Rapid Bromothymol Blue Screening to Determine the Composition of Cortical Bone Powder

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Cortical bone powder is often processed into three, lyophilized compositions: mineralized particulate (MIN), demineralized particulate (DEMIN), and a 70:30 blend of the two (BLEND). BLEND is a mix of MIN:DEMIN requiring both types of bone powder to be open in the same room during processing. MIN and DEMIN are indistinguishable from each other in a lyophilized state. As a result, errors in particulate packaging are difficult to detect and require expensive and time-consuming residual calcium testing to verify error.

Bromothymol blue (BTB) is a low-cost, pH color indicator that gradually shifts from yellow to blue as pH increases from 6.0 to 7.6. As a result, BTB can be used to detect small differences in pH around neutral. Due to differences in bone powder processing, DEMIN is expected to exhibit a different pH profile than MIN and is expected to allow for deeper penetration of the BTB stain compared to MIN. Consequently, BTB should be able to discriminate between the three compositions of particulate. The goal of the current study is to develop a rapid, qualitative method to determine bone powder composition using 0.04% BTB.

BTB exposure will reveal distinct visual differences for each type of bone powder. MIN will resist staining and indicate a higher pH while DEMIN will more readily uptake the stain and indicate a lower pH. BLEND will show a mix of these particles and indicate a pH between MIN and DEMIN.

MIN, DEMIN, and BLEND samples were obtained from nine research-consenting donors (one sample per donor, three samples per bone composition). MIN (0.25-1mm), DEMIN (0.125-0.85mm), and BLEND (0.25-1mm; 70:30, MIN:DEMIN) were processed by Community Tissue Services (CTS) according to established procedures. 0.5cc of bone powder was incubated in 1ml BTB for 1 hour. Samples were then placed on absorbent towels to remove excess BTB, then placed on glass microscopy slides and evaluated under no magnification and at 50X magnification.

MIN indicated a slightly basic pH (blue) and superficial staining (lighter tint). DEMIN indicated a more acidic pH (yellow-green) and deeper staining (darker tint). BLEND indicated a neutral pH (blue-green) and contained particles with superficial and deep staining leading to demineralized particles with dark blue-green coloring (Figure 1).

The use of bromothymol blue is a rapid and effective method to determine the composition of bone powder samples. MIN indicated a higher pH and only superficial staining while DEMIN indicated a lower pH and deeper staining of particulate. These differences are attributable to the demineralization process; acid exposure and subsequent buffering result is a lower particulate pH (between 6.5-7.0) compared to MIN and the removal of calcium and other minerals leaves the spongy collagen network that is more susceptible to staining. BLEND, as a mix of the two types of particles, showed a pH in between the MIN

and DEMIN groups and, more importantly, showed a mix of particles with superficial and deep staining. BTB is an effective method for the identification of bone particulate through a combination of pH color differences and stain penetration.

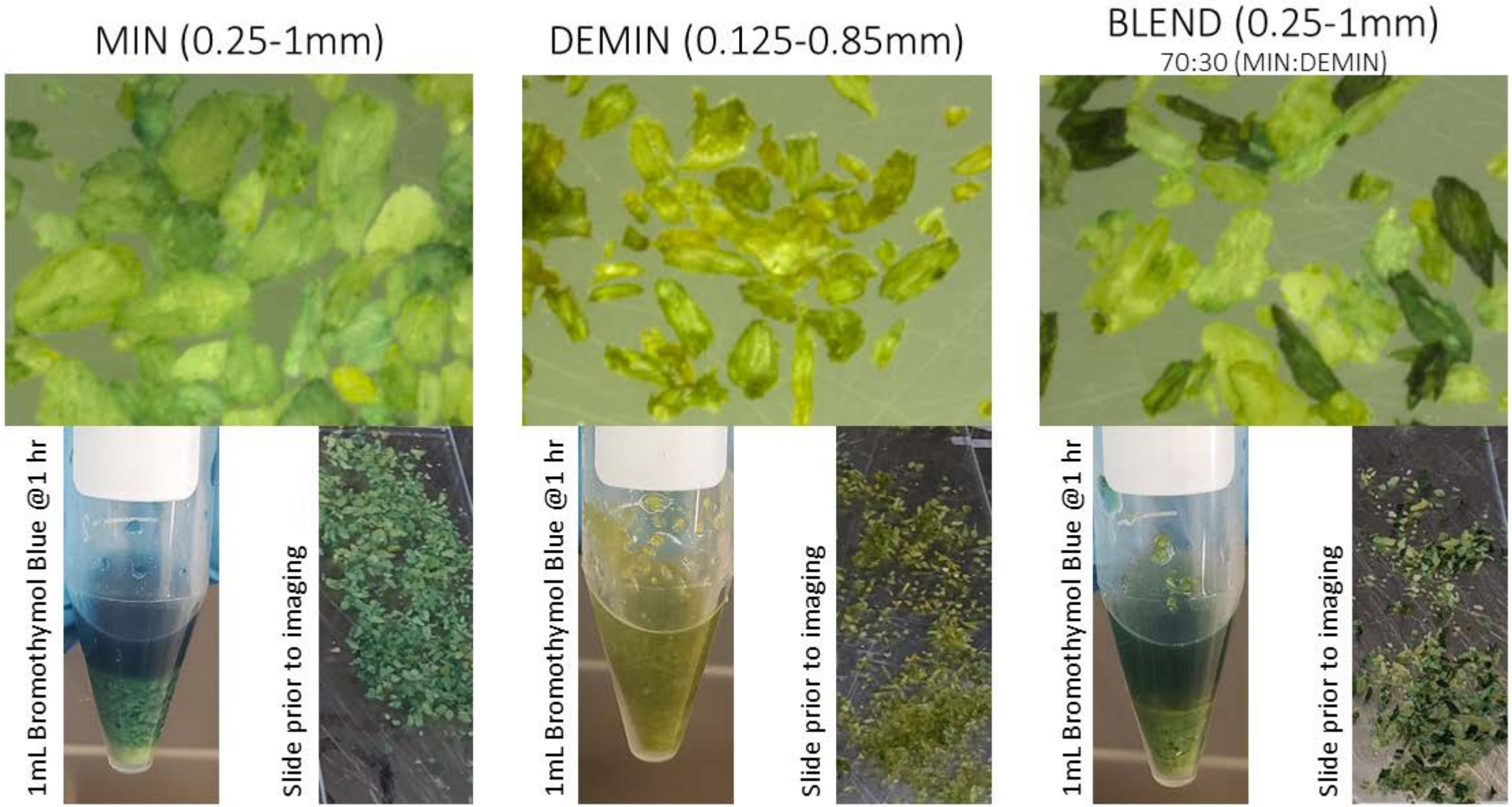

Figure 1: Representative bone powder appearance after exposure to BTB. Appearance of BTB after 1 hour incubation and unmagnified samples are also shown.