CONFOCAL LASER SCANNING MICROSCOPY (CLSM) FOR THE STUDY OF THE MORPHOLOGICAL CHANGES OF THE POST-EXTRACTION SITES

Brief title: CLSM and post-extraction changes

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ABSTRACT

A better understanding of the remodeling process of post-extraction sockets is essential in dental treatment planning. The aim of this study was to evaluate whether Confocal Laser Scanning Microscopy (CLSM) can be applied to imaging contour changes of post-extraction sites, as well as to its quantification with image analysis of obtained three-dimensional images. This work describes a new application of the CLSM technique. The system used was the OLS3100-USS, LEXT model (Olympus®). CLSM was used for the surface analysis of the extraction site. The measurements taken with CLSM were: 1) mesio-distal distance, 2) alveolar ridge thickness, and 3) vestibular and lingual alveolar ridge height. Results of study casts scanning at baseline, one and three months after tooth extraction, with CLSM are well-detailed images of post-extraction areas. The CLSM technique used in study casts is a valid method to measure the dimensional changes that happen in the edentulous area after tooth extraction. This technique allows the evaluation of changes in mesio-distal distance, thickness of the alveolar ridge and alveolar ridge height based on the measurements on the alveolar contours.

INTRODUCTION

Tooth extraction is one of the most common treatments in dentistry. The alveolar process is a tooth-dependant tissue that develops in conjunction with the eruption of the teeth. The volume as well as the shape of the alveolar process is determined by the shape of the teeth, their axis of eruption and eventual inclination. Therefore, when the tooth is extracted, the alveolar process undergoes atrophy, due to the loss of its function (Van der Weijden et al., 2009). Resorption of the alveolar process after tooth extraction may compromise the functionality and aesthetics of dental implants and prosthetic restorations. Consequently, a better understanding of the remodeling process of post-extraction sockets is essential in dental treatment planning, since this process generates changes in the clinical layout of the socket.

Morphological changes in extraction sockets have been described by cephalometric measurements (Atwood, 1963), study cast measurements (Pietrokovsky and Massler, 1967), radiographic analysis (Schropp et al., 2003), clinical contour changes (Lam, 1960) and direct measurements of the ridge following surgical re-entry procedures (Araújo and Lindhe, 2009, 2005; Botticelli et al., 2004; Fickl et al., 2008).

One of the main problems in the study of alveolar changes after tooth extraction is ionizing radiation generated by the radiographic analysis. Conventional radiographies do not permit three-dimensional studies. Computer Tomography (CT) generates three-dimensional images of the alveolar bone and is used for planning the implant surgery. However CT produce ionizing radiation that are harmful to the patient; consequently, CT is not recommended for the study of morphological changes after tooth extraction, because it would expose patients to unnecessary radiation dose.

Instead, the Confocal Laser Scanning Microscopy (CLSM) technique can be used to study contour changes of the edentulous area in study casts. This technique allows the visualization of the soft tissues of post-extraction sockets, which can not be collected by CT. Moreover, CLSM is a non-invasive and non-damaging technique and absolutely harmless for the patient.

CLSM is an optical microscope that includes a laser light as light source and an electronic system which helps on image collecting. A schematic diagram of the optical pathway in a CLSM system is illustrated in Figure 1. After passing through a first pinhole, the laser light is projected into the specimen by the dichroic mirror. The light emitted from the sample is collected by the objective lens and passes through pinhole 2. This pinhole discards rays that are reflected by out-of-focus planes. The main advantages of CLSM are: 1) removal of the veil that produces regions outside the in-focus plane, obtaining a greater contrast, 2) a greater resolution due to the use of a laser light as the light source, 3) the system can take images at different depths by varying the in-focus plane, 4) the images can be represented using tridimensional reconstruction and 5) it is possible to digitalize the image and use imaging techniques as morphometric measures (Martínez et al., 2008).

