Strontium Ranelate Effects on Inorganic Bone Grafts in Maxillary Sinus Floor Augmentation

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The maxillary sinus floor augmentation surgery is one of the techniques of restoration of bone quantity of the superior maxilla in partially or totally edentulous patients. However this technique could be associated with delays for final implant placement. Depending on the height of the residual bone, the period of osseointegration varies between 6 and 12 months. Therefore the aim of our study was to investigate if strontium ranelate improves osseointegration of maxillary sinus bone xenograft. Two surgery interventions (maxillary sinus augmentation) were performed on the same patient, at an interval of 6 months between them. The patient received strontium ranelate (Osseor, Servier Pharma, 2g/day, for 6 months) only after the first surgery. Cone-Beam CT exam (CBCT) of the maxilla was performed after each stage. After each surgery intervention including a 6-month healing period, the implants were inserted and bone core was harvested from both maxillary sinuses. The bone core was sent for histologic examination, automatic nuclear segmentation and microindentation tests. The results of the tests (histologic analysis, automatic nuclear segmentation and microindentation tests) were compared. We found out that the antiosteoporotic agent improved bone microarchitecture and bone mechanical properties. This study demonstrates that the osseointegration of the innorganic bovine bone graft can be influenced by Strontium ranelate (Osseor).

Keywords: bone graft, microindentation tests, automatic nuclear segmentation, strontium ranelate, sinus augmentation

The use of implant-prosthetic rehabilitation in the posterior maxilla is often complicated by insufficient bone offer, as a consequence of the extensive resorption and pneumatization of the maxillary sinus due to edentation. New surgical techniques have been developed to treat the loss of vertical bone height in the posterior maxilla. This involves sinus augmentation with elevation of the sinus mucosa and filling of the created space with innorganic bovine bone, but also with autogenous or mixed, autogenous and bone substitute materials [1].

Different drugs can be used to improve bone quality [2]. Strontium ranelate (SR) was shown to increase mechanical fixation of implants, on a rat model, when compared with controls, and improved the micro-architecture of the bone surrounding the implant. Additional effects of SR on biomechanical properties of bone tissue, led to excellent osseointegration, at both trabecular and cortical levels [3].

SR influences the phases of bone remodelling, stimulating bone formation and reducing bone resorption. SR has a dual action: it stimulates bone formation through its positive action on osteoblast differentiation and function, and decreases osteoclast differentiation as well as function. This enhances the equilibration of osseous turn-over in favour of bone formation [4]. Similar studies on the influence of various materials were done on bone grafts, having different impacts on these [5-9].

We have proposed to demonstrate that the administration of SR (Osseor) in sinus augmentation influences the processes of bone remodelling and that it improves the quality of the newly formed bone.

Experimental part

Materials and methods

In the study 6 patients were recruited for a bilateral sinus lift, two-step protocol, according to the folllowing inclusion criterias: totally edentulous or bilateral partially edentulous in the premolar/molar region, the height of the remaining alveolar bone 3-5 mm. The following exclusion criteria were established: smokers, cardiac pathology or any significant systemic disease, recent maxillary tooth extraction in the posterior region (less than 1 year), maxillary sinus disease. The mean age was 47.83 years (range 27 – 66 years).

The initial patient assessement included a detailed clinical examination and a preoperative ortopanto-mography.

Two surgical interventions (maxillary sinus augmentation) were performed on the same patient, at an interval of 6 months. The patient received SR (Osseor, Servier Pharma, 2g/day, for 6 months) only after the first operation.

This research project was conducted according to the World Medical Association Declaration of Helsinki — Ethical Principles for Medical Research Involving Human Subjects. The study received approval from the Ethics Committee of *Gr.T.Popa* University, Faculty of Medicine, Ia°i, Romania. The patient signed a detailed *informed* consent.

First stage surgery

Sinus augmentation was performed in the right maxillary sinus, using the lateral approach technique [10] and bovine bone substitute material for natural bone regeneration (Bio-Oss, Geistlich, 1g). The patient received SR (Osseor, Servier

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Pharma) from the day of the surgery 2g/day, for 6 months. CBCT of the maxilla was performed to evaluate bone volume.

