BAG-S53P4 as an Additive to Bone Allografts: A Laboratory Study Using an Uniaxial Compression Test

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ABSTRACT: We want to address the clinical issue of too sparse supply of allograft in total hip replacement and ambitions of controlling the grain size distribution. Bioglass BAG-S53P4 was evaluated as a bone graft additive to chemically treated allografts with controlled grain size distribution. Allografts were chemically cleaned (CG) and mixed with BAG-S53P4 additive (BG) for comparison. All samples were compacted with a dropped weight apparatus and then underwent a uniaxial compression test. The yield limit was determined by a uniaxial compression test and density was recorded while flowability was calculated. There was no difference between the yield stress limit of BG and CG after compaction ($p = 0.432$). Adding BAG-S53P4 reduced flowability and could indicate better interlocking mechanism between particles. Adding BAG-S53P4 seems to have no impact on the yield stress limit. The extended allografts withstand the compaction equally good which makes it a valid bone substitute in total hip replacement. An in vivo loaded study is needed before clinical use can be recommended. © 2015 Orthopaedic Research Society. Published by Wiley Periodicals, Inc. J Orthop Res

Keywords: BAG-S53P4; bioglass; additive to bone allografts; bone impaction grafting; bone graft extender

Compacted bone allografts are used to fill and reconstruct bone defects in orthopaedic and trauma surgery with good clinical long term results.[1–3] Allografts are compacted to prevent early massive subsidence of an implant.[4,5] The initial stability of the allografts also depends on the distribution of grain sizes,[3,5,6] as well as on the specific preparation method.[7–9] Different materials have been studied as bone graft extenders to overcome shortages of material from human donors.[10–14]

One such artificial substitute is bioglass.[15] Bioactive glasses are composed of SiO_2, Na_2O, CaO, and P_2O_5, all of which naturally occur in the human body.[16–18] The main advantage of bioglass is its bioactivity, while its disadvantages include its mechanical weakness and low fracture resistance. Bioglass S53P4 (BAG-S53P4) bonds firmly to bone and promotes new bone formation while naturally inhibiting bacterial growth.[19] The Young's modulus of BAG-S53P4 is very close to that of cortical bone.[20,21] Lindfors et al. proved clinically that the long-term performance is equivalent to that of autogenic bone.[19] It can be also used as a carrier material for antibiotics.[22]

BAG-S53P4 has similar mechanical properties as cortical bone tissue and could, therefore, be used as a bone graft extender in case only limited bone material is available.

The aim of this study was to evaluate the mechanical effects of adding BAG-S53P4 to chemically treated allografts with controlled grain size distribution.

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METHODS

Femoral heads were donated by patients who underwent total hip replacement surgery and gave their consent previously. The donated material was fresh-frozen at −80 °C and stored at −20 °C. Cortical and cartilage tissues were removed and the femoral heads were reduced to fragments smaller than 10 mm in diameter using a bone mill (Noviomagus, coarse milling drum, Spierings Medische Techniek BV, Nijmegen, The Netherlands) under sterile conditions.[6,23] Allografts were washed chemically, dried for 4 days at 37 °C and carefully mixed to minimize any patient-specific bone characteristics.[24] The allograft material was sieved (calibrated sieves; pore diameter between 0.063 and 16 mm; Shaker Amplitude 10 mm with 1 h application time; Haver and Boecker, Oelde, Germany) and divided into two groups of 30 samples each:

- Allografts with BAG-S53P4 (BonAlive® Biomaterials Ltd, Turku, Finnland) (BG)
- Control Group (CG)

In the BG group, particles smaller than 4 mm were substituted with BAG-S53P4, which is commercially available in three different grain sizes. The separated allograft material was reassembled according to the particle size composition specified in Table 1. Each sample was then carefully mixed and the weight recorded. All samples underwent a uniaxial compression test before and after being compacted with a dropped weight apparatus in a compaction chamber with an inner diameter of 40 mm and a resulting volume of 213.5 cm^3. The allograft material was compacted by dropping a weight of 1450 g 10 times from a height of 180 mm onto the allograft material.