CLSM has been used in dentistry for the characterisation of the composite-dentin interface in dental restorations (Bitter et al., 2009; Ding et al., 2010; Pioch et al., 2004), for the study of the performance of the materials used in dental restorations (Belli et al., 2009; Etman et al., 2009) and in the assessment of the cariostatic effect of restorative materials or fluoride compounds (Büyükyilmaz et al., 1997; Czochrowska et al.,1998; De Carvalho et al., 2009; González-Cabezas et al., 1998; Ogaard et al., 1996; Okuda et al., 2003; Umino et al., 2005). CLSM has also been used in the examination of the alveolar bone (Baschong et al., 2001; Favia et al., 2009; Iyama et al., 1997; Nishikawa et al., 2006; Pilolli et al., 2008; Suzuki et al., 1997; Traini et al., 2007) and the dental hard tissues (Crincoli et al., 2007; Girija et al., 2003; Goracci et al., 1999; Grötz et al., 1998; Kabasawa et al., 1995; Kagayama et al., 1997; Love and Chandler 1996; Lucchese et al., 2008; Radlanski et al., 2001; Saini et al., 2007; Sakakura et al., 1998; Scivetti et al., 2007; Sønju et al., 1997), in microbiological evaluation (Dige et al., 2007; Takenaka et al., 2001; Zaura-Arite et al., 2001) and in the study of the peridental soft tissues (Chantawiboonchai et al., 1998). Nevertheless, this technique has never been used in the study of the clinical contour changes following tooth extraction.

The aim of this study was to evaluate whether CLSM can be applied to imaging contour changes of post-extraction sites, as well as to its quantification through image analysis of obtained three-dimensional images. This article describes a new application of the CLSM technique.

MATERIALS AND METHODS

This study was approved by the ethical committee of Doctor Peset University Hospital of Valencia, Spain (Approval Protocol number 30/11).

Specimens

Patients who visited Doctor Peset University Hospital Dentistry Service for a tooth extraction were randomly selected. All selected patients signed informed consent. Patients with systemic diseases preventing dental outpatient treatment, medications that alter bone metabolism and pregnant women were excluded.

After tooth extraction, a silicone print of the post-extraction site was taken (fluid silicone Aquasil Ultra LV on putty silicone Aquasil Soft Putty, Dentsply®). After that, the silicone print was emptied with plaster (Vel-Mix Stone, ISO type IV, Kerr®) to obtain the study cast. When the plaster set down, the study of the post-extraction site was carried out by the CLSM technique. New silicone prints were taken to assess contour changes in the extraction area at one and three months after tooth extraction.

Finally, the sample was composed of 24 post-extraction sites at baseline and one month after tooth extraction. 16 specimens of post-extraction sites three months after tooth extraction were taken.

Confocal Laser Scanning Microscopy

The system used was the OLS3100-USS, LEXT model (Olympus®). This microscope allows the observation and the analysis of surfaces and microstructures of materials with a maximum resolution of 120 nm in X / Y and 10 nm in Z. The light sources of the LEXT system are a laser diode with a wavelength of 408 nm for confocal microscopy (CLSM) and non-confocal microscopy (LSM) and a 100W halogen lamp for conventional microscopy (bright or dark field, polarization and differential interference contrast (DIC) microscopy). It has a motorized stage that permits moving quickly to the interesting region.

Specimens were observed using a 2.5x lens, with a magnification of 25 increases. The observations were taken using a 4x6 grid (4 horizontal sections and 6 vertical sections). The sections were recorded at a size of 4.8x6.4 mm and a lateral resolution of 6.25x6.25 µm. Each section had a maximum depth of 10 mm.

Three-dimensional imaging allows the acquisition of transverse and longitudinal contours of the post-extraction site. All the measurements were taken at five points of the post-extraction site. Figure 2a shows the 5 longitudinal sections placed in: 1) the most vestibular part, 2) the fourth part of the length, in the vestibular part, 3) the central part, 4) the fourth part of the length, in the lingual part, 5) the most lingual part. Figure 2b shows the 5 transversal sections placed in: 1) the most mesial part, 2) the fourth part of the length, in the mesial part, 3) the central part, 4) the fourth part of the length, in the distal part, 5) the most distal part.

Measurements taken with the CLSM

1. *Mesio-distal distance:* it measures the mesio-distal distance between the teeth adjacent to the post-extraction site at baseline, one month and three months after extraction to assess possible movements of the teeth towards the edentulous space. The distances were obtained from the base of the tooth, near the top of the gingiva. Figure 3a shows the mesio-distal distance of the section number 2 of the Figure 2a.

2. *Alveolar ridge thickness:* it measures the vestibular-lingual width of the alveolar ridge in the post-extraction site at baseline, one month and three months after tooth extraction. The measurements were taken in the site where the gingiva was beginning to have a negative slope. Figure 3b show the mesio-distal distance of the section number 2 of the Figure 2b.

