Regeneration of Human Bone Using Different Bone Substitute Biomaterials

Tonino Traini, DDS, PhD;[*,†] Adriano Piattelli, MD, DDS;[‡] Sergio Caputi, MD, DDS;[‡] Marco Degidi, MD, DDS;[§] Carlo Mangano, MD, DDS;[¶] Antonio Scarano, DDS, MD;[**] Vittoria Perrotti, DDS, PhD;[††] Giovanna Iezzi, DDS, PhD[‡‡]

ABSTRACT

Purpose: The present study aimed to assess clinical and biological performances of several bone substitute biomaterials (BSBs).

Materials and Methods: The evaluation was conducted at 6 months and after several years on 295 patients undergoing sinus augmentation with 13 different BSBs; the data belonging to previously published studies have been analyzed using innovative mathematical models to evaluate the bone regenerative index (Br) and the structural density index (Ds).

Results: After 6 months, compared to the Ds index of native bone, the regenerated bone showed a D3 bone type; while, after several years, the regenerated bone type was D2, with an evident increase in the density of the regenerated bone over time. Moreover, the values of Br were higher for combined biomaterials indicating a fewer amount of residual particles and marrow spaces, while the values of Ds were higher for anorganic bovine bone indicating a greater new bone formation and a lesser amount of marrow spaces. After 20 years, the bone regenerated using hydroxyapatite still had a D4 bone quality.

Conclusions: After 6 months of healing, the regenerated bone had a composite structure resembling poor D3 bone type, and covered approximately one-third of the space filled by BSBs. None of the evaluated biomaterials seemed to be ideal.

KEY WORDS: allograft, biomaterials, bone, bone allograft

INTRODUCTION

Bone substitute biomaterials (BSBs) used in dentistry include inorganic or organic, natural or synthetic materials to compensate for a lack or loss of bone tissue. Ideally, a BSB should have specific biological and clinical peculiarities. Biologically, it could mediate recruitment of mesenchymal cells derived from the host site and have bioactive effects on ossification (osteoinduction). Furthermore, it must be osteoconductive, providing three-dimensional scaffolds for the ingrowth of vessels and osteoprogenitor cells. Finally, it should be resorbable. Clinically, a BSB should be easy to use, cost-effective and with an own density to allow an easy radiographic recognition during the entire healing process. This property is particularly important to follow the rate of resorption/substitution by means of radiographic evaluation.

Many biomaterials used in clinical practice have significant drawbacks, including a lack of resorbability, presence of animal or marine-derived components, and poor handling characteristics. Regarding the structure, it is known that a good biological integration requires pores that are greater than 100–150 μm in diameter to provide a blood supply to the host tissues.[1] The BSBs should degrade gradually with time until it is completely replaced with vital new bone tissue. Moreover, the

*Assistant professor, Department of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Chieti, Italy; †scientific consultant, Department of Dentistry, San Raffaele Hospital, Vita-Salute University, Milan, Italy; ‡full professor, Department of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Chieti, Italy; §oral surgeon, Private Practice, Bologna, Italy; ¶assistant professor, Department of Surgical and Morphological Sciences, University of Insubria-Varese, Varese, Italy; **professor, Department of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Chieti, Italy; ††researcher, Department of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Chieti, Italy; ‡‡aggregate professor, Department of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Chieti, Italy

Reprint requests: Dr. Tonino Traini, Department of Medical, Oral and Biotechnological Sciences, University of Chieti-Pescara, Via Dei Vestini 31, 66100 Chieti, Italy; e-mail: t.traini@unich.it

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resorption rate should be matched to the formation rate of new bone tissue;[2] indeed, a too fast degradation of the biomaterial can have a negative effect on the bone regeneration processes.[3] The presence of BSB residual grafted particles after bone healing may lead to the formation of a "*composite repair tissue*" rather than to a regenerated bone tissue. In an animal study, Johnson and colleagues[4] reported for both hydroxyapatite and tricalcium sulphate less biomechanical integrity than for cancellous bone. Recently, in an *in vitro* study on a porous hydroxyapatite (porHa), Dejaco and colleagues[5] reported an increase of local stress and a decrease of global stiffness due to the development of cracks inside the biomaterial granules. This fact points to the role of cracking in activating the bone cells able to initiate the biochemical cascade of bone formation[6] and to the lack of information about the role played by the residual grafted BSB particles inside the peri-implant bone under loading conditions.

Bone defects bring the dilemma of graft choice to the dental surgeon, taking into consideration that not all bone graft substitutes perform in the same way. A strategy to systematically choose the appropriate BSB properties should be guided by evidence-based results. From dental literature, we have confounding data, especially regarding the implant survival rate. For implants placed in augmented sinuses, it was reported[7] that the survival rate varied between 61.7 and 100%, with an average rate of 91.8%. Despite the very high mean results, it was evident that there is a 38.3% of variability on the implant survival rate. Furthermore, the data evaluating the residual crestal bone heights were usually limited.[8,9] The main objective of bone regeneration/augmentation in dentistry is related to implant placement. Inadequate long-term performance of grafts was often a result of a mismatch between the mechanical properties of the BSB and the surrounding bone, leading to tissue damage or failure of the restorations. The strength and fragility of bone as well as its structure are correlated factors.[10,11] Thus, it is of paramount importance to restore the three-dimensional organization and functionality of the newly formed tissue. Bone undergoes a constant renewal process, which helps to maintain its mechanical performance[12] and allows for adaptation to changes in mechanical requirements.[13] It is generally accepted that bone remodeling is controlled by a mechanosensory system.[14,15] In summary, the external load generates local strains in the bone architecture and by means of an osteocytes network; a signal reaches osteoclasts and osteoblasts, and activates the response of these cells in terms of bone resorption and deposition. From this point of view in the augmented peri-implant bone tissue, the amount of biomaterial residual particles and large marrow spaces impair or at least reduce the function of the mechanosensory system due to the lack of osteocytes network, which is present only inside newly formed bone.

