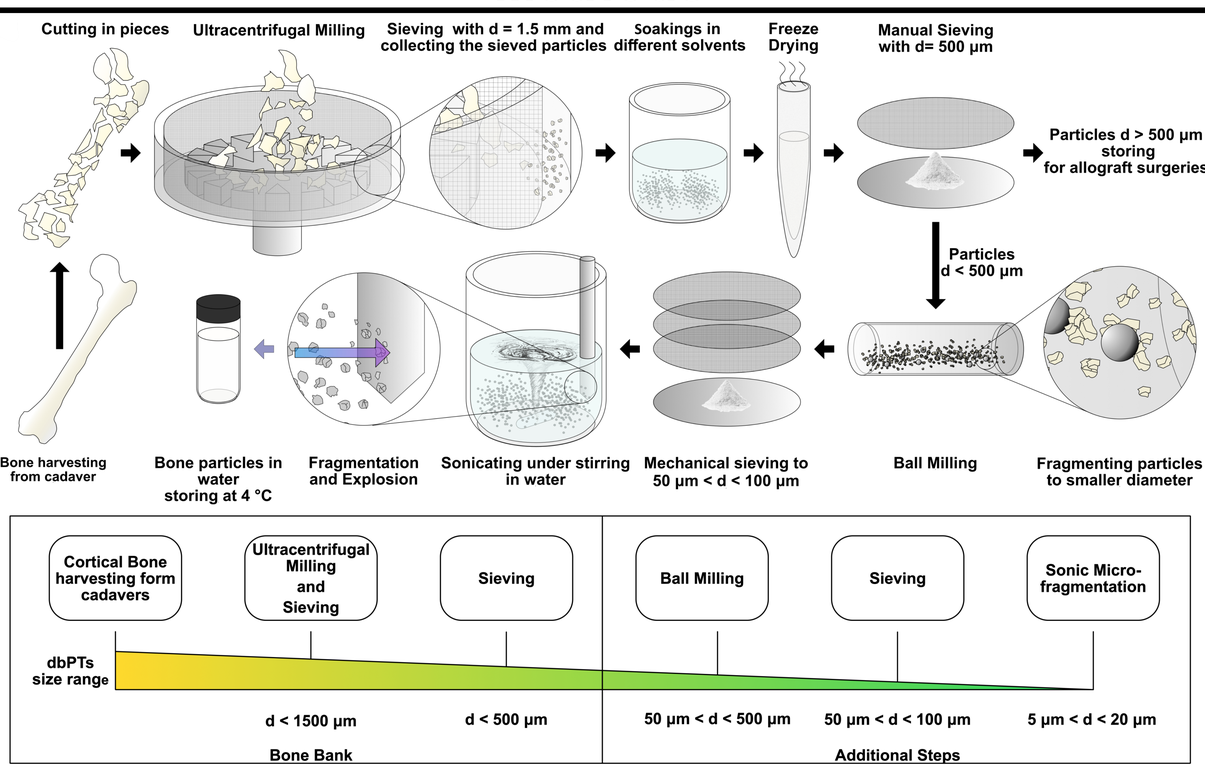
Improving Bone Regeneration Potential via Precision Ultrasonic Fragmentation of Decellularized Human Bone