Second stage surgery

After 6 months, a second sinus augmentation of the left maxillary sinus was performed following the same protocol (without postoperative administering of SR).

During the same stage, the implants (Tag Dental Implants) were inserted in the right upper quadrant. Bone core was harvested from the right maxillary sinus using a 4.7 mm diameter trephine under abundant saline irrigation. The biopsy was taken from the alveolar crest, from the future site of the implant. The core was obtained to a mean depth of 8 mm. The bone core was sent for histopathologic examination, automated image segmentation and microindentation tests. Maxillary CBCT was performed again.

Third stage surgery

After a healing period of 6 months, implants were inserted in the left upper quadrant. Bone core was harvested from the left maxillary sinus following the same protocol. The bone core was sent for the same analysis.

The bone cores were harvested by the same surgeon.

Cone – Beam CT analysis

All radiographic evaluations of patients were carried out with the use of Planmeca ProMax 3D Mid CBCT (Planmeca, Finland) with a field of view of 10x10 cm, voxel size 200μm, anode voltage 90 kV, anode current 10 mA, exposure time 12.08s and dose area product (DAP) 655.7mGy x cm^2. Using the software Planmeca Romexis Viewer 3.0.1, we assessed the integrity of the sinus mucosa obtained bone volume, the height of the total available bone and the thickness of the alveolar crest in order to insert the implants.

Tissue preparation and histologic examination

Collected samples were immediately fixed in 10% formalin, decalcified and embedded in paraffin according to standard protocol [11]. Sections of 4 μm were cut. The histological sections were stained using Hematoxylin and Eosin and van Gieson. In van Gieson staining, the viable bone tissue stained red, and the remanants of xenograft particles stained yellow. The histomorphometric analysis of the sections was performed as a real measurement using a standard light microscope (Leica DM 2500) at 50x magnification interfaced with a computerized morphometric system (Leica Application Suite version 4.0.0). Representative areas were photographied using a Leica DFC 500 camera and their surface was measured. Tissue preparation and the histomorphometric analysis were performed by the same pathologist, unaware of the stage in which SR was administered.

Automatic nuclear segmentation

The nuclear counting was performed using a digital light microscope (*Zeiss* AxioObserver *Z1)* and a PixeLINK PL-A622C camera. The specimens (not yet osseointegrated bone graft stained in yellow, revitalized bone graft or native bone stained in red, and stromal areas) were individually marked. Cellular data analysis were automatically performed by a computerized morphometric system (HistoQuest 3.5.3.0185, TissueGnostics).

Nuclear segmentation in HistoQuest is completely automatic after the input of a few starting values: average nuclear size, discrimination by area (exclusion of smaller

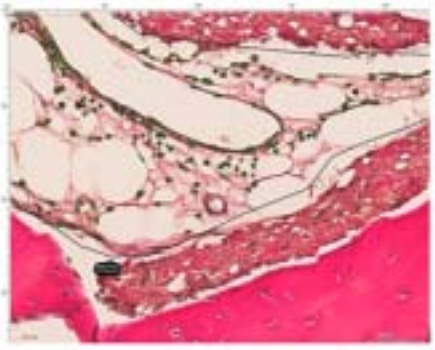


Fig. 1. Automatic nuclei segmentation image

nuclear sections), discrimination by gray value (exclusion of weakly stained nuclei), background threshold (default setting is automatic) [12].

The density of cells from the stromal area and from the newly formed bone, as well as the percentage of new bone formation were assessed.

Microindetation tests

Microindentation tests (The Rockwell hardness test, Young's modulus) assessed the intrinsic mechanical properties of bone tissue. A micro-hardness tester (CETR UMT-2 Multi-Specimen Test System) was used for this purpose. A conical diamond indenter (Rockwell indenter) was pressed into the bone block and force-displacement data were recorded. Specimens were kept in a saline solution before and after the testing. Bone blocks of 5x5x2mm were prepared using polishing strips. The Rockwell hardness test was performed through a test force, known as preload, which reflects the reference. The preload is applied to the bone sample with an indenter. Extra load will be applied in order to achieve the necessary test load (10N). After the force was applied, the maximum load was maintained at a pre-determined period of 15 seconds to permit elastic recovery. The major load was