3. *Vestibular and lingual alveolar ridge height:* it measures the distance that the lingual and vestibular alveolar ridges are placed in relation to the highest point of the gingiva in the mesial part of the extraction site. As the reference point of the gingiva could change at one and three months after the tooth extraction, the following measures were used:

H_0: it measures the distance from the top of the mesial tooth of the extraction site to the highest point of the gingiva in the mesial part of the edentulous space at baseline.

H_1: it measures the distance from the top of the mesial tooth of the extraction site to the highest point of the gingiva in the mesial part of the edentulous space at one month.

H_3: it measures the distance from the top of the mesial tooth of the extraction site to the highest point of the gingiva in the mesial part of the edentulous space at three months.

The height at which the ridge was at baseline was determined by measuring the distance between the reference point of the gingiva used in H_0 and the highest point of the lingual or vestibular alveolar crest in the different outlines, like it is shown at Figure 4.

To determine the height at one and three months, the distance between the reference point of the gingiva used in H_1 and H_3 and the highest point of the lingual or vestibular alveolar ridge was measured in the different outlines. Figure 5 shows the schematic illustration of the outlines at baseline and one month following tooth extraction.

Statistical Analysis

Statistical Analysis was performed using SPSS software (SPSS for Windows 17.0; SPSS Inc., Chicago, IL, USA). Nonparametric tests were used because the distribution of the sample was not known and the sample had less than 30 post-extraction sites. The measurements taken at baseline, one and three months after tooth extraction were compared using Friedman test. The one and three-months changes were performed using Wilcoxon test. The level of significance was set at 5% for all the tests.

RESULTS

Visualization of the post-extraction sites

Results of study casts scanning at baseline, one and three months after tooth extraction, with CLSM are well-detailed images of post-extraction areas. Figure 6 shows post-extraction areas after they were scanned with CLSM.

Measurement of post-extraction changes

The post-extraction changes one and three months after tooth extraction are shown in Table 1. Changes in vestibular and lingual ridge height are not provided in percentage because the measurements were obtained from the height of the tooth and not from the original height of the alveolar ridge.

The mesio-distal distance taken at baseline was higher than at one and three months after tooth extraction in the five measurement points. Significant differences were observed between the measurements taken at points 2, 3 and 4 at baseline, one and three months after tooth extraction ($p<0.05$; Friedman).

The alveolar ridge thickness at baseline was higher than one and three months after tooth extractions in the five measurement points. The smaller resorption occurred at the ends of the edentulous area, next to the adjacent teeth. Significant differences were observed in all measurement points at baseline, one and three months after tooth extraction ($p<0.05$; Friedman).

As regards the vestibular and lingual alveolar ridge height, point 1 was used to measure the distance from the top of the mesial tooth of the extraction site to the highest point of the gingiva in the mesial part of the edentulous area at baseline (H_0), one month (H_1) and three months after tooth extraction (H_3). Thus, one and three-months changes shown in Table 1 are similar in the vestibular and lingual parts. H_0 was significantly lower than H_1 and H_3 ($p<0.05$; Friedman). Therefore, there was a loss of height in the gingiva of the mesial tooth at the extraction site.

In the rest of the points measured on the lingual ridge, height loss was greater three months after tooth extraction and the differences were statistically significant at all points ($p<0.05$; Friedman). By contrast, in the vestibular ridge height loss was greater three months after tooth extraction, but only points 1, 2 and 5 were statistically significant ($p<0.05$; Friedman).

Finally, when comparing the height of the lingual and vestibular ridge, the vestibular ridge was placed apical to the lingual ridge at all measurement points and times. The vestibular ridge was located significantly apical ($p<0.05$; Wilcoxon), except for point 4 at baseline.

DISCUSSION

The use of addition silicone in study casts for further analysis by the CLSM is an accurate and stable technique, as is reported in many studies (Aguilar et al., 2010; Chen et al., 2004; Faria et al., 2008; Pereira et al., 2010; Thongthammachat et al., 2002). In addition, the significant differences obtained when comparing measures at baseline, one and three months after tooth extraction suggest that the variations are due to changes in the socket after tooth extraction and not to variations in the silicone morphology.

Moreover, when a tooth is lost, the structural integrity of the dental arch is interrupted, with the subsequent a re-alignment of the teeth to reach a new balance state. Frequently the teeth adjacent to the edentulous area move towards it. Specially the teeth placed in the distal part of the post-extraction site can move as a whole, although the inclination movement is more common (Shillingburg, 2002). The decrease in mesio-distal distance found, suggests the re-alignment of teeth discussed above. In addition, the significant differences in mesio-distal distances obtained at points 2, 3 and 4 indicate an inclination movement of the tooth distal to the edentulous area.