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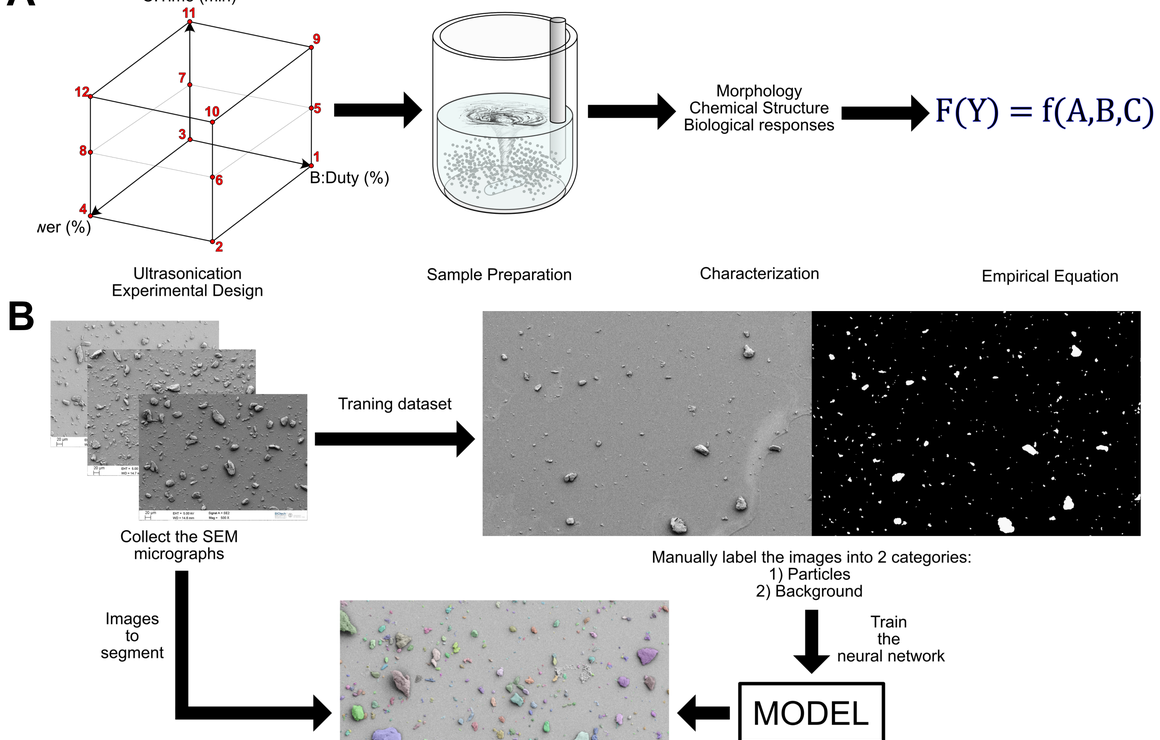
Introduction

Human decellularized bone particles (dbPTs) are commonly used in clinical practice to perform allograft surgeries. To reduce the immunological response in the recipient and ensure their safety, these fragments, undergo to decellularization, a procedure that greatly reduces their osteogenicity. In this work, by including an ultrasonication step to further fragment the human bone, control dbPTs size distribution, tuning their osteogenic potential. A Response Surface Method (RSM) was used to develop equations able to predict dbPTs properties based on different ultasonication factors (power, duty cycle and time).