The allograft material was initially loaded by a consolidation stress of 4 kPa in the vertical direction inducing an initial density d_1. With an electromechanical material testing device (Zwicki-Line Z 2.5, maximal load 2.5 kN, 320 kHz sample rate, accuracy ±0.04 N and ±2 μm, Zwick GmbH & Co. KG, Ulm, Germany), the samples were loaded with an increasing vertical stress at a constant compression velocity of 2 mm/min. A punch with a diameter of 15 mm was used, the preload was set to 5 N, and the data sample rate was

Table 1. Grain Size Distributions for the Two Groups are Reported

Group	Material	Particle Size (mm)	Quantity of Particles (%) in Weight
BG	Bone graft material	>4	63.50
		2.00–3.15	10.75
	BonAlive® BAG-S53P4	1.00–2.00	7.50
		0.50–0.80	18.25
CG	Bone graft material	>4	63.50
		2–4	10.75
		1–2	7.50
		<1	18.25

50 Hz. The yield limit YL, as well as the density at the yield limit d_{YL}, was determined at the failure point of the material (Fig. 1).

The coefficient of flowability (ffc) was calculated by dividing the unconfined yield strength, YL, by the consolidation stress. The coefficient of flowability provides a numerical classification of the interlocking mechanism between particles with a flowablity below 1 being classified as not flowing according to Jenike et al.[25]

The force displacement graphs measured by the electromechanical testing machine were analyzed in OriginPro8.5 (Origin Lab Corporation, Northampton, Massachusetts). A peak detection algorithm was used to determine the unconfined yield limit. The trend of the measured curves was eliminated by subtracting an exponential baseline. The baseline was calculated over 50 anchor points, which were determined after applying a smoothed 2nd polynomial order function (Savitzky Golay smoothing method with window size 10 and a threshold of 0.05). The peaks were then extracted by searching for the positive local maxima over 100 points after applying a smoothing window-sized 50 measurement points. The bulk density d_{YL} at the failure point was determined by considering the sample height at the instance when material failure occurred.

The sample size of $N = 52$ resulted from a power analysis performed for a two-tailed independent samples T-Test a priori with an expected effect size of $d = 0.8$ (G*Power 3.1.2, Universität Kiel, Germany, $\alpha = 0.05$, power = 0.80, number of groups = 2).

The two-tailed T-Test for dependent samples was used to compare the results before and after compaction within each group. The two-tailed T-Test for independent samples was used to compare BG and CG. All statistical calculations were conducted with SPSS v.20 (IBM, Chicago, Illinois). A p-value of <0.05 was considered statistically significant.

RESULTS

Adding BAG-S53P4 to the chemically treated allografts with controlled grain size distribution did not affect the yield limit after compaction (Fig. 2). No statistically significant difference regarding the yield limit could be found between CG and BG after compaction ($p = 0.432$).

Before compaction, a statistically significant increase was observed for BG compared to CG for the initial density ($p < 0.001$), the density at the yield limit ($p < 0.001$), at the yield limit itself ($p < 0.001$), and for the flowability coefficient ($p < 0.001$) (Table 2). After compaction, BG showed a statistically significant higher initial density d_1 ($p < 0.001$), a higher density at the yield limit d_{YL} ($p < 0.001$), and a lower flowabiltiy coefficient ffc ($p = 0.020$) when compared to CG (Table 3). The mean weight of the samples in BG was 8.03 ± 0.03 g, while in CG, the samples had a weight of

