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Research Paper

Bitemark analysis using an intraoral 3d scanner (iTero®): experimental study on human skin

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ABSTRACT

Bites can leave unique marks on the skin that may be useful to identify the perpetrator of a crime. Standard bitemark analysis is currently based on visual comparison, often conducted using photographs, and involving several operator-dependent measurements, but recently an experimental method using three-dimensional (3D) scanning and computer software to objectively analyze bitemarks on dentistry wax has been proposed. In the present study the same method was applied using 12 complete (upper and lower dental arches) plaster models of human dentitions to experimentally produce 24 bitemarks on human skin. The plaster models and the bitemarks were scanned using iTero® 3D scanner. Both the dental models and bitemarks were processed with MeshMixer™ software. For quantitative comparison, bitemarks were compared to their corresponding dentitions using CloudCompare software. A control group involved comparisons between bitemarks and random dentitions. Upper and lower dental arches of coincident comparisons were also compared to assess potential differences. The results revealed different distribution curve trends when comparing bitemarks to the biter dentition versus non-coincident dentitions. Instead, no difference emerged between upper and lower dental arches.

This method shows the potential of 3D scanning distinguishing between matched and non-matched dentitions under experimental conditions. Moreover, data from the 3D reconstruction of the dentition and the bitemark remain available for further in-depth study for second opinion. The promising results of digital comparisons between bitemarks and dental models suggest that this method could lead to a more accurate and objective approach to bitemark analysis in the future. However, further validation is required before forensic application.

1. Introduction

The bitemark is defined as "a circular or oval patterned injury consisting of two opposing (facing), symmetrical, U-shaped arches separated at their bases by open spaces", produced by the act of biting from human or animal dentition. Bitemarks are injuries involved in different criminal acts, such as aggression, child, elder or sexual abuse,[1,2] cannibalism, defense lesions, or even self-inflicted lesions.[3] The uniqueness of the human dentition,[4] considered specific for each individual, makes the bitemark on the skin of a victim an invaluable piece of evidence in the roster of investigations.[5] Current bitemark analysis primarily relies on visual comparison, often conducted using photographs, and involving several operator-dependent measurements.[6]

Microscopic examination has also been described in the literature as a complementary approach in bitemark analysis. In particular, the combined use of macroscopic pattern evaluation and microscopic assessment allows a more detailed examination of dental features, including both class and individual characteristics observable at higher magnification.[1,5] This integrated approach may improve the visualization of subtle morphological details that are not always detectable during macroscopic inspection alone.

Recently, three-dimensional (3D) technologies are increasingly employed in forensic science, from crime scene reconstruction to evidence analysis.[7,8] These techniques are applied in several forensic subdisciplines, including odontology, anthropology, and ballistics[9] and the technologies include scanning, modeling, software-based computer analysis and 3D printing of crime scenes, murder weapons or even the victims themselves, whether living or deceased.[10]

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The use of 3D scanners in bitemark analysis offers several advantages, including improved infection control in the process of data acquisition, simplified chain of custody, facilitated information exchange among forensic team members[11,12] and the availability of the samples for further in-depth study for possible second opinions. Intraoral scanners, commonly used in clinical dentistry, have been validated for bitemark analysis, with dental wax identified as the most suitable material for obtaining clear impressions.[13] Additionally, 3D scanning enables computerized analyses and quantitative comparisons between bitemarks and corresponding human dentitions, potentially enhancing the objectivity of forensic evaluations.[14] Moreover, a recent study demonstrated the value of 3D techniques as a supportive tool for subjects' identification through bitemark analysis as a supportive technique, but it outlines the need to produce more evidence to validate the digital technique.[15] This literature is part of a general effort of the scientific, forensic community to provide a robust, quantitative evidence in forensic dental analysis by leveraging advanced digital technologies, as showed in other recent works.[16]

Cadaveric models can serve as a practical first step when supplemented with validation studies using human donors[17,18] but they have significant limitations in forensic sciences, with absent circulation and inflammatory responses being the most critical constraints.[19,20]

Regarding elasticity, Thiel-embalmed cadavers showed elasticity patterns "similar" to living humans across multiple tissues.[21] However, it was demonstrated that freezing-thawing cycles altered tissue tensile strength unpredictably, since inflammatory responses and immune activation are central to trauma outcomes—processes entirely absent in cadavers.[22]

However, despite the inherent limitations of cadaveric studies, the evaluation of tools such as 3D scanners and computer-aided analytical methods necessarily requires an initial cadaver-based investigation. This approach represents an essential preliminary step before any potential in vivo application, given the ethical constraints that preclude the deliberate infliction of injuries on living individuals solely for the purpose of testing new detection and analysis methodologies.

This experimental study evaluates the suitability of a 3D intraoral scanner for identifying the biter's dentition, though the comparison between the 3D scans of bitemarks produced on human skin and the 3D scans of the producing plaster models. While traditional bitemark analysis has faced significant criticism due to subjective interpretation, our objective 3D approach may address some of these concerns. However, substantial additional validation is essential before the application in real forensic cases. This study does not aim to resolve the well-known controversies surrounding the analysis of bitemarks,[23] but rather to evaluate and apply, on an experimental basis, a new method through forensic analysis in areas where differing opinions persist.

2. Materials and methods

The study was approved by the Bioethics Committee of the local University (protocol no. 0388010).

The *a priori* statistical power of this study was calculated for the execution of a two-tailed *t*-test between two independent groups. An effect size of 0.8, a power of 0.8 and an α error of 0.05 were assumed. Considering these parameters, the suggested number of subjects for each group to measure a difference was 26.

Human skin was obtained from six amputated legs donated for scientific purposes, sampled by scalpel excision, preserving the superficial skin layer and, if present, any subcutaneous layer (i.e. fat). Muscle tissue was not included in the excided flap. The storage time of the limbs, from amputation to the execution of the study, ranged from a few days to a maximum of one month. From each leg, four square flaps of skin (with a different thickness for each one, depending on the quantity of subcutaneous tissue) were obtained, placed on a flat plastic tray and immediately used for the production of bitemark.

