

# Measuring Speed of Sound as a Safe Technique for the Investigation of Bone Graft Healing in Maxilla

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## Abstract:

**Background:** Depending on the insufficiency of radiographic techniques in investigating changes in grafted sites, this study aimed to evaluate the ability of measuring speed of sound (SOS) as a safe technique for the evaluation of bone graft healing.

**Materials and Methods:** A total of 20 patients in need of sinus augmentation were recruited for this study. Cone-beam CT scans were obtained presurgically, after 4 months, and after 6 months from surgery per patient. A hydroxyapatite HA and tricalciumphosphate TCP (75:25) grafts were applied for sinus elevation. Speed of Sound (SOS) measurements within the grafted sites were performed in bucco-palatal direction presurgically, directly after surgery, 6 weeks, 3 months, 4 months and 6 months after surgery. At the time of implant placement, bone core biopsies were harvested and sections from the graft core were histomorphometrically analyzed. The new vital bone formation %VB was correlated with SOS measurement outcomes using a statistical model.

**Results:** Twenty patients underwent sinus augmentation for a total of 20 sinuses. Two sinuses developed an infection after grafting resulting in a 90% success rate for the sinus grafting procedure. An 18 sinuses were used in the final statistical analysis. SOS measurements revealed a mean of 1538.18±28.02 m/s directly after surgery and 1794.21±43.54 m/s after 6 months. Histomorphometric analysis revealed that mean %VB was 20.64±5.36, mean percent of remaining graft material was 23.67±2.99, and average percent of non-mineralized connective tissue was 55.68±4.29. SOS values significantly correlated with %VB ( $r = 0.696$ ,  $P < 0.001$ ). No correlation found between radiographic density of the grafted sites after 6 months and SOS or histomorphometric results.

**Conclusions:** Measuring sound transmission velocity within the grafted sites is a safe and active technique for the expression of graft content changes and structure and evaluating graft healing.

**Keywords:** Cone-beam CT, speed of sound, graft, density.

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## INTRODUCTION

Alveolar bone density and mechanical properties were the most studied factors in many reports as an important determinants of host bone strength and available bone quality.<sup>1-3</sup> Posterior maxilla is the most interesting location studied for investigating bone quality prior to dental implant placement and reconstructive surgeries.<sup>4</sup> Bone quality is determined by range of factors, including bone density, bone mineralization, bone mechanical properties, and cellular or histologic factors that determine the internal architecture of the cancellous bone.<sup>5,6</sup> Computerized tomography CT is a common, adequate, and acceptable technique gives information about alveolar bone structure and density.<sup>7,8</sup> Cone-beam computed tomography CBCT was developed in 1998 and has become a reliable and simple technique and commonly used commercially since 2000.<sup>9</sup> CBCT has been shown to be a precise imaging modality and is a valuable tool for use in dental applications.<sup>10</sup> It was also used to measure bone density with dental implants.<sup>11</sup> This imaging technique is now used worldwide; it is a reliable and credible alternative to CT scan for dentomaxillofacial imaging.

CBCT offers less radiation than computed tomography (CT) in 3D image construction; this technique is compatible with dental implant software. The major diagnostic advantage is the exquisite spatial resolution while the main pitfall is the poor contrast of face and neck soft tissues, due to a lower density resolution compared to CT scan.<sup>8,12,13</sup> CBCT can provide identical information to multislice computed tomography (MSCT), with a considerable dose reduction when panoramic radiography is not sufficient in the study of the teeth and jaw bones.<sup>14</sup> The major disadvantages of all CT scanners is the high cost in installing the device itself and the cost implications on the patient as well.<sup>15</sup> In the other hand, these techniques are only available in hospitals or large scientific or educational centers. Despite the fact that CBCT reduces x-ray exposure approximately 51% to 96% of the radiation used in conventional CT scans, x-ray radiation dose used in currently available CBCT scanners is between 52 and 1025( $\mu$ S), depending on the type of device and imaging protocol; This is the dose required to obtain 4 to 77 digital panorama.<sup>16</sup> These disadvantages underline the need for a noninvasive and reliable method to assess the expected mechanical bone quality presurgically.

Despite the great advances in CT scanners, recent researchers showed insufficiency of radiographic techniques in the detection of graft material changes during healing period, and radiographic images give poor information about grafted site content and properties.<sup>17,18</sup> Histomorphometric studies still the gold standard but invasive technique in the assessment of grafts healing in human.<sup>7</sup>

In 1981, early steps started attempting the investigation of the internal characteristics of tissues by using ultrasound transmission velocity (UTV) measurement.<sup>19</sup> Njeh et al.<sup>20</sup> performed an in-vitro experimental study on animal bone fractures using a device for measuring speed of sound SOS. In 2003, Carstanjen et al.<sup>21</sup> studied the correlation between speed of sound values within the bone and bone mineral density using dual energy x-ray absorptiometry DXA on horses. It is concluded that measuring speed of sound is a valid technique for the assessment of mechanical properties of bone. Sarvazian et al.<sup>22</sup> studied changes in speed of sound values according to the type of studied tissue and its content of fluids. Rose et al.<sup>23</sup> evaluated the benefits of measuring speed of sound SOS to investigate the maturation of cortical bone compared with the mechanical properties in pig's mandibles and results were very promising. In 2006, AL-Haffar et al.<sup>24</sup> conducted a study to evaluate the use of speed of sound (SOS) as a safe ultrasound diagnostic tool to assess bone quality prior to dental implant placement. A total of 14 cadaver mandibles enrolled in this in-vitro study to investigate the correlation between local bone mineral density BMD and SOS or ultrasound transmission velocity UTV ( $R^2 = 0.68$ ,  $P < 0.0001$ ).

