Which are the main fluorophores in skin and oral mucosa? A review with emphasis on clinical applications of tissue autofluorescence

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ABSTRACT

Objectives: The present review provides information about which molecules appear to be the main fluorophores in skin and oral mucosa, together with their clinical applications.

Design: The MEDLINE database was searched, using “oral mucosa AND fluorophores”, “skin AND fluorophores”, “epidermal AND fluorophores”, “dermal AND fluorophores” and “cutaneous AND fluorophores” as entry terms. We searched the literature following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The level of evidence in the studies was assessed using the Classification of the Oxford Centre for Evidence-based Medicine (CEBM) Levels for Diagnosis.

Results: Five papers and 17 were primarily focused on description of fluorophores in oral mucosa and skin. Evidence exists that fluorophores of oral mucosa and skin are mainly proteins such as collagen, elastin, keratin and tryptophan. Other possible fluorophores identified are: porphyrins, advanced glycation end products, flavins, lipopigment, nicotinamide adenine dinucleotide, flavin adenine dinucleotide, pheomelanin, eumelanin and components of lipofuscin.

Clinical applications of oral mucosal autofluorescence (AF) are related to management of malignant and potentially malignant lesions. In the skin, AF has been used for acne assessment, diagnosis of sweat-gland pathologies, glycemic control and management of malignant lesions and as a marker for skin aging.

Conclusion: Fluorophores stimulated through AF devices are implied in different physiologic and pathologic processes. AF seems to be useful for several clinical applications, especially in skin department. Because most of the studies show a low level of evidence, further studies are necessary in such a promising and fascinating field.

1. Introduction

Fluorophores are molecules that can absorb and re-emit specific wavelengths of light and are therefore responsible for the phenomenon of fluorescence. Fluorophores can be classified into endogenous, when produced within the body, and exogenous, if they originate from outside the organism. Endogenous fluorophores are either intracellular or extracellular, both the cause of biological tissue autofluorescence (AF). AF is defined as a peculiar visual property of some tissues directly associated with the concentration and distribution of specific fluorophores. A diagram illustrating the excitation of molecules and the following phenomenon of fluorescence (Jablonski diagram) is shown in Fig. 1. Cells and extracellular compartments within dysplastic and malignant lesions may display modifications of the amount, distribution and physicochemical properties of some endogenous fluorophores (Monici, 2005). Malignant and potentially malignant changes may therefore result in AF variations, potentially useful for diagnostic purposes (Fig. 2).

AF evaluation might improve the sensitivity and specificity of cancer diagnosis, having been tested on several organs, such as colon, lung, cervix and oesophagus (Schantz, Savage, Sacks, & Alfano, 1997). Evidence has recently emerged that supports the usefulness of AF for head and neck cancer diagnosis, at both the mucosal and cutaneous levels (Monici, 2005; Schantz et al., 1997).

However, even though some fluorophores have been identified and...
described in detail, there is currently a gap of knowledge about other specific molecules responsible for tissue AF as well as about the mechanisms through which they cease to emit fluorescence in cases of malignant or pre-malignant lesions (Schantz et al., 1997).

The present review provides information about which molecules show evidence of being the main fluorophores in skin and oral mucosa, together with their excitation and emission wavelengths and the devices used for inducing fluorescence, as well as a discussion of clinical applications of AF.

1.1. Methods for the literature search

The MEDLINE database was searched, using “oral mucosa AND fluorophores”, “skin AND fluorophores”, “epidermal AND fluorophores”, “dermal AND fluorophores” and “cutaneous AND fluorophores” as entry terms.

To avoid accidental loss of relevant studies, we searched the literature following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement published in 2009 (Moher, Liberati, & Tetzlaff, 2009).

Reference lists of reviews were screened to identify papers potentially not identified in the database search.

1.1.1. Study selection and data extraction

1.1.1.1. Inclusion criteria

- Papers focused on identification and characterisation of endogenous fluorophores, reporting studies performed in vivo (humans, animals), ex vivo and in vitro models
- No time limits

1.1.1.2. Exclusion criteria

- Papers with unavailable abstract
- Papers not in English language
- Congress proceedings
- Review papers
- Papers not clearly detailing the fluorophores investigated
- Papers not indicating excitation wavelength used for inducing fluorescence
- Studies evaluating fluorescence induced by exogenous fluorophores

A PRISMA flow diagram was used to summarise the study selection process. The flowchart followed for selection of studies of oral mucosa is shown in Fig. 3. The flowchart for selection of studies on fluorophores in skin is shown in Fig. 4.

