Manufacture of Lyophilized Cortical Bone Particles for Bone Filling Applications by Machining Methods

Lina M. Bernal and Fabio A. Rojas
Universidad de los Andes, Bogotá, Colombia
lm.bernal72@uniandes.edu.co, farojas@uniandes.edu.co

***Abstract*— Initially was conducted a study of the properties that bone particles for bone filling applications must fulfill and the influence of these properties on the shape and size of the particles. Then we studied the shape and size of commercial particles and determined a geometry that can satisfy these properties with a better assessment than previously studied particles. Then a factorial experiment is design where we studied the parameters that are influential in the process of cutting and the particles that best meet the preselected geometry were selected.**

***Index Terms*—bone particles, cortical femoral bone, manufacture, machining.**

I. Introduction

The use of bone grafts as an alternative for reconstruction of bone defects, whether congenital or caused such trauma, oncology and infectious sequelae, are intended to restore the anatomical and functional integrity of an altered structure[1]. This is achieved by the process of Guided Bone Regeneration, which consists in obtaining bone defect filling volume or increasing crestal bone loss using a combination of membrane and membrane simply graft or blood clot.

The high cost of the material currently used grafts necessitates the search for better techniques using traditional materials, able to maintain the high quality of the grafts known on the market. That's why in this paper aims studied the machining process of bovine bone in order to obtain a process to manufacture bone grafts with better properties. Despite the widespread use that bone powder have had in the last decades, there is little information on the effect of the geometry of the particles in the bone regeneration [2], therefore, the methodology that have been used describes a selected geometry that is presupposed to have a better performance in the bone regeneration.

In this spirit, the first step, is to characterize the shape of bone particles commercially available and then by the same methods the ones obtained by machining process and the variables that govern it. In this sense, the machining processes known in greater depth are related to singlepoint cutting tools, in this study only turning processes would be used.

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The properties which are important in bone graft and its relation to the manufacturing process shown below:

Particle size: the particles must be sized large enough to have a mechanically strong structure and small enough to allow a high concentration in the cavities where it will be implemented. A review of the literature shows some consensus on the recommendation of using cortical particles of sizes between 300 and 800 microns [3].

Size and pore structure: interconnectivity is desirable as close to 100% to avoid the limitation of the overall biological system and vascularization necessary for tissue growth [4].

Surface Topography: This suggests that a greater surface area promotes more zones of material for bone formation while maintaining more fibrin clots in the early stage of bone regeneration [5].

Chemical Composition and Degradation: The biocompatibility of the material is guaranteed to contain the same chemical composition as the original material.

Mechanical strength: Need the ability to respond to relatively high loads [6].

After that, the implementation of a method of manufacture is described in which we specify all process variables to produce bone particles that promote bone regeneration process by meeting the characteristics of improving the relationship between the area and volume of the particles currently commercially available and achieving the characteristics of being statistically of the same size between them (which earns one step in the industrial processing, the sieving) and to have dimensions that are deemed suitable according to the literature for being absorbed by the body.

II. Materials and Methodology

A. Femoral Cortical Bone

It was machined by turning femoral bovine bone obtained in the local butchery market and cleaned with commercial detergent and lyophilized by immersion in salt for a period of four months for achieving the homogeneity in the element. The subjection of the bone was made by a structural device created for the particular problem and the collection of the particles was made by an acetate cage with absence of color allowed to monitor the cutting process.

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B. Commercial Products

Since we want to achieve bone particles whose properties match those found in the literature which provide the best conditions for osteoinduction and osteoconduction processes for grafting, it is necessary to measure the characteristics of the particles that are currently implemented. Therefore particles of the following brands were characterized: Bio-oss, lyophilized human bone particles obtained from the Bank of Bones and Tissues Cosme and Damian, Hydroxyapatite Bionnovation and Cerasorb.

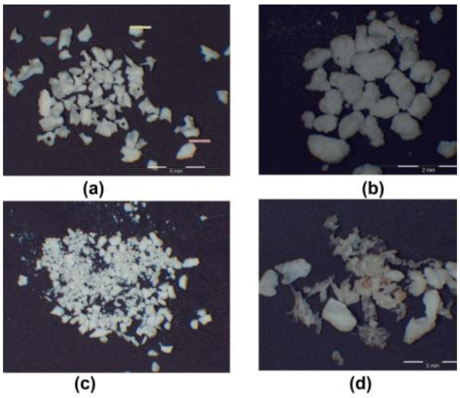

Figure 1. Ommercial particles (a) Bio-oss, (b) Cerasorb, (c) Bionnovation hydroxyapatite and (d) Human bone particles from cosme and damian bone and tissue bank. scale shows 2 mm.

