

Viability, Particle Size, and Cell Outgrowth of Bone Particulate Collected Into the GraftNet™ Device

Arthrex Research and Development

OBJECTIVE

This study aimed to assess whether bone particulate collected by various burs can contain viable cells and results in osteoblastic outgrowth that promotes osteogenesis.¹

METHODS AND MATERIALS

In a porcine spine model, an incision was made along the spinous process at the midline of each spine using an aseptic technique. Soft tissues were properly dissected and retracted until the right and left lamina were fully exposed. The following bur types were used to drill the lamina and facet at the highest speed (60,000 rpm) to collect bone particulate into GraftNet autologous tissue collector (Arthrex): 2.5 mm match head (2.5 MH), 3.0 mm match head (3 MH), 6.0 mm ball/round (6 R), 3.0 mm ball/round (3 R), and 5.0 mm diamond (5 D).

Table 1. Bur types used per study with associated sample size.

Bur Type	Sample Size (n)	Donor/Study #
2.5 MH	4	1-4
3 MH	4	1-4
6 R	4	1-4
3 R	2	1-2
5 D	1	1

To assess undigested samples, the bone particulate was cultured in 0.15 cc portions in osteoblast growth media (OGM) at 37° C under humidified conditions. A portion of the undigested sample was stored for particle analysis, and additional samples were plated for collagenase digestion.

To assess digested samples, bone particulate was incubated in collagenase in OGM (1.2 mg/mL) on a rotating shaker set to 200 rpm at 37° C for 25 minutes. The supernatant was collected into conical tubes, and samples were rinsed with Dulbecco's phosphate-buffered saline (DPBS) and added to the tubes.

The samples were centrifuged at 200 g for 5 minutes, and the resulting pellet was resuspended in OGM after aspirating the liquid.

Cell suspensions were plated in T-25 flasks, with all digestions consolidated into designated flasks. The digestion process was repeated, alternating between collagenase solution and 5 mM EDTA in DPBS, for a total of six collagenase digestions and three EDTA digestions. The culture flasks were incubated for 10 days in OGM at 37° C under humidified conditions and then cultured in osteogenic differentiation media (ODM) for 14 days.²

The following analyses were performed on undigested samples (refer to Table 1 for sample size per bur type). Viability analysis was performed at 21 days of culture using fluorescent staining for calcein AM and ethidium homodimer. Enzymatic alkaline phosphatase (ALP) analysis was performed at 24 hours, 8 days, and 21 days of OGM culture, and after 21 days in differentiation media. Alizarin red (AR) and calcium quantification via acid elution were performed after 21 days in differentiation media. Particle analysis was performed using fluorescent DTAF staining.

Digested sample analysis was performed for three donors, except for the 3 R bur (n = 1) and the 5 D bur, which were not analyzed. After 14 days in ODM, the samples were stained for colony-forming units via alkaline phosphatase (CFU-ALP).

To better compare bur sample types, ALP activity data were indexed to a known outcome through normalization to the positive control (2 mm bone pieces collected with a Kerrison rongeur). Image J was used to analyze particle size. Particle sizes of less than 15 µm were excluded from analysis and not used in the average size calculation. The maximum size value for the positive control was used to accurately report the largest piece of the sample.