The assessment of ultrasound transmission velocity (UTV) or speed of sound SOS has been introduced as a fast, reliable and noninvasive method to analyze mechanical properties of bone as a fundamental criterion prior to dental implantation.<sup>19</sup> In this technique, the speed of an ultrasonic signal or pulse conducted through the bone is calculated. This signals need a transmitting medium such as water, muscles, or bone and don't cross in the air.<sup>24</sup>

In a human study, Klein et al.<sup>19</sup> used a small UTV device for the recording of intra-oral UTV values in a large and heterogeneous patient collective for the first time. Measurements of speed of sound conducted within the alveolar bone in 6 different sites of interest selected in 108 patients. SOS values categorized according to age, gender, jaw and measured site. Veltri et al.<sup>25</sup> studied the relationship between SOS values and the implant final insertion torque

values in rabbits and concluded that the measurement of speed of sound is a safe and useful presurgical tool in evaluating bone mechanical properties. Kumar et al.<sup>26</sup> concluded that types of bone can be discriminated according to speed of sound values when correlating resonance frequency analysis RFA values with SOS measurements on different types of bone. Kammerer et al.<sup>27</sup> conducted a study to evaluate ultrasound transmission velocity (UTV) for the assessment of mechanical bone quality, an ex vivo comparison of different bone types measured with UTV, 2-dimensional (2D) histomorphometry and with 3-dimensional (3D) radiology (cone-beam computerized tomography [CBCT], computerized microtomography [ $\mu$ CT]). It is concluded that UTV is able to discriminate between different bone types ex vivo.

Depending on the insufficiency of radiographic techniques in detecting changes in the properties of graft material, and the absence of the application of SOS technique in the assessment of bone graft healing in the medical literature, this study aimed to evaluate the ability of measuring speed of sound (SOS) as a safe technique for the evaluation of bone graft healing after maxillary sinus augmentation.