Each plaster model (for a total of 12), representing an upper and lower dental arch (for a total of 24 arches), was placed on a skin sample, which was previously placed flat on a plastic tray. Since the skin came from amputated limbs stored at - 20 °C, it was noted that prolonged application of the bitemark or the application of an excessive weight tended to cause lacerated injuries on the surface layer. However, excessive low weights did not produce any visible bitemark, given the elasticity of the tissues and their tendency to return to their initial shape. After several attempts with different weights and timing, a standard weight of 55 kg, placed on the plaster model for 10 s, resulted to be the best solution to produce a visible a durable bitemark. After producing the bitemark, the corners of the skin squares were secured to the tray with needles and subsequently scanned, right after production.

According to a previous study,[14] both plaster models and bitemarks were individually scanned with the iTero® 3D scanner (version 1.13.0.704), a device capable of capturing volumetric surface data, with an accuracy level less than 1 mm.

All scans were imported into MeshMixer™ software from Autodesk (version 3.5 of 2023), a 3D surface processing tool, and redundant scanned areas were removed, preserving areas where the bitemark was visible.[24] According to the method developed in a previous study,[14] incisors, canines and first premolars were considered, while posterior teeth scanned areas were excluded, in order to preserve only the teeth commonly visible in a bitemark.

Subsequent analyses for bitemark-dentition compatibility assessment were performed using CloudCompare software (version 2.12.4),[25] which allows the comparison between two or more three-dimensional points clouds located in a virtual space. The "point sampling on Mesh" function was used in order to simplify the points of the Mesh, and point clouds were obtained. The scan of the dentition plaster model was simplified with a function based on the density of points in the space, and a cloud consisting of 30,000,000 points per square meter was obtained. For the subsequent analysis, the number of points at which the bitemark is simplified must remain within a certain range defined in advance. Therefore, some bitemarks were simplified with 30,000,000 points per square meter, obtaining values around 16,000 points: all the scans of the bitemark on the skin were simplified with a function based on the total number of points (16,000 points) and a cloud of about 16, 000 points was obtained. Then, the bitemark cloud was manually aligned in virtual space, along the three axes (x, y, and z) to bring it as close as possible to the dentition model to assess the compatibility between the dentition itself and the lesion. When the best fit was reached, the distance between the points of the two compared surfaces was calculated with the "Cloud-To-Cloud Distance" function, which provides a colorimetric scale that indicates with different colors the relative distance between the two objects (blue, green, yellow and red). The cloud obtained from the dentition model was set as the reference, while the one obtained from the bitemark on the skin was set as the one to analyze. Then, the charts reporting the distance, measured as point numbers, between the two clouds with a colorimetric parameter were exported divided into 12 classes. The Gaussian curve indicating both the distance at which 50% of the points were included, along with the standard deviation, divided into 110 categories of increasing distances (without allowing a different number of categories) was also exported.

The lesion and dental models were scanned by a single trained operator. Subsequent digital processing of the files and the comparison between the dentition and the bitemark were independently conducted by four different operators. When relevant discrepancies emerged among the four operators, the case was jointly re-evaluated in dedicated calibration sessions. During these sessions, the digital models were reviewed collectively, the sources of disagreement were discussed, and additional digital processing was performed when required. This procedure allowed the operators to harmonize their interpretation criteria, and the final assessment was recorded only after a consensus had been reached.

Statistical analysis was conducted using IBM SPSS Statistics [Software]. Armonk, NY: IBM Corp. For our purposes, the mean distance between the dentition and the bitemark, measured with CloudCompare, was considered the quantitative variable of interest. A parametric test was used to compare mean distances in case of normal distribution, otherwise a non-parametric test was used. The Kolmogorov-Smirnov test and the Shapiro-Wilk test were used to verify normal distribution. As a parametric test, the *t*-test was used, with Levene's test for equality of variances. As a non-parametric test, the Mann-Whitney test was used. A p-value <0.05 was considered statistically significant.

Formerly, the plaster model dentitions and their corresponding lesion (coincident comparisons, study group) were compared. Thus, 10 bitemarks were randomly compared to 10 non-corresponding dentitions scanned in the study (non-coincident comparisons, controls).

The difference between the study group and the control group was assessed. Within the study group (coincident comparisons), the difference between upper and lower dental arches was also assessed.

3. Results

3.1. Measurements and semi-quantitative subdivision into color classes

After multiple comparisons, we obtained a distribution of the 16,000 points for each bitemark mesh into 12 classes, divided by color, based on the distance of each point from the dental model, ad shown in Fig. 1. In case of coincident comparisons, most points fell within the first three classes (blue and green shades), while in the case of non-coincident comparisons the points were more distributed among the 12 classes. From the comparisons it was then possible to obtain Gaussian curves with a distribution of the points in 110 classes, which also provided the mean values of the distance between the model and the bitemark, with the relative standard deviation. For each comparison, the mean and standard deviation were calculated as the index of dispersion of values from the mean value.

3.2. Statistical analysis: difference between coincident and non-coincident comparisons

The mean distance values for each measurement and the relative standard deviations, both for coincident (study group) and non-coincident (control group) comparisons, are reported in Tables 1 and 2. The distribution of mean values in relation to the standard deviations are reported in Figs. 2 and 3.