C. Definition of Particles

For the formation of a particle capable of being grafted, there are some fundamental properties previously described in the introduction to favor osteoconduction and osteoinduction, therefore it was necessary to formulate a particle geometry that we were able to reproduce and that could meet those needs in a more palatable form than those currently on the market. To accomplish this end, were taken shavings from previously achieved machining processes [7], and selected the one that best met with the features that are necessary to fulfill the role of bone graft with the assessment of medical specialists according to the shape of the osteocytes, the original cells involved in the be regeneration process.

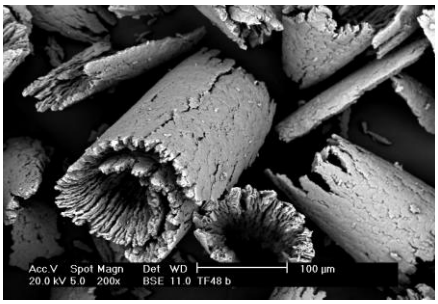

Figure 2. SEM of spiral bone particles at 200x.

The selected particle is one that is seen in Fig. 3. In the figure, the characteristics of a particle formed by a porous sheet become evident and coiled to form a spring-like structure with a certain number of turns which was defined to be >1.According to that, we wanted to design a manufacture process to recreate the geometry of this particles as much as possible to be able to that could possibly met the requirements for improving the possibility of bone regeneration in a defect.

D. Machining Process

For the processing of the new particles, we proceeded to design a factorial experiment for three different machining processes: turning, turning with an angle of incidence and bucking with corresponding parameters to each experiment which were selected according to some preliminary tests. The three processes are illustrated in Fig. 2. The tool used to cut was a monocrystalline synthetic diamond insert in shape of diamond of 60 ° of Technodiamant brand with a radius of 0,75 mm and a rake angle of 0 °

Preliminary Test: Since bone is a new material from the manufacturing point of view, besides the small literature on the machining of this material, it was on the little information available [3], [4] in order to define initial conditions that allowed us to evaluate the response of the material to the machining process.

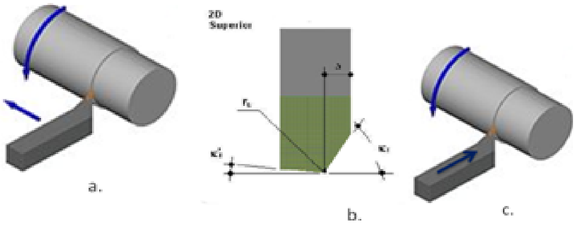

Figure 3. (a) Traditional turning process, (b) Superior 2D view of the cutting tool where K’r is the incidence angle we varied, (c) Traditional buckling process

The preliminary test included a huge range of values for the parameters in order to be able to select the ones that behaved the best and create smaller intervals for the following trials. The factors considered for the turning process where cutting speed, depth and advance and for turning with incidence angle we added the factor of the incidence angle. Each of the levels considered are showed on Table I and Table II for the turning and turning with incidence levels respectively.

TABLE I. FACTORS AND LEVELS TURNING PRELIMINARY TESTS

Factor	Units	Level 1	Level 2	Level 3	Level 4
Cutting Speed	rpm	102 4	512	352	
Depth	mm	0,15	0,3	0,4	0,5
Advance	mm/rev	0,2			

TABLE II. FACTORS AND LEVELS TURNING WITH INCIDENCE ANGLE PRELIMINARY TESTS

Factor	Units	Level 1	Level 2	Level 3	Level 4
Incidence Angle (Kr)	degrees	5	10	15	
Cutting Speed	rpm	128			
Depth	mm	0,3	0,5	0,8	**1**
Advance	mm/rev.	0,7			

Trials: Once some approximated ranges in which the performance of the tools and the material was acceptable where obtained, we proceed to perform the real trials with the following factors and levels for each one of the processes explained before.

Also, as each one of the processes yields to some typical characteristics in the geometry of the particles, each one of them is accompanied by a figure of the typical geometry of the particles obtained in each process according to all the samples obtained.