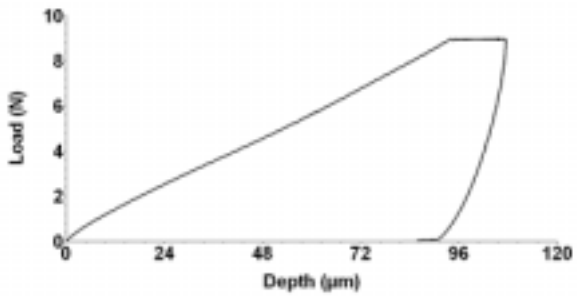


Fig. 2. The graphic shows the resulting curve that consists of three parts. In part 1, the indenter tip was loaded into the sample. At maximum force, the load was held constantly. When the force on the tip was released, the elastic response of the material was recorded (Part 3)

released and the resulting position was measured against the preload position [13].

The mechanical tests included three indents on each bone core at the level of the graft and three indents on alveolar bone crest. All the tests were performed by a technician unaware of the stage in which SR was administered.

Statistical analysis

Wilcoxon's-signed rank test for paired samples was used to calculate statistical differences between the 2 sides.

Results and discussions

There were no postoperative complications besides normal swelling and inflammation at the surgical site.

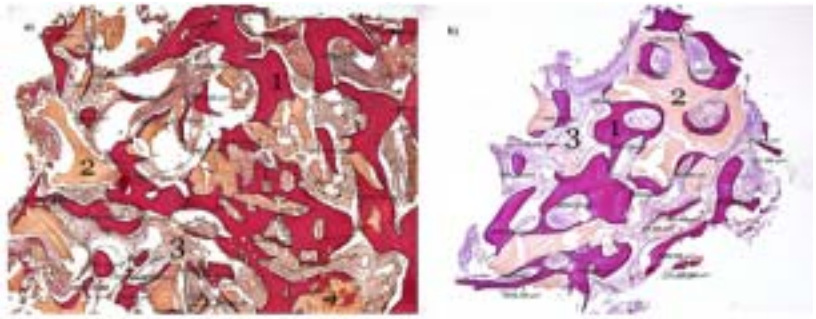


Fig.3. Specimens retrieved at 6 months postoperatively, 50X magnification. Van Gieson staining. (1 – newly formed bone 2 - xenograft particles, 3 - fibrous tissue/stromal area): a) specimen after SR administration (black arrow - area of ongoing graft colonization); b) specimen without SR

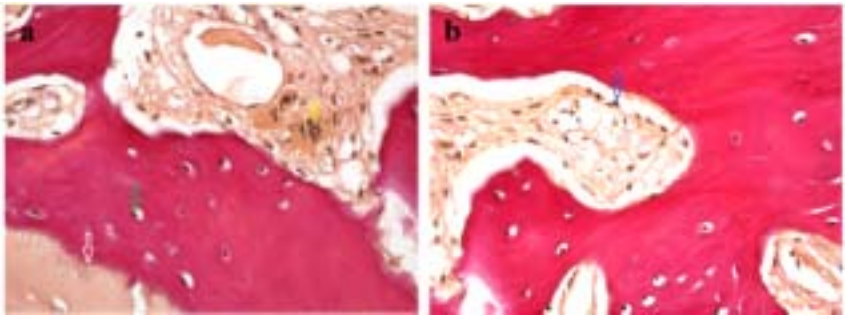


Fig.4. Histological sections, 200X magnification, showing: a) osteocyte (green arrow), osteoclast (yellow arrow), stelate form appearance of the repopulated graft – revitalized area interface (white arrow); b) line of osteoblasts (blue arrow)

One patient was excluded from the study because he was diagnosed with osteoporosis.

Effects of SR on bone microarchitecture

In the SR treated specimens we noted areas of ongoing graft colonization (fig. 3).

Lines of osteoblast were found around some of the newly formed bone in both specimens. Occasionally, osteoclasts can be seen around the bone graft particles (fig. 4).