The level of evidence in the studies was assessed using the Classification of the Oxford Centre for Evidence-based Medicine (CEBM) Levels for Diagnosis, updated in March 2009.
Specific data extracted from each study is shown in Table 1. Molecules identified as fluorophores of the oral mucosa are: collagen, the reduced form of nicotinamide adenine dinucleotide (NADH), flavin, adenine dinucleotide (FAD), porphyrins, elastin and keratin. Details of studies and molecules are shown in Table 1.

**2. Fluorophores of oral mucosa**

Five papers evaluating fluorophores in oral mucosa were identified (Gurushankar, Nazeer, Jayasree, & Krishnakumar, 2015; Jayanthi et al., 2009; Lane et al., 2006; Nazeer, Asish, Venugopal, Anita, & Gupta, 2014; Vedeswari, Jayachandran, & Ganesan, 2009).

Molecules identified as fluorophores of the oral mucosa are: collagen, the reduced form of nicotinamide, adenine dinucleotide (NADH), flavin, adenine dinucleotide (FAD), porphyrins, elastin and keratin. Specific data extracted from each study is shown in Table 1. Collagen, NADH and FAD were indicated as fluorophores in four studies, porphyrins in two studies and elastin and keratin in one study.

Excitation wavelengths used for the identification of the presumptive fluorophores ranged from 320 to 460 nm (including ultraviolet [UV] radiations). Instead, the emission spectra ranged from 350 to 750 nm.

**2.1. Reported clinical applications**

Data on clinical applications was available in all five of the studies selected for the present review.

In two studies AF was investigated as a potential aid for improving cancer diagnostic accuracy (excitation wavelength used: 400–460 nm) (Jayanthi et al., 2009; Lane et al., 2006). In one study, AF spectroscopy was used for monitoring the therapeutic response and for characterising tissue reaction after treatment of oral submucous fibrosis (excitation wavelength used: 330 nm) (Vedeswari et al., 2009). Another study demonstrated a variation in AF spectra between the oral mucosa of habitual tobacco users and the oral mucosa of a group of volunteers without any habits (excitation wavelength used: 410 nm) (Nazeer et al., 2014). In the only animal study identified, fluorescence spectroscopy was proposed for monitoring or potentially predicting response to cancer therapy (excitation wavelength used: 320 nm) (Gurushankar et al., 2015).

**2.2. Level of evidence**

Four out of the five studies on oral mucosa and fluorophores were in vivo studies, performed on humans. One study was a randomised trial in an animal model (Gurushankar et al., 2014). The Oxford CEBM and National Institutes of Health (NIH) Quality Assessment classifications were therefore applicable for four studies (Jayanthi et al., 2009; Lane et al., 2006; Nazeer et al., 2014; Vedeswari et al., 2009).

Level of evidence was 2b for all studies evaluated.

**3. Fluorophores of skin**

Fifteen papers were identified by using “Skin AND Fluorophores” as entry terms, five papers by using “Epidermal AND Fluorophores” and one paper by using “Dermal AND Fluorophores”. Four papers were repetitions. The database search using “Cutaneous AND Fluorophores” as entry terms did not identify papers dealing with the topic of the present review. Seventeen papers were eventually included (Fig. 4) (Breunig, Studier, & König, 2010; Dimitrov, Riemann et al., 2009, 2009b; Eny et al., 2015; Gillies, Zonios, Anderson, & Kollias, 2000; Krasieva et al., 2013; Laiho, Pelet, Hancewicz, Kaplan, & So, 2005; Leupold et al., 2011; Miyamoto & Kudoh, 2013; McMullen, Chen, & Moore, 2012; Na, Stender, Henriksen, & Wulf, 2011; Patalay et al., 2011; Richter, Trojahn, Dobos, Blume-Peytavi, & Kottner, 2016; Stirban, Nandrean, Negrean, Koschinsky, & Tschoepe, 2008; Sandby-Møller, Thieden, Philipson, Heydenreich, & Wulf, 2004; Yu et al., 2012; Zhao et al., 2016).

Molecules identified as possible fluorophores in the skin were porphyrins, advanced glycation end products (AGEs), flavins, lipopigment, the reduced form of nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FAD), phomelanin, eumelanin, collagen, elastin, tryptophan, components of lipofuscin and keratin. Details of studies and molecules are shown in Table 2.

