Production and characterization of a decellularized human bone scaffold for skeletal reconstruction

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Objective

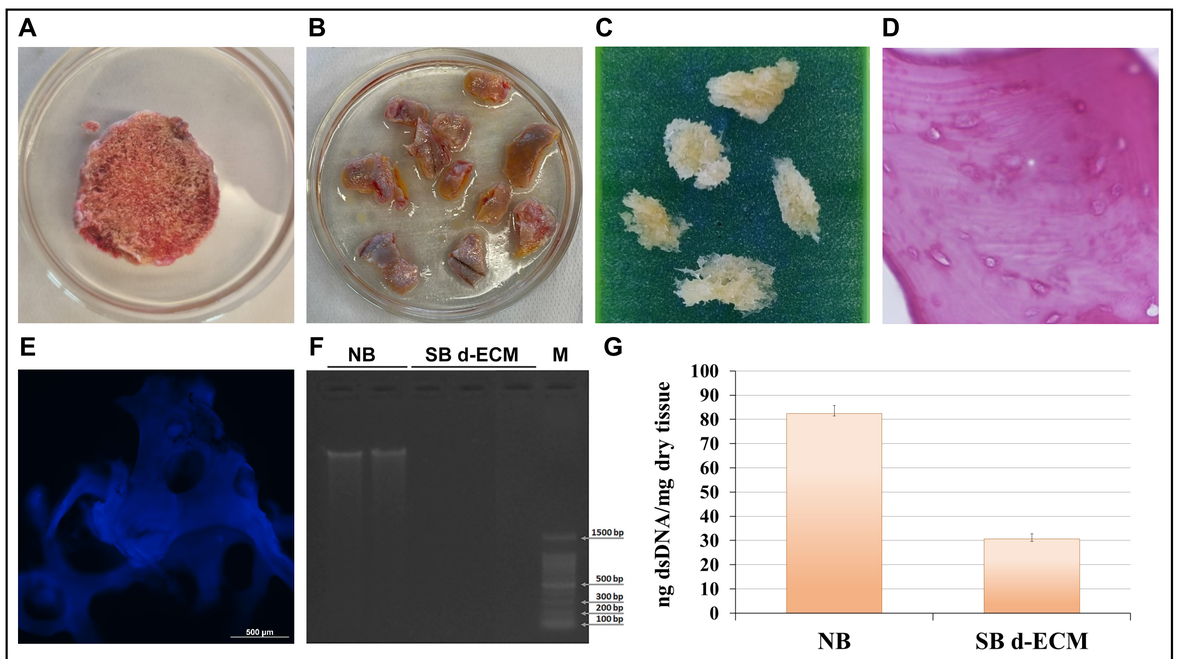
The extracellular matrix (ECM) provides structural support and regulates cellular behavior. Bone ECM is the noncellular material that represents 90–95% of bone volume, surrounds cells, provides strength and rigidity, and is responsible for its three-dimensional architecture. Although reproducing the biological and structural characteristics of bone ECM is crucial for bone engineering for regenerative purposes, synthetic substitutes that mimic the complexity of native bone ECM have not yet been developed. To this end, natural scaffolds derived from native tissue represent a promising strategy for skeletal regenerative medicine. In this study, we propose an effective and highly reproducible method for producing decellularized ECM (d-ECM) from trabecular bone for tissue engineering, regenerative medicine, and orthopedic research applications.