This movement should be taken into account when an extracted tooth is going to be replaced by any prosthetic restoration, because if the edentulous space is greatly reduced, its placement will be difficult.

Regarding the alveolar ridge thickness, CLSM permits the assessment of the horizontal resorption that generates a narrower alveolar ridge. Schropp et al. (2003) estimated bone healing and soft-tissue contour changes following single-tooth extraction. They assessed the resorption at three, six and twelve months following the removal of the tooth. Schropp et al. (2003) stated that approximately two thirds of the reduction of the width of the alveolar ridge occurred within the first three months after tooth extraction. Their results would agree with the value of 38.6% thickness reduction found at three months after tooth extraction in the central part of the post-extraction site. Pietrokovsky and Massler (1967) measured the alveolar resorption in plaster models by overlying the contralateral quadrant of the edentulous area. The mean value of resorption in the central part was 5.39 mm. These results are similar than those obtained in this study at the central point three months after tooth extraction: 5 mm. But Pietrokovsky and Massler did not indicate the time between tooth extraction and the study measurements.

The alveolar ridge thickness will determine the possibility of placing a dental implant as well as the aesthetic of dental restorations. If the alveolar ridge is narrow after tooth extraction it will be necessary the use of techniques to increase the alveolar ridge thickness to replace the missing tooth.

About the alveolar ridge height, the difference between H_0 - H_1 and H_0 – H_3 can be measured by CLSM and suggests that there is a gingival retraction in the tooth mesial to the post-extraction site. This retraction may cause aesthetic and clinical alteration like disturbing feelings. The changes obtained in lingual ridge height by the CLSM technique indicate that the lingual alveolar ridge experiences a vertical decrease after tooth extraction. By contrast, the vestibular ridge shows less resorption after three months than after one month. This could be due to the way of healing of the post-extraction site and the measuring procedure. The heights of the alveolar ridges were taken from a fixed reference point to the highest point of the alveolar ridge. After tooth extraction, the socket is reepithelialized and filled with new bone, and the old bone is remodelled. Since the lingual ridge is coronal than vestibular, the plane of the socket is inclined. Therefore, the horizontal resorption of the vestibular ridge could cause the highest point of the vestibular ridge to be more coronal and lingual than before extraction.

The study of the clinical profiles after tooth extraction will improve the design of clinical protocols after tooth extraction. The clinical profiles of the socket are very important in the aesthetics of dental restorations that will be placed in the position of the missing tooth.

In conclusion, the use of CLSM for cast analysis is a valid method to measure the dimensional changes that occur in the edentulous area after tooth extraction. This technique allows the evaluation of changes in mesio-distal distance and alveolar ridge thickness and height by taking measurements on the alveolar contours.

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Table 1: Changes obtained at the five measurements points one and three months after tooth extraction.

	Point of measurement	One-month changes				Three-months changes			
		µm		%		µm		%	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
Mesio-distal distance	**1**	-359	921	-1.9	6.7	-597	1270	-4.1	8.6
	2	-490	724	-3.9	6.4	-586	845	-5.5	7.6
	3	-354	458	-3.8	4.9	-613	698	-6.4	8.1
	4	-98	760	-0.9	7.6	-470	831	-4,6	8.7
	5	-104	954	-1.1	7.0	-727	1250	-5.9	8.9
Alveolar ridge thickness	**1**	-1668	2015	-15.2	19.2	-2266	1854	-21.0	15.9
	2	-3546	1634	-29.5	11.2	-5047	2188	-41.5	13.4
	3	-3517	2336	-26.4	15.2	-5071	2418	-38.6	14.8
	4	-3269	2086	-25,2	13.9	-4897	1713	-37.8	10.3
	5	-2352	2184	-18.7	17.6	-3366	1932	-26.5	17.2
Vestibular ridge height	**1**	967	474			1333	395		
	2	566	1735			452	1626		
	3	786	1747			465	1360		
	4	866	1671			672	1099		
	5	800	1595			759	1125		
Lingual ridge height	**1**	967	474			1333	395		
	2	916	853			1084	624		
	3	897	742			823	638		
	4	835	742			935	709		
	5	876	858			915	640		