Excitation wavelengths used for inducing AF ranged from 260 to 1000 nm (including UV). Wavelengths used with two-photon tomography and femtosecond laser ranged from 720 to 1000 nm. Wavelengths used with other devices ranged from 260 to 488 nm.

**3.1. Reported clinical applications**

Clinical applications of skin AF displayed a great heterogeneity.

In four of the selected studies, skin AF was proposed as a possible new diagnostic tool for malignant melanoma. In all these papers, a two-photon tomography instrument was the device used for investigation (Dimitrov, Riemann et al., 2009, 2009b; Krasieva et al., 2013; Miyamoto & Kudoh, 2013).

In two out of 17 papers, skin AF was investigated as a marker for photo-aging, using UV wavelengths (330 and 370 nm) (Na et al., 2011; Sandby-Møller et al., 2004).

Other applications reported for skin AF were measurement of acne severity (excitation wavelength: 400 nm) (Richter et al., 2016); diagnosis and therapy of eccrine sweat gland disease (excitation wavelength: 488 nm) (Zhao et al., 2016); screening for patients with undetected diabetes or cardiovascular risk (excitation wavelength used: 300–420 nm) and assessment of the effectiveness of anti-proliferative agents (excitation wavelength used: 260–480 nm) (Gillies et al., 2000; Stirban et al., 2008).

**3.2. Level of evidence**

Seven out of 17 studies on skin/epidermal fluorophores were in vivo studies performed on humans; four out of 17 studies selected were ex vivo and in vivo studies, and six out of 17 studies were ex vivo or in vitro studies (Breunig et al., 2010; Dimitrov, Riemann et al., 2009, 2009b; Eny et al., 2015; Gillies et al., 2000; Krasieva et al., 2013; Leupold et al., 2011; McMullen et al., 2012; Na et al., 2011; Patalay et al., 2011; Richter et al., 2016; Stirban et al., 2008; Laiho et al., 2005; Sandby-Møller et al., 2004; Yu et al., 2012; Zhao et al., 2016).

The Oxford CEBM guidelines were applicable in 11 studies (Breunig et al., 2010; Dimitrov, Riemann et al., 2009, 2009b; Eny et al., 2015;...
The level of evidence was 1b for one study, 2b for six studies and 3b for four studies.

4. Commercial devices used to induce and visualise fluorescence

Commercial devices reported in the literature are usually based on simple setups, (e.g., peculiar excitation and emission wavelength bands, obtained by using excitation light sources [light editing diode LED]). Such devices have been optimised for specific applications, (e.g., Visiopor® (Courage + Khazaka Electronic GmbH, Germany), including the quantitative measurement of the AF emitted by bacterial porphyrins in patients with acne (Richter et al., 2016).

A qualitative analysis of tissue AF is possible through devices similar to those described. The VELscope™ system (LED Medical Diagnostics Inc., Barnaby, Canada) has been widely employed for diagnostic purposes, particularly for oral mucosa.

Other devices are based on multi-photon tomography (e.g., DermaInspec® [JenLab GmbH, Germany]). The technique, used for in vivo non-invasive tomography of human skin (Breunig et al., 2010; Dimitrow, Riemann et al., 2009, 2009b; Miyamoto & Kudoh, 2013; Patalay et al., 2011), is based on the near-infrared femto laser beam scanning technology. Devices employing multi-photon tomography allow optical biopsies with subcellular spatial resolution. The technology generates an image based on the AF intensity and time decay of fluorophores, including NADH, flavins, porphyrins and elastin at different tissue depths.