Methodology

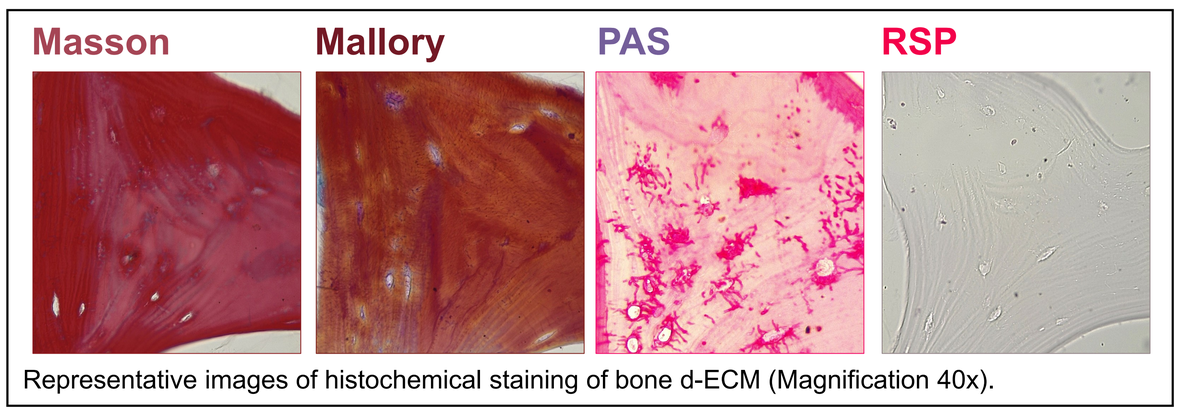
Bone fragments obtained from humeral heads of patients undergoing orthopedic surgery (n = 8) were decalcified in 14% EDTA solution, trimmed to fit embedding cassettes, and decellularized under constant agitation using a combination of SDS, Triton X-100, and antibiotics. The efficacy of the decellularization procedure was assessed by histological analysis and quantitative measurement of residual dsDNA content, while d-ECM integrity was assessed by histochemistry, immunohistochemistry, and quantitative assays. In vitro cytocompatibility was assessed by measuring cell viability in the Trypan Blue exclusion assay on human Adipose-Derived MSCs (hADMSCs) obtained from patients undergoing abdominoplasty (n = 16) and mouse MSCs (mMSCs, MC3T3-E1 cells) (n = 3) used to repopulate bone d-ECM scaffolds.

Results

The color change from reddish to white was the macroscopic evidence of the thorough decellularization. The absence of nuclei in H&E staining and a residual dsDNA content below the accepted standards of 50 ng/mg of dry tissue (30.6 ± 2.14 ng/mg) confirmed the effectiveness of the decellularization procedure.

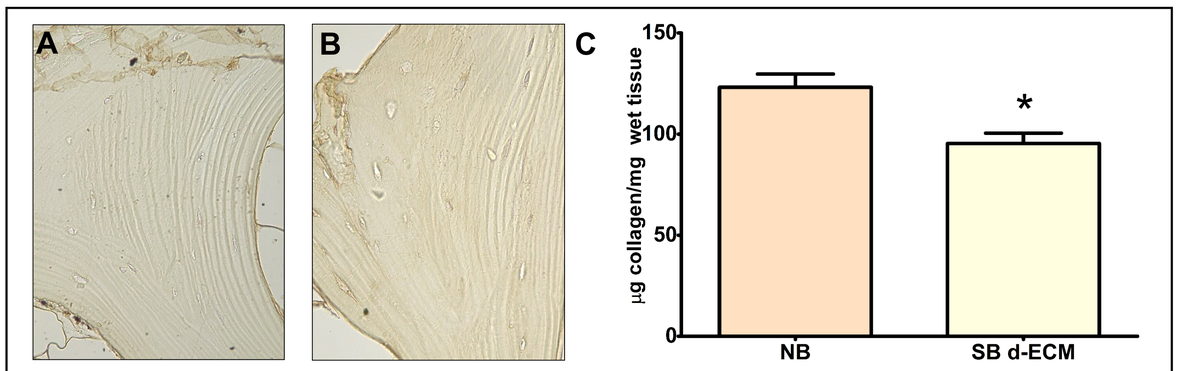


Human humeral head specimen (A) and bone fragments before (B) and after (C) the decellularization, as evaluated macroscopically (B and C) and microscopically (D, magnification 40x). DAPI staining showing the absence of nuclei in bone d-ECM scaffold (E, scale bar = 500 µm), agarose gel electrophoresis (F) and graphical representation (G) of dsDNA quantification in native (NB) and decellularized bone (SB d-ECM) (n = 5, M = Marker).



