Matrix vesicle-embedded biopolymeric scaffolds: a model for in vivo bone mineralization studies

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Bone mineralization is a highly orchestrated and dynamic process that has been extensively studied from a structural, chemical, and biological point-of-view. The bone tissue can be described by the action of mineralization-competent cells embedded in an extracellular matrix (ECM) composed mainly by collagen and other non-collagenous macromolecules. Those cells release a special class of extracellular vesicles, named matrix vesicles (MVs) which are the responsible for the deposition of an inorganic phase called biological apatite on collagen fibrils, giving rise to the mineralized tissue. Biomimetic materials able to reproduce the complexity of the bone tissue represent an outstanding strategy for both the treatment of bone defects caused by trauma or diseases and biomineralization studies. In this sense, the built of 3D-biopolymeric scaffolds embedded with MVs offers alternative strategies to develop cell-free therapies. Moreover, the use of collagen in the production of mineralized biomimetic matrices replicates the microenvironment of the native tissue. In the present study, self-assembled 3D-matrices were obtained by slow evaporation of high concentrated type I collagen solutions. Induction of fibrillogenesis concomitant with the stabilization of the supramolecular order was then obtained by exposure to $NH_{3(g)}$ for 24 hours. Scanning electron microscopy revealed the organization of superficially uniform fibrils compacted and intertwined in a dense network. Side view images revealed a parallel alignment of collagen fibrils, which is characteristic of arrangements in dense connective tissues. The incorporation of non-collagenous macromolecules, such as -carrageenan, that mimics the structure of sulphated glycosaminoglycans, to the collagen matrices result in a significant change in the surface morphology characterized by a highly rugged irregular and non-periodic pattern. 3D osteoblasts cultures revealed the potential of the scaffolds in increase the differentiation and mineralization of pre-osteoblastic MC3T3 cells, as revealed by increased alkaline phosphatase activity and enhanced expression of RUNX-2 and OCP genes. MVs previously isolated from the cells were also adhered to the biomimetic matrix, simulating the process observed in vivo. Thus, reproducing the condensed state of collagen fibrils embedded with MVs in vitro is a suitable method to design mimetic matrices inspired by the native bone ECM.