For more specific ex vivo or in vitro AF measurements, particular microscopes (e.g., Olympus IX71®) are used. Depending on their setup, such microscopes allow high-resolution imaging of reflected or
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<th>Application fields or conclusions</th>
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<tr>
<td>K. Gurushankar et al</td>
<td>2015</td>
<td>Animal scientific study</td>
<td>- Collagen</td>
<td>320 nm</td>
<td>ranging from 350 to 550 nm</td>
<td>Datamax software (Datamax, Round Rock, Texas) and the inbuilt double-grating monochromator.</td>
<td>Fluorescence spectroscopy for monitoring or potentially predicting response to cancer therapy.</td>
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<tr>
<td>SS. Nazeer et al</td>
<td>2014</td>
<td>Clinical cohort study</td>
<td>- FAD</td>
<td>410 nm</td>
<td>ranging from 450 to 750 nm</td>
<td>Instruments consists of a 450-W Xenon arc lamp, a double excitation monochromator, and a photosmultiplier tube.</td>
<td>The study demonstrated a variation in autofluorescence spectra from the oral mucosa of habitual tobacco users with that of a group of volunteers without any habits in a clinical setup.</td>
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<tr>
<td>CP. Vedenswari et al</td>
<td>2009</td>
<td>Clinical cohort study</td>
<td>- Collagen</td>
<td>330 nm</td>
<td>ranging from 350 to 600 nm</td>
<td>A monochromator with a 150 W ozone-free Xenon lamp</td>
<td>Autofluorescence spectroscopy for monitoring the therapeutic response and characterize the tissue transformation after treatment of oral submucous fibrosis.</td>
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<tr>
<td>JL. Jayanthi et al</td>
<td>2009</td>
<td>Clinical cohort study</td>
<td>- Collagen</td>
<td>404 nm</td>
<td>- peak in healthy patient: 500 nm - peaks in malignant lesions: 635, 685 and 705 nm</td>
<td>A 404 nm, 50 mW diode laser (Stocker Yale, Montreal, Canada)</td>
<td>Autofluorescence can improve diagnostic accuracy.</td>
</tr>
<tr>
<td>PM. Lane et al</td>
<td>2006</td>
<td>Clinical pilot study</td>
<td>- Collagen</td>
<td>400-460 nm</td>
<td>&gt; 475 nm</td>
<td>The light source X-Cite 120, EXFO used a 120-W metal-halide arc lamp with integral elliptical reflector optimized for near-UV/blue reflection.</td>
<td>Direct fluorescence visualization device has potential as a simple, cost-effective screening device for the early detection of oral premalignant lesions.</td>
</tr>
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<td>Eny KM et al</td>
<td>2015</td>
<td>Clinical study</td>
<td>Advanced glycation end products (AGEs)</td>
<td>SIF 1: 375 nm; SIF 1: 415-455 nm</td>
<td>–</td>
<td>SCOUT i8 SF spectrometer (Veralight, Inc., Albuquerque, NM)</td>
<td>Skin intrinsic fluorescence is associated with caffeine consumption</td>
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<tr>
<td>Zhao HL et al</td>
<td>2015</td>
<td>Ex vivo study</td>
<td>Flavin; Lipopigment</td>
<td>near-infrared (760 nm)</td>
<td>–</td>
<td>Olympus Microscope IX71, OLYMPUS CORPORATION, Tokyo, Japan</td>
<td>Autofluorescence for diagnosis and therapy of eccrine sweat glands disease</td>
</tr>
<tr>
<td>Krasieva TB et al</td>
<td>2013</td>
<td>In vitro scientific study</td>
<td>Pheomelanin; Eumelanin</td>
<td>900-1000 nm</td>
<td>Pheomelanin: 615-625 nm Eumelanin: 640-680 nm</td>
<td>Meta detector of the Zeiss LSM 510 Meta NLO microscopy system; Chameleon-Ultra femtosecond pulsed tunable laser (Coherent Inc.)</td>
<td>Two-photon excited fluorescence (TPF) spectral index and phasor fluorescence lifetime imaging microscopy (FLIM) used for rapid melanin ratio characterization both in vivo and in vitro.</td>
</tr>
<tr>
<td>Yu Y et al</td>
<td>2012</td>
<td>In vitro + ex vivo scientific study</td>
<td>Keratin; NADH; FAD; Melanin; Collagen; Elastin (human skin) - Collagen - Tryptophan</td>
<td>ranging from 730 to 920 nm</td>
<td>Keratin: 485-525 nm NADH: 460 nm FAD: 550-543 nm Melanin: 579 nm Collagen: 458-675-510 nm Elastin: 454-472-508 nm</td>
<td>UVA lamp</td>