For coincident comparisons, the minimum mean value was 0.6733 and the maximum mean value 1.6926 with an overall sample mean of 1.2424 and standard deviation of 0.2909. The median value for coincident comparisons was 1.3186 and the variance 0.085. These values resulted lower when compared to those of the control group, suggesting a better correspondence between the dentition and the bitemark in this group. In fact, for the non-coincident comparisons, the minimum mean value was 2.3969, and the maximum 5.7086; the overall mean value was 3.7094, with a standard deviation of 1.0436; the median was 3.4141 and the variance was 1.0890. Descriptive statistics of both groups (study and control group) are reported in detail in Table 3. Fig. 4a presents a bar graph which illustrates the averages for both the study and the control group, with the relative standard deviations. Fig. 4b represents a boxplot with the median values and quartiles of the two groups and shows the lower median value for the study group. The boxplot does not highlight any outlier, suggesting that the mean is an appropriate measure of central tendency.

The Kolmogorov-Smirnov test showed a $p < 0.05$ for both the study and the control group, suggesting that the values of interest are not normally distributed in our sample. The Shapiro-Wilk test confirmed a not normal distribution for the non-coincident comparisons, whereas suggested a normal distribution for coincident comparisons. The details of the normality tests are reported in Table 4.

The Mann-Whitney test showed that the distance between the

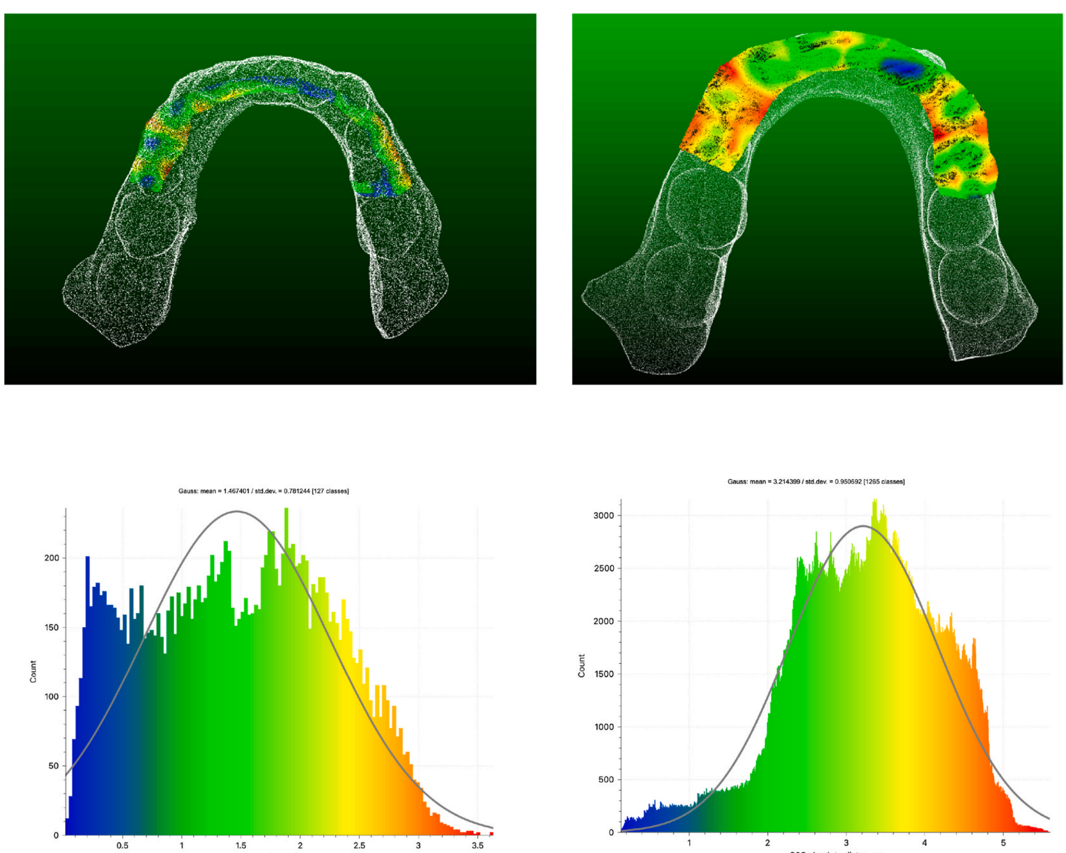


Fig. 1. Example of coincident comparisons (left) and non-coincident comparisons (right).

Table 1
Results of the comparison between the plaster model dentition and their corresponding lesion (coincident comparisons). Each plaster model, divided in upper and lower dentition model, was identified with a letter while each bitemark was identified with a number. For each comparison are reported mean values and standard deviation (S.D.) of distance between the model and the bitemark.

Coincident comparisons					
Dentition model		Bitemarks	Mean	S.D.	CV (%)
a	Upper	1	0.9675	0.5427	56.09%
	Lower	2	1.5914	0.9288	58.36%
b	Upper	3	0.89180.	0.4509	50.56%
	Lower	4	1.3129	0.6203	47.25%
c	Upper	5	1.3572	0.5775	42.55%
	Lower	6	1.6926	0.7299	43.12%
d	Upper	7	1.3776	0.9030	65.55%
	Lower	8	1.5443	1.0697	69.27%
e	Upper	9	0.6733	0.3706	55.04%
	Lower	10	0.7169	0.4595	64.10%
f	Upper	11	0.8170	0.4757	58.23%
	Lower	12	1.4315	1.0105	70.59%
g	Upper	13	1.5131	0.9066	59.92%
	Lower	14	1.1097	0.4759	42.88%
h	Upper	15	1.2610	0.5442	43.16%
	Lower	16	1.4674	0.7812	53.24%
i	Upper	17	1.0899	0.6982	64.06%
	Lower	18	1.2678	0.8148	64.27%
j	Upper	19	1.3329	0.7552	56.66%
	Lower	20	1.4482	0.5825	40.22%
k	Upper	21	1.3236	0.5965	45.07%
	Lower	22	1.5027	0.5175	34.44%
l	Upper	23	1.3135	0.6941	52.84%
	Lower	24	0.8138	0.4982	61.22%

C.V. Coefficient of variation for coincident comparisons.