This paper aims to evaluate and compare the in vivo behavior of different biomaterials, placed in humans, by means of two mathematical indexes, one used to examine bone regeneration processes and the other for the assessment of bone density structure obtained after regeneration. Both indexes, computed on histomorphometric data, used weighted associated variables to compare the results and give an indication for the clinical use of the different biomaterials.

MATERIALS AND METHODS

The present study was conducted considering 295 patients, ranging in age 42–69 years with a mean age of 54.9 ± 3.3 years, and a male/female ratio with a slight predominance of females (Table 1). Almost all the cases were sinus augmentation procedures; one case was of alveolar socket regeneration and one case of an implant retrieved for fracture. The residual bone between the sinus floor and alveolar ridge was in mean 2.9 ± 1.1 mm (Table 1). Thirteen different BSB were considered in the present analysis (Table 2). The data of the present study belong to 10 previously published studies conducted at the Department of Medical, Oral and Biotechnological Sciences of the University of Chieti-Pescara since 2004 (Table 1). Each one of the protocols involved had received an Ethical Committee approval; for details in specimen processing, surgical treatments and materials characteristics, the readers are referred to the original publications.[16–25]

Special Procedures

To compare and evaluate the *in vivo* efficiency of different biomaterial this equation was used (1)

$$Br = \sqrt{\frac{\left[(NB - SD_{NB}) + (Ms - SD_{Ms}) + (Rp - SD_{Rp})\right]^2}{\left[Rp - SD_{Rp} + \frac{1}{2}(Ms - SD_{Ms})\right]^2}} \quad (1)$$

where Br is the bone regenerative dimensional index, NB is the mean rate of newly formed bone, Ms is the

TABLE 1 Summary of the Data

Patient Number	Male	Female	Age Range (Years)	Mean of Age (Years)	Residual Alveolar Ridge Height (mm)	Number of Implants Placed	Study	Time
15	9	6	51–67	55	2–3	82	Iezzi et al 2012[16]	6 months
121	50	71	51–63	54	nr	nr	Scarano et al 2011[17]	4–6 months
10	6	4	54–65	59	2–3	23	Scarano et al 2012[18]	6 months
94	nr	nr	52–68	61	3–5	362	Scarano et al 2006[19]	6 months
7	3	4	48–69	58	1–4	33	Degidi et al 2004[20]	6 months
40	18	22	42–67	52	3–5	100	Mangano et al 2007[21]	6 months
5	3	2	47–58	51	1.5–3	15	Traini et al 2008[22]	20 months
1	1	–	–	54	Implant fracture	1	Degidi et al 2012[23]	8 years
1	1	–	–	52	3 mm	nr	Traini et al 2007[24]	9 years
1	1	–	–	53	Alveolar socket	nr	Mangano et al 2008[25]	20 years
295	92	109	42–69	54.9 ± 3.3	2.9 ± 1.1	616		

nr, not reported.

bone marrow spaces and Rp is the mean rate of residual particles of the implanted biomaterial. SD is the standard deviation rate of the mean.

The Br index was estimated following the relation among the amount of single mean corrected with each SD value and posing an inverse relation with the mean of Rp corrected with half amount of Ms. The correction of Rp with $^1/_2$ Ms was made considering that the Ms, in any case, belonged to both conditions of area completely filled by NB or completely filled by Rp; in other words 100% of success and 100% of failure of the regeneration.

Moreover, an index for the structural density, defined Ds, was calculated and was applicable for both augmented and native bone. To evaluate the range of Ds in native bone tissue the modified equation (1) was used.

$$Ds = \sqrt{\frac{[(B - SD_B) + (Ms - SD_{Ms})]^2}{\left[\frac{1}{2}(Ms - SD_{Ms})\right]^2}} \qquad (2)$$

As reported in equation (2) the bone tissue rate (B) was used instead of NB and the Rp variable disappeared.

Native Bone Core Processing for Ds Evaluation

A total of 15 bone cores harvested during the implant bed preparation using a trephine bur of 3.0 mm of

TABLE 2 Summary of the Biomaterials Used and Manufacturers

Bone Substitute Biomaterials	Manufacturers
Anorganic bovine bone (ABB)	BioOss ®; Geistlich Pharma AG, Wohlhusen, Switzerland
Dense hydroxyapatite (dHA)	DAC; Dense Apatite Ceramic, Novaxa, Milan, Italy
Phycogene hydroxyapatite (pHA)	Algipore®; Dentsply Friadent, Mannheim, Germany
Porous hydroxyapatite (porHA)	Fingranule®; Finceramica, Faenza, Italy
Collagenized porcine bone (collPB)	Apatos®; Tecnoss, Turin, Italy
Cortical/cancellous porcine bone (cortPB)	Apatos®; Tecnoss, Turin, Italy
Macroporous biphasic calcium phosphate (Ca2PO4)	MBCP®; Leone, Florence, Italy
Demineralized freeze-dried bone allograft (DFDBA)	LifeNet, Virginia Beach, VA, USA
Calcium carbonate (CaCO3)	Biocoral®; Inoteb, St. Gonnery, France
Bioactive glass (BGlass)	Bioglass®; US Biomaterials, Alachua, FL, USA
Polymer of polylactic and polyglycolide acids (PLL/PLG)	Fisiograft®; Ghimas, Bologna, Italy
Anorganic bovine bone with synthetic peptide P-15 (P-15)	PepGen P-15™; Dentsply Friadent CeraMed, Lakewood
Calcium sulphate (CaSO4)	Surgiplaster sinus; ClassImplant, Rome, Italy