The results are provided in microns (µm) and in the percentage change from baseline (%).
SD: Standard deviation

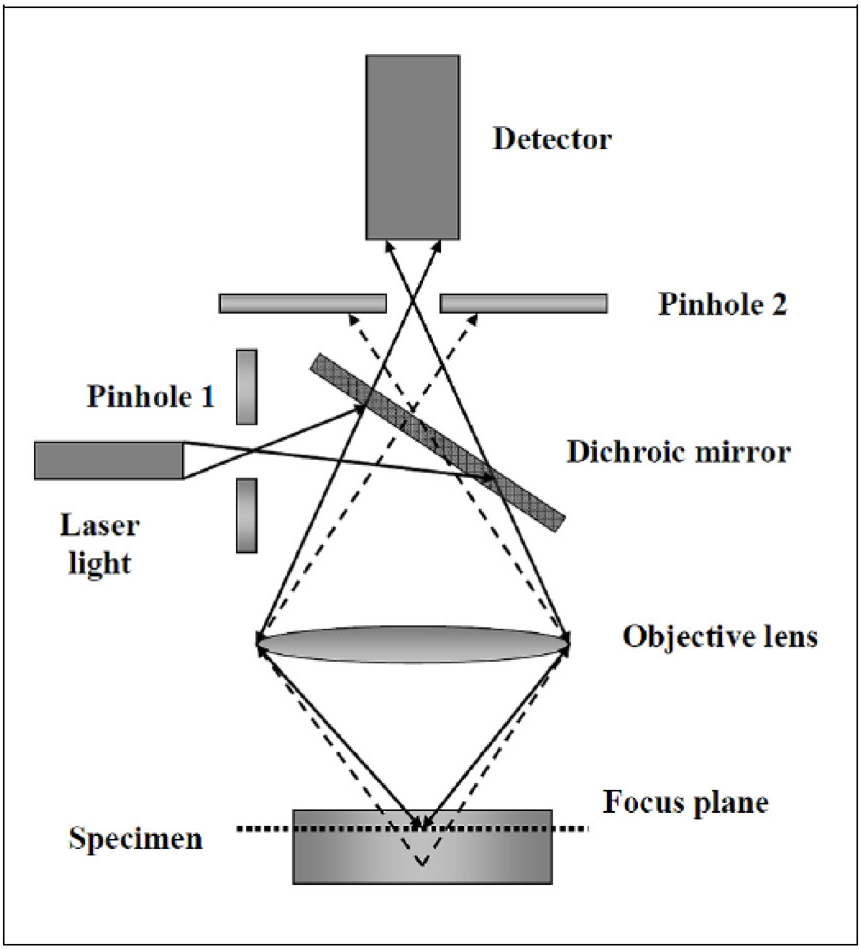


Figure 1: Schematic diagram of the optical pathway in a CLSM system (Martínez et al., 2008)