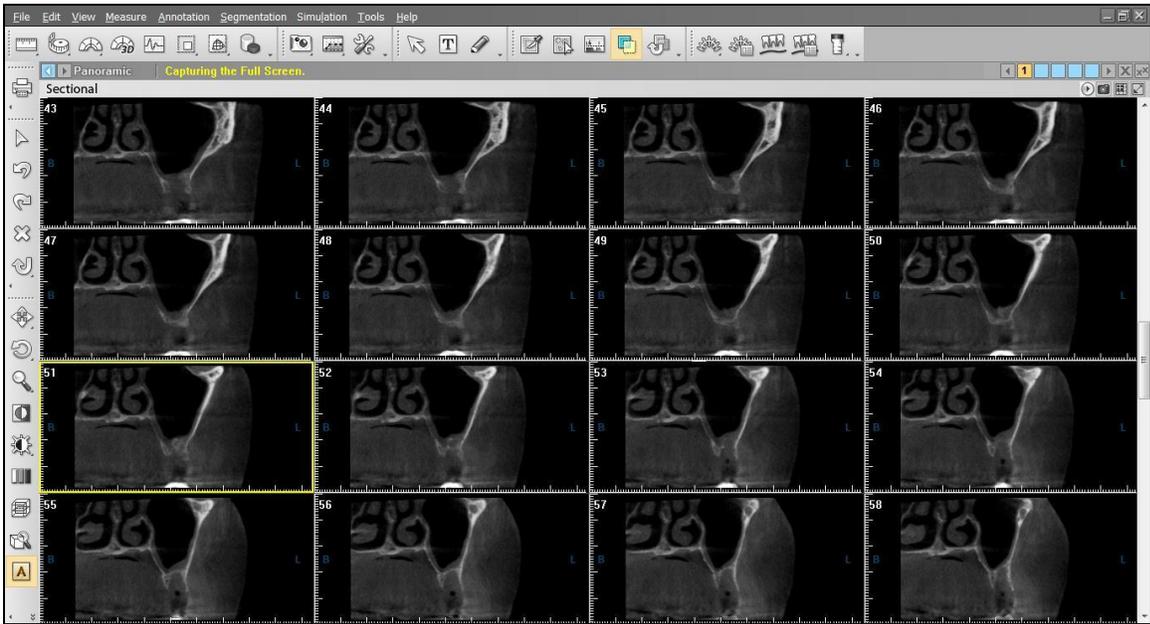
## MATERIALS AND METHODS

### *Case Selection*

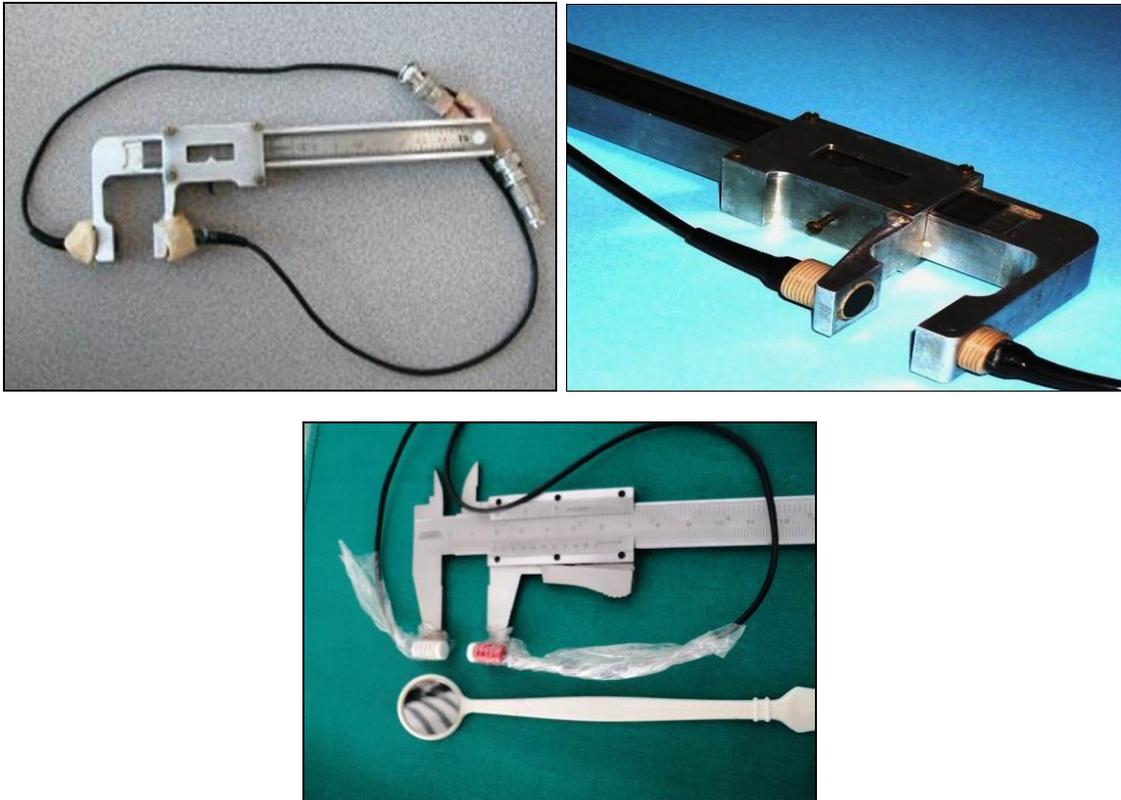
Patients from Damascus University attended in the Implantology Clinic in the Faculty of Dentistry. Patients were in need of either unilateral or bilateral sinus augmentation and were included in the study according to the following criteria: 1) adult patients 18-60 years in need of sinus augmentation, 2) residual bone height RBH  $\leq$ 5mm assessed in cone-beam CT scans obtained presurgically, 3) classification of physical status of I or II according to the American Society of Anesthesiologists. Exclusion criteria included long-term use of antibiotics in the past 3 months; use of medications known to affect bone metabolism; smoking >10 cigarettes per day; alcoholism and pregnancy; severe acute or chronic sinus pathology. Patients who met the criteria and agreed to participate in the study were required to sign an informed consent. The study was conducted from October 2011 to April 2013.

### *Preoperative Sequence*

A cone-beam computed tomography (CBCT) scan was made with a cone-beam machine (Vatech Uni3D device, Korea) for the quantitative and qualitative assessment prior to surgery (fig. 1). Ultrasound transmission values UTV or speed of sound SOS values were measured bicortically in buccal–palatal direction at the site of interest SOI (grafting site) using a prototype measuring instrument designed especially for Al-Haffar et al. 2006 study<sup>24</sup>. This instrument consists of ultrasonic generator device with a continuous 1.6-MHz ultrasound waves. The generator is attached to ultrasonic transmitter and detector fixed on a proper caliper (Fig. 2). A processor attached to a computer with specialized program used to calculate the velocity of the ultrasonic waves between the transmitter and the detector. For each measurement site, the mean value of three individual SOS-measurements were calculated with repositioning of the device. Presurgically, SOS measurements revealed the value of 0 m/s since the ultrasonic signals don't cross air within the maxillary sinus. Patients were requested to take an antibiotic (amoxicillin + clavulanic acid for 10 days, starting 2 days before the surgery or clindamycin in case of allergy to penicillins).