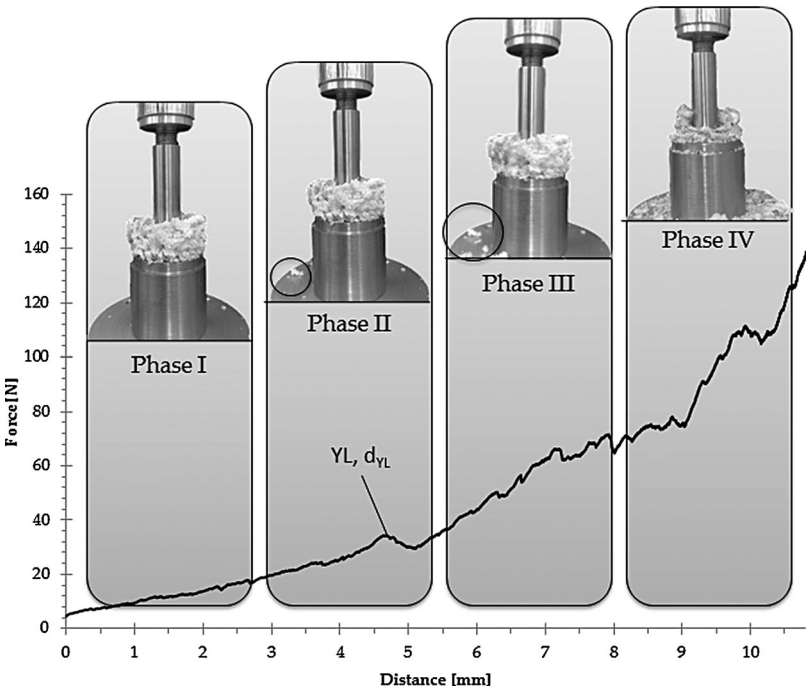

Figure 1. An example of the force and distance variations while carrying out an uniaxial compression test. In Phase I, the punch is lowered into the consolidated allografts. In Phase II, a significant peak indicates the YL and the corresponding d_{YL} of the material under a compressive load. The load is now bigger than the cohesive forces and first fragments of the consolidated bone mass are getting pulled out of the sample (circle). In Phase III, more and more fragments are getting pulled out (circle) and the bone mass is successive destroyed by the compressive force. In Phase IV, finally the sample was completely disintegrated and the measured compressive force rises as some bone fragments are blocked under the punch.

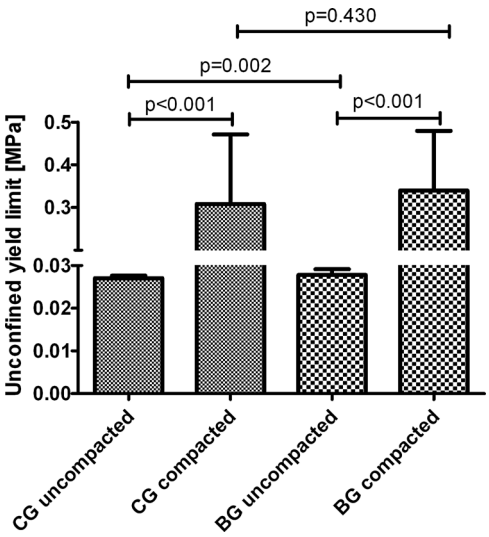

Figure 2. Comparison of the yield limit before and after a standardized compaction for the two groups under evaluation.

8.01 ± 0.01 g. There was no statistical significant difference in weight for the two groups ($p = 0.269$).

The coefficient of flowability was <1 for both groups before compaction which can be classified as not flowing (Fig. 3) according to Jenike et al.[25] The flowability coefficient decreased in both groups by a factor of 10 after compaction. BG had the smallest value of flowability, while CG had the highest value before and after compaction.

Pair-wise comparisons within each group for the values before and after compaction with the fall hammer apparatus returned statistically significant differences ($p < 0.001$) for all parameters (initial density d_1, the density at the yield limit d_{YL}, the yield limit YL, and flowability coefficient ffc). In both groups, compaction increased the initial density by 33% after compaction ($p < 0.001$ for BG and CG), while the density at the yield limit increased by 42% for CG ($p < 0.001$) and 39% for BG ($p < 0.001$) (Fig. 4). The yield limit YL showed an increase of approximately 96% in CG ($p < 0.001$) and 93% in BG ($p < 0.001$), while the flowability coefficient decreased by approximately 95% for CG and 94% for BG, which confirms the importance of impacting bone chips used for load bearing applications like in hip arthroplasty (Fig. 5).