RESULTS

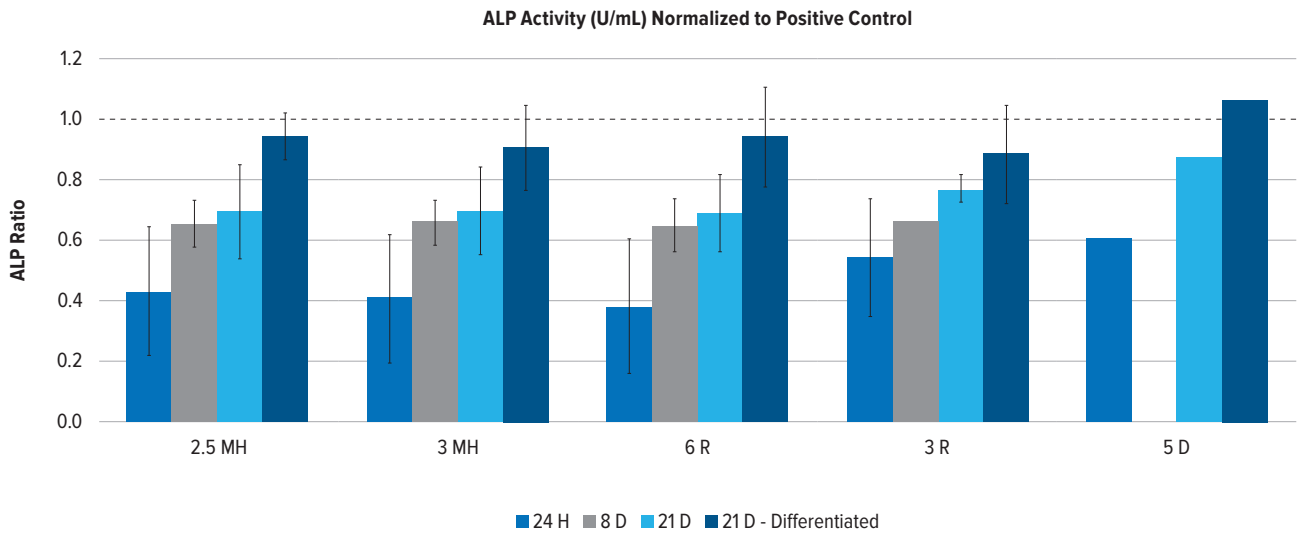


Figure 1. Ratio of alkaline phosphatase (ALP) measured in enzymatic activity (U/ml) when normalized to positive control pieces at 24 hours, 8 days, and 21 days for undigested samples cultured in OGM vs 21 days cultured in ODM. The dashed line represents a ratio of 1.0, indicating equivalency to the control. The 5 D group (n = 1) was not measured at 8 days.