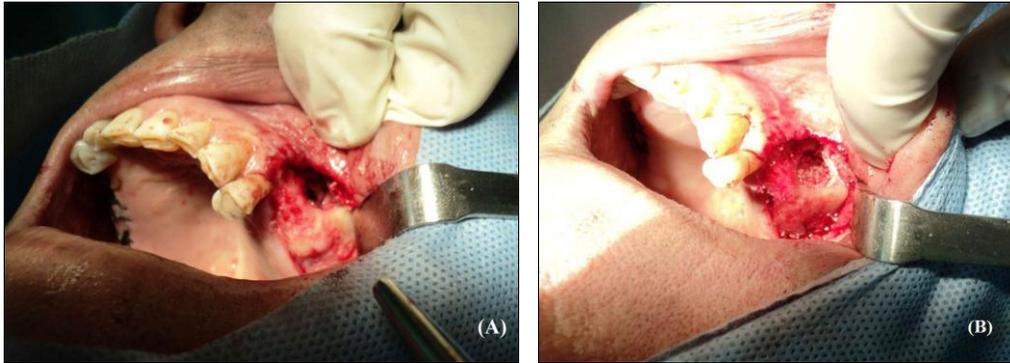
**Fig. 1: Presurgical diagnostic CBCT.**



**Fig. 2: Prototype SOS-measurement instrument used in the study.**

### *Surgical Procedure*

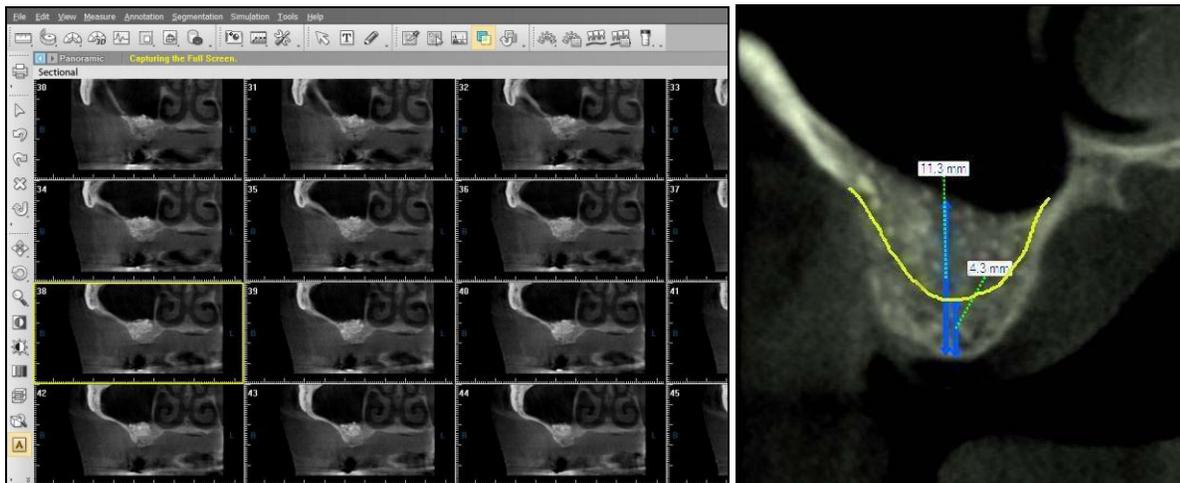
Surgeries were performed under local anesthesia (Lidocaine HCL 2% with Epenephrine 1:80000, Kwang Myung Pharm, Korea). The incision extended between the remaining teeth or from the remaining teeth to the tuberosity in cases of edentulous distal extension. A releasing incision was made when necessary to gain appropriate access. A full thickness mucoperiosteal flap was elevated. Then, a window was delineated with a round diamond bur and careful elevation of the sinus membrane was performed using sinus membrane elevators (fig. 3 A). An alloplast consists of 75% hydroxyapatite HA and 25% tricalciumphosphate TCP (adbone@BCP, Medbone, Medical Devices, Portugal) with a particle size ranging from 0.1 to 0.5 mm, was used to fill the sinus cavity to obtain a minimum height of 12 mm from the alveolar crest, and to fill up completely to the borders of the lateral window (fig. 3 B). In all cases, an absorbable collagen material (Biocollagen, BioTeck®) was placed over the window. Soft tissues were sutured using 3-0 silk incision.



**Fig. 3: Bone window and a perforation in sinus membrane can be noticed (A). Alloplast was applied to fill the elevated sinus and the bone window completely filled up (B).**

### *Postoperative Care And Follow-Up*

Patients were provided instructions for surgical procedures and continued the antibiotic regimen for 7 days. A prescription for analgesic and anti-inflammatory medication (potassium declofenac 50 mg 3 times a day ) was given to all patients. Patients were seen 10 days after the surgery for suture removal. Cone-beam CT scans performed per patient 4 and 6 months after the surgery (fig. 4). SOS measurements conducted in the sites of interest SOIs directly after surgery, 6 weeks, 3 months, 4 months, and 6 months after the surgery (fig. 5).