Table 1

RATIO OF CELL DENSITY IN REVITALISED BONE SR/WITHOUT SR

Ratio of cell density in revitalised bone SR/without SR	
Small-sized nucleush	Large-sized nucleush
1.64	1.62
2.39	1.01
2.59	46.03
2.51	32.49
1.45	3.12

Percentage of newly formed bone (%)	
SR	without SR
19	34
35	31
29	30
1	12
21	22

Table 2

PERCENTAGE OF NEWLY FORMED BONE

Table 3

RATIO OF CELL DENSITY IN STROMAL AREA SR/WITHOUT SR

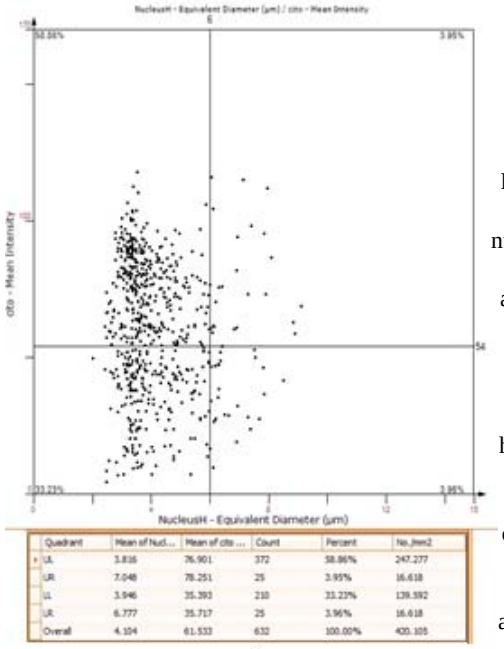
Ratio of cell density in stromal area SR/without SR	
Small-sized nucleush	Large-sized nucleush
0.44	0.05
1.89	1.03
1.51	22.53
0.95	7.94
1.72	3.75

The results from digital microscopic evaluation are shown in table 1, 2 and 3.

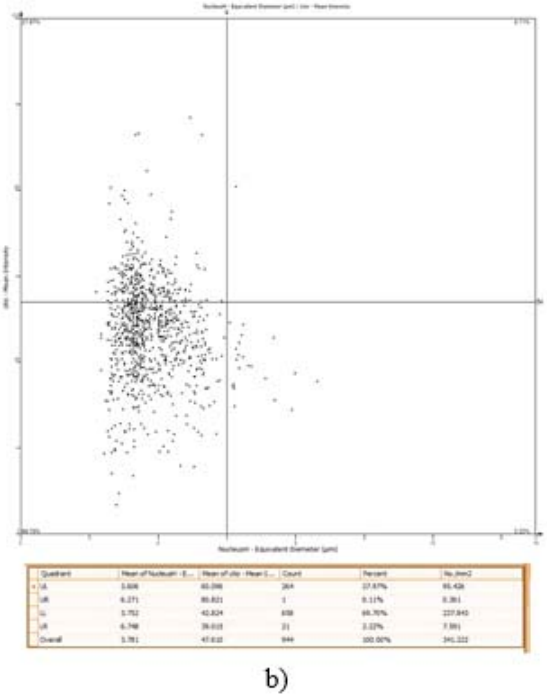
Effects of SR on intrinsic bone tissue quality

SR administration was associated with an increase of hardness values in all patients (table 4)

Although, there was no statistically significant difference between first and second surgical intervention in each variable tested, systematic higher values can be seen, for the mean density of cells (osteocyte-like cells) from the



a)



b)

Fig. 5. Graphic showing the number of cells/ mm² in the areas of newly formed bone (Upper left - small-sized nucleush and high values for cytoplasm intensity, osteocyte-like cells) with SR treatment (a) and without SR treatment (b)

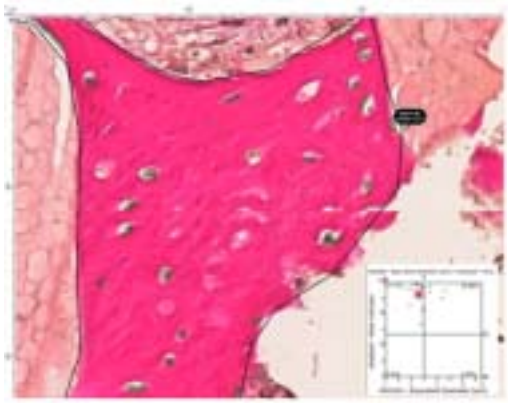


Fig.6. Image from automatic nuclei counting and the localisation of an osteocyte-like cell in the graphic