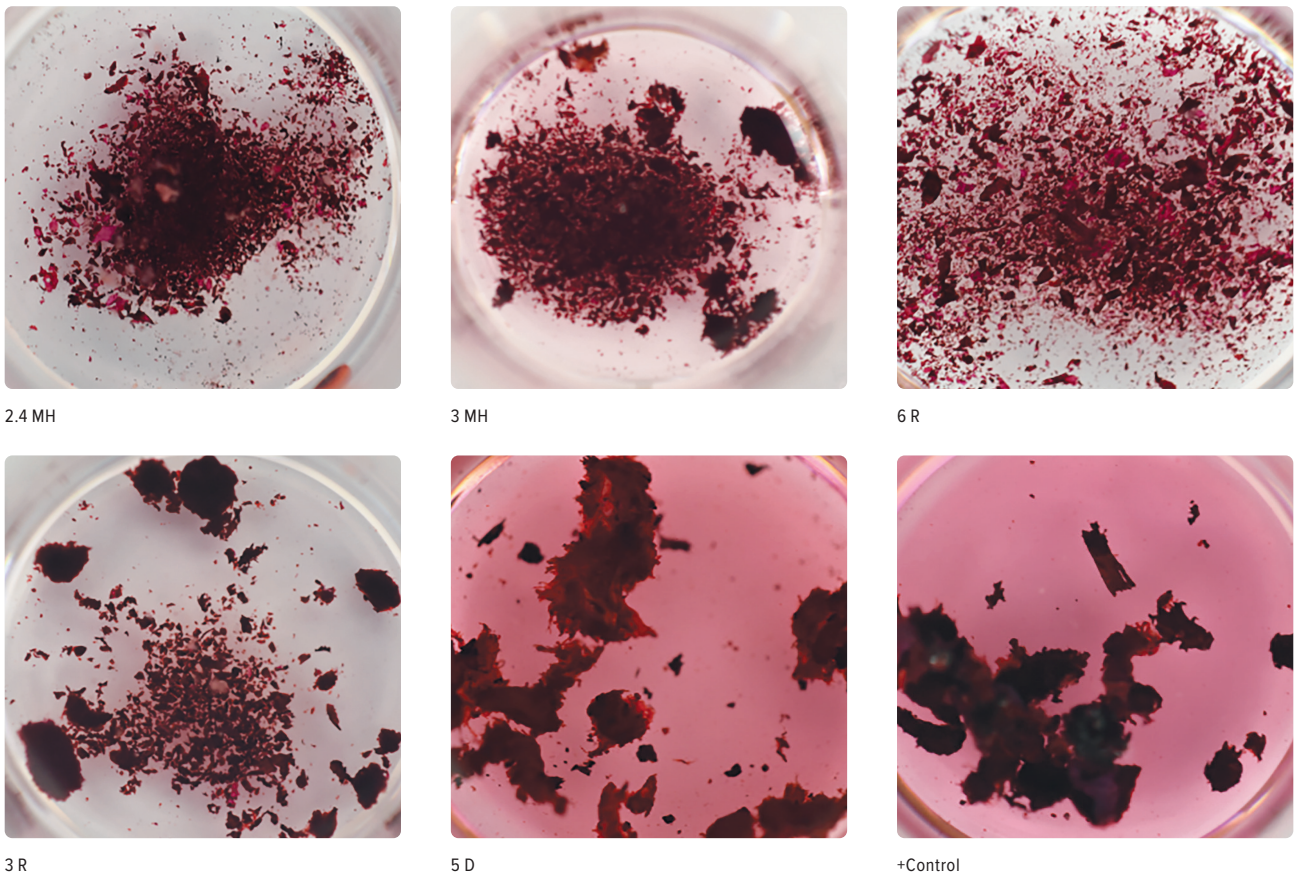


Figure 2. AR staining for calcium deposition in undigested bone samples after 21 days of culture in ODM. Red staining indicated calcium.

