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Synthesis of hydroxypatite-based bioceramics using a mechanochemical-thermal approach and biocompatibility testing

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The aim of our work was to prepare a hydroxyapatite (HA)-based bioceramic material in powder form via mechano-chemical synthesis, using eggshells as a source of calcium. The products were characterized using X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and thermal analysis (TG/DTA). The biocompatibility of the synthesized samples was subsequently tested *in vivo* using a chicken embryo (*Gallus gallus domesticus*) model, during which we observed morphological and developmental responses to the presence of the material. We used the CHEST II methodology to assess embryotoxicity and teratogenic potential. We used 32 fertilized hatching eggs, divided into 4 groups. The first group was the control group, without the application of HA or saline (n = 9); the second group was the control group, with the application of 100 µl of saline (n = 6). The third group was the experimental group, with the application of 50 mg of HA (n = 9). The fourth group received 50 mg of hydroxyapatite in the form of a suspension in 100 µl of saline solution (n=8). The combined group allowed us to observe differences in the bioavailability and diffusion behavior of HA when applied in dry form and in suspension form. The test substance was applied to the surface of the embryo, to the intact inner sub-shell layer (*membrana papyracea*), while maintaining the integrity of extraembryonic structures. We used the CHEST II methodology to assess embryotoxicity and teratogenic potential. In the evaluation we monitored parameters such as embryo weight, heart weight, and liver weight, which varied depending on the substance administered. It was found that no significant differences were observed between the individual groups in either total embryo weight or heart weight. However, liver weight increased significantly following the administration of hydroxyapatite in saline, indicating better diffusion and bioavailability of HA in suspension form. We also concluded that the prepared biomaterial in powder form exhibits neither embryotoxicity nor teratogenicity.

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