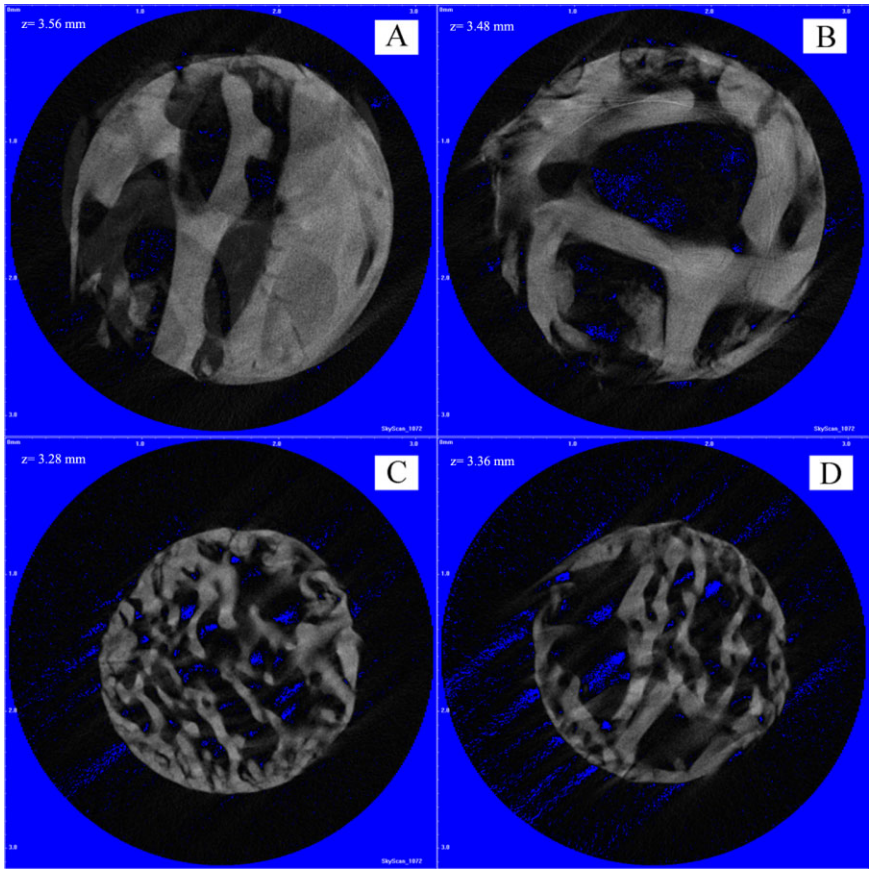

Figure 1 Three-dimensional images from stacks of two-dimensional micro-computed tomography images. (A) Reconstruction of maxillary bone of density D1 (z = 3.56 mm). (B) Reconstruction of maxillary bone of density D2 (z = 3.48 mm). (C) Reconstruction of maxillary bone of density D3 (z = 3.28 mm). (D) Reconstruction of maxillary bone of density D4 (z = 3.36 mm).

diameter were collected from both the maxillary and mandibular bones. The specimens were fixed in a 10% buffered formalin solution, dehydrated in a graded series of alcohol, and then they were scanned using a micro-computed tomography (μCT) instrument (SkyScan 1072, SkyScan, Aartselaar, Belgium). The scanning procedure was completed using 10W, 100 kV, 98 μA, a 1 mm-thick aluminium plate and 15 × magnification with 5.9 seconds exposure time and 0.45° rotation step, resulting in a pixel size of 19.1 μm ×19.1 μm. The bone cores were divided into three groups of five specimens each on the basis of the bone densities D1, D2-D3, and D4.[26] The measurements were performed on three-dimensional reconstruction from stacks of the two-dimensional images collected by means of μCT (Figure 1).

Measuring Procedures

Both the Br and Ds results were used to compare the histological results among the different BSBs at different times. The Br index, more than the rates of newly formed bone, marrow spaces or residual particles itself, took into account the relationship among the variables. The effects of single variable fluctuation were minimized by Br index giving back the level of weight of residual particle rate during the comparison of different BSB grafts.

The Ds index was preliminarily evaluated for bone D4, D3-D2 and D1 to have a range of comparison in physiological condition. Ds was also calculated for each group of BSB to classify the type of regenerated bone as function of the BSB used.

In an ideal condition, the values of Br and Ds indices should be coincident with values included into the range of 4.30–6.95.

