

Comparison of Bone and Periosteal Extracellular Matrix Processing for the Development of Osteoregenerative Biomaterials

Giovanna Pires Marzola

João Paulo Ruiz Lucio de Lima Parra (Laboratory of Regenerative Medicine; University of Marília;
Gabrielly Pires Marzola (Laboratory of Regenerative Medicine; University of Marília and School of
Medicine; University of Marília); Rodrigo Paolo Flores Abuna (Laboratory of Regenerative Medicine;
University of Marília)

RESUMO

Introduction: Decellularized extracellular matrix (dECM) has been widely explored in tissue bioengineering because it preserves structural and bioactive components involved in cell adhesion, migration, proliferation, and differentiation. In bone regeneration, both bone tissue and periosteum are relevant ECM sources; however, their structural composition strongly influences processing requirements. Bone tissue is highly mineralized, rigid, and rich in type I collagen, requiring prior decalcification before downstream processing. In contrast, the periosteum is a specialized connective tissue associated with bone repair, composed of a fibrous layer and a cambial layer related to osteogenic progenitors, vascularization, and regenerative signaling. We hypothesized that periosteal ECM would be more efficiently processed than bone ECM and would present biological advantages for future osteoregenerative biomaterial development. **Objective:** This study aimed to compare bone and periosteal ECM processing by evaluating tissue preparation time, histological preservation, residual genomic DNA content, and the qualitative relative presence of major ECM proteins associated with bone repair and homeostasis. **Methodology:** Bone samples were first decalcified before decellularization using EDTA for approximately three months or nitric acid for approximately two months. After decalcification, bone fragments and periosteal fragments were subjected to a three-day physical-chemical and detergent-based decellularization protocol. Processing efficiency was evaluated by hematoxylin and eosin staining, focusing on nuclear removal and matrix preservation. Residual genomic DNA was quantified and compared between decellularized bone and periosteal matrices using Student's t-test. When applicable, one-way ANOVA followed by Tukey's post hoc test was also performed. Statistical significance was set at $p < 0.05$. In parallel, a literature-based qualitative review was conducted in PubMed using the descriptors "periosteum extracellular matrix," "bone extracellular matrix," "extracellular matrix proteins," and "bone regeneration"; original articles, systematic reviews, and meta-analyses published between 1993 and 2025 were included. **Results/Discussion:** Bone tissue required prolonged decalcification before decellularization, reflecting its mineralized composition and structural rigidity. In contrast, periosteal tissue did not require decalcification and was directly processed through the three-day decellularization protocol. Hematoxylin and eosin analysis showed marked cellular reduction in both tissues after decellularization. However, periosteum showed more efficient processing, preserved connective matrix organization, and lower residual genomic DNA content compared with decellularized bone tissue. The literature-based analysis indicated that both tissues share important ECM components, including type I collagen, fibronectin, biglycan, decorin, BMPs, and TGF- β . However, periosteal ECM is relatively enriched in periostin, tenascin-C, type III collagen, and type VI collagen, supporting roles in stem-cell niche regulation, mechanosensing, immune modulation, and rapid repair. Bone ECM is characterized by high mineralization, abundant type I collagen, SIBLING proteins such as BSP, osteopontin, DMP1, DSPP, and MEPE, as well as osteonectin/SPARC and osteocalcin, supporting mineral homeostasis, remodeling, and load-bearing function. **Conclusion:** The periosteum demonstrated methodological and biological advantages over bone tissue, including the ability to bypass prolonged decalcification, efficient genomic DNA removal, and preservation of a connective

matrix associated with regenerative signaling. These findings support periosteum-derived dECM as a promising source for future injectable or moldable osteoregenerative biomaterials.

Palavras-chave: Decellularized Extracellular Matrix; Periosteum; Bone Tissue; Genomic DNA; Biomaterial.