TABLE III. FACTORS AND LEVELS TURNING

Factor	Units	Level 1	Level 2	Level 3	Level 4
Cutting Speed	rpm	128	224	352	
Depth	mm	0,03	0,05	0,08	0,1
Advance	mm/rev	0,2	0,325	0,45	

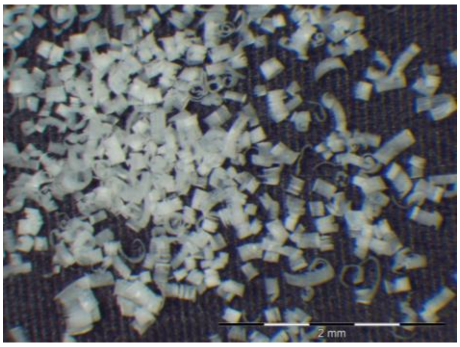

Figure 4. Typical geometry of particles obtained by turning

TABLE IV. FACTORS AND LEVELS TURNING WITH INCIDENCE ANGLE

Factor	Units	Level 1	Level 2	Level 3	Level 4
Incidence Angle (κ_r)	degrees	5	10	15	
Cutting Speed	rpm	128			
Depth	mm	0,3	0,5	0,8	1
Advance	mm/rev.	0,7			

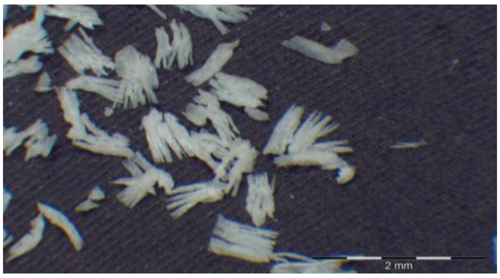

Figure 5. Typical geometry of particles obtained by turning with an incidence angle

TABLE V. FACTORS AND LEVELS BUCKING

Tool	Advance (mm/rev)	Cutting Speed (rpm)
Steel	0,2	128
	0,4	254
	0,7	352
Diamond	0,2	96
	0,3	144
	0,4	208

E. Particle Characterization

Once all the particles had been produced they were characterized by an Olympus Optical Stereoscopy and software for image manipulation without further treatment AnalySis finish condition and this corresponds to low feeds and depths of cut low.

During this characterization of the particle is particularly interesting in the same geometry, size, morphology, homogeneity and dispersion thereof.

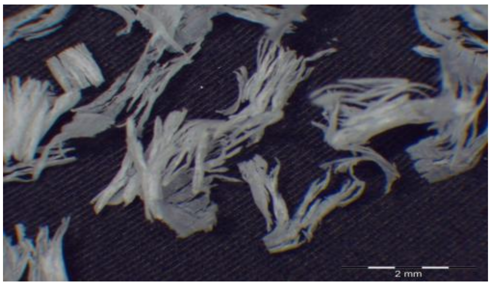

Figure 6. Typical geometry of particles obtained by buckling.

Through Fitter Polygon function Figure Editor, defined the area and perimeter of a polygon of 10 sides approximate as closely as possible to the contour of the particle as shown below.

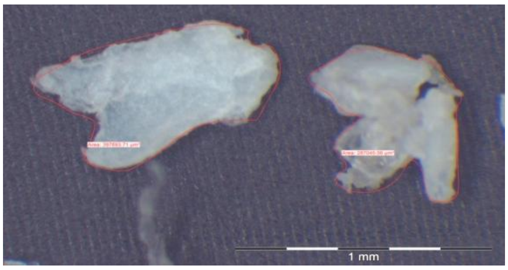

Figure 7. Particle characterization by figure analysis. The analysis function fitted polygon shows the contour of the particles.

Thus, dimensionless ratios were defined for the form of particles according to the area and perimeter thereof and the relationship between the radius that the particle would have if it were spherical according to its area and the radius that the particle would have if it were spherical according to its perimeter as:

$$Ratio = \frac{Area}{Perimeter} = \frac{r_a}{r_p} \quad (1)$$

where

$$r_a = \sqrt{\frac{Area}{\pi}} \quad (2)$$

and

$$r_p = \frac{Perimeter}{2\pi} \quad (3)$$

This way, a value of 1 in the ratio relationship indicates that the particle is completely spherical and 0 indicates that the particle shape lacks completely.