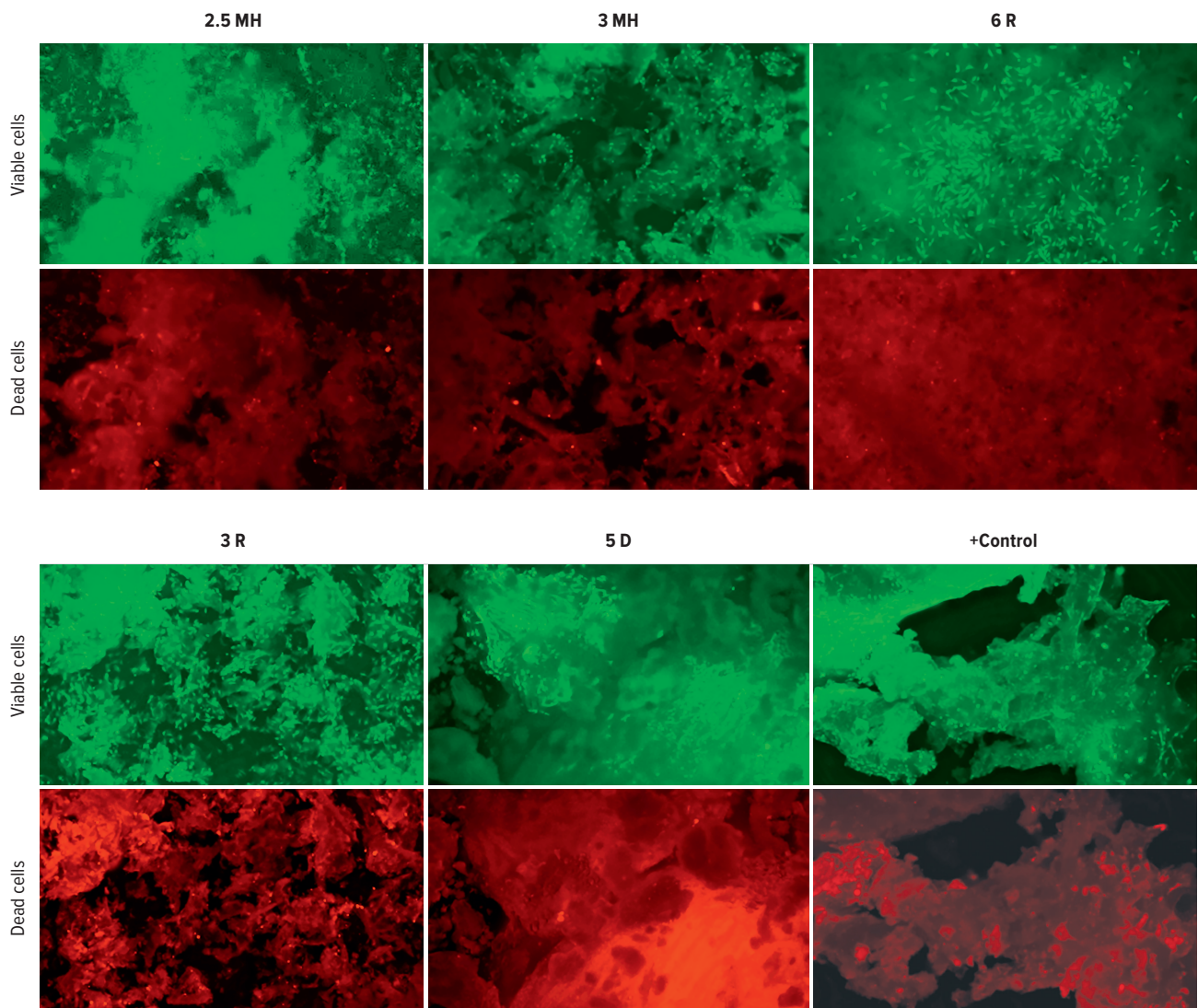
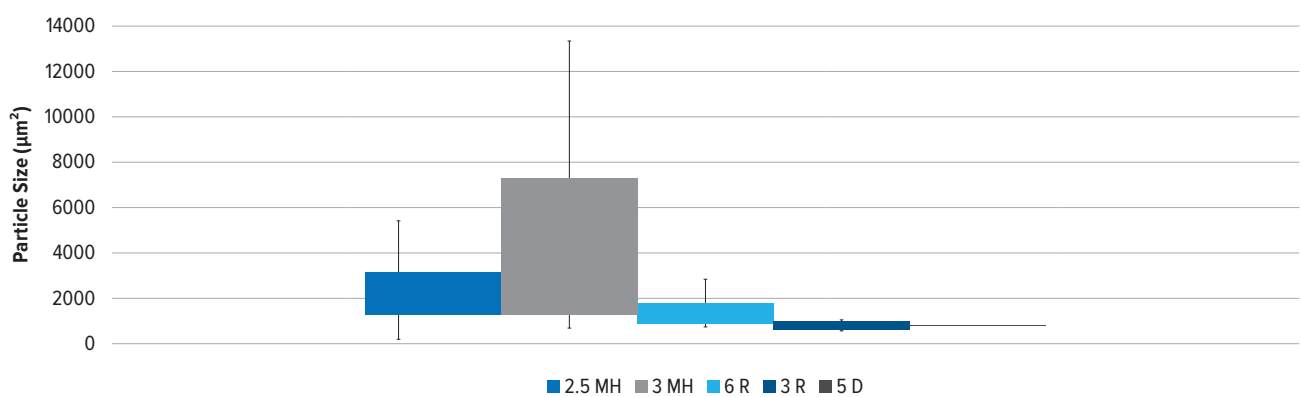


Figure 3. Images from viability analysis show living cells (green) and nonliving cells (red) for undigested bur samples and positive control pieces at 21 days of culture.



	AVG ± SD (µm ²)					AVG ± SD (mm ²)
	2.5 MH	3 MH	6 R	3 R	5 D	+Control
	2120 ± 1316	4106 ± 3607	1222 ± 564	716 ± 195	702 ± 5	14 ± 11

Figure 4. Box and whisker plot representing surface area of bone particulate obtained from each bur with outliers removed. 3 R and 5 D have low sample size (for reference only).

