Talanta 134 (2015) 514–523

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Fourier transform spectral imaging microscopy (FT-SIM) and scanning Raman microscopy for the detection of indoor common contaminants on the surface of dental implants

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ARTICLE INFO

Article history:
Received 14 October 2014
Received in revised form
22 November 2014
Accepted 24 November 2014
Available online 2 December 2014

Keywords:
Dental
Inplant
Contamination
Spectroscopy
Analysis

ABSTRACT

Endosteal dental implants are used routinely with high success rates to rehabilitate the integrity of the dentition. However if implant surfaces become contaminated by foreign material, osseointegration may not occur and the dental implant will fail because of the lack of mechanical stability. Detection and characterization of dental implant surface contaminants is a difficult task. In this article we investigate the application of several spectral microscopy methods to detect airborne contaminants on dental implant surfaces. We found that Fourier Transform Spectral Imaging Microscopy (FT-SIM) and scanning Raman microscopy provided the most useful information. Some implants possess weak and homogeneous auto-fluorescence and are best analyzed using FT-SIM methods, while others are Raman inactive and can be analyzed using scanning Raman microscopy.

1. Introduction

Endosseous osseointegrated dental implants are used routinely in dentistry and generally have a long-term success rate [1,2], providing functional and aesthetically pleasing implant-borne dental restorations [3]. Osseointegration can be defined as a healing process of bone around implants, the outcome of which is the establishment and maintenance of a clinically asymptomatic rigid fixation of an alloplastic material in bone under functional loading [4]. However, uncommonly implants fail to osseointegrate because of a variety of causes including contamination of the implant surface by foreign material [5]. These contaminants may affect cellular responses in the peri-implant microenvironment immediately after implant placement, resulting in fibrous encapsulation of the implant without adequate mechanical stability [6].

Most dental implant surgical procedures are carried out in dental surgeries, in which the air is neither filtered nor pre-treated. Under such conditions it is reasonable to assume that the concentration of airborne particulates is similar to that found in other rooms in the same area. The indoor concentration of airborne particulates in common residential areas strongly depends on the outdoor conditions [7] and is of the order of 10^2–10^4 particles per cubic centimeter. The airborne particles are usually classified by their size, and about 40% of them are coarse particles (1–10 μm) and about 60% are fine particles (0.1–1 μm). The ultrafine particles (<100 nm) are difficult to monitor but their concentration is probably higher. The chemical composition of the indoor airborne particles is affected by the local outdoor environment, and it includes inorganic compounds (such as salts, oxides and calcium carbonate), biological species (such as spores, pollens and viruses) and organic matter (such as soot and polycyclic aromatic hydrocarbons). The concentration of bioaerosols is of the order of a few thousands of particles per cubic meter. [8–10]

Detection of particulate contamination on surfaces is a challenging task. [11–14] Usually, optical analysis methods are preferred because they provide fast and reliable results [11]. Several techniques have been developed and validated for analysis of aerosols adhered to surfaces, including fluorescence [14,15] and multi-photon ionization [16]. When surface imaging is of interest, Fourier Transform Spectral Imaging Microscopy (FT-SIM) [17–24] and Raman spectroscopies are known to provide detailed chemical information [25,26]. Some of these methods have the capacity to provide important information about characteristics of oral hard and soft tissues [27–30]. These methods can be applied to a large variety of materials; however, they are especially suitable to investigate contaminants of organic nature because they have

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http://dx.doi.org/10.1016/j.talanta.2014.11.060
0039-9140/

the capacity to detect chemical modifications of the contaminants. [31,32]

2. Instrumental and methodological section

2.1. Instrumental

Several spectroscopic instrumental setups have been tested in this research for analysis of particulate contamination on dental implants:

1. FT-IR microscopy system, (Nicolet™ iS™5 FT-IR Spectrometer, Thermo Scientific, USA), coupled to Nicolet™ iN™10 Infrared Microscope and equipped with micro-ATR sampling device. It covers the spectral range of 7600–450 cm^{-1} at a resolution of 0.4 cm^{-1}.
2. Luminescence spectrometer (AMINCO-Bowman Series 2, Thermo Scientific, USA) equipped with two monochromators and with a front surface accessory for inspecting small surfaces. The excitation was performed using a Xenon flash lamp, in the wavelength range of 200–600 nm, and the emission was measured in the range 220–850 nm.
3. The following two systems were found to be the best suited to analyze contaminations on dental implant surfaces and provided the best performance for this application:
4. Raman microscopy system (Renishaw, 2000, U.K.). The experimental setup used for Raman spectral imaging of dental implants is depicted in Fig. 1**a**. It consists of a Raman spectrometer from Renishaw, coupled to a scientific imaging microscope (Leica DM-LM) and a high sensitivity and low noise Charge Coupled Device (CCD) detector. The latter was thermo-electrically cooled to −70 °C, reaching noise level of 7 e^{-1}pixel^{-1} and dark current of 0.0005 e^{-1} pixel^{-1} s^{-1}. The microscope was equipped with several objectives (all figures presented here were obtained using the x20 objective). The implants were manipulated using a piezoelectric stage (RGH22) of 0.1 μm resolution. For imaging purposes, a circular area on the sample was illuminated and a tunable filter was used to image the light from a selected Raman band directly onto the detector in a single step. Detailed Raman spectra at points of interest were acquired afterward, using a grating spectrometer. Excitation was performed by an air-cooled HeNe laser, emitting at 632.8 nm in a single mode (TEM000, vertically polarized). The output power was 17 mW. An air-cooled diode laser, emitting 17 mW output at 785 nm, with true single-mode operation and a line width of 0.1 cm^{-1} was also applied.
5. FT-SIM system (Green Vision, Israel). The experimental setup is described in Fig. 1**b**. A UV fluorescence microscope was coupled to a spectral imaging unit and a CCD camera. The microscope (Axiolab AB0100W/2, Carl Zeiss, Germany) was equipped with UV transparent objectives (Zeiss Fluar and Ultrafluar) providing several magnifications (x10, x20 and x40). Samples were irradiated by a mercury lamp, through the microscope objectives. Fluorescence was excited at 365 nm using a narrow band filter (10 nm). A dichroic mirror placed in the optical path was used for cutting off the reflected light at wavelengths below 390 nm. Microscope images were transferred to an imaging Fourier transform spectrometer and to a CCD camera for simultaneously recording the fluorescence spectra at each image pixel. The electro-thermally cooled CCD detector consisted of 480 × 640 pixels of 10 × 10 μm^2 each. (Hamamatsu, 4880).

2.2. Methodology

There are many potential methods that have the capacity to detect particulate contaminants on implant surfaces. The most relevant are the optical technologies, which allow for direct inspection without any pre-treatment. Obviously, in view of the size of the relevant contaminants, microscopy methods are needed. However, not all microscopic surface analysis methods are applicable to implants, because of their morphological structure. They are spirally shaped and possess large sprockets that prevent scanning and make focusing difficult.

Optical microscopy is available almost in every laboratory, however it only provides structural data, and the lack of spectral information does not allow for insight into the chemical nature of the contaminants. Therefore, microscopes equipped with fluorescence, Raman and IR spectrometers are the best candidates for inspecting dental implant surfaces. Instrumental setups that allow for surface imaging with spectral resolution in each image pixel are available. The capacity of these instruments to detect and identify particulates on dental implant surfaces were tested in this study. The results obtained using FT-IR microscopy, in the range 450–7500 cm^{-1}, were poor compared to other methods, so in the following we only report on fluorescence and Raman microscopies.

Dental implants differ in their surface properties, morphological characteristics, chemical composition and in surface treatments.

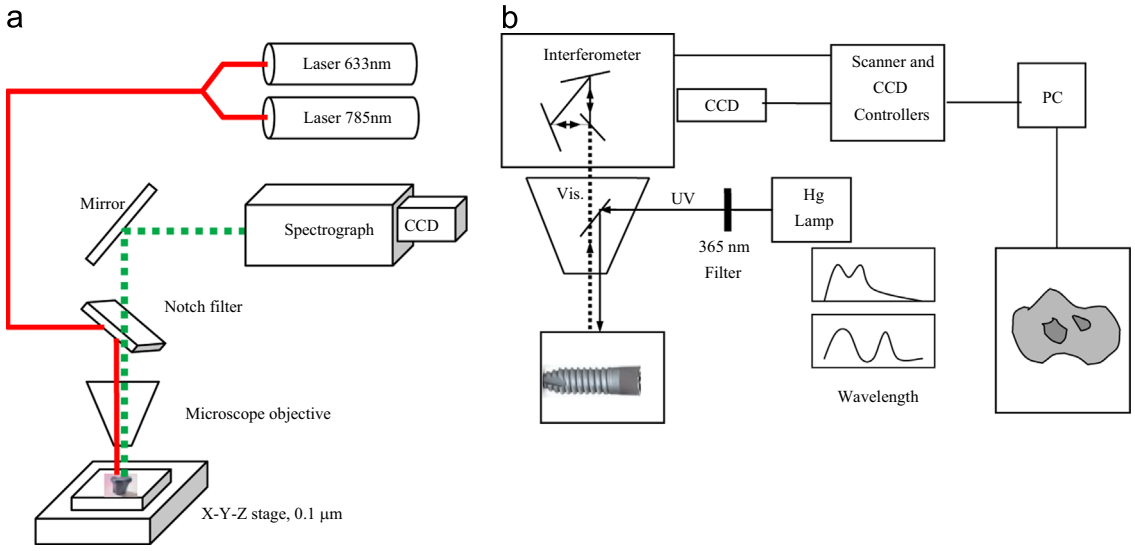

Fig. 1. The experimental setups of micro Raman (a-left) and FT-SIM imaging (b-right) used for inspecting dental implants.