III. RESULTS

A. Shape Particle Analysis

The dimensionless ratio gives us information of how these particles are similar to a sphere. This is wanted as it promoted more density in the graft and better regeneration probabilities. In Fig. 8 we can see for commercial particles that the closest to a sphere where Bionnovation, followed by Cerasorb, Bio-Oss and particles from the bone bank. This is entirely consistent with the fact that the two above mentioned are the synthetic products while the two biological products have the worst value for the index.

In Fig. 9 the ratio for turned particles is illustrated with an sphericity average levels similar to those produced commercial with a biological raw material. Moreover, we see that the relationship is not symmetrical for all the particles, if asymmetric this asymmetry is upward, implying that certain particles have a larger sphericity index, which is considered positive. The ratio for particles produced with incident angle are shown in Fig. 10, which average value is significantly lower than for the particles without this angle displacements. However in this case we see that the dispersions are lower. The tendency of buckled particles is retained, where a clear difference is seen in Fig. 11 between the first 9 and the following experiments 9, where the ratio performed better for particles produced with steel, not only by having greater values but also with a lower dispersion in almost all cases.

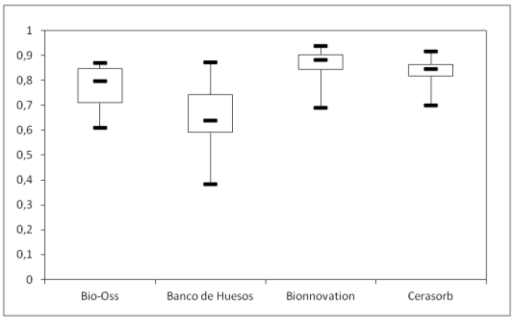

Figure 8. Box-and-whisker plot for the area Figure perimeter relation of the commercial particles

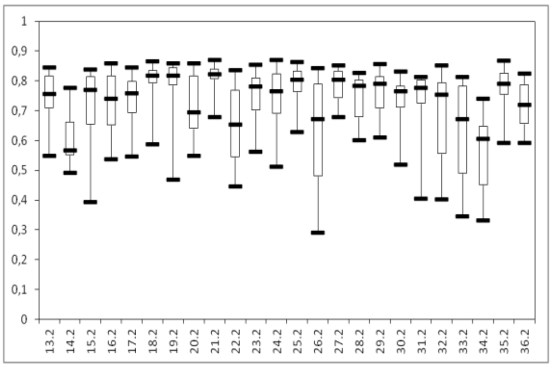

Figure 9. Box-and-whisker plot for the area/perimeter relation of the turned particles.

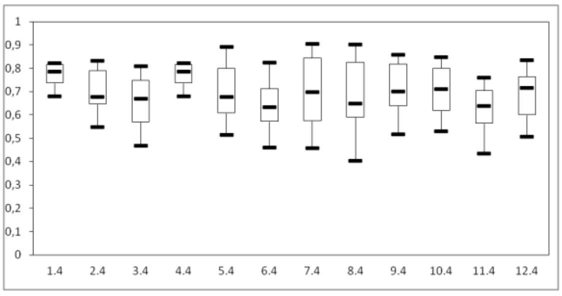

Figure 10. Box-and-whisker plot for the area/perimeter relation of the turned with an incidence angle particles.

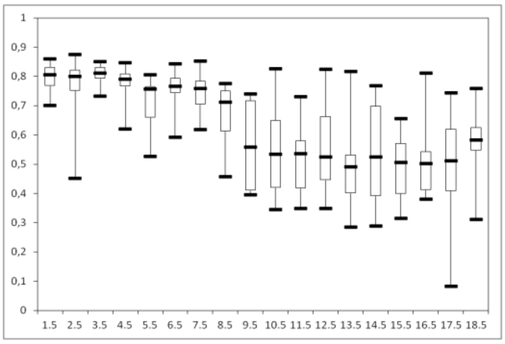

Figure 11. Box-and-whisker plot for the area/perimeter relation of the buckled particles.

B. *Particle Analysis*

ANOVA was performed to identify the relevant factors in obtaining the spiral geometry in the particles. Therefore, we studied the ratio $\frac{number\ of\ particles\ in\ spiral\ shape}{number\ of\ particles\ in\ the\ sample}$ for a sample of 80 to 120 particles in all cases.