Propagation of Uncertainty

To get an estimate of the propagation of errors in both the equation (1) and the equation (2), we used the equation (3). Under the circumstances of equation (2),

TABLE 3 Summary of Data for Native Maxillary Bone

Bone Densities	*n*	Bone % (SD)	Marrow spaces % (SD)	Ds	±SD
D1	5	74.6 (2.7)	25.4 (1.7)	8.06	0.77
D2 and D3	5	52.9 (4.3)	47.1 (4.9)	4.30	0.64
D4	5	42.3 (3.1)	56.8 (2.8)	3.45	0.32

the uncertainty was evaluated changing the variables σ_{Br} / *Br* in σ_{Ds} / *Ds* the variable *NB* in *B* and finally eliminating both *Rp* and SD_{Rp} variables.

$$\sigma_{Br}/Br = \sqrt{\sum_{NB,Ms,Rp=1}^{N} (SD_{NB}/NB)^2 + (SD_{Ms}/Ms)^2 + (SD_{Rp}/Rp)^2 + \frac{1}{2}(SD_{Ms}/Ms)^2 + (SD_{Rp}/Rp)^2} \quad (3)$$

Statistical Evaluation

Statistical analysis was performed by means of a computerized statistical package (Sigma Stat 3.5, SPSS Inc., Ekrath, Germany). One-way analysis of variance and Holm-Sidak tests were used to evaluate the overall significance and to perform all pairwise comparisons of the mean responses, respectively. A *p*-value of <.05 was considered statistically significant.

RESULTS

The results of measurements for native maxillary bone were reported in Table 3. The Ds index for bone D1 was 8.06 ± 0.77, for D2-D3 was 4.30 ± 0.64 and for D4 was 3.45 ± 0.32. The Br indexes for the different BSB were summarized in Table 4. Statistical evaluation for Br index indicates a significant difference among the biomaterials ($p < .001$) (Table 5). Among the 210 pairwise comparisons procedures, significant differences were related only to the polymer of polylactic and polyglycolide acids versus porHA, ABB, demineralized freeze-dried bone allograft and cortical/cancellous porcine bone ($p < .05$) (Table 6). The Ds index also indicates significant differences among the biomaterials ($p < .001$) (Table 7). The pairwise comparisons procedure versus control groups of maxillary bone D1, D2-D3, and D4 showed significant differences ($p < .05$) mainly with groups D1 and D2-D3 and less when compared to group D4 (Table 8). The overall mean of Br index calculated for all BSBs after 6 months was 2.1 ± 0.3, while the overall mean of Ds index was 3.8 ± 0.5. Comparing these results with the Ds index of native bone, it was evident that after 6 months, the bone regenerated using the different BSBs had generally a poor bone structure, such as a D3/D4 bone (Figure 2). Moreover, the values of Br were higher for combined biomaterials, indicating a lesser amount of residual particles and marrow spaces, while the values of Ds were

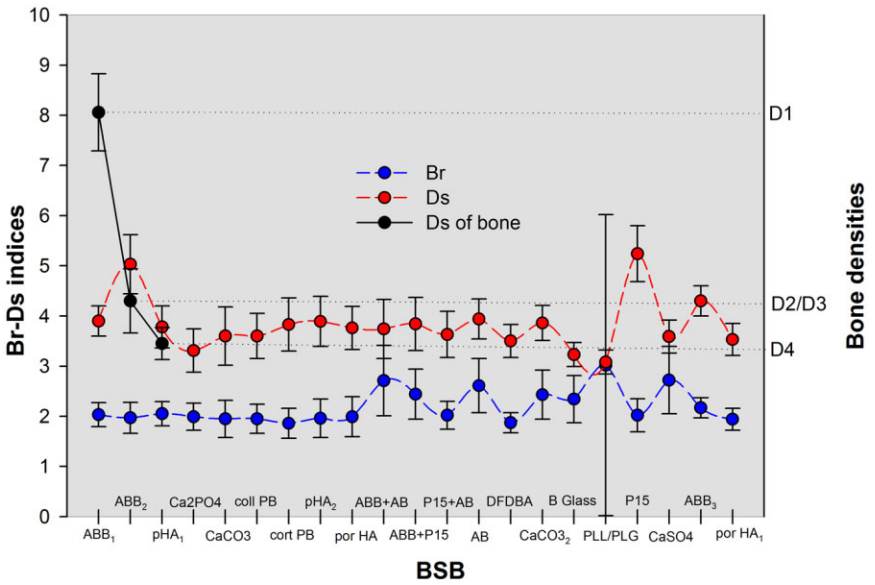

Figure 2 Means of Br and Ds indices versus bone substitute biomaterials (BSB) compared to Ds means of maxillary bone densities D1, D2-D3, and D4.