<td>Imaging guided TPF excitation-emission-matrix (EEM) spectroscopy provides useful information for developing clinical devices for skin disease diagnosis</td>
</tr>
<tr>
<td>McMullen RL et al</td>
<td>2012</td>
<td>Ex vivo animal study + in vivo human study</td>
<td>Keratin; NADH; FAD; Melanin; Collagen; Elastin (human skin) - Tryptophan</td>
<td>ranging from 720 to 450 nm</td>
<td>human skin) - Collagen: 466 nm - Tryptophan: 350 nm</td>
<td></td>
<td>Collagen fluorescence has different characteristics depending on type of skin tissue investigated.</td>
</tr>
<tr>
<td>Patalay R et al</td>
<td>2011</td>
<td>Ex vivo scientific study</td>
<td>Keratin; NADH; FAD; Melanin; Collagen; Elastin; Keratin; Porphyrins; NADPH; Flavins</td>
<td>760 nm</td>
<td>NADPH: 460-460 nm (main fluorophore in the blue channel) Melanin: 550 nm Flavins: 523 nm (main fluorophores in the green channel)</td>
<td>(modified) Two-photon tomography (DermalInspect/MPTflex, JenLab GmbH, Jena, Germany)</td>
<td>Fluorescence for differential diagnosis from dysplastic nevi and malignant nodular basal cell carcinoma.</td>
</tr>
<tr>
<td>Leupold D et al</td>
<td>2010</td>
<td>Ex vivo scientific study</td>
<td>Keratin; NADH; FAD; Melanin; Collagen; Elastin</td>
<td>810 nm</td>
<td>Common melanocytic nevi: band peaking 590 nm Melanoma: band peaking 640 nm</td>
<td>5 ns-pulsed via 810 nm wo-photon</td>
<td>Two-photon excited fluorescence is a possible new diagnostic tool on the basis of the red-shift of melanin fluorescence in melanoma.</td>
</tr>
<tr>
<td>Breunig HG et al</td>
<td>2010</td>
<td>Clinical study</td>
<td>Keratin; NADH; FAD; Melanin; Collagen; Elastin</td>
<td>ranging from 710 to 920 nm</td>
<td>–</td>
<td>Sapphire laser (Mai Tai XF, Spectra Physics)</td>
<td></td>
</tr>
<tr>
<td>Dimitrov E et al</td>
<td>2009</td>
<td>In vivo + ex vivo scientific study</td>
<td>Keratin; NAD(P)H; metal-free porphyrins; components of lipofuscin; melanin; elastin; collagen; keratin</td>
<td>760 nm and 800 nm</td>
<td>–</td>
<td>Two-photon tomography (DermalInspect/MPTflex, JenLab GmbH, Jena, Germany)</td>
<td>Early detection of black skin cancer.</td>
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<tr>
<td>Dimitrow E et al.</td>
<td>2009</td>
<td>In vivo + ex vivo scientific study</td>
<td>- NAD(P)H - metal-free porphyrins - components of lipofuscin - melanin - elastin - collagen - keratin</td>
<td>750 nm - 850 nm</td>
<td>-</td>
<td>Two-photon tomography (DermalInspect/ MPTflex, JenLab GmbH, Jena, Germany)</td>
<td>Multiphoton laser tomography for malignant melanoma diagnosis</td>
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<td>Stirban A et al</td>
<td>2008</td>
<td>Clinical study</td>
<td>AGEs ranging from 300 to 420 nm</td>
<td>ranging from 300 to 420 nm</td>
<td>-</td>
<td>AGE Reader (Diagnoptics BV, Groningen, The Netherlands)</td>
<td>Skin Autofluorescence as a non-invasive tool for screening people for undetected diabetes or cardiovascular risk. Basis for future in vivo skin spectroscopy studies and clinical examinations.</td>
</tr>
<tr>
<td>Laiho LH et al</td>
<td>2005</td>
<td>ex vivo study</td>
<td>Tryptophan; NADPH; Melanin; Elastin; Collagen</td>
<td>EX1 370 nm EM 1: 455 nm; EM 2: 455 / 370 nm</td>
<td></td>
<td>Quarz Y-fiber (wavelength allowance range: 120-1600 n. Volpi AG, Zurich, Switzerland)</td>
<td>Collagen-linked autofluorescence decreased with UVR exposure. F ratio might be the best measure of individual photodamage and a marker of individual cumulative UVR dose. 375 nm skin autofluorescence as a biologic marker of skin aging in vivo.</td>
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<tr>
<td>Sandby-Møller J et al</td>
<td>2004</td>
<td>Clinical study</td>
<td>-</td>
<td>EX1 330 nm EM 1: 375 nm; EM 2: 455 nm</td>
<td></td>
<td>Quarz Y-fiber (wavelength allowance range: 120-1600 n. Volpi AG, Zurich, Switzerland)</td>
<td></td>
</tr>
<tr>
<td>Na R et al</td>
<td>2001</td>
<td>Clinical study</td>
<td>-</td>
<td>EX1 330 nm EM 1: 375 nm; EM 2: 455 nm</td>
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transmitted fluorescence, by selecting the chosen excitation and emission bands, followed by digital image processing (Ericson et al., 2008).