Table 2
Results of random comparisons between the bitemarks and non-corresponding dentitions (controls). Each plaster model, divided in upper and lower dentition model, was identified with a letter while each bitemark was identified with a number. For each comparison are reported mean values and standard deviation (S.D.) of distance between the model and the bitemark.

Random comparisons (controls)					
Dentition model		Bitemarks	Mean	S.D.	CV (%)
c1	Upper	1	5.7086	2.1367	37.43%
	Lower	2	4.2554	2.4505	57.59%
c2	Upper	3	4.4049	1.6237	36.86%
	Lower	4	3.5936	1.4997	41.73%
c3	Upper	5	3.0435	1.0250	33.68%
	Lower	6	3.4546	1.1856	34.32%
c4	Upper	7	5.6119	2.4930	44.42%
	Lower	8	3.7848	2.4588	64.96%
c5	Upper	9	5.1706	2.5506	49.33%
	Lower	10	3.0945	0.8039	25.98%
c6	Upper	11	3.0933	1.0526	34.03%
	Lower	12	2.8578	1.0095	35.32%
c7	Upper	13	3.5423	1.2689	35.82%
	Lower	14	3.3735	1.1129	32.99%
c8	Upper	15	5.4345	2.7530	50.66%
	Lower	16	2.3969	0.7434	31.01%
c9	Upper	17	3.1896	1.2264	38.45%
	Lower	18	2.4206	0.8974	37.07%
c10	Upper	19	2.9018	1.0010	34.49%
	Lower	20	2.8559	0.8573	30.02%

C.V. Coefficient of variation for non-coincident comparisons.

dentition and the bitemark is averagely lower in the study group respect to the control group (U = 0.001, z = - 5.657, $p < 0.001$), suggesting that, as expected, there is a better correspondence between the bitemark and the model that produced it rather than in the control group (random comparisons). The detailed results of the Mann-Whitney test are reported in Table 5.

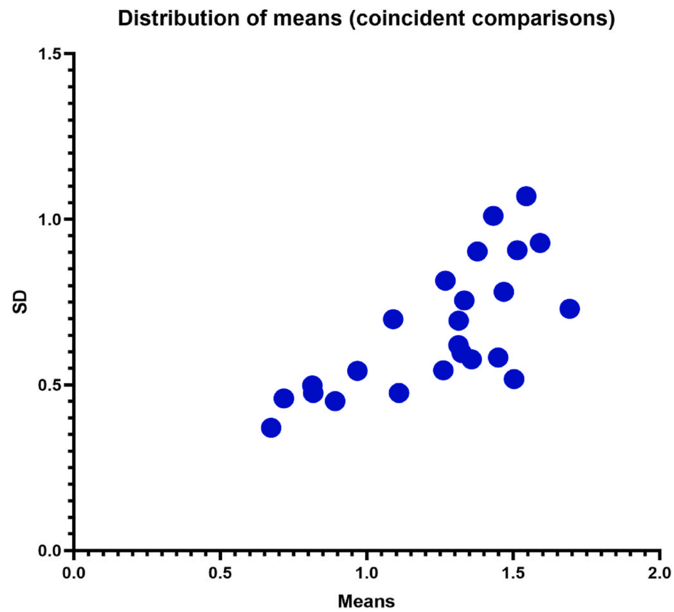


Fig. 2. Distribution of means and standard deviations (SD) for coincident comparisons.

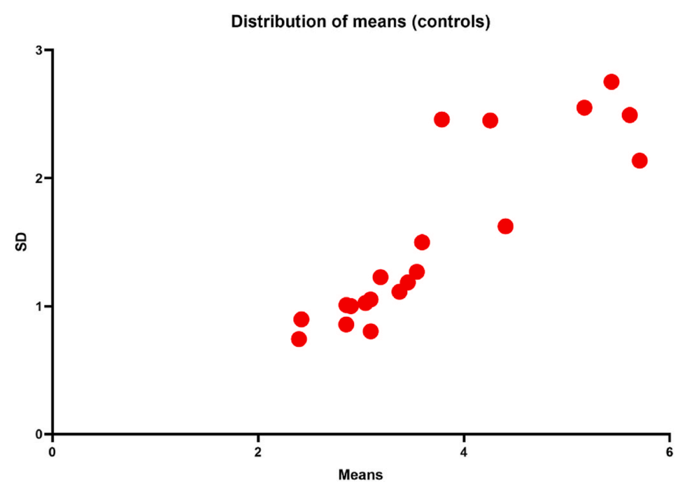


Fig. 3. Distribution of means and standard deviations (SD) for non-coincident comparisons (controls).

For Coincident comparisons, the CV values are quite high (nearly all exceeding 40-50%), indicating a high degree of dispersion among the bitemark measurements. Instead, compared to the "Coincident comparisons" group, the control group (non-coincident comparisons) generally showed lower CV values. While still high by absolute standards, many values fall within the 30-35% range, suggesting a slightly more stable distribution around the mean for random matches. It is noteworthy that the mean values in this table are significantly higher (mostly >3.0) than in the coincident comparisons' group (mostly around 1.0-1.5). This indicates, as expected, that random comparisons produce much larger differences than coincident ones.

3.3. Statistical analysis: comparison between upper and lower dental arch within the study group

For upper dental arch, the minimum mean value was 0.6733 and the maximum mean value 1.5131 with an overall sample mean of 1.1599 and standard deviation of 0.2645; the median value was 1.2873 and the variance 0.070. For the lower dental arch, the minimum mean value was

Table 3
Descriptive statistics of coincident comparisons (study group) and non-coincident comparisons (control group). C.I. = confidence interval; Inf. = inferior limit; Sup. = superior limit; S.D. = standard deviation.