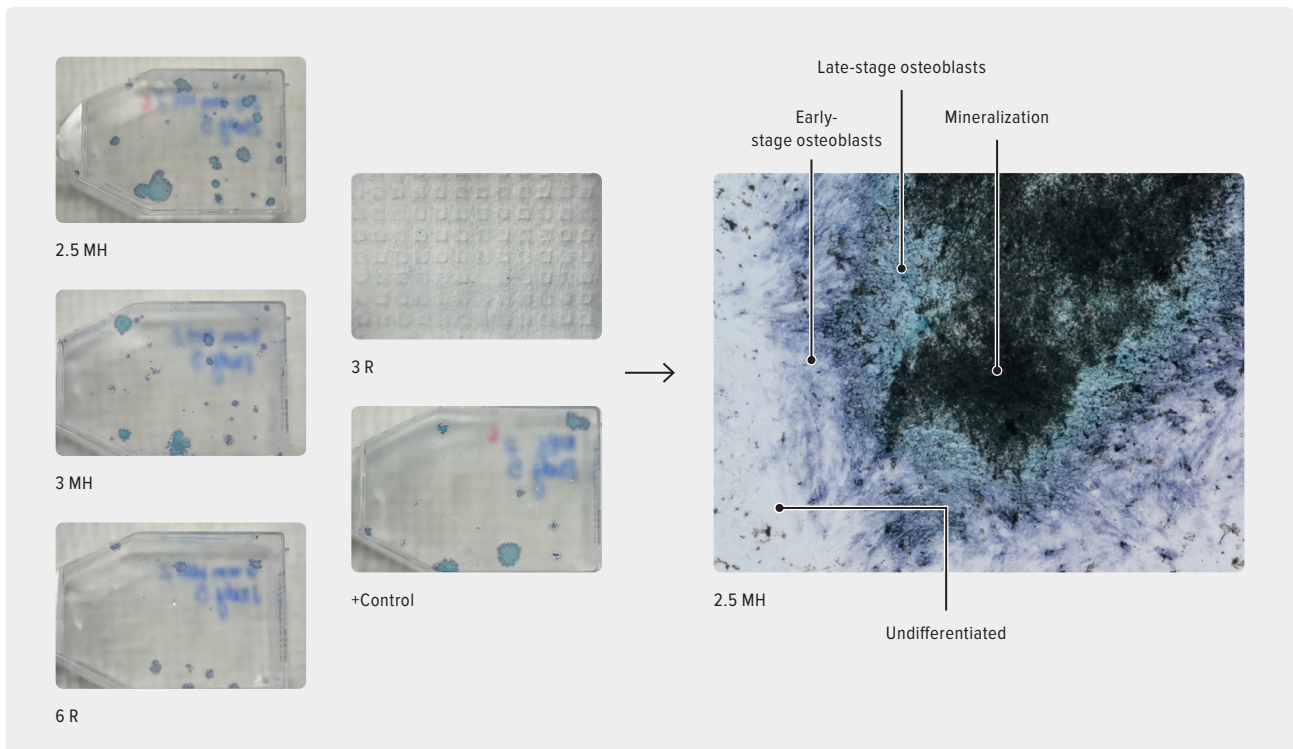


Figure 5. Colony-forming units staining for CFU-ALP after 14-day culture of digested sample in ODM.

DISCUSSION AND CONCLUSION

ALP activity increased throughout the 21-day period, which indicates increased osteoblast activity (Figure 1).³ After 21 days in ODM, the samples stained with AR showed dark red staining for calcium deposition within the bone matrix (Figure 2). After 21 days of culture, it could be demonstrated that undigested samples from each bur group resulted in viable cells (Figure 3).

Samples from the 3 MH group have the largest particulate size on average, followed by the 2.5 MH and 6 R groups (Figure 4). Match-head designs produce particles with greater variation in size and result in larger particulates on average compared to the 6.0 mm round cutting design. This difference may be due to the multi-fluted tip of the round bur, which minces the tissue, whereas the match head functions with a shaving mechanism.

Digested samples from the 2.5 MH, 3 MH, 6 R, and control groups stained positively for ALP. All samples that stained for CFU-ALP demonstrated osteoblastic growth and osteogenesis via mineralization (Figure 5).

The 3 MH group had the most positive staining for ALP and CFU-ALP, at an average count of 100 ± 105 . The positive control group had an average count of 67 ± 34 . Samples from the 3 MH and 6 R groups had similar average counts, at 42 ± 70 and 50 ± 52 respectively.

The data suggests that bone particulate collected by variable burs can contain viable cells and result in osteoblastic outgrowth that can promote osteogenesis. Limitations include sample size variation due to limited bur availability and reduced sample volume due to tissue damage from the dull tip design of the diamond bur. The drilling process is also inherently disruptive to tissue, and bone particulate tends to congeal, preventing particle size analysis in some cases; however, this pilot study suggests that autologous bone particulate could be used as a source of viable autologous osteoblasts and osteoprogenitors to facilitate bone healing.⁴

References

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