Scout DS© (Veralight) performs a non-invasive automatic quantitative fluorescence spectroscopy to detect the levels of advanced glycation end products (AGEs) in the skin (Eny et al., 2015) of patients with diabetes.

Among the optical components more frequently used in clinical settings, the monochromator is a tunable optical filter that enables selection and transmission of wavelengths of only a specific, narrow band of the incident light (Gurushankar et al., 2015; Na et al., 2011; Nazeer et al., 2014; Vedeswari et al., 2009). Such a device can be used to filter narrow-band light, starting from a wide-band source to elicit tissue AF (Breunig et al., 2010; Drakaki, Dessinioti, Stratigos, Salavasstu, & Antoniou, 2014; Gurushankar et al., 2015; Lane et al., 2006; Nazeer et al., 2014; Na et al., 2011; Sandby-Meller et al., 2004; Vedeswari et al., 2009; Yaroslavsky, Neel, & Anderson, 2004). A second monochromator can be used to select the wanted emission narrow-band light by removing the unwanted reflected excitation light. In some cases, photomultiplier tubes, which are extremely sensitive detectors of light signals, can be used (Breunig et al., 2010; Dimitrow, Riemann et al., 2009, 2009b; Gurushankar et al., 2015; Krasieva et al., 2013; Leopold et al., 2011; Nazeer et al., 2014; Vedeswari et al., 2009; Yu et al., 2012).

5. Discussion

Innovative, non-invasive visual tools, possibly improving diagnostic accuracy, have recently gained interest. Among these, devices evaluating tissues’ auto-fluorescence (AF) are emerging (Drakaki et al., 2014; Giovannacci, Vescovi, Manfredi, & Meleti, 2016).

It seems likely that malignant or potentially malignant changes in soft tissues may induce variations of the tissue AF spectra, which can be visualised in real time by non-invasive methods.

On the basis of the present literature review, some evidence exists that endogenous fluorophores of skin, stimulated by ultraviolet or blue excitation wavelengths, are proteins such as collagen, elastin, keratin and the amino acid tryptophan. On the other hand, the very limited number of papers (5) investigated for fluorophores in the oral mucosa does not allow drawing any robust conclusion on such an anatomical compartment. Data on AF molecules in oral mucosa should, therefore, be considered preliminary and presumptive, and they should be confirmed by further research.

Even though skin and oral mucosa share some similarities in terms of overall histological architecture and molecule distribution, it should be borne in mind that specific features (e.g., width of the keratin layer, presence of peculiar glands, etc.) may be associated with different behaviours in terms of AF (light absorption and emission). Nevertheless, it would seem that both in skin and oral mucosa, dysplastic and neoplastic processes induce some alteration of fluorophores’ structure and/or concentration, thus producing a variation of normal AF (Croce & Bottirolì, 2014; Jayanthi et al., 2009).

Among fluorophores identified in the present review, localised either in the oral mucosa or skin, there are molecules involved in tissue metabolism such as the reduced forms of NAD (NADH) and NAD phosphate (NADPH) and FAD. Metabolic activity of the relative amounts of NADH, NADPH and FAD and the microenvironment of these metabolic electron carriers can apparently be used to monitor changes in metabolism non-invasively, which is one of the hallmarks of carcinogenesis (Gurushankar et al., 2015; Skala et al., 2007). Such coenzymes are the main ones responsible for AF rising from cell cytoplasm, with their amount, redox and bound-free state in close relationship with their engagement in energetic metabolism, cell oxidative defence, reductive biosynthesis, and signal transduction (Croce & Bottirolì, 2014).

Particularly, NADH is more fluorescent than the oxidised form, NAD⁺ (Miyamoto & Kudoh, 2013). Depending on the metabolic status of cells (e.g., prevalence of reactions of oxidation or reduction), the oxidised or reduced form of the coenzyme will be prevalent in the cytoplasm, thus leading to a higher or lower degree of fluorescence (Croce & Bottirolì, 2014). It has been widely demonstrated that neoplastic cells display an increase in oxidative processes, and it is therefore reasonable to hypothesise a shift in the NAD⁺/NADH equilibrium toward a predominance of NAD⁺ (Drakaki et al., 2014; Gao & Schöttker, 2017).