TABLE 4 Summary of the Results for Br and Ds after 6 Months

Biomaterial (6 Months)	*N*		NB	Ms	Rp	Br (Ds)	±SD	Authors
ABB $_1$	12	Mean	32.9	36.4	32.8	2.03 (3.90)	0.24 (0.30)	Iezzi et al.[16] *Clin Oral Implants Res* 2012
		SD	0.5	2.3	2.1			
ABB $_2$	20	Mean	36.2	25.2	39.0	1.97 (5.03)	0.31 (0.59)	Mangano et al.[21] *Int J Oral Maxillofac Implants* 2007
		SD	1.4	2.3	2.9			
pHA $_1$	12	Mean	33.2	39.3	30.1	2.05 (3.78)	0.24 (0.42)	Iezzi et al.[16] *Clin Oral Implants Res* 2012
		SD	1.2	3.4	0.9			
Ca2PO4	12	Mean	30.5	43.6	28.1	1.99 (3.31)	0.27 (0.43)	Iezzi et al.[16] *Clin Oral Implants Res* 2012
		SD	3.4	2.5	0.9			
CaCO3 $_1$	12	Mean	28.1	45.6	27.1	1.95 (3.17)	0.37 (0.58)	Iezzi et al.[16] *Clin Oral Implants Res* 2012
		SD	3.9	4.5	1.0			
Coll PB	12	Mean	31.8	38.7	33.1	1.95 (3.60)	0.29 (0.45)	Iezzi et al.[16] *Clin Oral Implants Res* 2012
		SD	2.9	2.7	1.9			
Cort PB	32	Mean	31.4	34.3	37.6	1.86 (3.83)	0.30 (0.53)	Scarano et al.[17] *Clin Implant Dent Relat Res* 2011
		SD	2.6	3.1	2.2			
pHA_2	10	Mean	35.2	35.6	37.1	1.96 (3.89)	0.38 (0.50)	Scarano et al.[18] *Oral Maxillofac Surg* 2012
		SD	3.6	2.3	3.8			
porHA	20	Mean	34.7	38.1	35.9	1.99 (3.76)	0.40 (0.43)	Mangano et al.[21] *Int J Oral Maxillofac Implants* 2007
		SD	3.1	2.2	4.2			
ABB + AB (50%)	7	Mean	38.7	45.6	14.4	2.71 (3.74)	0.70 (0.59)	Degidi et al.[20] *J Oral Implantol* 2004
		SD	3.2	5.0	2.1			
ABB + P15 (50%)	7	Mean	36.7	39.7	19.6	2.44 (3.84)	0.50 (0.53)	Degidi et al.[20] *J Oral Implantol* 2004
		SD	3.3	3.4	2.1			
P15 + AB (50%)	7	Mean	32.2	38.0	28.8	2.02 (3.63)	0.28 (0.46)	Degidi et al.[20] *J Oral Implantol* 2004
		SD	3.2	2.5	1.1			
AB	16	Mean	40.1	40	18	2.61 (3.94)	0.54 (0.40)	Scarano et al.[19] *Implant Dent* 2006
		SD	3.2	2.1	2.3			
DFDBA	16	Mean	29	37	34	1.87 (3.50)	0.20 (0.33)	Scarano et al.[19] *Implant Dent* 2006
		SD	2.3	1.6	1.2			
$CaCO3_2$	16	Mean	39	40	22	2.43 (3.86)	0.49 (0.35)	Scarano et al.[19] *Implant Dent* 2006
		SD	3.1	1.5	2.8			
BGlass	16	Mean	31	49	18	2.34 (3.23)	0.47 (0.24)	Scarano et al.[19] *Implant Dent* 2006
		SD	1.9	1.8	2.4			
PLL/PLG	16	Mean	33	59	3	3.02 (3.08)	3.0 (0.24)	Scarano et al.[19] *Implant Dent* 2006
		SD	2.1	2.3	2.1			
P15	16	Mean	37	23	37	2.02 (5.24)	0.33 (0.56)	Scarano et al.[19] *Implant Dent* 2006
		SD	2.3	1.6	3.2			
CaSO4	16	Mean	38	45	13	2.72 (3.59)	0.67 (0.33)	Scarano et al.[19] *Implant Dent* 2006
		SD	3.2	1.3	2.1			
ABB_3	16	Mean	39	34	31	2.17 (4.30)	0.20 (0.30)	Scarano et al.[19] *Implant Dent* 2006
		SD	1.6	1.6	1.4			
$porHA_1$	16	Mean	32	40	34	1.94 (3.53)	0.22 (0.32)	Scarano et al.[19] *Implant Dent* 2006
		SD	2.5	1.6	1.6			

higher for anorganic bovine bone (ABB), indicating a greater amount of newly formed bone and less marrow spaces. Calcium sulphate showed the highest amount of residual particles while the polylactic and polyglycolide acids showed the greatest quantity of marrow spaces and the fewest amount of residual particles. In addition, the polylactic and polyglycolide acids showed almost coincident values for Br and Ds.

TABLE 5 One-Way Analysis of Variance for Br Index

Group Name	*N*	Missing	Mean	SD	SEM
ABB_1	12	0	2.030	0.240	0.0693
ABB_2	20	0	1.970	0.310	0.0693
pHA_1	12	0	2.050	0.240	0.0693
Ca2PO4	12	0	1.990	0.270	0.0779
$CaCO3_1$	12	0	1.950	0.370	0.107
Coll PB	12	0	1.950	0.290	0.0837
Cort PB	32	0	1.860	0.300	0.0530
pHA_2	10	0	1.960	0.380	0.120
porHA	20	0	1.990	0.400	0.0894
ABB + AB	7	0	2.710	0.700	0.265
ABB + P15	7	0	2.440	0.500	0.189
P15 + AB	7	0	2.020	0.280	0.106
AB	16	0	2.610	0.540	0.135
DFDBA	16	0	1.870	0.200	0.0500
$CaCO3_2$	16	0	2.430	0.490	0.123
BGlass	16	0	2.340	0.470	0.117
PLL/PLG	16	0	3.020	3.000	0.750
P15	16	0	2.020	0.330	0.0825
CaSO4	16	0	2.720	0.670	0.168
ABB_3	16	0	2.170	0.200	0.0500
$porHA_1$	16	0	1.940	0.220	0.0550

One-Way Analysis of Variance

Source of Variation	DF	SS	MS	*F*	*p*
Between groups	20	33.320	1.666	2.723	<0.001
Residual	286	174.948	0.612		
Total	306	208.268			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($p \leq .001$).
Power of performed test with alpha = 0.050: 0.966.