Group	Statistics			Standard error
Coincident comparisons (study group)	Mean	1.2424		0.0594
	C.I. 95% (mean)	Inf.	1.1196	
		Sup.	1.3652	
	Median	1.3186		
	Variance	0.085		
	S.D.	0.2909		
	Minimum	0.6733		
	Maximum	1.6926		
Non-coincident comparisons (controls)	Mean	3.7094		0.2334
	C.I. 95% (mean)	Inf.	3.2210	
		Sup.	4.1979	
	Median	3.4141		
	Variance	1.0890		
	S.D.	1.0436		
	Minimum	2.3969		
	Maximum	5.7086		

0.7169, and the maximum 1.6926; the overall mean value was 1.3249, with a standard deviation of 0.3035; the median was 1.4399 and the variance was 0.092. The results did not show an apparently different trend in the two groups. Descriptive statistics for upper and lower dentitions are reported in detail in Table 6. Fig. 5a and b illustrates the difference between the mean values and the median values. In this case the boxplot did not show any outlier either, therefore the mean value was considered an appropriate measure of central tendency.

The Kolmogorov-Smirnov test showed a $p > 0.05$ for both the upper and lower dental arches groups, suggesting that the values of interest are normally distributed in our sample. The Shapiro-Wilk test confirmed the normal distribution for both the groups. The details of the normality tests are reported in Table 7.

The results of the *t*-test (Table 8) showed that there is not a statistically significant difference between the mean distances for upper and lower dental arches ($t = -1.420$ (22); $p = 0.170$). Moreover, Cohen's d was 0.28, suggesting a small effect (negligible practical effect) in our samples.

4. Discussion

Based on a previous experiment conducted on wax,[14] this study aimed to test the suitability of a 3D intraoral scanner to identify the dentition responsible for bitemarks inflicted on human skin. The use of human skin obtained from amputated limbs made it possible to incorporate into the method under examination all the challenges associated with the biological properties of skin (even post-mortem) such as elasticity, extensibility and resistance.

First, even though the statistical power computed for this study indicated a number of 26 subjects for each group (study and control), the creation of the groups themselves depended strictly on the amount of skin obtainable from the amputated legs. Considering the legs available, only smaller groups could be created (study = 24 and control = 20). In any case, given the limitations of the experimental conditions, the number of subjects included in the two groups can be considered reasonably close to that indicated by the statistical power calculation.

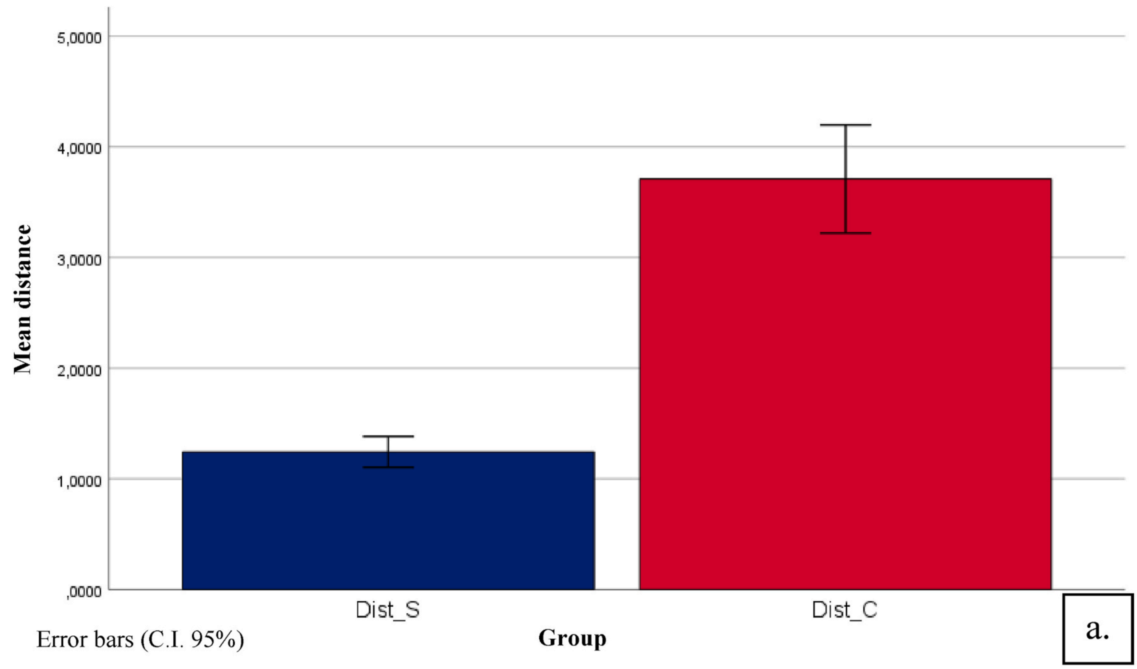
Under the experimental conditions of this study, the 3D intraoral scanner proved suitable for identifying bitemarks inflicted on human skin, although its performance was lower than that observed on dental wax. A comparison of mean distances for coincident (matching) comparisons reveals a value of 1.2424 for bitemarks on skin versus 0.24433 for those on wax; for non-coincident (non-matching) comparisons, the mean distances were 3.7094 for skin and 1.31324 for wax. This disparity, indicating a greater mean distance in the experiment on human skin, is expected, as dental wax is designed to faithfully reproduce bitemarks,[13] whereas skin is a less uniform substrate. Future perspectives include applying this method in real cases to verify the results and its applicability, as well as optimizing the instrumentation and the method itself.