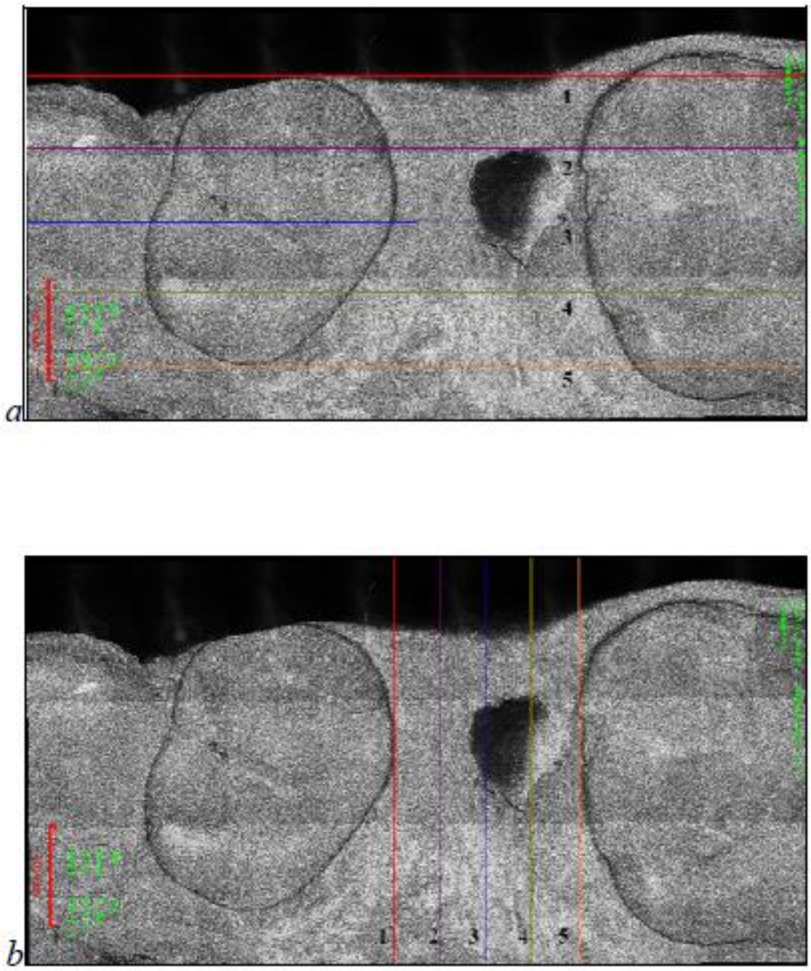
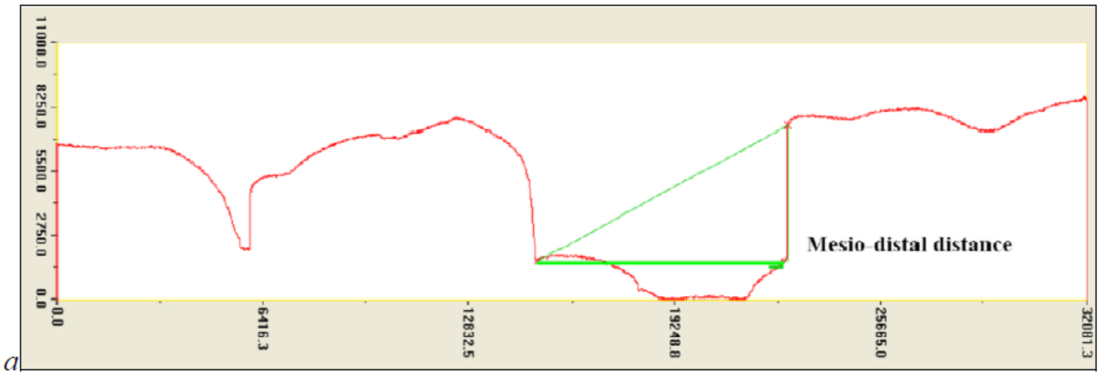


Figure 2: a) 5 longitudinal sections of the post-extraction area; b) 5 transversal sections of the post-extraction area.



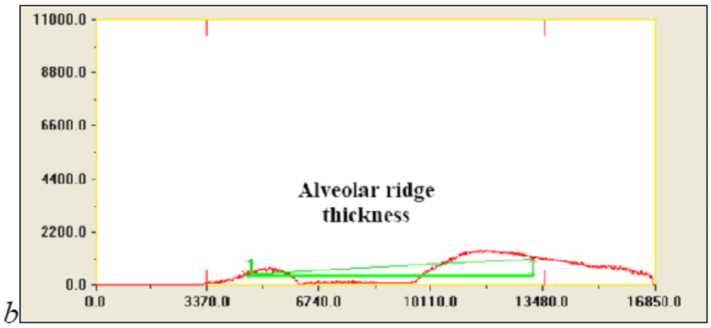


Figure 3: a) Outline of the section number 2 of the Figure 2a, where it is possible to obtain the mesio-distal distance; b) Outline of the section number 2 of the Figure 2b, where it is possible to obtain the alveolar ridge thickness.