Methods

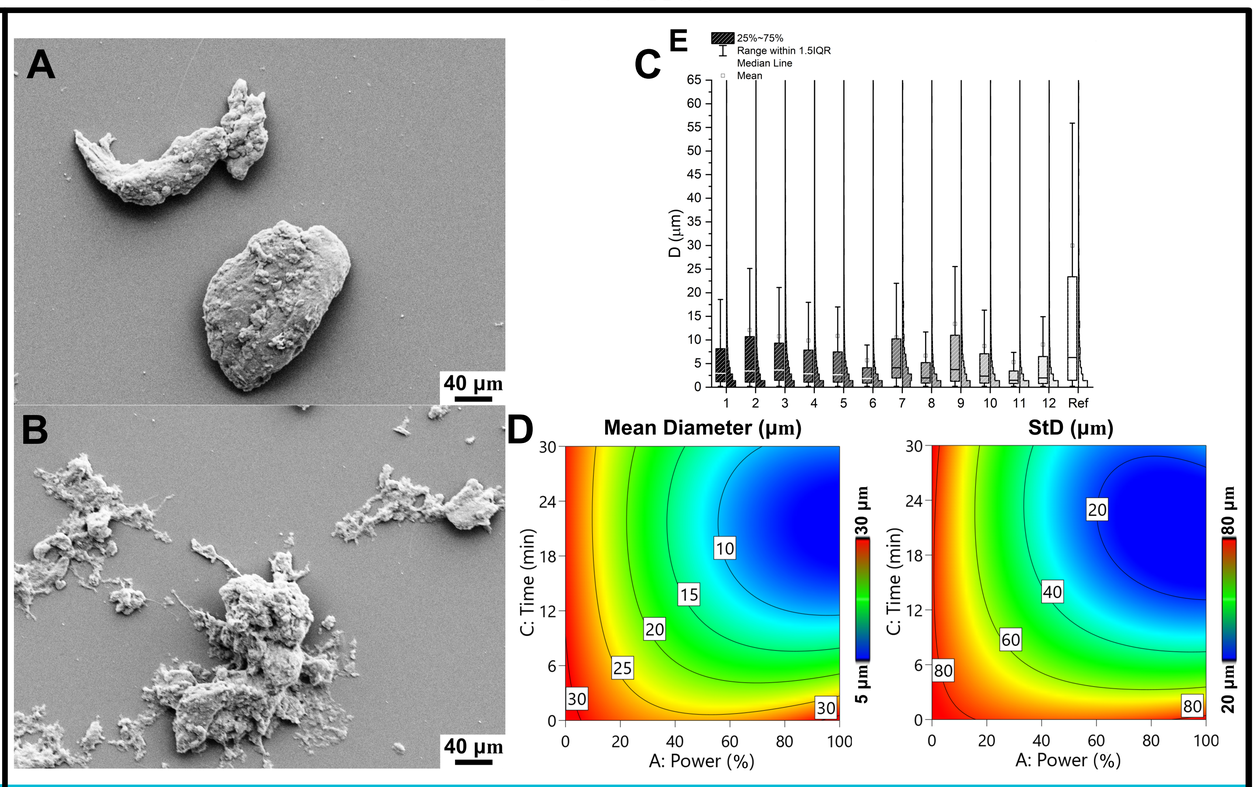


dbPTs manufactig process from cortical bone harvested from human donors. The size was progressively reduced to a mean diameter between 5 µm and 20 µm by centrifugal milling, ball milling and then ultrasonication. This first two steps are conventionally performed at the Musculoskeletal Tissue Bank while the last two were additional steps here introduced.



(A) The ultrasonication process was studied with a RSM, changing 3 factors (sonication power, duty cycle, process time) and studying their effect on the resulting dbPTs in term of morphology, chemical structure, and biological response. (B) The collected SEM images were used to develop a deep-learning model able to segment the dbPTs and determine their dimensional distributions.