To evaluate the behavior after a long time, some BSB (ABB, dense hydroxyapatite and ABB+P15) were considered, notwithstanding the fact that they were single case reports. The results summarized in Table 9 appeared to be of interest. The mean of Br index was 3.1 ± 1.5, while the mean of Ds index was 4.2 ± 0.7. Again comparing these results with the Ds index of native bone a slight increase in the density of the regenerated bone was noted, and its structure was similar to bone type D2 (Figure 3).

TABLE 6 Multi-Comparison Procedure for Br Index

Comparisons	Diff. of Means	*t*	Unadjusted *P*	Critical Level	Significance
PLL/PLG versus cort PB	1.160	4.844	0.00000209	0.000	Yes
PLL/PLG versus DFDBA	1.150	4.159	0.0000423	0.000	Yes
PLL/PLG versus ABB_2	1.050	4.003	0.0000799	0.000	Yes
PLL/PLG versus porHA	1.030	3.926	0.000108	0.000	Yes
PLL/PLG versus $porHA_1$	1.080	3.906	0.000117	0.000	Yes

Holm-Sidak multiple comparison procedure. Overall significance level = 0.05. Only statistical significant comparisons were reported among 210 pairwise.

TABLE 7 One-Way Analysis of Variance for Ds Index

Group Name	*n*	Missing	Mean	SD	SEM
ABB_1	12	0	3.900	0.300	0.0866
ABB_2	20	0	5.030	0.590	0.132
pHA_1	12	0	3.780	0.420	0.121
Ca2PO4	12	0	3.310	0.430	0.124
$CaCO3_1$	12	0	3.600	0.580	0.167
Coll PB	12	0	3.600	0.450	0.130
Cort PB	32	0	3.830	0.530	0.0937
pHA_2	10	0	3.890	0.500	0.158
Por HA	20	0	3.760	0.430	0.0962
ABB + AB	7	0	3.740	0.590	0.223
ABB + P15	7	0	3.840	0.530	0.200
P15 + AB	7	0	3.630	0.460	0.174
AB	16	0	3.940	0.400	0.1000
DFDBA	16	0	3.500	0.330	0.0825
$CaCO3_2$	16	0	3.860	0.350	0.0875
Bglass	16	0	3.230	0.240	0.0600
PLL/PLG	16	0	3.080	0.240	0.0600
P15	16	0	5.240	0.560	0.140
CaSO4	16	0	3.590	0.330	0.0825
ABB_3	16	0	4.300	0.300	0.0750
$PorHA_1$	16	0	3.530	0.320	0.0800
Bone D1	5	0	8.060	0.770	0.344
Bone D2-D3	5	0	4.300	0.640	0.286
Bone D4	5	0	3.450	0.320	0.143

One-Way Analysis of Variance

Source of Variation	DF	SS	MS	*F*	*P*
Between Groups	23	177.126	7.701	39.389	<0.001
Residual	298	58.263	0.196		
Total	321	235.389			

The differences in the mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($p \leq .001$).
Power of performed test with alpha = 0.050: 1.000.

After 20 years, bone regenerated using HA still had a D4 bone structure, while after 9 years of using the ABB, the bone structure appeared in density as D2-D3 bone type. This trend was confirmed for bone regenerated with ABB in combination with P15. Of interest seemed to be the observation relative to ABB after 20 months, which appeared not substantially different from the Ds index after 6 months (Figure 3).

DISCUSSION

In clinical practice, the main purpose of the bone augmentation procedures is the formation of bone, where the implants will be positioned to best support the prosthetic rehabilitation. On the other hand, in sites with no implant insertion, the use of incomplete resorbable bone substitutes did not produce any significant effects since the primary objective of the therapy was the space-filling maintenance not related to the mechanical support. The bone tissue around dental implants must be mechanically competent after the augmentation procedures. Sites augmented to receive implants should, possibly, be treated with complete resorbing biomaterials. The relationship between foreign material inclusion into the living bone and the possible reduction

TABLE 8 Multi-Comparison Procedure for Ds Index

Comparison	Diff. of Means	*t*	Unadjusted *P*	Critical Level	Significance
D4 versus P15	1.790	7.901	5.373E-014	0.002	Yes
D4 versus ABB_2	1.580	7.147	6.875E-012	0.002	Yes
D4 versus ABB_3	0.850	3.752	0.000211	0.003	Yes
D2-D3 versus PLL/PLG	1.220	5.385	0.000000147	0.002	Yes
D2-D3 versus BGlass	1.070	4.723	0.00000358	0.002	Yes
D2-D3 versus Ca2PO4	0.990	4.206	0.0000344	0.003	Yes
D2-D3 versus P15	0.940	4.149	0.0000436	0.003	Yes
D2-D3 versus DFDBA	0.800	3.531	0.000479	0.003	Yes
D2-D3 versus $PorHA_1$	0.770	3.399	0.000769	0.003	Yes
D2-D3 versus ABB_2	0.730	3.302	0.00108	0.003	Yes
D2-D3 versus CaSO4	0.710	3.134	0.00190	0.003	Yes
D2-D3 versus Coll PB	0.700	2.974	0.00318	0.004	Yes
D2-D3 versus $CaCO3_1$	0.700	2.974	0.00318	0.004	Yes
D1 versus PLL/PLG	4.980	21.983	2.524E-064	0.002	Yes
D1 versus BGlass	4.830	21.320	6.710E-062	0.002	Yes
D1 versus Ca2PO4	4.750	20.182	1.079E-057	0.002	Yes
D1 versus DFDBA	4.560	20.129	1.700E-057	0.003	Yes
D1 versus $PorHA_1$	4.530	19.996	5.278E-057	0.003	Yes
D1 versus Cort PB	4.230	19.894	1.271E-056	0.003	Yes
D1 versus CaSO4	4.470	19.731	5.105E-056	0.003	Yes
D1 versus Por HA	4.300	19.450	5.730E-055	0.003	Yes
D1 versus $CaCO3_1$	4.460	18.950	4.238E-053	0.003	Yes
D1 versus Coll PB	4.460	18.950	4.238E-053	0.004	Yes
D1 versus $CaCO3_2$	4.200	18.539	1.457E-051	0.004	Yes
D1 versus AB	4.120	18.186	3.079E-050	0.004	Yes
D1 versus pHA_1	4.280	18.185	3.121E-050	0.005	Yes
D1 versus ABB_1	4.160	17.675	2.570E-048	0.005	Yes
D1 versus pHA_2	4.170	17.218	1.341E-046	0.006	Yes
D1 versus P15 + AB	4.430	17.110	3.411E-046	0.006	Yes
D1 versus ABB + AB	4.320	16.686	1.351E-044	0.007	Yes
D1 versus ABB_3	3.760	16.597	2.901E-044	0.009	Yes
D1 versus ABB + P15	4.220	16.299	3.821E-043	0.013	Yes
D1 versus ABB_2	3.030	13.705	1.739E-033	0.017	Yes
D1 versus P15	2.820	12.448	6.328E-029	0.050	Yes