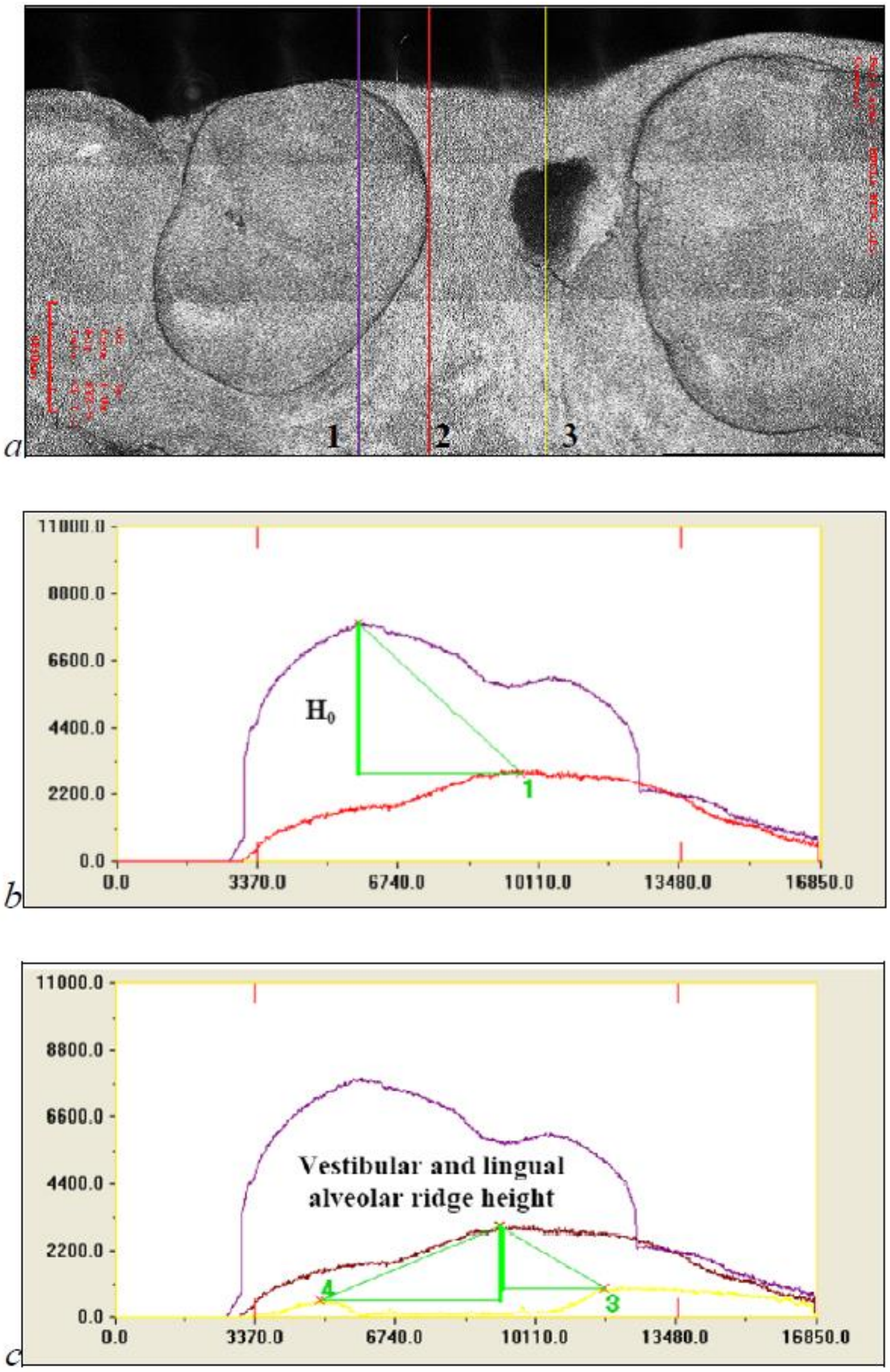


Figure 4: a) CLSM image with the transversal sections of the post-extraction area: 1- passing through the top of the mesial tooth of the extraction site, 2- passing through the highest point of the gingiva in the mesial part of the edentulous space and 3- transversal section of the edentulous space; b and c) Outlines of theses sections, where it is possible to obtain the H_0 and the vestibular and lingual alveolar ridge height.