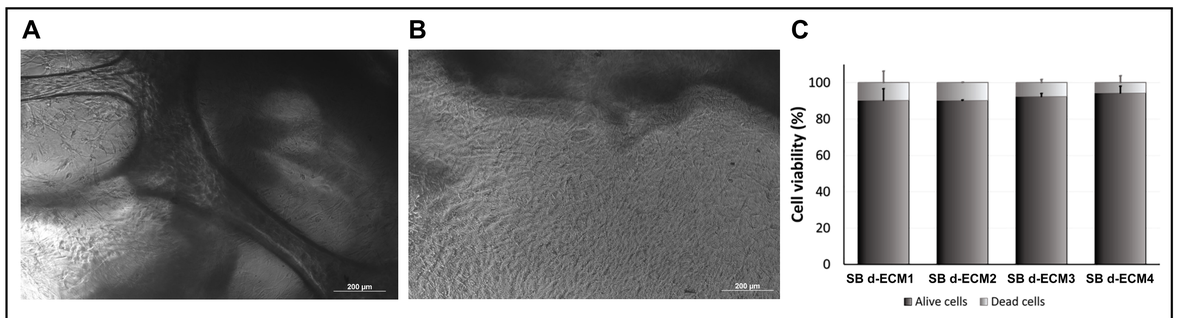
Representative images of histochemical staining of bone d-ECM (Magnification 40x).

Histochemical and immunohistochemical analysis was performed to analyze ECM composition and architecture, revealing that the decellularization did not affect the 3D architecture of d-ECM. Masson's, Mallory's and Sirius Red staining documented the collagen preservation while Periodic Acid Schiff showed partial retention of non-collagenous proteins. The presence of the main components of bone ECM, namely fibronectin and collagen I, was further confirmed by immunohistochemistry. Collagen content was also quantified by dye-binding specific assay that confirmed the retention of the insoluble collagen in bone ECM.

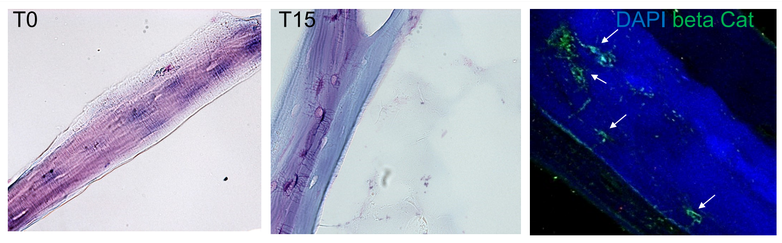


Representative images of immunodetection and distribution of fibronectin (A) and type I collagen (B) in bone d-ECM by immunohistochemistry (Magnification 40x). Collagen content in native and decellularized bone, quantified by specific dye-binding assay (C) (n = 4).

Finally, to assess the cytocompatibility of the bone d-ECM, hADMSC and mouse mMSCs were seeded on scaffolds and cultured for 15 days. Cell viability rate, as evaluated by trypan blue exclusion test, exceeded 90%.



Representative images of hADMSC (A and B) on SB d-ECM. hADMSCs on SB d-ECM viability, as measured by Trypan blue exclusion test (C) (n = 4).



May-Grunwald-Giemsa staining showing the SB d-ECM at T0 and the recellularization after 15 days of culture of mMSCs. Cells showed a stellate morphology typical of active osteoblastic cells after 15 days and immunofluorescence for β-Catenin validated the cell engraftment (white arrows).

Conclusion

Overall, our results support the efficacy of the proposed decellularization protocol in producing a biocompatible and highly preserved d-ECM of human trabecular bone, with the potential for exploitation in bone reconstruction and regeneration. Further studies are needed to evaluate mechanical properties and osteogenic differentiation.