Table 2. Comparison of Mean and Standard Deviation of the Yield Limit (YL), Flowability Coefficient (ffc), Initial Density d_1, and Density at the Yield Limit d_{YL} for the Uncompacted Samples

	CG	BG	p-Value
YL (MPa)	0.013 (0.009)	0.023 (0.012)	$p < 0.001$
ffc	3.0 (1.6)	1.6 (1.1)	$p < 0.001$
d_1 (g/cm^3)	0.424 (0.052)	0.554 (0.050)	$p < 0.001$
d_{YL} (g/cm^3)	0.462 (0.061)	0.595 (0.062)	$p < 0.001$

Table 3. Comparison of Mean and Standard Deviation of the Yield Limit (YL), Flowability Coefficient (ffc), Initial Density d_1, and Density at the Yield Limit d_{YL} for the Compacted Samples

	CG	BG	p-Value
YL (MPa)	0.308 (0.163)	0.339 (0.140)	$p = 0.432$
ffc	0.13 (0.08)	0.10 (0.04)	$p = 0.020$
d_1 (g/cm^3)	0.639 (0.081)	0.830 (0.078)	$p < 0.001$
d_{YL} (g/cm^3)	0.801 (0.308)	0.986 (0.084)	$p < 0.001$

DISCUSSION

In BG, bone chips smaller than 4 mm were substituted with BAG-S53P4. The initial density as well as the density at the yield limit was statistically significant higher for BG when compared to CG. The difference of the initial density before and after compaction was equal for CG, which means that the volume reduction was similar. Therefore, BAG-S53P4 showed a similar resistance to the compression force of the fall hammer apparatus. The density at the yield limit was statistically significant higher for BG than CG, still both groups had the same grade of compaction of approximately 40%.

The higher initial density as well as the higher density at the yield limit before and after compaction of BG can be explained by the adding of smaller BAG-S53P4 particles (>3.15) equally in weight as the allografts (Table 1). The allografts in CG contained also particles between 3.15–4 mm. In BG, a tighter "packaging" was obtained filling out spaces between particles.

BAG-S53P4 has similar mechanical properties as cortical bone tissue.[20,21] Allografts, however, consist mainly from spongious tissue. We would have expected

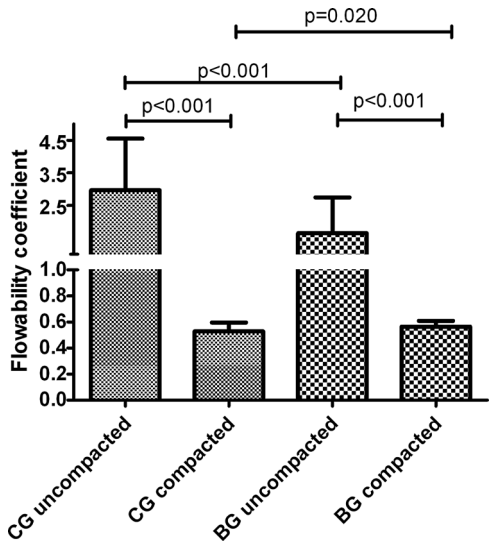

Figure 3. Comparison of the flowability coefficient before and after a standardized compaction for the two groups under evaluation.

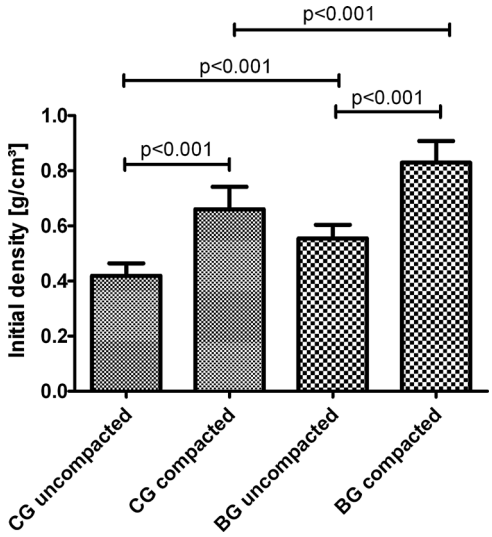

Figure 4. Comparison of the initial density before and after a standardized compaction for the two groups under evaluation.

