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Review Article

# Osteogenic Differentiation of Bone Marrow-Derived Mesenchymal Stromal Cells on Demineralized Bone Scaffolds: A Review

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## Abstract

**Background:** Demineralized bone matrix (DBM) is the only widely used allograft that retains osteoinductive activity after processing, but contains no living cells. Seeding it with bone marrow-derived mesenchymal stromal cells (BM-MSCs) is the most direct route to a living osteogenic construct.

**Objective:** To review how demineralized bone scaffolds influence osteogenic commitment of human BM-MSCs, and what determines whether it is reproducible.

**Findings:** Residual calcium near 2% of dry weight is repeatedly optimal, and partially demineralized scaffolds outperform both fully demineralized and untreated bone — consistent with the 25-40 kPa stiffness window for osteogenesis. Against this, fusion rates across seven commercial DBM products in one rodent model ranged from 0% to 100%.

**Conclusions:** Partial rather than complete demineralization is the more favourable preparation. Translation is limited less by the biology than by lot-to-lot variability and the lack of a validated potency assay.

**Keywords:** mesenchymal stromal cells; demineralized bone matrix; osteogenic differentiation; osteoinduction; bone scaffold

## Introduction

Autogenous iliac crest remains the reference graft because it supplies osteogenic cells, an osteoconductive lattice and osteoinductive signal in one material, but harvest carries donor-site morbidity and the volume is finite. Among the alternatives, demineralized bone matrix is the only widely used allograft that retains genuine osteoinductive activity after processing.

That activity was first shown by Urist, who found that bone matrix decalcified in hydrochloric acid and implanted into rodent muscle induced new bone where none would otherwise form [1]. Acid extraction removes the mineral phase while leaving a collagen type I network and non-collagenous proteins, among them bone morphogenetic proteins liberated from mineral sequestration. The material is therefore osteoconductive and osteoinductive but not osteogenic: it contains no living cells. Bone marrow-derived mesenchymal stromal cells, defined by the standard identity criteria [2], supply the missing component. The combination has been studied for three decades, yet results are less consistent than the rationale predicts.

## Materials and Methods

Literature was retrieved from PubMed, Europe PMC and Crossref for the terms *demineralized bone matrix*, *mesenchymal stromal cell*, *osteogenic differentiation* and *residual calcium*, with priority to studies reporting human BM-MSCs on demineralized bone with quantified endpoints. Every cited record was confirmed against Crossref, OpenAlex or Europe PMC.

## Demineralization and the residual mineral window

Demineralization is conventionally performed in 0.5-0.6 N hydrochloric acid, a compromise rather than an optimum. Rate constants of 0.811, 1.655 and 2.558 h  at 0.6 M, 1.2 M and 2.4 M [3] show the rate rising with concentration but not proportionally, so higher acid buys progressively less speed while raising the risk of protein denaturation.

How much mineral should remain is the central question, and the literature converges on a narrow answer. Zhang and colleagues reported approximately 2% residual calcium as optimal, with both under- and over-demineralized preparations worse [4]. Turonis and colleagues tested this in a critical-sized rat calvarial defect against allograft at 1%, 2%, 3-6% and 23% residual calcium; the 2% group showed significantly greater bone fill at twelve weeks than every other group [5]. Commercial DBM retains 1-6% [6], so products sit across rather than within the optimum. Measurement compounds this: against neutron activation analysis, titrimetry recovered only 21% of the true calcium value and atomic absorption 61% [7], so a laboratory reporting 2% by titration may hold several times that.

## Scaffold mechanics

Bone demineralized only at its surface retains a mineralized core, and the resulting stiffness matters more than the liberated matrix proteins alone. Mauney and colleagues cultured human bone marrow stromal cells on partially demineralized scaffolds and observed elevated alkaline phosphatase activity, osteocalcin and later bone sialoprotein, with the cuboidal morphology of mature osteoblasts against fibroblastic morphology in controls [8]; a companion study established partial demineralization as the more favourable preparation [9].