Holm-Sidak multiple comparison procedure. Overall significance level = 0.05. Comparisons for factor: bone D4.

of the bone mechanical competence is still not very clear. Most of the dental literature on implant survival in sites augmented with bone substitutes is based either on histomorphometrical or clinical evaluations.[4,5,16–25] Obviously, the biological behavior of the BSB and the histomorphometrical evaluation are of primary interest. As reported by Kirkpatrick and colleagues[27] the regenerative processes have the teleological purpose of bringing the affected tissue to a state of low entropy with "restitutio ad integrum" while repair is a tissue structural adaptation to function tasks. The entire biological process of bone regeneration was summarized by a drawing (Figure 4). Biomaterial residual particles were present in the T2 phase of the process producing a composite tissue that should be called "bone repair" instead of "bone regeneration." Only the T3 phase should be considered "bone regeneration," since it was free of biomaterials residual particles.

The implant osseointegration process has been found to be obtained and maintained also in augmented

TABLE 9 Summary of the Results of Case Reports after Several Years

Biomaterial	*n*		NB	Ms	Rp	Br (Ds)	±SD	Authors	Time
ABB + P15 (50%)	1	Mean	51.4	40.0	8.6	5.28 (4.83)	1.35 (1.14)	Degidi et al.[23] *Int J Periodontics Restorative Dent* 2012	8 years
		SD	4.8	7.1	0.6				
ABB_1	1	Mean	46.0	38.0	16.0	3.26 (4.84)	1.94 (1.47)	Traini et al.[24] *J Periodontol* 2007	9 years
		SD	4.6	8.9	5.8				
ABB_2	1	Mean	38.0	36.0	29.0	2.19 (4.06)	0.25 (0.29)	Traini et al.[22] *J Periodontol* 2008	20 months
		SD	2.1	1.3	1.8				
dHA	1	Mean	25.4	41.3	38.1	1.77 (3.22)	0.44 (0.64)	Mangano et al.[25] *J Periodontol* 2008	20 years
		SD	3.2	5.2	4.1				

sinuses where residual grafted particles of BSB were still present, and no untoward effect on peri-implant bone regeneration was found to be derived from the presence of residual grafted particles of BSB.[28–31] Unfortunately, the reported studies were mainly case reports and no definitive conclusions could be made.

The present results showed, after 6 months, an increase of NB in mean (± SD) of 34.2% (3.5) while by 9.6% (7.6) years, the NB increased to 40.2% (11.2). The Rp, was in mean (± SD) 27.3% (9.7) after 6 months and decreased to 22.9% (13.1) by 9.6 years. These observations were supported by Tadjoedin and colleagues[32] who reported a total bone volume increase of 38% after 6 months.

These results have led to the observation that the bone healing processes in presence of BSB had a "*bone-gain-threshold*." In details, following the surgical procedure for BSB implantation, a regional acceleratory phenomenon (RAP) process will take place[33] accelerating the biochemical events and the de novo bone formation to reach a stable (low entropy) composite-space-filling structure. During the early phase of the bone healing, the modeling process was responsible for de novo bone formation, while, later on, the increase in NB volume took place by remodeling processes, therefore by resorption and replacement of the BSB. The data of the present study showed that the decrease of Rp by 9.6 years was 4.4% (3.4) and the increase of NB was 6% (7.7). After the