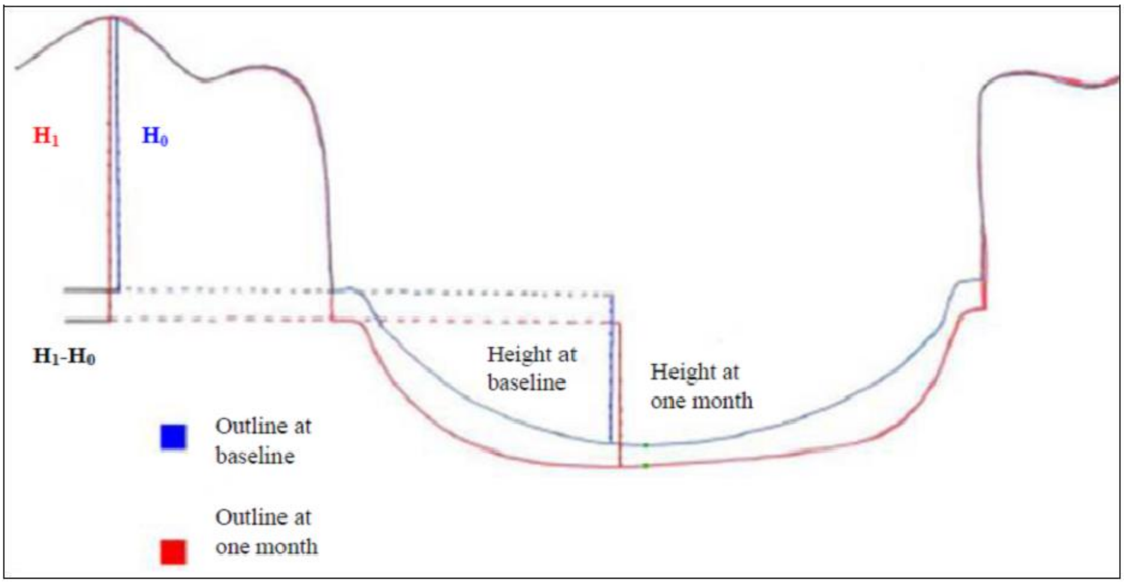
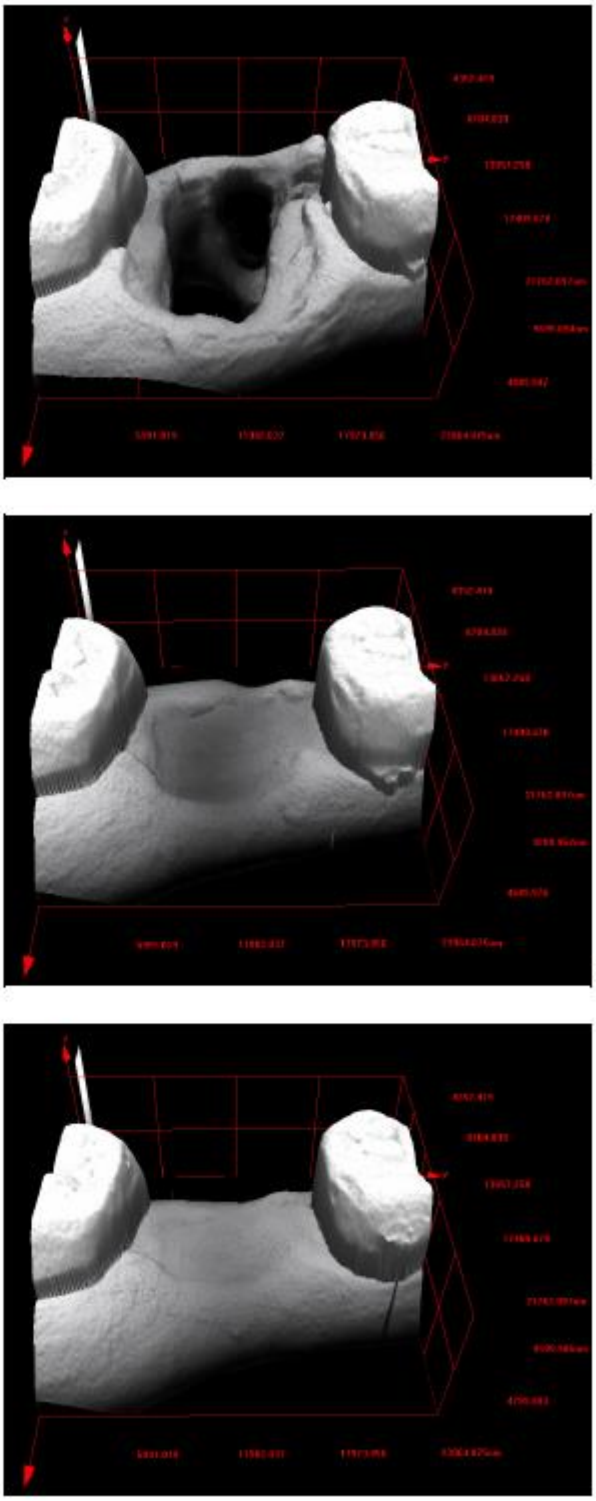


Figure 5: Schematic illustration of H_0 and H_1 measurements in the baseline and one month outlines of the post-extraction site.



a b c

Figure 6: Three-dimensional CLSM images of the post-extraction site at baseline (a), one (b) and three months (c) after tooth extraction.