Tabel 4
MICROINDETATION TEST RESULTS

Patient	Young (SR)	Rockwell (SR)	Young	Rockwell
1	2.05	75.58	1.08	69.89
2	1.51	69.18	1.73	64.05
3	1.22	60.48	1.19	38.01
4	1.97	135.03	2.24	120.41
5	1.71	73.65	1.12	39.96

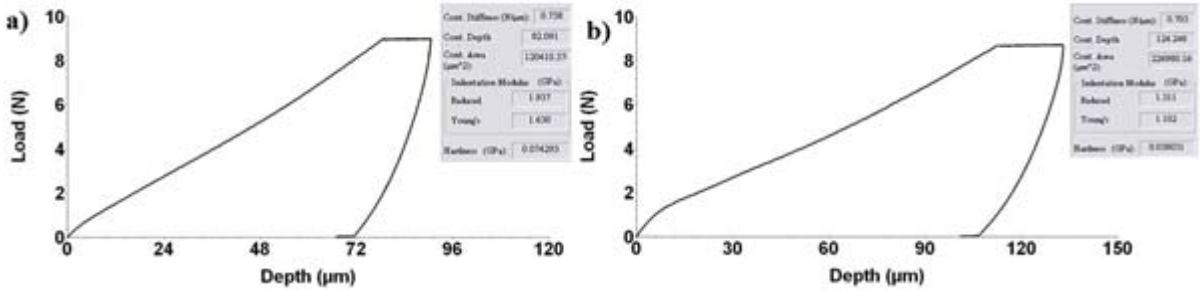


Fig. 7. Microindentation tests: a)The highest resulting curve and the smallest force displacement can be seen for after SR treatment. b) Bone graft without SR treatment

newly fomed bone and for the Rockwell hardness, in the graft under SR treatment (table 1).

To the author's knowledge, this is the first case to assess the effects of SR on bone grafts. The present study demonstrates that SR is able to improve the osseo-integration of maxillary sinus bone graft.

This study demonstrates the effects of SR on maxillary sinus augmentation. SR was administered only after ther first maxillary sinus augmentation. The fact that strontium contained in bone is rapidly eliminated after withdrawal of treatment [14] suggests that the antiosteoporotic agent did not have any influence on the second surgery intervention.

This is the first time when, using automatic nuclear segmentation, the number and type of cells seen in bone grafts are assessed. SR treated bone grafts showed systematic higher values for the density of the osteocyte-like cells, altough the percentage of newly formed bone was similar. In vitro, SR was shown to enhance bone cell replication and bone formation [15].

The effects of SR on bone tissue quality at the level of the maxillary sinus bone graft were analyzed. Young's modulus, also known as the tensile modulus or elastic modulus, is a measure of the stiffness of an elastic material [16] and the Rockwell test determines the hardness of the material. Higher values of Rockwell test were observed in SR treated patients. These facts suggest that SR influences positively the intrinsic bone tissue quality. This is in agreement with other studies [17]. Our results suggest that administering SR after sinus augmentation, we can obtain a better mechanical fixation of titanium implants.

The results from automated nuclei counting are in agreement with the results from microindentation tests – higher values for the Rockwell hardness of the graft and for the density of the osteocyte-like cells in the newly formed bone are seen in SR treated bone grafts. No statistical significant difference could be seen between first and second surgical intervention, because of the reduced number of patients.

Despite the fact that the processing technique followed standard protocol, retraction artefact was noticed. This can be due to the fact that conventional histology slides were used, and nod grip slides, and because of the fast transition between hydration/deshydratation phases of the sections in the staining process.

There is a different color gradient between slides, because staining occured in different sessions. This is the reason why intensity parameters were determined, individually, for each slide.

Conclusions

Taken together, these data suggest that SR improves osseointegration of the innorganic bovine bone graft and that it improves the intrinsic bone tissue quality. A longer-term follow-up investigation, including a larger group of patients, is necessary to further discriminate the specific effects of SR. Specific insights and clues describing the imunophenotype of the cellular types involved in graft osseointegration (provided for example by imuno-histochemical chemistry targeting some typical molecular cellular markers) could provide more effcient data and targets for a better ossseointegration.

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