**Fig. 4: Cone-beam CT scan after 6 months from sinus augmentation.**



**Fig. 5: Application of SOS instrument in region of interest.**

### *Bone Biopsy Harvesting*

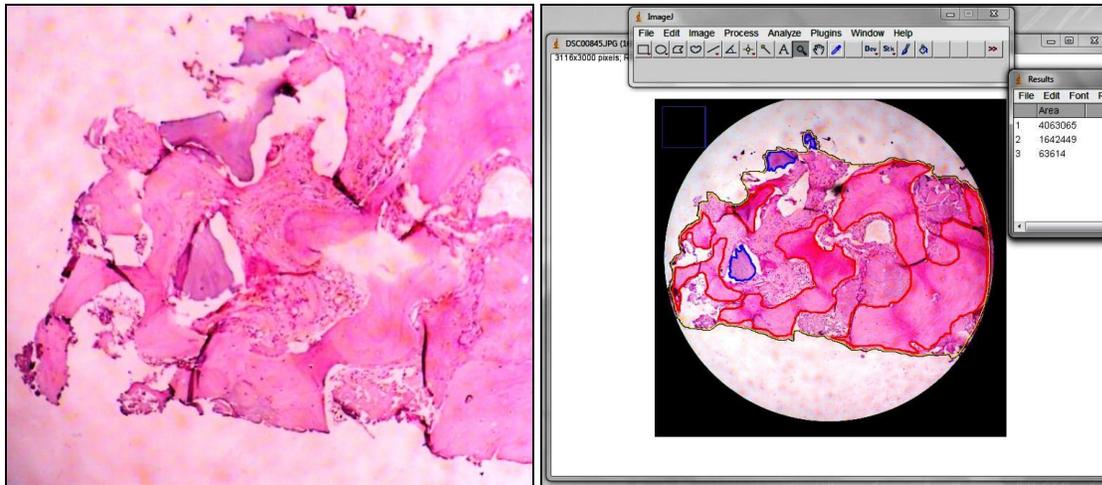
Implant placement was performed approximately 6 months after sinus augmentation. A total diameter of 2.2 mm and 1.8 mm internal diameter trephine (Medicon<sup>®</sup>, Germany) was used for the harvesting of a core biopsy from the graft material for each implant location after the use of pilot drill in residual alveolar bone (fig. 6). The drilling sequence continued to the planned implant length and diameter.



**Fig. 6: Trephine used for graft core harvesting.**

### *Histomorphometric Analysis*

After the histologic preparation of graft core biopsies, samples were analyzed using a bright-field optical microscope with a digital camera (Kyowa<sup>®</sup> Medilux-20, Japan). Three slides of each core specimen were randomly selected and analyzed. Images of the samples were captured at the same magnification (x4). A quantification of the vital bone %VB, remaining graft material (%RG), and soft tissue (%ST) was performed using specialized software (ImageJ<sup>®</sup>, Bethesda, USA) in each slide and the mean of the three slides results is recorded as a final variable (fig. 7).



**Fig. 7: Analyzing the percent of new vital bone formation %VB, soft tissue %ST, and residual graft material %RG by measuring areas using Image J software.**

### *Statistical Analysis*

In this study, the unit of analysis is the patient. The association of SOS with the %VB was quantified as a correlation coefficient. a *P* value <0.05 was considered statistically significant. Data analyses were performed using SPSS v.16 and Excel 2010 statistical softwares.

## RESULTS

### *Study Population*

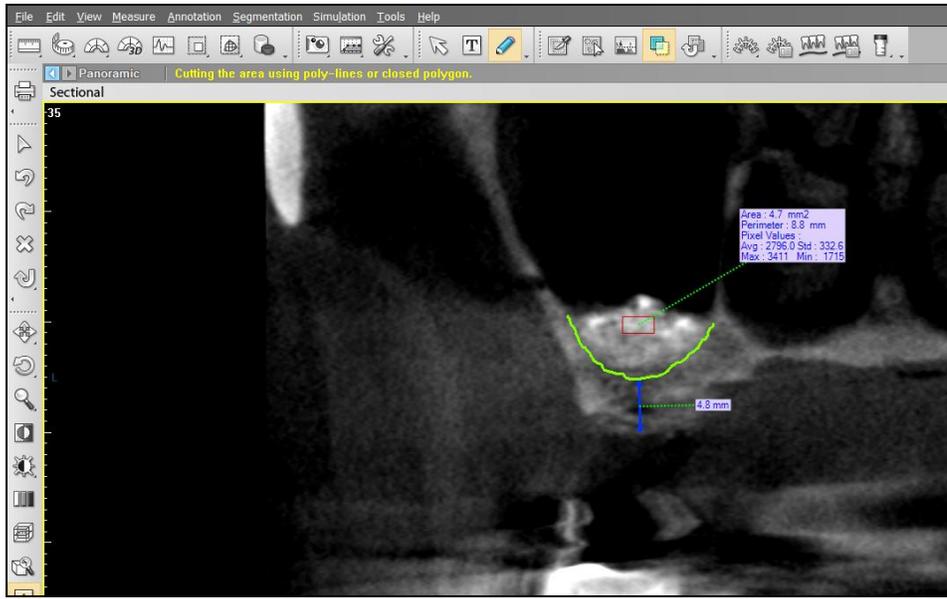
A total of 20 patients attended in the Implantology Clinic at the Faculty of Dentistry, Damascus University enrolled in this study. Two patients dropped out after developing sinus infection; 18 sinuses for 18 patients (11 males and 7 females) with mean age  $41.83 \pm 5.5$  years (range, 33 to 52 years) were included in this study.

