

Early differentiating osteoclast interactions with a well suitable bone-like composite

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Osteoclasts, as well as preosteoclasts, show different adhesion features in relation to the substrate on which cells are grown, i.e. the formation of either podosomes belt or sealing zones. Podosomes belt forms on non bone substrates, i.e. when cells interact with glass coverslips or culture plates, whereas sealing zones form when cells grow on bone-like substrates. Podosomes belt corresponds to numerous F-actin columns arranged at the cell periphery, whereas the sealing zone could be defined as a unique large band of actin [1]. In the study of bone resorption mechanisms, the employ of bone slices is not perfectly suitable to investigate actin rearrangement due to cell-extracellular matrix (ECM) interaction, since it doesn't allow to obtain high quality preparations to be examined both by light and electronmicroscopy (TEM and SEM). In particular, TEM preparation requires demineralization which could influence the chemical properties of either bone slices or bone-like composites. Moreover, the use of bone slices as scaffold, although extensive, doesn't allow ultrastructural details that are necessary in the study of mineral resorption by monocytes or preosteoclasts [2,3]. The aim of the present study was to set up an experimental model for the study of cell-ECM interaction between either monocytes or early differentiating osteoclasts and a mineralized ECM. RAW 264.7 cells (a monocyte-macrophage cell line that can differentiate in osteoclasts) were cultured on a composite constituted by calcium phosphate and type I collagen to investigate actin polymerization and podosome formation. This bone-like composite doesn't present the mechanical bone properties, but it is constituted by the main bone components and exhibits the advantage that collagen glues the mineral phase in clusters that can be either added to cell cultures or applied on coverslips, as well as to the culture medium. Light and fluorescence microscopy, as well as TEM and SEM techniques were employed. Results showed that the use of this bone-like composite allowed to obtain useful morphological information about the resorption activity of RAW 264.7 cell line differentiating towards the osteoclastic phenotype.

References

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