Despite these biological complexities and lower performance, the primary finding aligns with the previous experiment: a significantly higher mean distance was recorded for non-coincident comparisons relative to coincident ones. On human skin, the mean distance for coincident comparisons was nearly three times lower than that of the control group. This result suggests that 3D technology may not only distinguish between a causative and a non-causative dentition on wax classically used for dental assessment but could also make it possible to detect this difference on actual human skin samples. These satisfactory results are expected to be further improved by optimizing the 3D scanning and processing workflow. The iTero® 3D scanner is primarily engineered for intraoral applications, which typically involve capturing the topography of protruding dental elements rather than depressions within a relatively smooth surface. However, this tool is equipped with technology capable of capturing any real surface with very high precision, so its application in forensic contexts, such as analysing bitemarks on skin, may not be a hardware limitation *per se*, but rather an issue of software optimization. A prospective avenue for future research would therefore be to enhance its software capabilities or develop dedicated post-processing protocols to better suit its use for forensic documentation and analysis. While the current findings already hold significant value, these future implementations could bridge the performance gap between skin and dental wax, potentially yielding comparable or even superior results. Compared to other 3D image acquisition techniques, such as photogrammetry or laser scanning, the structured light scanner, like iTero®, performs better on detailed surfaces such as bitemarks.

Anyway, one of the main limitations of this method is that the experience and the precision of the forensic specialist during the software elaboration and digital comparison of the scans can affect the final result. More specifically, the most difficult phase resulted to be the processing of the scan on MeshMixer™, and it should be highlighted that all the further comparisons depend heavily on the results of this phase where the area affected by the bite is selected. Different operators might cut the bitemark scans in different ways, leading inevitably to different results during the comparing session. Similarly, the subsequent comparison phase in CloudCompare is also subject to operator subjectivity, particularly during the manual alignment of the models in 3D space. For this reason, when conducting the analysis, the expertise of the operator in the use of the software is essential and a qualitative interpretation ultimately needs to be provided regarding the compatibility or incompatibility of the bitemark with the suspect's dentition.

There is also another limitation in this study, which is strictly dependent on the method applied. In fact, the bitemarks were produced simply by applying a standard weight on a model: this method of production clearly does not consider, nor does it reply, the action of the temporomandibular joint and the whole complex dynamics involved in the production of a bitemark in real cases. Despite it represents a major limitation, in this study these variables were deliberately excluded: firstly because the main aim of the experiment was to assess whether the 3D scanner was able to detect differences in the scansion on different lesions and whether the digital comparison allowed to detect significant differences in simple wounds; secondly, because the dental arches in the dentition models were not articulated with a temporomandibular joint. The reproduction of the complex dynamics of a real bite would have entailed the use of more complex models and the analysis of more complex wounds. Therefore, considering the aim to assess this method on simpler models, these variables (the action of the temporomandibular joint and the reproduction of the dynamics involved in the production of a bitemark) were not taken into consideration. However, these are essential further steps to be made in future studies.

Compared to the first experimental study conducted on wax, human skin gave worse results in terms of data compatibility, thus highlighting



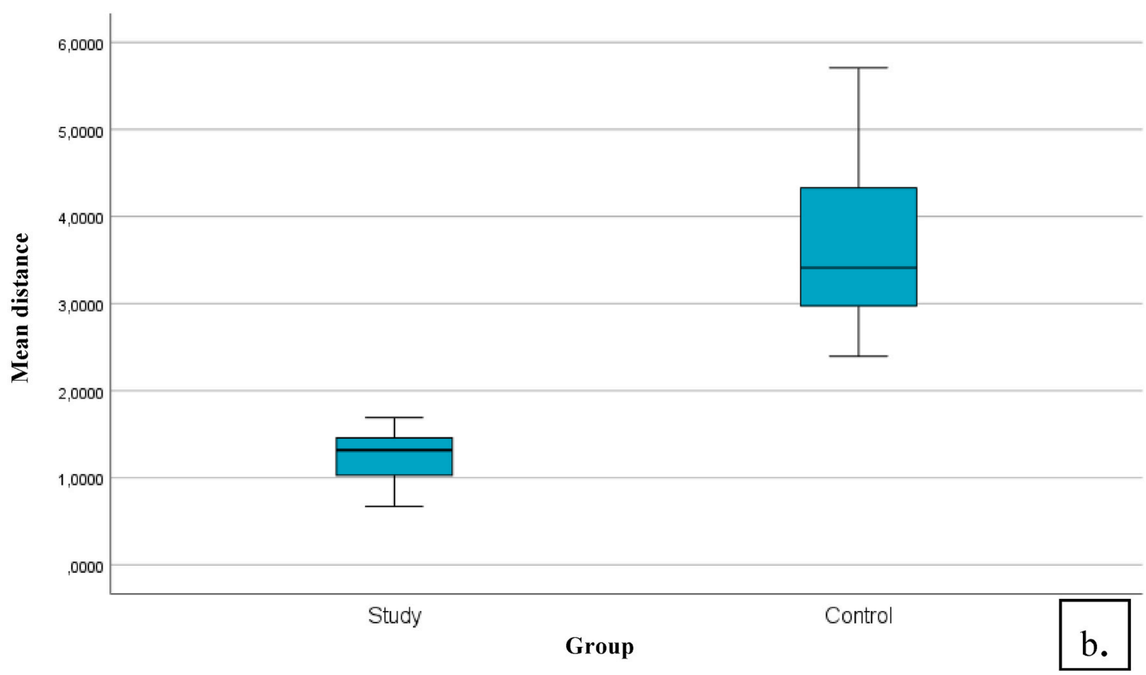


Fig. 4. a) presents a bar graph illustrating the average distance between the dentition and the bitemark, as measured using CloudCompare. The graph reveals that the average distance in the study group (coincident comparisons, Dist_S) is notably smaller than that in the control group (non-coincident comparisons, Dist_C). This indicates that comparisons between a bitemark and its causative dentition yield lower average distances than non-coincident comparisons. Furthermore, the inclusion of error bars (95% confidence interval) on the graph demonstrates the higher precision of measurements obtained when comparing a bitemark with its producing dentition. b) displays a boxplot illustrating the average distance between dentition and bitemark, as measured using CloudCompare. The plot highlights the difference in median average distances between the study group and the control group. The absence of outliers suggests that the mean of the average distances is an appropriate measure of central tendency.

the need to study all confounding factors (e.g., the contribution of the temporomandibular joint, the time elapsed between the production of the bitemark and the acquisition of the scan, etc.) before moving on to real-world applications of the method.