### *Clinical Findings*

A 20 sinus augmentation surgery were performed (all patients received unilateral sinus augmentation). Two patients developed an infection after surgery and excluded from final measurements. The incidence of sinus membrane perforation was 10% (two sinuses). The remaining 18 patients completed the study. 31 implants were applied after approximately 6 months from first surgery.

### *Radiographic Findings*

Cone-beam CT scans were obtained per patient presurgically and at the follow-up times of 4 and 6 months after surgery. Radiographic densities at the grafted sites were measured in all section plans and revealed a very high mean values of  $2511.04 \pm 217.48$  and  $2639.62 \pm 401.91$  for the follow-up times 4 and 6 months respectively. (fig. 8) and (Table 1).



**Fig. 8: Cone-beam CT scan after 6 months from sinus augmentation reveals very high radiodensity values within the grafted site.**

**Table. 1: Mean radiographic density at the grafted sites in all plans at the follow-up times 4 and 6 months after sinus augmentation**

Patient ID	Mean SOI radiodensity after 4 months	Mean SOI radiodensity after 6 months
1	2356.7	2468.8
2	2458.4	2348.5
3	2156.8	2286.5
4	2632.5	2379.8
5	2463.9	2358.8
6	2534.1	2421.9
7	2436.5	2731.8
8	2231.5	2391.8
9	2928.3	2342.1
10	2435.4	3127.5
11	2647.5	3104.8

12	0	0
13	2634.8	3514.8
14	2431.6	2394.8
15	0	0
16	2736.4	3526.4
17	2683.6	2698.9
18	2463.5	2465.8
19	2834.6	2493.8
20	2132.6	2456.4

### *SOS Measurement Results and Histomorphometry*

Table 2 shows SOS values in all follow-up times. Figure 9 shows that SOS values increase from the first follow-up point (directly after surgery) and decrease 3 months after sinus augmentation. A gradual, but sharp increase noticed in the period between 3-6 months after surgery in a similar pattern in all cases. A total of 31 biopsies were harvested. Seven of these biopsies could not be analyzed because of deterioration but 18 biopsies from the 18 patients were obtained successfully. Histomorphometric analysis revealed that mean %VB was  $20.64 \pm 5.36$ , mean %RG was  $23.68 \pm 2.99$ , and average %ST was  $55.68 \pm 4.29$  (Table. 2)

**Table. 2: SOS values at the follow-up times and histomorphometric results after 6 months**

Patient ID	SOS Presurgically	SOS directly after surgery	SOS after 6 weeks	SOS after 3 months	SOS after 4 months	SOS after 6 months	Histomorphometry		
							%VB	%RG	%ST
1	0	10.4.8	107.0	104.8	109.7	18.3.4	27.11	20.23	47.77
2	0	1028.4	1073.3	1097.7	1789.4	170.7	20.71	27.91	47.38
3	0	1027.2	109.1	170.3	171.7	180.7	28.01	22.49	49
4	0	1039.1	1044.9	103.7	177.7	178.7	21.78	24.77	03.47
5	0	1073.9	1074.2	10.9.7	1097.4	189.7	24.88	19.37	00.70
6	0	1019.4	1078.2	17.4.8	170.4	1703.4	14.29	27.04	08.17

7	0	1000.1	1701.7	1098.4	1732.2	1789.7	17.82	27.94	00.24
8	0	1079.4	1044.9	1093.0	1784.4	1709.4	17.12	23.11	70.77
9	0	1048.2	1024.0	1079.0	1737.4	1779.7	10.73	24.22	70.00
10	0	1009.2	1701.7	1098.3	1707.9	1837.9	24.91	19.44	00.70
11	0	1033.8	1079.4	1037.4	1780.4	1789.7	13.71	27.19	70.2
12	0	1043.3	1033.0	1001.8	.	.	0	0	0
13	0	1073.8	1079.2	1089.4	1784.9	1737.0	13.07	20.29	71.10
14	0	1072.1	1047.4	1073.7	1738.9	1809.7	20.78	18.12	07.1
15	0	1079.9	1097.7	.	.	.	0	0	0
16	0	1033.4	1071.1	1037.4	1777.7	1788.8	17.18	27.2	07.72
17	0	1021.7	1048.2	1084.9	1703.3	1843.1	27.27	19.88	02.87
18	0	1022.2	1071.8	1001.3	1078.1	1842.3	24.07	21.11	04.83
19	0	1010.4	1709.3	1700.4	1799.9	1708.8	18.33	23.81	07.87
20	0	1097.3	1000.4	1077.9	1700.0	1741.3	10.93	24.08	09.49