Architecture acts alongside stiffness. Pore size and interconnection govern nutrient transport, cell infiltration and vascular ingrowth, and the porosity required for bone formation is higher than that needed for the scaffold to survive handling [10]. Scaffold density behaves the same way, with an optimum rather than a monotonic effect: across three densities of decellularized bone, the intermediate one produced both the highest cell density and the most new matrix, a trade-off between transport,

infiltration space and mechanical competence [11]. Retained mineral increased bone-marker expression in the same study, which is an independent line of evidence that full demineralization is not the optimum.

This aligns with matrix elasticity as a lineage cue. Engler and colleagues showed soft matrices of 0.1-1 kPa favouring neurogenic commitment, 8-17 kPa myogenic and rigid bone-like matrices of 25-40 kPa osteogenic commitment, with myosin II blockade abolishing the effect [12]. In three dimensions the window shifts down, osteogenesis predominating at 11-30 kPa and governed by integrin-mediated traction rather than cell shape [13]. Complete demineralization moves a scaffold below this range; a retained mineral core keeps it within.

## Endpoints and what they measure

Several endpoints used to declare differentiation fail in a direction that inflates positive findings. The transcriptional cascade is well established — RUNX2 is required at all stages, osterix/SP7 acts downstream, and BMP signals through SMAD1/5/8 onto the same genes [14] — so marker expression reports position in a sequence, provided the time points capture it: RUNX2 peaks at days 9-14 while COL1A1 rises to day 9 then falls [15].

Mineralization assays are less trustworthy. Alizarin red S and von Kossa staining detect calcium and phosphate deposits but cannot distinguish bone-specific hydroxyapatite from dystrophic mineral, so cultures incapable of osteogenic differentiation can stain positive, particularly at β-glycerophosphate concentrations at or above 2 mM [16]. Bonewald and colleagues showed this directly: three cell types all stained von Kossa-positive under some conditions, yet Fourier-transform infrared spectroscopy found no calcium phosphate mineral in MC3T3 cultures at all [17]. Where mineral must be quantified, the alizarin red extraction assay is linear between roughly 50 µM and 2 mM and about threefold more sensitive than cetylpyridinium chloride extraction [18] — better precision on a signal whose specificity remains the problem.

Culture format matters independently: calcium deposition in three-dimensional collagen sponges runs roughly fourfold higher than in matched monolayers [15].

## Donor, lot and processing variability

The most consequential finding here is how much of the observed variation has nothing to do with the biology under study. Bone morphogenetic protein content differs between commercial DBM brands and between lots of the same brand [19]. The clearest demonstration is in vivo: seven commercial fiber and putty products in an athymic rat posterolateral fusion model gave fusion rates spanning the entire possible range, from 100% for two fiber products to 0% for two others, correlating inversely with carrier proportion [20]. An earlier comparison in the same model found 78% fusion with Osteofil, 65% with Grafton and 0% with Dynagraft, the last significantly lower than both ($P = 0.0001$) [21]. Carrier composition is not incidental: DBM content ranges from 17-31% in glycerol-based products to 40-86% in calcium sulfate-based ones [6].

**Table 1: In vitro endpoints of osteogenic differentiation, what each measures, and its principal limitation.**

| Endpoint | Measures | Principal limitation | Ref. |
|---|---|---|---|
| Alkaline phosphatase | Early commitment | Peaks and declines; the time point chosen determines the answer | [8,13] |
| Alizarin red S | Calcium deposits | Cannot distinguish hydroxyapatite from dystrophic mineral | [14] |
| Von Kossa | Phosphate deposits | Positive in cultures where FTIR shows no calcium phosphate | [15] |
| Calcium assay | Deposited calcium | Per-well values conflate differentiation with cell number | [13] |
| RUNX2 / SP7 | Lineage commitment | Transient; RUNX2 peaks at days 9–14 | [12] |
| Osteocalcin, COL1A1 | Matrix maturity | Sequence-dependent; COL1A1 falls after day 9 | [12,13] |

| Endpoint | Measures | Principal limitation | Ref. |
|---|---|---|---|
| FTIR / XRD | Mineral identity | Rarely performed; the only specific confirmation | [15] |

Processing is frequently misattributed. Ethylene oxide at 55 °C almost completely extinguishes osteoinductivity while at 40 °C it causes only slight alteration, gamma irradiation at 25 kGy reduces it by roughly 40%, and ethanol has no measurable effect [22] — temperature, not ethylene oxide as such, is the variable. Hydration governs radiation damage: DBM irradiated dry retains activity, whereas water-containing composites lose substantially more at lower doses [23].