Future perspectives may include the application of different parameters on bitemark production and the development of dedicated software for each step of the procedure, in order to reduce the inter-operator variability. The implementation of artificial intelligence (AI) algorithms could automate the most critical and subjective phase identified in the study: processing the scan to isolate the bitemark area and alignment between the dentition and bitemark 3D scans. This would mitigate the variability introduced by manual segmentation and alignment though a standardized and automated workflow enhancing the method's objectivity and repeatability and making it more robust for future validation and forensic application.[26] However, AI may also introduce new systematic errors into the analysis if sufficient consensus among experts is not reached on the parameters provided to the system during the training phase.

Table 4
Normality tests for both groups (study group and control group). a = Lilliefors significance correction; D.f. = degree of freedom; Sign. = significance.

Group	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistics	D. f.	Sign.	Statistics	D. f.	Sign.
Coincident comparisons (study)	0.192	24	0.022	0.926	24	0.078
Non-coincident comparisons (controls)	0.194	20	0.047	0.882	20	0.019

Table 5
Mann-Whitney test. Comparison between the mean distance in the two groups. Sign. = significance.

Group	N	Mean rank	Sum of ranks
Coincident comparisons (study)	24	12.50	300.00
Non-coincide nt comparisons (control)	20	34.50	690.00
Total	44		
			Mean distance
Mann-Whitney *U* test			0.000
Wilcoxon W test			300.000
Z			−5.657
Sign. (two-tailed)			<0.001

Table 6
Descriptive statistic within the study group (coincident comparisons), considering upper and lower dental arches. C.I. = confidence interval; Inf. = inferior limit; Sup. = superior limit; S.D. = standard deviation.

Dental arch	Statistics			Standard error
Upper dental arch	Mean	1.1599		0.0764
	C.I. 95% (mean)	Inf.	0.9918	
		Sup.	1.3280	
	Median	1.2873		
	Variance	0.070		
	S.D.	0.2645		
	Minimum	0.6733		
	Maximum	1.5131		
Lower dental arch	Mean	1.3249		0.0876
	C.I. 95% (mean)	Inf.	1.1321	
		Sup.	1.5178	
	Median	1.4399		
	Variance	0.092		
	S.D.	0.3035		
	Minimum	0.7169		
	Maximum	1.6926		

It is acknowledged that an experimental model cannot fully adhere to the complexity of real-world scenarios. In practice, the dynamics of bitemark production are often complex, whereas real cases frequently yield only partial marks, such as an impression from only the upper or lower arch. However, our results seem encouraging: in our sample, there was not a statistically significant difference between the upper and lower dental arch within the study group. This means that the small difference between the means was randomly determined; moreover, it should be acknowledged that the Cohen's d was 0.28. This means that the practical effect of the difference between the groups is negligible and it should not produce concrete effects in forensic scenarios, where the conclusions of an assessment require robust support from scientific evidence that is certain or, at the very least, highly probable.

Although these results should be verified also in real casuistry, it is an important finding, since it shows that, potentially, there is no difference between an identification based exclusively on an upper or a lower dental impression on the skin of a subject.

4.1. Limitations of the experimental model

The present experimental model has several biomechanical limitations that should be considered when interpreting the results. The use of amputated cadaveric limbs does not fully replicate the physiological and biomechanical conditions of living tissue; however, it represents the only ethically acceptable approach for generating controlled bitemarks prior to potential in vivo research.[17] Post-mortem changes in tissue properties, such as reduced elasticity and altered hydration, may affect the morphology of the resulting impressions. Additionally, the absence of physiological responses—including muscle tone, active perfusion, and defensive movement—may influence the dynamics of bite application [[20],[22]]. Finally, the controlled experimental setting may not fully reproduce the variability and complexity of real-life biting events.

In any case, when considering the overall limitations of the study and the numerous potential confounding variables (e.g., operator experience, differences between flaps' thickness related to subcutaneous tissue), all these results should be interpreted with appropriate caution. Although, within an experimental model, the data reveal a statistically significant difference between coincident and non-coincident comparisons, they do not allow the identification of a threshold value capable of determining when a comparison is truly indicative of correspondence between the dentition and the injury, nor do they permit an estimation of the probability that a given dentition produced the bitemark. Therefore, the proposed methodology should be regarded as a preliminary approach that requires further investigation, including refinement of technical and procedural standards and assessment on larger samples to establish an average-distance threshold for classifying a comparison as a true positive. These limitations are typical of this area of research. However, while traditional bitemark analysis has faced significant criticism due to subjective interpretation, this objective 3D approach may address some of these concerns, even though substantial additional validation is needed before forensic application. It is not only applicable to the forensic context of assaults, but also to personal identification, and it represents an effort to bring a quantitative, reliable and reproducible method in forensic dental analysis, as previous works.[16]

5. Conclusions

This study aimed to apply a previously tested method to assess the compatibility between bitemarks and dentitions on wax to evaluate its accuracy on bitemarks produced on human skin samples. The results indicate that bitemarks artificially produced on human skin from amputated limbs are visible and can be scanned using an intraoral 3D scanner. This proves to be an effective method for acquiring morphological data from the wound, as it enables rapid recording and preservation of the three-dimensional characteristics of bitemarks on human skin. Moreover, under our experimental conditions, it was possible to distinguish between a dentition that is likely responsible for the wound and one that probably did not, also considering incomplete bitemarks (i.e. upper or lower dental arch). This finding may lead to significant implications for forensic odontology, as it advances the precision of bitemark analysis beyond traditional two-dimensional methods, expanding the possibility of obtaining more accurate representations of the complex spatial relationships and surface deformations present in soft tissues through 3D imaging and modeling.

Further researches are needed to validate these methods across a wider range of tissue types and in alive people and in post-mortem conditions and, eventually, to establish 3D scanning as a standard tool for bitemark analysis within multidisciplinary forensic investigations.