a higher yield stress limit for BG compared to CG after compaction. As no significant difference was found between the two groups before and after compaction, it proves that BAG-S53P4 has a similar mechanical behavior under loading conditions as allograft despite it mainly consist of spongiosa. Also, the amount of how much the yield stress limit improved before and after compaction was similar in both groups. It is, therefore, not possible to draw any conclusions as to which group shows a more favorable outcome regarding mechanical stability.

The coefficient of flowability before compaction was lower in BG than and in CG with CG displaying twice the value of BG. After compaction, the flowability of both groups was reduced by a factor of 10, with CG having a higher flowabilty than BG. In CG, a much larger reduction was observed. This indicates that the BAG-S53P4 enhances the cohesion between the allograft particles. After applying a compaction force, the interlocking mechanism is not as evident anymore. On the other hand, the allograft mixed with BAG-S53P4 had the highest densities in all cases compared to CG. This could be due to the tight packaging of the material reducing the flowability of the graft and the resulting increased interlocking between the particles.

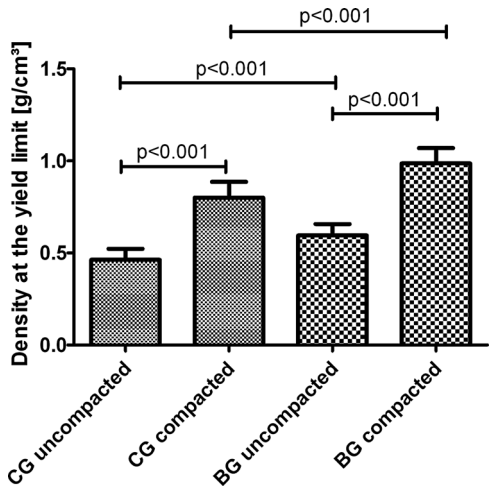

Figure 5. Comparison of the density at the yield limit before and after a standardized compaction for the two groups under evaluation.

A limitation of the study is that the bone quality was not measured in a densitometry analysis; however, only non-osteoporotic patients were selected for bone tissue donation.

In BG, bone chips smaller than 4 mm were substituted with BAG-S53P4 particles smaller than 3.15 mm, which may have influenced the compaction behavior and density measurements. The mechanical results are applicable to cleaned dry bones and dry BAG-S53P4. Mechanical properties will change when the materials come in contact with liquids. As the dry bone material and the BAG-S53P4 were very brittle, a high standard deviation was observed in the yield limit during mechanical testing. Further studies should determine the quantity of liquids necessary to achieve a satisfying result regarding the primary stability.

Achieving a high density may not be the major goal for the bone remodeling process as it may actually obstruct new osteocytes from growing into the allograft material.[26] The influence of the density on the bone remodeling process should be evaluated in further studies to come for better conclusions on the bone impaction technique. Also, the bone remodeling process of cleaned, well graded allograft mixed with BAG-S53P4 should be evaluated in terms of osteoconductivity and osteointegration.

We believe that using allografts in bone stock reconstruction is a valid and proven method specifically beneficial for younger patients who are at risk of facing another revision during their life.[27–29] In 18% of all hip revision cases performed in our institution, we used allografts that have the potential to recreate bone. Shortages in providing enough allografts in specific gradation can be avoided using alternative bone graft additives.[30,31]

In conclusion, the BAG-S53P4 extended allografts withstand the compaction equally good as the allograft alone, as no statically significant difference was found for the yield stress limit. Many of the aspects discussed indicate that the BAG-S53P4 is ready to be tested in an experimental animal model as a allograft extender in vivo.

AUTHORS' CONTRIBUTIONS STATEMENT

All authors have seen and concur with the contents of the manuscript. All authors have made substantial contributions and were involved in the study as well as the preparation of the manuscript. The authors declare that the material within the submitted paper

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