In addition, Drakaki et al., in their review on laser-induced fluorescence for diagnosis of basal cell carcinoma, confirmed that AF variation in malignant tissues was caused by decreased NADH levels and by a shifted equilibrium between the highly fluorescent NADH and the less-fluorescent oxidised-form NAD⁺ (Drakaki et al., 2014).

Similar to NAD, NADP is fluorescent in its reduced form (NADPH); the AF emission properties were strictly dependent on the bound/free condition (Miyamoto & Kudoh, 2013). The typical energetic anaerobic metabolism was observed in most neoplastic cells results, in general, in lower values in the NADPH bound/free ratio with respect to non-neoplastic cells (Croce & Bottirolì, 2014; Salmon et al., 1982). Such a low value of the bound/free ratio is, most probably, one of the biological features associated to the loss of AF observed in tumour cells.

As part of the group of flavin free derivatives, FAD exhibits absorption/excitation and emission properties at 440–450 nm and 525 nm (Penzer, 1980; Wolfbeiss, 1985). The fluorescence emission from FAD has been reported to be strongly affected by the nature of the protein to which the prosthetic group is bound (Kunz & Kunz, 1985; Kunz, 1986). Interestingly, the first description of FAD fluorescence was performed in 1962, in intracellular granules of eosinophils (Grossi & Zaccheo, 1963). It seems worthy to mention here that further studies demonstrated an increase in AF amplitude and a shift from the blue to the yellow region when the circulating cells settled in the tissues (Barnes, Aggarwal, Thomsen, Fitzmaurice, & Richards-Kortum, 1992; Mayeno, Hamann, & Gleich, 1992; Weil &. 1981).

Porphyrins are indicated as fluorophores both in oral mucosa and in skin tissues, with an excitation wavelength of about 400 nm and emission wavelength around 630–690 nm. Variation in porphyrin concentration is considered an important marker for differentiating malignant disorders from healthy tissues (Jayanthi et al., 2009). According to Bagri-Manjrekar et al., the mechanism responsible for such a phenomenon seems to depend on one or more of the following possibilities: (1) porphyrins of host origin and excreted from the circulating blood by the tumour; (2) the tumour itself produces porphyrins and (3) porphyrins are the product of microbial activity restricted to the ulcerated surface of the tumour (Bagri-Manjrekar et al., 2018).

In particular, Nazeer et al. demonstrated a more elevated level of porphyrins in the tissues of patients with oral leukoplakia and among tobacco users compared to non-tobacco users. Jayanthi et al. reported that the level of porphyrins increases with the increase in pathological grading, and Croce et al. reported that an excess of protoporphyrin IX (PpIX) occurs naturally in cancers and their metastases (Croce et al., 2011; Jayanthi, Subhash, Stephen, Philip, & Beena, 2011).

Interestingly, Inaguma et al. demonstrated that when exposed to 410 nm wavelength light, 85% of oral carcinomas returned porphyrin-like fluorescence spectra, whereas the normal mucosa of the oral cavity did not display such a feature (Inaguma & Hashimoto, 1999).

Moreover, the Mallia et al. study highlighted that the peak observed at 685 nm, especially in neoplastic and dysplastic tissues, could depend on the accumulation of the endogenous fluorophore coproporphyrin III, which is a precursor of PpIX in the heme synthesis. This peak was absent in tissues from healthy volunteers, which confirmed such findings (Mallia et al., 2008).

Among less-reported fluorophores, the present review identified flavin, lipopigment, advanced glycation end products (AGEs), pheomelanin and eumelanin.

Zhao et al. reported flavin and lipopigment as endogenous fluorophores of eccrine sweat glands. Following irradiation with a 488 nm wavelength, a single eccrine sweat gland isolated from normal skin tissue under a microscope showed AF with two peaks: 530 and 590 nm,
corresponding to flavin and lipopigment peaks. Such molecules are coenzymes in many metabolic pathways. AF measurement could therefore be performed to monitor cells and tissue energy metabolism (Zhao et al., 2016).

AGEs are a heterogeneous group of molecules resulting from non-enzymatic glycation of proteins, lipids, and nucleic acids. The measure of AGE residues provides information on glycomic control in diabetic patients (Eny et al., 2015; Stirban et al., 2008). It would seem that their concentration can be indirectly measured by evaluating skin AF. Stirban et al., using an excitation light source between 300 and 420 nm, demonstrated that skin AF increases after lunch in both healthy and diabetic patients (Stirban et al., 2008).