TABLE VI. ANOVA TESTS RESULTS

Experiment	Influence Factor	p-value	Influence Factor	p-value
Turning	Cutting Speed	0,4495	Interaction Cutting Speed and Depth	0,0276 0,0153
	Depth	0,0464	Interaction Cutting Speed and Advance	0,0086 0,2585
Buckling	Tool	0,0069	Interaction Advance and Cutting Speed for Steel	0,0638 0,0709
	Cutting Speed	0,0273	Interaction Advance and Speed for Diamond	0,5789 0,6975
	Advance	0,7051		
Incidence Angle	Incidence Angle	0,0407	Interaction Angle and Depth	0,0477 0,3340
	Depth	0,5929		

In general we can formulate the null hypotheses for each of these tests that the factor is significant and alternate hypothesis that there is insufficient evidence to prove that the factor is significant. Thus the interpretation of these results can give them according to the null hypothesis is rejected if the p-value associated with the observed result is equal to or less than the significance level set, for this case it was 0.05.

The result of each of these analyzes of variance is reported in Table VI where the second column expresses the factor being tested individually, the third column gives the p-value for this test and the fourth column lists the interactions between two factors and the last column shows the p-value for the interaction between these factors in the proposed order.

Thus we found that in a process of turning the cutting speed and the interaction between this and the progress and advancement and depth are relevant. In a turning process with an incidence angle, the depth and the interaction between the depth and the incidence angle were proved to be statistically relevant.

Finally, we see that in a buckling process progress the interactions between the advance and the tool and the tool and the advance are relevant.

Having ruled out the factors that are not relevant to our analysis and retaining only the factors identified as affecting the result produced, we select particles having the best ratio of number of particles in the form that we need on the total number of particles as the particles selected to continue the production process specimens. The particles that meet these characteristics are shown in Fig. 12, which shows that almost all particles are spirals. They also have a length dimension of 650µm in average, which is between the sizes 250 and 1200 µm, characteristic of particles commercially available and therefore susceptible of being grafted.

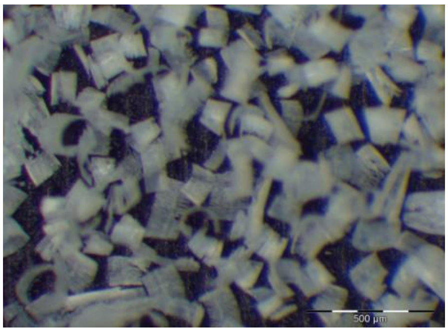

Figure 12. Stereoscopy of selected particles for the manufacture of the compressed tablets

IV. Conclusions

We conducted a manufacturing process based on the construction of bone particles from bovine specimens for which a factorial experiment was designed and conducted and for which the analysis of variance ANOVA provides information about the relevant factors in a cutting process. After addressing the ideal parameters for the conformation of a particle with a special geometry which provide technological advantages versus commercially available loose particles to the generation of new bone.

It is remarkable the high level of controllability that was achieved in this process, not only for the result obtained which corresponded to the design process for the geometry of the particles but by the ability to relate the parameters in the cutting process to the change of geometry in the particles which allows us to repeat this design process to a different geometry, and to create expectations about the possibility of achieve different geometries that can solve problems of bone regeneration in other parts of the body and under different load conditions.

The particles constructed and selected by the information provided by the statistical techniques where of a size that makes them suitable for being implanted and that improved the properties of the studied commercial products to some extent by a special design of the particle geometry which can promote regeneration processes within bone. This has been standardized for a production process by creating adimensional indexes of the area and perimeter relations from which important information is obtained about the sphericity and dispersion of the particle, and also allows defining the homogeneity between the particles in terms of statistical distributions of the same size, which is important in a production process.

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Lina M. Bernal was born in Bogotá D.C. – Colombia the 6th of July of 1989. She completed two different bachelors, which are mechanical engineering and industrial engineering at Universidad de los Andes, in Bogotá D.C. – Colombia in 2012 Actually she´s the CEO of a Colombian start-up developing, manufacturing and marketing medical devices such as screws, tacks for structural and membrane fixation and biomaterials as the one whose development was achieved as showed in this article. Current research interests include biomechanics, biomaterials and bioengineering. Eng. Bernal is an actual member of IIE and ASME.