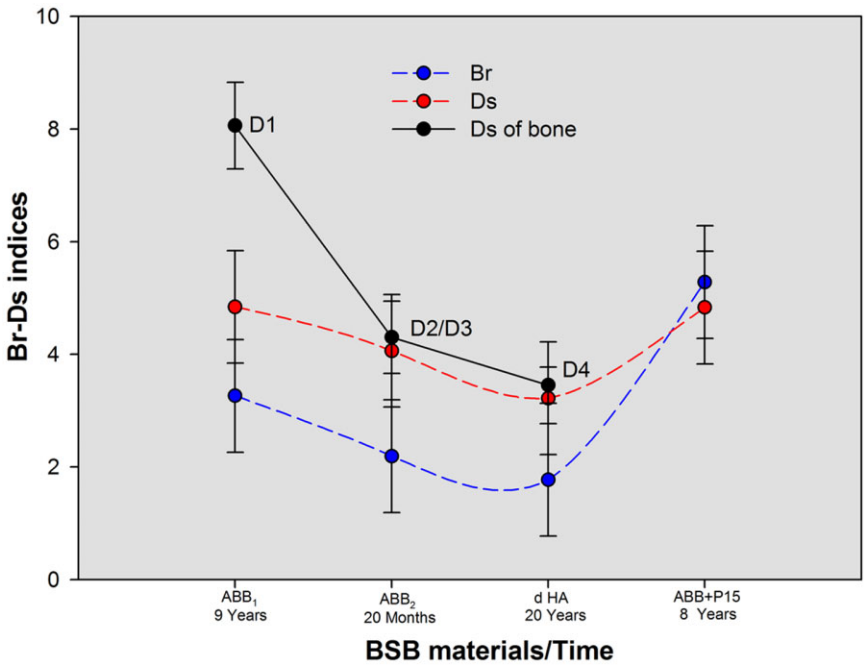

Figure 3 Br and Ds indices versus bone substitute biomaterials (BSB)/time in comparison to Ds means bone D1, D2-D3, and D4.

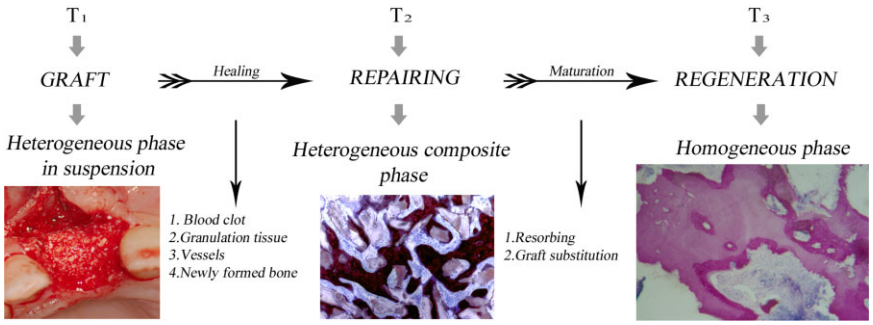

Figure 4 Schematic drawing of the healing process after grafting procedure. T_1 time of grafting. T_2 time of repairing. This step will last also 20 years and more as demonstrated by the present results. T_3 time of regeneration. This step appeared to be not obtainable before 6 months even after 20 years with some BSB considered in the present study.

healing period, most of the increase of NB could be related to the resorption/substitution processes. The resorption capabilities of the different BSB should be taken into the consideration.

What Are the Clinical Results for Implants Placed in Augmented Sites?

Evaluating the data from literature on implant survival rate, after maxillary sinus grafting, several systematic reviews were considered. Wallace and Froum[7] reported for 5267 implants a mean (± SD) survival rate of 91% (5.2); Del Fabbro and colleagues[8] reported, for 4378 implants placed in 1321 patients, a mean (± SD) survival rate of 91.6% (8.1), after a mean of 45 months. The same authors[34] reported, for 13162 implants placed in 4019 patients after 45.3 months, a mean (± SD) survival rate of 92.55 (7.4); more recently, Cabezas-Mojón and colleagues[35] reported, for 3975 implants placed in 1318 patients after 28 months, a mean (± SD) survival rate of 95.5% (5.2).

However, these results were only based on implant survival time, while the bone quality in the grafted area was not considered. Moreover, when analyzing in depth the reviews, evaluating the distribution of the data in each study, surprisingly it was possible to note a wide difference in confidence intervals (DCI). In fact, DCI was 38.8% for Del Fabbro and colleagues[8,34] and Wallace and Froum,[7] while for Cabezas-Mojón and colleagues[35] it was 19%. No final conclusion could be drawn. Considering the time of failure, it was reported that more than 80% occurred during the first 6 months of loading, and 97.1% within the first year of loading, while the late implant loss (up to 2 years from the abutment connection) was significantly affected by the bone quality.[36]

More investigations should be performed on this topic.[37] The low histological performances of most BSBs appeared clear when it was considered that, on average, slightly more than one-third of the grafted space was filled by NB after 6 months. The present results showed that none of the BSBs evaluated performed better than any of the others. Moreover, the augmented bone had a density structure resembling poor D3 type bone. These results are supported by those of Gisep and colleagues[38] who showed, in a mechanical ex vivo investigation on sheep, a mechanical behavior of materials comparable to that of cancellous bone. As a consequence, the clinical implant loading protocol should be progressive and related to the number and the dimensions of the inserted implants. Some limitations of the present paper were related to both the limited number of cases evaluated after long term, and the limited types of BSBs examined. The use of data retrieved from our histological laboratory helped, probably, to minimize many of the procedural bias. Further investigations on the biomechanical performances of regenerated bone will help to determine the appropriate clinical use of these materials.

In conclusion, the clinical implications of the present observation appeared to be irrelevant in cases for which the BSBs were used with the aim to restore or augment bone for aesthetic/prosthetic reasons without implant placement. Instead, for those cases in which the use of BSBs was an essential pretreatment for implant prosthetic restorations, it was necessary to take into consideration that the augmented bone, after 6 months of healing, had on average a structure like poor D3 type bone and represented one-third of the space filled by BSBs. Finally, none of the evaluated biomaterials seemed to be an ideal BSB.

DISCLOSURE

The authors have not received remuneration or other perquisites for personal or professional use from a commercial or industrial agent in direct or indirect relationship to their authorship.

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