Light absorption behaviour of melanin is completely different from the absorption of other organic fluorophores (Hoffmann, Stucker, Altmeyer, Teuchner, & Leupold, 2001). In fact, melanin AF in the spectral region of 450–650 nm is extremely weak in human skin tissue and is completely hidden from the main endogenous fluorophores (e.g., NADPH e flavins) (Teuchner et al., 1999; Zeng, MacAulay, McLean, & Palic, 1995). Such a disadvantage has been overcome only recently, by using special fluorescence techniques such as one-photon excitation and two-photon absorption (Weinberger et al., 2006; Teuchner et al., 1999).

Upon irradiation with 800-nm laser pulses, melanin absorbs two photons in a stepwise process through an intermediate excited electronic state (Teuchner et al., 1999).

The multiphoton laser tomography (MLT), developed on the basis of the aforementioned technologies, enables a three-dimensional optical sectioning with a subcellular spatial resolution, thus allowing selective melanin imaging (Koemg & Riemann, 2003).

Investigation of melanin fluorescence, within a different set of pigmented lesions of the skin, showed a marked difference in AF behaviour of benign pigmentation (e.g., melanocytic nevi) with respect to melanoma (Hoffmann et al., 2001). Leupold et al. confirmed that through the use of a two-photon excitation technology with 2.5-ns pulses, melanin fluorescence peak is 590 nm in all types of benign melanocytic nevi, although it shifts to red light with 640 nm in melanoma (Leupold et al., 2011). Such observation led to the hypothesis that malignant transformation of melanocytes is associated with changes in melanogenesis and in the amounts of cellular eumelanin and pheomelanin (Leupold et al., 2011).

The reason for the red-shift of melanin fluorescence in melanoma is the modification of the eumelanin/pheomelanin ratio with a higher concentration of pheomelanin in malignant lesions (Leupold et al., 2011; Simon, Peles, Wakamatsu, & Itô, 2009; Yaroslavsky et al., 2004). Dimitrow et al. further demonstrated that MLT can differentiate between benign and malignant melanocytic lesions both in vivo and ex vivo. In fact, among 83 pigmented lesions of the skin, the authors highlighted distinct morphological differences in melanoma compared with melanocytic nevi. In particular, six characteristic features of AF malignant melanoma were specified (architectural disarray, pleomorphic cells, poorly defined keratinocyte cell borders, large intercellular distance, ascending melanocytes and dendritic cells). The technique showed an overall sensitivity of 75% in vivo and 93% ex vivo (Dimitrow, Ziemer, et al., 2009).

All clinical applications of oral mucosal AF reported in the papers included in the present review were related to the diagnosis and follow-up of malignant and potentially malignant lesions (Gurushankar et al., 2015; Jayanthi et al., 2009; Lane et al., 2006; Vedeswari et al., 2009).

It should be borne in mind that a potential risk of malignant transformation associated with the use of UV-emitting devices exists (D’Orazio, Jarrett, Amaro-Ortiz, & Scott, 2013). Therefore, caution should be used in clinical settings, when fluorescence is excited through UV wavelengths. It would, however, seem improbable that the limited time of exposure necessary in the reported techniques is sufficient to generate some damage at the cellular and tissue levels. AF detection in skin seems to cover a wider range of applications than in oral mucosa. Several papers are focused on the diagnosis and follow-up of malignant and potentially malignant lesions, both non-melanoma skin cancers and melanoma. Skin AF has also been proposed for assessing the severity of acne, for the diagnosis of sweat gland pathologies, for monitoring glycemic control in diabetic patients and as a marker for skin aging.

6. Conclusion

Some evidence exists for the usefulness of AF in the management of potentially malignant and malignant lesions of the skin. On the other hand, the very limited number of papers (5) investigating fluorophores in oral mucosa, and their behaviour in physiologic and pathologic conditions, does not allow any robust conclusions about such anatomical compartmentalisation. Further to diagnosis of malignant lesions, potential fields of application of skin and mucosal AF have been explored. However, on the basis of the weak level of evidence, more well-designed studies are needed to confirm such potentialities.

The heterogeneity of AF devices as well as their applications makes it difficult to compare the results of the studies included in the present review.

References