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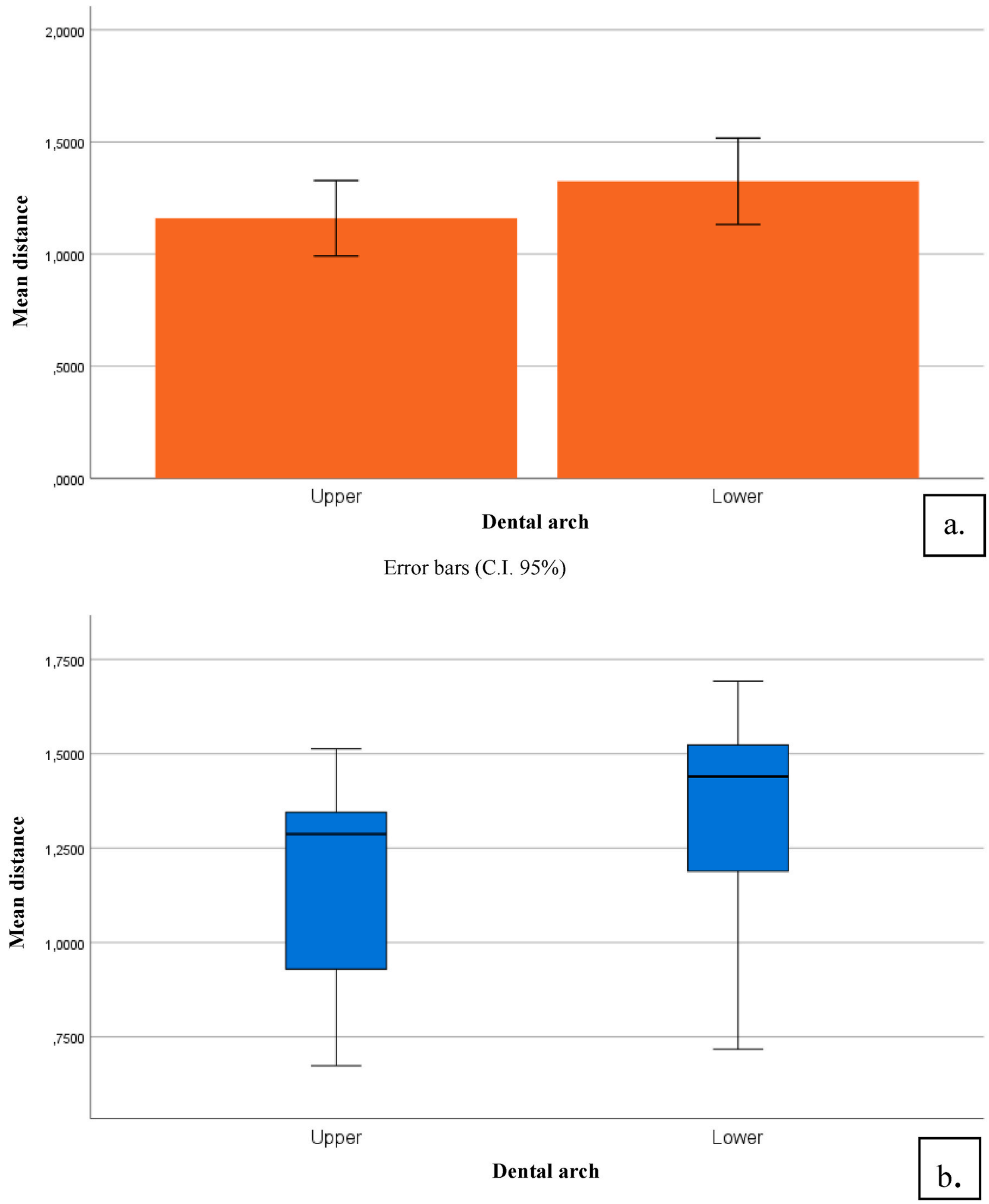

Fig. 5. a) presents a bar graph illustrating the average distance between the upper and lower dentition and the bitemark, as measured using CloudCompare. The graph reveals that the average distances for the upper and the lower groups seem to be comparable. This indicates that the comparisons between a bitemark and its causative dentition averagely does not yield a different result depending on the dental arch. b) displays a boxplot illustrating the average distance between dentition and bitemark, as measured using CloudCompare. The plot highlights the comparability of median average distances between upper and lower dental arches. The absence of outliers suggests that the mean of the average distance is an appropriate measure of central tendency.

Table 7
Normality tests the two groups of upper and lower dental arches, within the study group. a = Lilliefors significance correction; D.f. = degree of freedom; Sign. = significance.

Dental arch	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
	Statistics	D.f.	Sign.	Statistics	D.f.	Sign.
Upper	0.232	12	0.073	0.909	12	0.208
Lower	0.221	12	0.111	0.884	12	0.100

Declaration of competing interest

This research did not receive any specific grant from funding agencies in the public, commercial or not-for-profit sectors.

The authors have no competing interests directly or indirectly related to the work submitted for publication.

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Table 8
T-test. Comparison between the mean distance of the upper and lower dental arches within the study group.

	Levene's test for equality of variances					*t*-test for equality of means		C.I. of the difference (95%)	
	F	Sign.	t	D.f.	Sign. (two-tailed)	Diff. of the mean	Diff. S.E.	Inf.	Sup.
Equal variances assumed	0.014	0.908	−1.420	22	0.170	−0.1651	0.1162	−0.4061	0.0760
Equal variances not assumed			−1.420	21.597	0.170	−0.1651	0.1162	−0.4064	0.0762

	Standardizer	Point estimate	C.I. 95%	
			Inf.	Sup.
Cohen's D	0.2847	−0.580	−1.392	0.245
Hedges' correction	0.2949	−0.560	−1.344	0.236
Glass's delta	0.3035	−0.544	−1.364	0.299

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