## From construct to clinic

Clinical experience with DBM-plus-marrow composites is longer than the tissue-engineering framing suggests. Tiedeman and colleagues reported union in 30 of 39 patients, a 77% success rate [24]; more recently, DBM enriched with concentrated bone marrow aspirate achieved solid posterolateral fusion in 81.3% and interbody fusion in 92.5% of 80 lumbar fusion patients [25]. What separates these results from routine practice is potency standardization. In vitro surrogates such as the C2C12 alkaline phosphatase assay were developed to replace the athymic rodent bioassay [26], but the predictive relationship is not established [27,28].

## Discussion

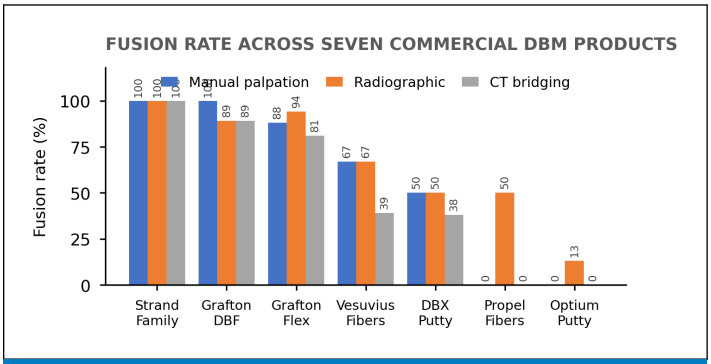


**Figure 1: Fusion rate of seven commercially available demineralized bone matrix products in a rat posterolateral fusion model, by three assessment methods. Data from Russell et al. [17].**

Two conclusions survive. First, partial demineralization outperforms complete demineralization, and two independent lines of evidence converge on it: the direct scaffold comparisons, and the elasticity work showing that osteogenic commitment needs a stiffness fully demineralized collagen cannot provide.

Second, and more limiting, the spread attributable to donor, lot, carrier and processing is comparable to or larger than that attributable to the variables under study. A fusion rate range of 0% to 100% across products sharing a mechanism is not subtle. Until potency is characterized per lot by a validated assay, such studies measure cells and material together without separating them. This is a narrative review without systematic search or risk-of-bias assessment, and the values above are from individual studies rather than pooled estimates.

## Conclusion

Demineralized bone scaffolds support osteogenic differentiation of BM-MSCs, and the evidence favours partial over complete demineralization, with residual calcium near 2% keeping stiffness within the osteogenic range. The biology is not the bottleneck. Reproducibility is limited by lot-to-lot variability, by mineralization assays that cannot distinguish bone-like mineral from dystrophic deposits, and by the absence of a validated potency measure. Studies should state the demineralization endpoint and the assay used.

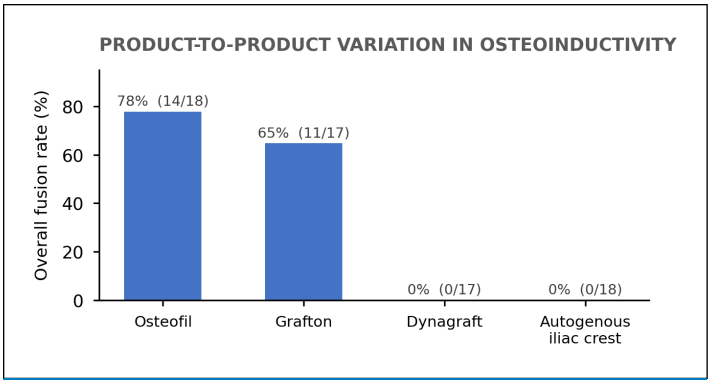


**Figure 2: Overall fusion rate for three commercial DBM preparations and an autogenous iliac crest control in an athymic rat posterolateral fusion model. Data from Wang et al. [18].**

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## Conflict of Interest

The authors declare that they have no competing interests relevant to the content of this review.

## Ethics Statement

This article reviews previously published studies and contains no new studies with human participants or animals performed by any of the authors. Ethical approval was therefore not required.

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