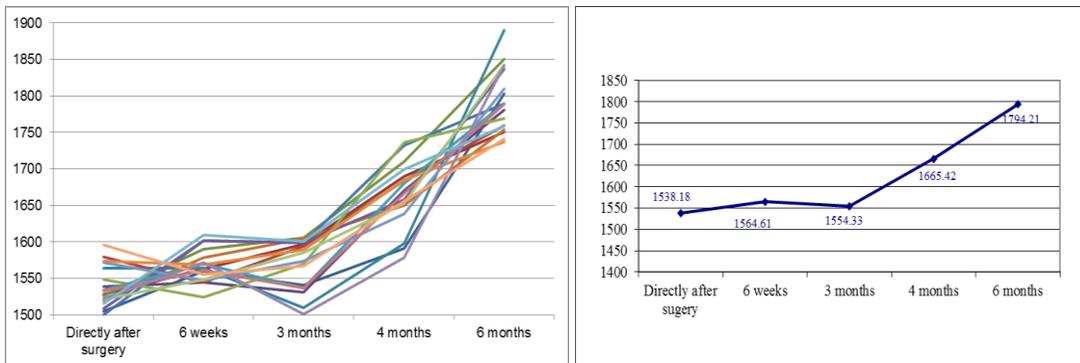


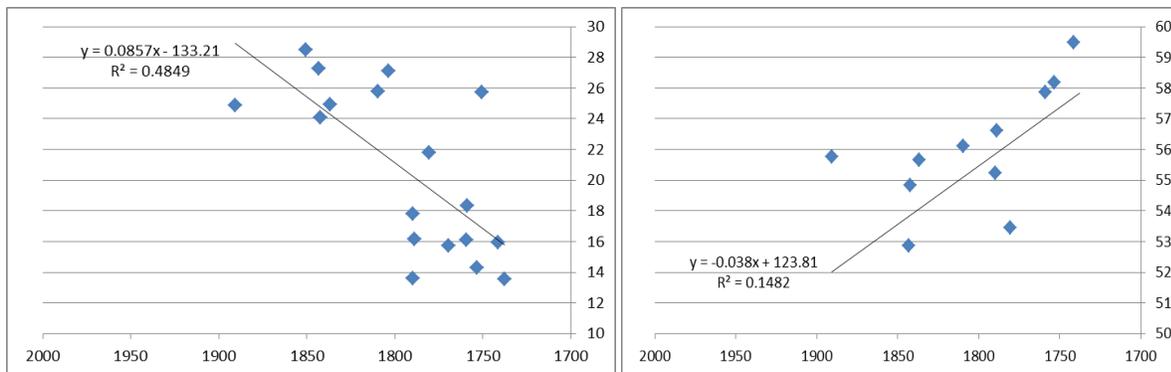
Fig. 9: Changes patterns of SOS values in all studied patients within follow-up periods (left); Changes of SOS averages (right).

## Correlation

Analysis of the correlation between SOS values and %VB by Pearson's correlation coefficient, showed a strong positive association ( $r = 0.696$ ,  $P < 0.001$ ). A negative association recorded when correlating SOS values and soft tissue %ST presented within the histologic samples ( $r = -0.385$ ,  $P < 0.001$ ). This means that as %VB increases, SOS increases; and as %ST increases, SOS decreases (Table 3) (fig. 10). No correlation found between radiographic density of the grafted sites after 6 months and SOS or histomorphometric results.

**Table. 3: Pearson's correlation coefficient between SOS values and %VB and %ST**

Variable 2	Variable 1: SOS after 6 months			Association
	Pearson (r)	n	Significance	
%VB	0.696	18	<0.001	Positive
%ST	-0.385	18	<0.001	Negative



**Fig. 10: A diagram shows positive correlation between SOS and %VB (left); negative correlation between SOS and %ST (right).**

## DISCUSSION

Placement of implants after bone grafting in posterior maxilla poses a challenge due to poor bone quantity and quality, and difficulties that merge during diagnosis phase or within the surgical procedure.<sup>4</sup> Current CBCT scanners are considered the most accurate, reliable, and gold standard diagnostic tools for the quantitative and qualitative presurgical assessment of the target site.<sup>28,29</sup> Meanwhile, Stoppie et al.<sup>30</sup> concluded that with the current CT scan technology, predictions of the mechanical properties of trabecular jaw bone based on Hounsfield values were only valid for jaws with a thin layer of cortical bone. For jaws with a thicker cortical layer, the prediction of the mechanical properties decreased significantly. Until now, no valid presurgical, noninvasive method has been clinically established to assess mechanical bone properties before implantation, including applications based on dental CT. Furthermore, CT techniques are insufficient in investigating bone graft healing due to the lack in the ability to detect graft material changes.<sup>17,18</sup> Histomorphometric study is another gold standard, but invasive technique for the evaluation of bone density and bone graft healing in human.<sup>29</sup>

Padilla et al.<sup>31</sup> investigated at the proximal femur the relationships between SOS values and bone properties measured by x-ray CT and  $\mu$ -CT. He suggested that SOS measured at the proximal femur are appropriate to assess bone status and structure (connectivity). In 2008, Woolf and Akesson mentioned the importance of high-frequency

ultrasound in giving information about bone mass, bone structure, and bone material features.<sup>32</sup> Taken together, previous facts made us speculate that SOS is a valid tool in assessing the internal structure and the connectivity within the graft after sinus augmentation. We conducted this study to evaluate SOS technique in such goal by correlating SOS values with histomorphometric outcomes.