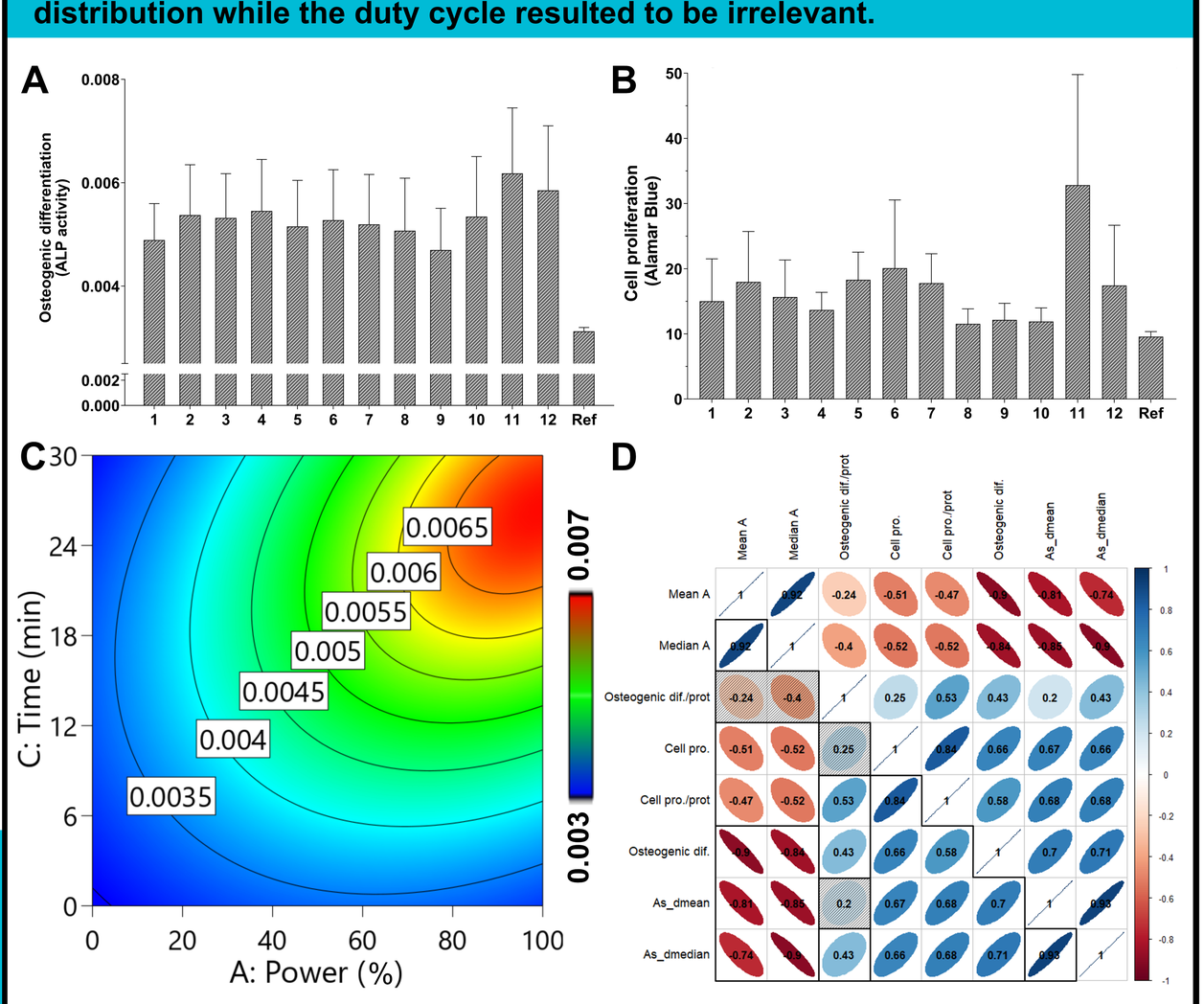
Results



(A-B) SEM ANALYSIS: The morphology produced by ultrasonication (B) was different from the morphology obtained by ball milling (A). The ultrasonication was effective in exposing the organic matrix (B) as highlighted by the presence of filaments.

(C) DIMENSIONAL DISTRIBUTIONS: The ultrasonication was also able to reduce the dbPTS mean diameter to a range between 5 µm and 20 µm compared to the sole ball milling (Ref) and contemporary decrease its standard deviation, shrinking the diameter distribution.

(D) RSM CONTOUR PLOTS: RMS revealed the influence of both ultrasonication power and process time on the particles dimensional distribution while the duty cycle resulted to be irrelevant.



(A-B) ALP AND ALAMARBLUE QUANTIFICATION: Ultasonication affected both the osteogenic differentiation (A) and the cell proliferation. In particular, the treatments that were able to reduce the particles size were also the most effective in increasing the biological response.

(C) RMS CONTOUR PLOT: The increasing trend of the ostogenic differentiation modeled by RMS was similar to the trend revealed for the decrease in particles' dimensions.

(D) CORRELATION MATRIX: A strong inverse correlation between the osteogenic differentiation and the dbPTs dimension (and a direct correlation with the total exposed surface).

Take home messages

- **The ultrasonication process was effective in both exposing the organic matrix and reducing the particles' dimensions and the standard deviation of their distributions.**
- **The biological outcome is linearly dependent to the particles' size.**
- **The sonication process was modelled using a Response Surface Method, allowing the control and tuning of the resulting material properties.**
- **Overall, this process is easly scalable to large production of material and thus applicable to bone tissue banks and medical device manufacturers.**

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