Citotoxicity assay of gelatin/chitosan and hydroxyapatite membranes obtained by in situ precipitation

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**Resumo:**
Guided Tissue Regeneration (GTR) uses physical barriers typically constituted of polymeric membranes. Among them, gelatin (G) and chitosan (CS) are interesting for this purpose, due to their biodegradability and high similarity with adjacent tissues. The incorporation of hydroxyapatite (HA) in the polymeric matrix aims to induce osteogenesis on the damaged region, in addition to grant mechanical resistance to the formed composites. In this work, the membranes were produced by an in situ precipitation technique of Ca(OH)2 and H3PO4 to form HA directly in an aqueous solution of gelatin. Thus, the formation of HA in the polymeric matrix is homogeneous, lowering possible inflammations or surgical failure. The amount of CS was varied, forming composites with 20/80, 50/50 and 80/20 of G/CS and 0.3M of HA. Lastly, crosslinking steps with sodium tripolyphosphate (TPP) and glutaraldehyde (GTA) were performed. The composites were characterized by Fourier Transform Infrared Spectroscopy (FTIR) and X-ray Diffraction (XRD) and subjected to swelling essay in a phosphate buffer saline solution (PBS). Furthermore, cytotoxicity assays were carried out using the Neutral Red (NR) incorporation method. The FTIR spectra show good agreement amongst all membranes and through the XRD analysis, HA and beta-TCP (tricalcium phosphate) peaks were identified. The swelling essay pointed out that the crosslinked membranes with GTA exhibited higher resistance to the aqueous medium; however the cytotoxicity assay showed that the membranes crosslinked with TPP had better cellular viability. In conclusion, the in situ precipitation method was efficient to produce membranes of G/CS with HA with reasonable results concerning cellular viability to the membranes crosslinked with TPP, evincing its use in GTR.