērošu pētījuma protokolu, Pasaules medicīnas asociācijas Helsinku deklarāciju par ētikas principiem medicīnas pētniecībai ar cilvēkiem, Eiropas padomes Ovjedo konvenciju par cilvēktiesību un cieņas aizsardzību bioloģijā un medicīnā, un uz klīniskiem pētījumiem attiecināmos Latvijas Republikā spēkā esošus likumdošanas nosacījumus. Man ir pienākums ziņot par protokola izmaiņām un klīniskā pētījuma rezultātiem kompetentām pētniecības iestādēm un komisijām.

Datums __09.10.2018.__ Paraksts ____________

Šo vietu aizpilda LU KRMI Zinātniskās izpētes Ētikas komisija

**VII. PARAKSTI** APSTIPRINĀTS _x_ NEAPSTIPRINĀTS ___

Datums ~~22.10.2018.~~ 26.02.2019. Paraksts, atšifrējums ____________, L. Plakane
LU KRMI Zinātniskās izpētes Ētikas komisija

Datums ~~22.10.2017.~~ 26.02.2019. Paraksts, atšifrējums ____________, T. Arenhalofs
LU KRMI Zinātniskās izpētes Ētikas komisija

**DOCUMENTATION PAGE**

This Diploma Thesis **„The assessment of microcirculation in oral mucosa by means of imaging photoplethysmography”**
was developed at the Faculty of Medicine of the University of Latvia.

With my signature, I attest, that this research has been carried out without aid or assistance. Used information was obtained only from indicated sources and the electronically submitted copy of this diploma work complies with printout.

Author Muckle, Robert Andrianirina________________________________________
*(name, surname)* *(signature)*

I recommend the work for presentation.

Supervisor: LU BF assoc. Prof. Marcinkevičs, Zbignevs, Dr. biol.______________________
*(position, name, surname, degree)* *(siganture)* *(date)*

Reviewer: ____________________________________________________________________
*(position, name, surname, degree)* *(signature)* *(date)*

The diploma thesis was submitted to the Faculty of Medicine on: _________________
*(date)*

International students' coordinator, __________________________________________
*(signature)*

The diploma thesis is presented at the meeting of the State Examination Commission of Second Level Higher Professional Study Program „Dentistry”_____________2019. Protocol No. _________________

Secretary of Commission: _______________________________ _________________________
*(position, name, surname, degree)* *(signature)*