Data from our study show that mean %VB obtained was  $20.64 \pm 5.36$ , mean %RG was  $23.67 \pm 2.99$ , and average %ST was  $55.68 \pm 4.29$  after 6 months using 75% HA mixed with 25% TCP. These findings are in agreement with studies that evaluated sinus augmentation outcomes using an alloplasts. Cordaro et al.<sup>33</sup> reported a mean %VB of  $21.6 \pm 10.0\%$ , Kurkcu et al.<sup>34</sup> showed a proportion of  $21.09 \pm 2.86\%$  using TCP alone, whereas Franken et al.<sup>35</sup> reported a value of  $27.3 \pm 4.9\%$  after 6 months using 60% HA with 40% TCP, Friedmann et al.<sup>36</sup> showed a mean %VB of  $38.8 \pm 5.89\%$  after 6 months. Differences in %VB between studies could be explained either by the variability in the waiting time to harvest the samples after sinus augmentation or the differences in graft mixture contents ratio.

In this study, mean SOS measured directly after surgery was  $1538.18 \pm 28.02$  m/s,  $1564.61 \pm 20.87$  m/s after 6 weeks,  $1554.32 \pm 34.53$  m/s after 3 months,  $1665.42 \pm 44.3$  m/s after 4 months, and  $1794.21 \pm 43.54$  m/s 6 months after surgery. A diagram in figure 9 shows the same pattern of SOS changes during follow-up times in all studied patients. SOS values increase from the first follow-up point and decrease 3 months after sinus augmentation. These low SOS values directly after surgery could be explained by the loose structure of HA and TCP granules mixed with blood directly after application. TCP granules are rapidly resorbed after 3 months making a space for a non-mineralized tissue within a hydroxyapatite scaffold.<sup>37</sup> After that, sharp increase occur in SOS values in the period between 3-6 months after surgery. Histologic samples showed a new bone formation within the graft material. This means the existence of bone bridges within the hydroxyapatite scaffold after 6 months leading to the increase in SOS measurements.

No study found in the literature using SOS within the graft material for the assessing of bone graft healing. In the Klein et al.<sup>19</sup> study, a small SOS instrument (DBM Sonic 1200, Italy) with 1.25 MHz pulsed ultrasonic waves was used for the assessment of intra-oral SOS. This study showed significantly higher values both for mandibular side regions (female  $1713 \pm 153$  m/s, male  $1734 \pm 221$  m/s) and the maxillary frontal region (female  $1665 \pm 189$  m/s, male  $1648 \pm 82$  m/s) than for maxillary side regions (female  $1538 \pm 177$  m/s, male  $1583 \pm 90$  m/s). Al-Nawas et al.<sup>38</sup> showed that SOS values less than 1767 m/s reveals a compromised mechanical properties in cadaver mandibles.

Statistical analysis of the correlation between SOS and %VB in our study showed that as the proportion of VB increases, SOS increases ( $r = 0.696$ ,  $P < 0.001$ ), and as the proportion of ST increases, SOS decreases ( $r = -0.385$ ,  $P < 0.001$ ). These results confirm the hypothesis that measuring speed of sound is appropriate to assess the internal structure and the connectivity within grafted sites.

Cone-beam CT scans are used to measure radiographic density at the grafted sites. It revealed a very high values of  $2511.04 \pm 217.48$  and  $2639.62 \pm 401.91$  for the follow-up times 4 and 6 months respectively. This can be explained by the prolonged existence of hydroxyapatite particles which need longer time to resorb. No significant correlation found between graft radiodensity after 6 months and %VB or SOS values. These results support the fact that radiographic techniques give poor information about the internal structure of grafted sites. Bridges of new woven bone with a considerable density start to form after 6 months of sinus augmentation.<sup>39</sup> In this phase, bone remodeling starts for the formation of mature lamellar bone.<sup>37</sup> This explains the increasing values of SOS 6 months after surgery and suggests more waiting time for more follow-up points.

## CONCLUSIONS

Measuring ultrasound transmission velocity UTV or speed of sound SOS within the grafted sites is a free x-ray, safe, simple, and active technique for the expression of graft content changes and structure and to evaluate graft healing. Considering the limitations of this study, it is recommended to conduct a similar study on a large-sized population or longitudinal studies using different types of graft materials.

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